Headspace Solid Phase Microextraction for the Analysis of Volatiles in a Meat Product: Dry-Cured Iberian Ham

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The application of solid-phase microextraction (SPME) combined with gas chromatography/mass spectrometry for the analysis of dry-cured Iberian ham volatile compounds has been studied. To determine the optimal conditions to extract volatile compounds, several extraction times and temperatures conditions were tested. The majority of the 82 volatile compounds tentatively identified coincided with those reported for different types of dry-cured ham in the literature. In addition, SPME successfully extracted high molecular weight compounds, some of them being reported for the first time in this type of meat product. Both extraction time and temperature dramatically increased less volatile compounds' chromatographic area and consequently total area ($P < 0.0001$).

Keywords: *Dry-cured Iberian ham; solid-phase microextraction (SPME); gas chromatography/mass spectrometry; volatiles*

INTRODUCTION

Dry-cured ham from Iberian pig is an expensive meat product with a first-rate consumer acceptance in Spain. The overall acceptance of dry-cured hams highly depends on their flavor quality, which is mainly determined by taste and odor compounds.

A considerable amount of research has been devoted to characterize the volatile profile of Iberian ham (García et al., 1991; López et al., 1992), Parma ham (Barbieri et al., 1992; Hinrichsen and Pedersen, 1995; Bolzoni et al., 1996), French ham (Berdagué et al., 1991, 1993; Buscailhon et al., 1993), American country-style ham (Piotrowski et al., 1970), and Spanish "Serrano" dry-cured ham (García-Regueiro and Díaz, 1994; Flores et al., 1997). In addition, the biochemical reactions involved in the formation of flavor compounds in Iberian ham have been considered in previous studies (Antequera et al., 1992; Ventanas et al., 1992; Cordoba et al., 1994). All of this quantitative and qualitative information is desired to monitor flavor quality and to provide quality control for processed products.

Various methods such as steam distillation (Lillard and Ayres, 1969), vacuum distillation (Berdagué et al., 1991; García et al., 1991), and the widely used purge and trap method (Barbieri et al., 1992; Berdagué et al., 1993; Buscailhon et al., 1994; Hinrichsen and Pedersen, 1995) have been used to measure the volatile profile of cured hams.

The recently developed absorption technique solid phase microextraction (SPME) (Arthur and Pawliszyn, 1990; Arthur et al., 1992) has been successfully used to analyze the volatile compound composition of different foods and drinks (Xiaogen and Peppard, 1994; Pelusio et al., 1995; Steffen and Pawliszyn, 1996; Chin et al., 1996; Lay-Keow et al., 1996; Elmore et al., 1997; Song et al., 1997). However, its use on meat samples has been reported only for hexanal determination in minced pork (Nielsen et al., 1997). This technique fits the conditions required for quality control analysis in the meat industry because it is solvent-free, cheap, easy to use, and relatively fast. However, extraction conditions using SPME to analyze meat flavor volatiles require further research.

During the analysis of headspace by SPME, two equilibria should be reached by the analytes: between the matrix and the headspace and between the headspace and the coating of the fiber (Zhang and Pawliszyn, 1993). Agitation and heating have been proposed to reduce the equilibration time for less volatile compounds (Zhang and Pawliszyn, 1993). However, when one is working with solid samples, effective stirring is not possible (Chin et al., 1996). In solid samples, therefore, temperature is the main factor in reducing equilibrium time and analysis time. On the other hand, the use of a high temperature for a prolonged time during sampling could increase the formation of thermally generated compounds, showing artifacts in volatile profile. Compounds derived from lipid oxidation processes are the major ones in cured ham headspace (García et al., 1991) and are strongly related to the flavor of meat products (Flores et al., 1997). Therefore, the formation of such artifacts during sampling due to heating should be checked.

The aims of the present study were to evaluate the feasibility of using SPME for the analysis of volatile compounds of dry-cured ham and to assess the influence of extraction time and temperature on the volatile profile of this meat product.

MATERIALS AND METHODS

Dry-Cured hams. Hams were produced using thighs from Iberian pigs fed on concentrate feeds. They were processed according to the traditional prolonged method (Córdoba et al., 1994).

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Sampling. A sample of dry-cured ham (~50 g) was taken from the semimembranosus muscle, vacuum-packaged, and stored frozen for 5 months at −80 °C before analysis. Thin slices of ~1 cm^2 surface and between 0.3 and 1 mm thickness were obtained from the frozen samples using a scalpel the day before analysis; 2.00 g of ham was weighed into a 10 mL (20−21 mm diameter × 56−57 mm height) headspace vial (Hewlett-Packard) and sealed with a PTFE butyl septum (Perkin-Elmer) in an aluminum cap. Samples were kept at −80 °C overnight before analysis.

Headspace SPME. An SPME (Supelco Co., Bellefonte, PA) fiber (10 mm length) coated with poly(dimethylsiloxane) (100 μm thickness) was used to extract the headspace volatiles of dry-cured ham. Prior to analysis the fiber was preconditioned at 220 °C for 45 min in a GC injection port. The SPME fiber was inserted into a sample vial through the septum and then exposed to headspace. The extractions were carried out in an oven to ensure a homogeneous temperature for samples and headspaces. Effects of different extraction temperature (40 and 60 °C) and time (20, 40, and 60 min) conditions were tested. Prior to extraction, samples were equilibrated for 15 min at the same temperature used for extraction. Each temperature × time combination was repeated five times.

Gas Chromatography/Mass Spectrometry (GC/MS). Analyses were performed using a Varian 3400CX gas chromatograph coupled with a Saturn 3D ion-trap mass spectrometer. Volatiles were separated using a 5% phenyl−95% dimethyl polysiloxane column (30 m × 0.25 mm id, 1.0 μm film thickness; Restek). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6 mL min^{-1} at 40 °C. The SPME fiber was desorbed and maintained in the injection port at 220 °C during the whole chromatographic run. The injection port was in a splitless mode. The temperature program was isothermal for 10 min at 40 °C and then raised to 250 °C at a rate of 7 °C min^{-1} and held for 5 min. *n*-Alkanes (Sigma R-8769) were run under the same conditions as the samples to calculate the Kovats index values of the compounds.

The GC/MS transfer line temperature was 270 °C. The mass spectrometer operated in the electron impact mode with an electron energy of 70 eV and a multiplier voltage of 1650V; date were collected at a rate of 1 scan s^{-1} over a range of *m*/*z* 40−300. Compounds were tentatively identified by comparing mass spectral data of volatiles with those of the NIST/EPA/NIH library and by comparing Kovats indices of volatile compounds with those reviewed by others in the literature (Berdague et al., 1991, 1993; Buscailhon et al., 1993; Hinrichsen and Pedersen, 1995; Kondjoyan and Berdagué, 1996; Acree and Arn, 1997).

Statistical Analysis. The effects of extraction time, extraction temperature, and their interaction on the chromatographic area of volatile compounds were studied by an analysis of variance using the General Linear Model procedure (GLM) (Statistical Analysis Systems Institute, 1997). Statistical analyses were performed on results expressed in arbitrary units (peak area). Significant differences among means were separated using Tukey's multiple-range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Headspace Volatile Profile. A total of 82 compounds (Table 1) were tentatively identified using a SPME−GC/MS method. Molecular weights (MW) of compounds tentatively identified ranged from 48 (methanethiol) to 266 (octadecenal). SPME extracted a broader range of MW compounds compared with the MW ranges obtained in other studies on dry-cured ham volatiles using vacuum distillation or purge and trap. Studies carried out using vacuum distillation detected high MW compounds but not compounds with a Kovats index (KI) <700 and a MW <72 (Berdagué and García, 1990; Berdagué et al., 1991; García et al., 1991; Charrier, 1992). This is most likely due to losses of low MW compounds during the concentration step this method involves prior to the injection of the volatiles extracted in the gas chromatograph.

On the other hand, studies carried out using purge and trap hardly detected compounds of >1100−1200 KI, not only in dry-cured ham but in other dry-cured meat products (Barbieri et al., 1992; Charrier, 1992; Berdagué et al., 1993; Buscailhon et al., 1993; Hinrichsen and Pedersen, 1995; Flores et al., 1997).

These observations agree with studies about other food materials, such as fruits, in which SPME extracted high MW compounds in a much shorter time than purge and trap (Matich et al., 1996).

Major compounds (Table 1) such as 2-propanol, acetic acid, a branched pentane, chloroform (a laboratory contamination), 3-methylbutanal, hexanal, the coelution of octanal and trimethylpyrazine, nonanal, diethyl phthalate (a package contamination), tetradecanol, and hexadecanol were basically the same as those found in higher quantities in Serrano ham (Flores et al., 1997), French ham (Berdagué et al., 1993; Buscailhon et al., 1993), or Parma ham (Hinrichsen and Pedersen, 1995), except for the high MW ones.

SPME successfully extracted compounds that several studies have related to dry-cured ham flavor characteristics, such as rancidity (hexanal and 2,4-decadienal) or aged flavor(2- and 3-methylbutanal and 2- and 3-methylbutanoic acids) (Careri et al., 1993; Hinrichsen and Pedersen, 1995; Flores et al., 1997); thus, it appears to be a promising technique for future research on the flavor of dry-cured meat products.

Some of the low volatile compounds identified for the first time in this study could influence the flavor of dry-cured ham: some long-chain alcohols, such as dodecanol, tetradecanol, and hexadecanol, possess waxy and coconut notes (Acree and Arn, 1997); long-chain aldehydes, such as dodecanal or 2-undecenal, possess herbaceous and fruity notes (Badings, 1970); and long chain esters, such as ethyl tetradecanoate and ethyl decanoate, possess ethereal and grape notes (Miranda-Lopez et al., 1992).

Due to the low volatility of these high MW compounds, they should have a limited effect on meat product flavor. However, further research on their influence on dry-cured ham flavor is presently ongoing in a parallel experiment.

Effect of Time and Temperature. A clear time and temperature effect on the chromatographic area of the extracted compounds could be noticed when the chromatograms of the extraction performed at 40 and 60 °C for 20, 40, and 60 min were compared (Figure 1). There was an evident increase in the height of peaks eluted at the end of the chromatographic process with increasing extraction time and temperature, whereas the peaks eluted within the first 15 min showed only slight changes.

When an ANOVA is performed using volatiles as variables, extraction time and temperature and their interaction showed statistical significance in a great number of peaks identified (Table 1). In most compounds the highest values of peak area were found in the samples extracted at 60 °C for 60 min.

Both extraction time and temperature strongly affected total chromatographic area and high MW compounds, such as tetradecanol and hexadecanol: the higher the temperature and the longer the time, the greater the chromatographic area. In addition, both

Table 1. Effect of Time and Temperature on Volatile Compound Areas from Dry-Cured Iberian Ham Using SPME−GC/MS[a]

compound	rel[b]	40 °C			60 °C			temp[c] *P*	time[c] *P*	T × T *P*
		20 min	40 min	60 min	20 min	40 min	60 min			
methanethiol	MS	9.16 (50)^{ab}	10.35 (27)^{ab}	16.52 (30)^{a}	9.32 (18)^{b}	9.64 (47)^{ab}	10.22 (18)^{ab}	0.0768	0.0494	0.1249
2-nitrobutane	MS	91.06 (75)^{ab}	121.59 (73)^{ab}	234.60 (74)^{a}	16.82 (76)^{b}	28.88 (76)^{a}	116.99 (69)^{ab}	0.0133	0.0221	0.8827
2-propanol	MS, KI	502.23 (16)^{ab}	429.58 (32)^{b}	522.55 (20)^{ab}	740.78 (29)^{a}	414.47 (25)^{b}	435.87 (26)^{b}	0.3549	0.0076	0.0280
2-methylpropanal	MS, KI	5.66 (32)^{bc}	4.74 (19)^{c}	5.82 (7)^{bc}	9.09 (29)^{b}	14.55 (11)^{a}	16.90 (16)^{a}	0.0001	0.0003	0.0002
acetic acid	MS, KI	232.56 (15)^{b}	242.30 (8)^{b}	247.54 (9)^{b}	248.01 (6)^{b}	257.87 (10)^{b}	339.67 (8)^{a}	0.0001	0.0001	0.0021
branched pentane	MS	169.88 (22)	193.75 (7)	476.51 (74)	154.17 (12)	138.99 (12)	174.80 (17)	0.2389	0.3495	0.4787
chloroform	MS, KI	307.52 (38)^{ab}	415.62 (33)^{a}	303.81 (22)^{ab}	296.96 (19)^{ab}	182.94 (30)^{b}	364.69 (37)^{ab}	0.1133	0.6969	0.0094
methylcyclopentane	MS, KI	8.27 (18)	8.89 (39)	7.40 (26)	7.55 (15)	6.83 (25)	10.39 (21)	0.9319	0.4811	0.0369
3-methylbutanal	MS, KI	196.83 (9)^{b}	178.30 (22)^{b}	230.31 (17)^{ab}	191.32 (16)^{b}	259.29 (5)^{a}	271.00 (14)^{a}	0.0023	0.0018	0.0170
2-methylbutanal	MS, KI	36.84 (7)^{e}	39.47 (7)^{de}	49.31 (9)^{d}	62.62 (13)^{c}	82.01 (8)^{b}	106.90 (8)^{a}	0.0001	0.0001	0.0001
pentanal	MS, KI	13.98 (60)^{ab}	12.09 (24)^{ab}	19.42 (70)^{ab}	11.06 (17)^{b}	18.28 (38)^{ab}	27.46 (28)^{a}	0.2024	0.0132	0.2700
1-pentanol	MS, KI	15.50 (20)	16.23 (46)	13.32 (20)	13.09 (22)	12.98 (25)	14.66 (22)	0.3500	0.9462	0.4299
dimethyl disulfide	MS, KI	15.77 (14)^{b}	17.23 (25)^{b}	26.21 (20)^{b}	23.76 (14)^{b}	38.50 (20)^{a}	39.20 (20)^{a}	0.0001	0.0001	0.0376
toluene	MS, KI	5.02 (18)^{bc}	6.12 (7)^{abc}	6.60 (16)^{ab}	4.33 (23)^{c}	5.69 (20)^{abc}	7.89 (24)^{a}	0.8924	0.0002	0.1330
2,2-dichloroethanol	MS, KI	16.77 (4)^{b}	17.99 (16)^{b}	17.68 (8)^{b}	19.65 (7)^{ab}	19.96 (14)^{ab}	22.99 (10)^{a}	0.0002	0.0862	0.1967
butanoic acid	MS, KI	11.60 (23)^{c}	12.68 (13)^{bc}	11.01 (15)^{c}	13.09 (8)^{bc}	15.33 (9)^{ab}	17.13 (9)^{a}	0.0001	0.0562	0.0161
octane	MS, KI	27.88 (45)	20.34 (22)	36.41 (53)	22.48 (25)	32.05 (37)	29.30 (19)	0.9485	0.2713	0.1389
hexanal	MS, KI	113.74 (29)^{b}	160.06 (20)^{b}	109.68 (14)^{b}	102.24 (21)^{b}	140.93 (18)^{b}	241.46 (31)^{a}	0.0263	0.0026	0.0003
4-methylpentanenitrile	MS	2.95 (80)	4.12 (5)	3.25 (20)	1.87 (58)	3.01 (33)	3.26 (13)	0.1055	0.0992	0.4945
3-methylbutanoic acid	MS, KI	8.98 (18)^{c}	9.87 (3)^{bc}	13.56 (16)^{a}	8.43 (3)^{c}	12.07 (14)^{ab}	9.66 (12)^{bc}	0.1567	0.0002	0.0002
2-methylbutanoic acid	MS, KI	1.98 (37)^{b}	2.30 (9)^{b}	2.54 (24)^{b}	2.39 (68)^{b}	5.60 (16)^{a}	3.61 (47)^{ab}	0.0006	0.0057	0.0181
m-xylene	MS, KI	3.40 (33)	3.74 (30)	3.69 (31)	2.15 (23)	2.16 (43)	3.65 (10)	0.0087	0.0881	0.1598
p-xylene	MS, KI	14.30 (22)	13.29 (22)	13.07 (52)	11.94 (30)	13.20 (19)	15.41 (29)	0.9807	0.8064	0.4597
propylcyclopentane	MS, KI	3.93 (9)^{b}	4.02 (23)^{b}	4.01 (13)^{b}	3.28 (40)^{b}	4.67 (15)^{b}	6.69 (11)^{a}	0.0061	0.0003	0.0005
2-heptanone	MS, KI	8.60 (7)^{b}	11.29 (10)^{b}	9.33 (3)^{bc}	8.73 (6)^{c}	7.43 (12)^{c}	13.93 (18)^{a}	0.5248	0.0001	0.0001
heptanal	MS, KI	16.95 (7)^{b}	22.81 (12)^{b}	19.36 (7)^{b}	22.60 (20)^{b}	29.68 (11)^{b}	61.43 (26)^{a}	0.0001	0.0001	0.0001
methional	MS, KI	8.59 (7)^{bc}	6.67 (10)^{c}	10.16 (12)^{ab}	9.24 (5)^{b}	11.73 (18)^{a}	11.81 (13)^{a}	0.0001	0.0019	0.0015
2,6-dimethylpyrazine	MS, KI	11.77 (13)^{ab}	13.52 (14)^{ab}	16.06 (16)^{a}	10.03 (35)^{b}	14.26 (9)^{ab}	11.36 (15)^{b}	0.0280	0.0097	0.0387
α-pinene	MS, KI	5.94 (7)^{a}	4.38 (13)^{bc}	5.54 (13)^{ab}	4.25 (18)^{c}	6.56 (9)^{a}	4.18 (15)^{c}	0.2121	0.1065	0.0001
benzaldehyde	MS, KI	17.85 (9)^{d}	16.11 (12)^{d}	19.60 (5)^{d}	27.83 (6)^{c}	35.44 (13)^{b}	44.80 (13)^{a}	0.0001	0.0001	0.0001
dimethyl trisulfide	MS, KI	5.15 (11)^{c}	4.87 (21)^{c}	5.74 (28)^{c}	6.95 (22)^{c}	12.21 (11)^{b}	18.00 (12)^{a}	0.0001	0.0001	0.0001
1-octen-3-ol	MS, KI	30.62 (8)^{b}	37.85 (7)^{b}	33.11 (3)^{b}	27.50 (4)^{b}	31.13 (10)^{b}	68.24 (21)^{a}	0.0011	0.0001	0.0001
4-hydroxybenzenesulfonic acid + 2,3-octanedieone	MS + MS, KI	18.64 (24)^{c}	32.96 (25)^{ab}	20.91 (18)^{bc}	19.58 (27)^{c}	25.02 (21)^{bc}	42.60 (25)^{a}	0.0580	0.0008	0.0002
6-methyl-5-hepten-2-one	MS, KI	13.28 (27)^{ab}	6.50 (21)^{c}	15.45 (31)^{a}	9.19 (22)^{bc}	11.82 (21)^{abc}	7.55 (15)^{c}	0.0449	0.1606	0.0001
2-pentylfuran	MS, KI	8.11 (42)^{b}	10.88 (37)^{b}	12.44 (39)^{ab}	9.50 (31)^{b}	15.33 (35)^{ab}	20.65 (28)^{a}	0.0090	0.0320	0.2580
decane	MS, KI	30.59 (61)	30.79 (74)	35.36 (39)	37.80 (78)	51.12 (64)	38.53 (72)	0.2869	0.8408	0.7405
octanal + trimethylpyrazine	MS, KI	61.77 (10)^{b}	66.66 (16)^{b}	67.92 (5)^{b}	55.85 (17)^{b}	66.12 (10)^{b}	100.71 (28)^{a}	0.0862	0.0009	0.0073
3-carene	MS, KI	4.50 (18)	3.81 (22)	3.73 (44)	3.72 (21)	5.34 (25)	3.50 (39)	0.6874	0.2093	0.0926
limonene	MS, KI	9.07 (34)^{a}	2.89 (46)^{c}	4.96 (50)^{bc}	8.08 (10)^{ab}	9.23 (8)^{a}	4.40 (17)^{c}	0.0223	0.0002	0.0001
isopropyl hexanoate + benzyl alcohol	MS, KI	12.62 (13)	15.81 (15)	14.29 (34)	11.59 (8)	10.74 (9)	13.94 (22)	0.0355	0.2523	0.1195
phenylacetaldehyde	MS, KI	64.78 (19)^{ab}	42.81 (21)^{c}	57.83 (18)^{ab}	59.90 (7)^{b}	77.53 (7)^{a}	80.51 (6)^{a}	0.0001	0.0620	0.0001
5-ethyldihydro-2(3*H*)-furanone	MS	7.31 (12)^{abc}	8.09 (9)^{a}	7.59 (17)^{ab}	5.30 (16)^{c}	5.88 (12)^{bc}	7.16 (20)^{abc}	0.0004	0.0830	0.1296
1-octanol	MS, KI	15.16 (10)^{b}	15.95 (6)^{b}	15.01 (16)^{b}	13.30 (6)^{b}	13.47 (10)^{b}	26.56 (13)^{a}	0.0032	0.0001	0.0001
2,6-dimethyl-3-ethylpyrazine	MS, KI	6.80 (9)^{a}	6.40 (12)^{ab}	7.91 (15)^{a}	7.45 (6)^{a}	7.38 (17)^{a}	4.75 (24)^{b}	0.1521	0.1777	0.0001
2-nonanone	MS, KI	3.88 (14)^{b}	3.84 (7)^{b}	4.34 (18)^{ab}	4.75 (9)^{ab}	4.88 (18)^{ab}	5.63 (16)^{a}	0.0002	0.0668	0.7830
nonanal	MS, KI	139.79 (14)^{b}	187.65 (15)^{b}	150.77 (12)^{b}	157.56 (24)^{b}	164.62 (14)^{b}	318.47 (23)^{a}	0.0007	0.0001	0.0001
phenylethyl alcohol	MS, KI	50.19 (27)	56.75 (58)	101.98 (76)	38.80 (41)	53.50 (25)	68.51 (37)	0.4393	0.2582	0.8232
an acetamide	KI	14.14 (27)^{bc}	13.14 (16)^{c}	23.96 (20)^{ab}	17.48 (22)^{bc}	17.84 (19)^{bc}	30.52 (35)^{a}	0.0236	0.0001	0.8086
2-nonenal	MS, KI	4.54 (35)	6.10 (42)	3.57 (44)	3.33 (29)	4.82 (44)	5.95 (39)	0.9560	0.2320	0.0733
a lactone	MS	17.46 (20)^{bc}	24.93 (25)^{a}	21.32 (3)^{ab}	13.28 (17)^{c}	15.35 (10)^{bc}	18.66 (10)^{abc}	0.0001	0.0041	0.0620

decanal	MS, KI	27.71 (43)^{ab}	29.42 (26)^{ab}	29.92 (64)^{ab}	12.80 (16)^{b}	12.13 (8)^{b}	38.15 (41)^{a}	0.0732	0.0225	0.0416
propyloctanoate	MS	25.56 (5)^{b}	30.85 (16)^{b}	32.51 (11)^{b}	29.99 (9)^{b}	27.91 (9)^{b}	42.59 (13)^{a}	0.0107	0.0001	0.0033
dimethyl tetrasulfide	MS, KI	0.39 (22)	0.75 (32)		0.20 (46)	0.36 (58)	0.92 (76)	0.9918	0.2594	0.8389
2-decenal + 5-butyldihydro-2(3*H*)-furanone	MS, KI + MS	22.74 (15)^{bc}	22.61 (8)^{bc}	28.75 (10)^{ab}	19.09 (15)^{c}	17.56 (12)^{c}	31.83 (22)^{a}	0.1807	0.0001	0.0493
undecanal	MS, KI	4.33 (34)^{bc}	3.23 (34)^{bc}	5.34 (26)^{b}	4.58 (11)^{bc}	2.53 (14)^{c}	8.59 (14)^{a}	0.0266	0.0001	0.0012
an indene	MS	12.21 (6)^{bc}	10.96 (7)^{c}	12.74 (8)^{bc}	18.25 (28)^{a}	16.40 (17)^{ab}	14.63 (12)^{abc}	0.0001	0.3124	0.1695
2,4-decadienal + methyl decanoate	MS, KI	7.91 (26)^{ab}	13.92 (41)^{a}	10.01 (38)^{ab}	4.96 (30)^{b}	6.21 (22)^{b}	8.71 (40)^{ab}	0.0036	0.0563	0.1101
2-undecenal	MS, KI	5.79 (24)^{b}	11.29 (27)^{b}	11.29 (24)^{b}	11.40 (49)^{b}	15.28 (30)^{b}	38.87 (46)^{a}	0.0003	0.0004	0.0048
dihydro-5-pentyl-2(3*H*)-furanone	MS	14.30 (34)^{b}	28.52 (27)^{a}	29.61 (12)^{a}	28.33 (15)^{a}	24.25 (33)^{ab}	35.73 (21)^{a}	0.0281	0.0017	0.0110
ethyl decanoate	MS, KI	3.34 (13)^{c}	5.54 (9)^{bc}	6.69 (12)^{b}	5.18 (29)^{bc}	5.79 (25)^{bc}	10.01 (28)^{a}	0.0030	0.0001	0.0917
dodecanal	MS, KI	2.66 (12)^{b}	3.88 (32)^{b}	3.81 (29)^{b}	3.59 (37)^{b}	4.13 (15)^{b}	10.24 (14)^{a}	0.0001	0.0001	0.0001
propyl decanoate	MS, KI	28.10 (13)^{d}	44.13 (27)^{cd}	60.89 (10)^{bc}	66.28 (19)^{b}	62.27 (10)^{bc}	98.82 (16)^{a}	0.0001	0.0001	0.0671
6,10-dimethyl-(E)5,9-undecadien-2-one	MS	11.75 (12)^{d}	22.02 (15)^{cd}	26.19 (29)^{bc}	19.77 (20)^{cd}	34.55 (26)^{ab}	42.90 (18)^{a}	0.0001	0.0001	0.3070
dimethyl phthalate	MS	13.61 (7)^{c}	14.06 (18)^{c}	19.25 (25)^{bc}	23.92 (32)^{ab}	27.94 (10)^{a}	15.46 (16)^{c}	0.0002	0.1632	0.0002
2,6 bis(1,1-dimethyl)-2,5-cyclohexadiene-1,4-dione	MS, KI	11.55 (13)^{c}	26.14 (13)^{bc}	41.18 (59)^{ab}	32.77 (32)^{abc}	43.24 (29)^{ab}	57.81 (38)^{a}	0.0028	0.0019	0.9320
BHT	MS, KI	2.17 (27)^{b}	3.95 (28)^{b}	4.10 (45)^{b}	5.52 (41)^{ab}	5.80 (20)^{ab}	9.82 (53)^{a}	0.0006	0.0327	0.2456
diethyl phthalate	MS	79.16 (8)	62.73 (19)	134.24 (68)	104.69 (15)	122.88 (5)	90.86 (10)	0.3237	0.4101	0.0186
dodecanol	MS, KI		7.76 (7)^{c}	12.85 (20)^{bc}	18.14 (21)^{bc}	26.11 (14)^{b}	50.99 (30)^{a}	0.0001	0.0001	0.0071
butyl decanoate	MS	1.28 (12)^{ab}	2.43 (32)^{ab}	2.01 (36)^{ab}	2.86 (29)^{ab}	3.87 (55)^{a}	3.67 (37)^{a}	0.0013	0.1274	0.9784
cycloalkene	MS	1.29 (12)^{c}	3.15 (23)^{bc}	3.59 (22)^{bc}	3.88 (39)^{bc}	7.78 (18)^{b}	18.15 (30)^{a}	0.0001	0.0001	0.0001
pentadecanal	MS, KI	0.74 (35)^{b}	2.92 (38)^{b}	4.53 (23)^{b}	18.33 (17)^{b}	40.27 (24)^{b}	142.92 (40)^{a}	0.0001	0.0001	0.0001
2,4-diphenyl-4-methyl-2(*Z*)pentene	MS	1.31 (16)^{d}	2.14 (27)^{cd}	1.90 (35)^{cd}	4.17 (10)^{bc}	6.98 (21)^{a}	5.96 (43)^{ab}	0.0001	0.0122	0.2320
ethyl tetradecanoate	MS, KI	ND[d]	ND	ND	ND	ND	7.61 (24)			
tetradecanol	MS, KI	75.85 (39)^{c}	189.47 (28)^{c}	513.10 (11)^{c}	3555.41 (16)^{bc}	7623.68 (25)^{b}	15475.63 (39)^{a}	0.0001	0.0001	0.0002
hexadecanonitrile	MS	ND	ND	ND	ND	ND	12.35 (13)			
octadecenal	MS, KI	7.47 (16)^{b}	8.47 (30)^{b}	17.35 (46)^{b}	31.42 (22)^{b}	70.87 (18)^{b}	311.44 (51)^{a}	0.0001	0.0001	0.0002
hexadecanol	MS	1.15 (24)^{b}	0.95 (37)^{b}	7.10 (19)^{b}	103.17 (38)^{b}	374.76 (28)^{b}	1374.26 (54)^{a}	0.0001	0.0003	0.0003
total area		2764.21 (8)^{c}	3148.80 (11)^{c}	4081.98 (19)^{c}	6698.65 (11)^{bc}	11011.90 (17)^{b}	21261.11 (31)^{a}	0.0001	0.0001	0.0001

[a] Values are means (area counts × 1000) of five analyses with percentage coefficient of variance shown in parentheses. Means with different superscripts within the same row are significantly different ($P < 0.05$). [b] MS, mass spectrum tentatively identified using NIST, EPA, NDH library; KI, Kovats index in agreement with literature values. [c] *P* values for the different studied factors (extraction time and temperature and their interaction). [d] ND, not detected.

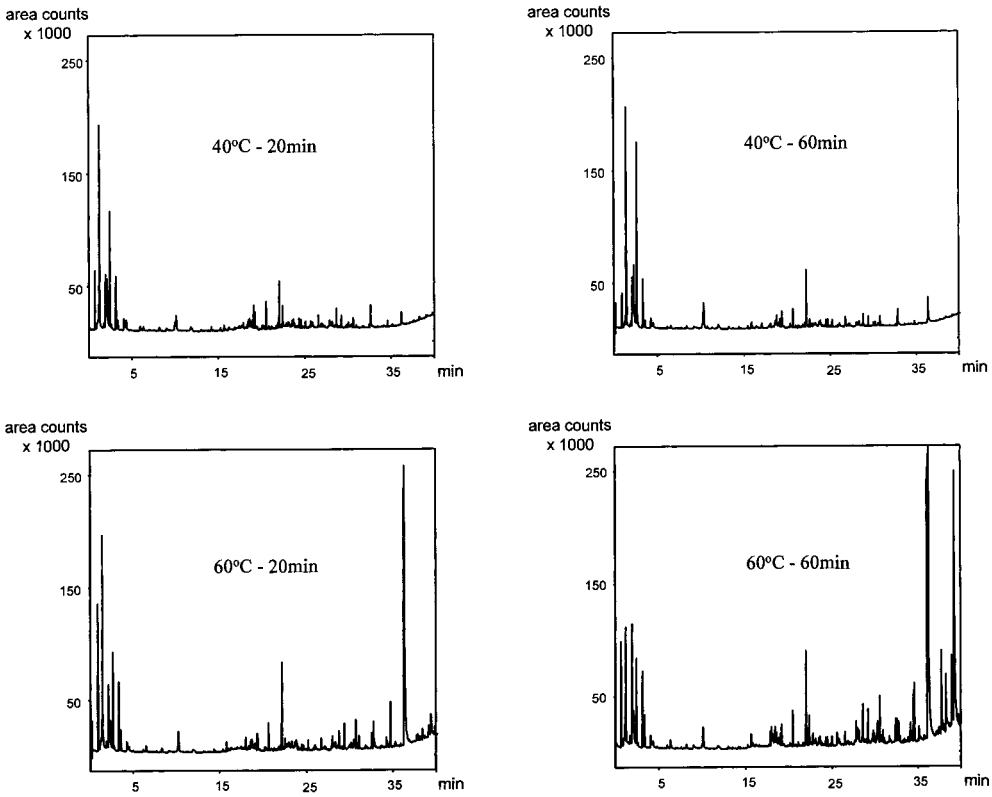

Figure 1. Comparison of chromatograms obtained by SPME−GC/MS at different extraction temperatures and times.

effects showed a significant interaction (Table 1): the higher the extraction temperature, the higher the extraction time effect, as can be observed in Figure 2. However, the effect of both factors on the most volatile compounds (e.g., 3-methylbutanal) was not as marked. Moreover, two compounds (ethyl tetradecanoate and hexadecanenitrile) were detected on the samples when the extraction was performed at 60 °C for 60 min but not in the other conditions tested. These data suggest that less volatile compounds need a longer time to achieve equilibrium. Nilsson et al. (1996) studied volatile metabolites emitted by *Penicillium* species and concluded that in polidimethylsiloxane fibers 30 min was enough to reach equilibrium. However, most researchers using SPME headspace analysis have observed the same trend as in the present study regarding the time required for high MW compounds to reach equilibrium. In this sense, Zhang and Pawliszyn (1995) reported that the sensitivity of the fiber to less volatile compounds is high, but low partition coefficients for these compounds between the sample and the headspace would result in long equilibration times. Matich et al. (1996) observed a similar effect in apple volatiles: compounds with a low MW (ethyl butanoate or propyl butanoate) achieved equilibrium on the fiber within 5−10 min, whereas the higher MW compounds had not equilibrated after 90 min. These authors suggested that slow equilibrium of around 200 MW compounds may be a general phenomenon.

Heating provides energy for analyte molecules to overcome energy barriers that tie them to the matrix (Alexandrou et al., 1992), enhances the mass transfer process, increases vapor pressure of the analytes (Robbat et al., 1992), and therefore facilitates the release of analytes into the headspace (Zhang and Pawliszyn, 1995). However, the absorption of analytes by the fiber coating is an exothermic process, which means that although the high temperature is good for the release of analytes for their matrix, it can adversely affect the absorption of analytes by the coating due to a decrease of the partition coefficients, so that if the fiber is not at a lower temperature, there is not an enhancement in extraction (Zhang and Pawliszyn, 1995). Results from the present study are not in agreement with this affirmation, because during the extraction not only were the sample and headspace heated but the fiber was also, and there was an increase in the peak chromatographic area, especially in the low volatile compounds.

Some compounds that could have a thermal generation origin, such as hexanal or dimethyl trisulfide, showed an increase in area produced by longer extraction times at 60 °C, but not at 40 °C (Figure 2). It might be that there was thermal generation of these compounds during extraction. Therefore, the increase in area could be due not only to an enhancement in extraction, because of the higher temperature and the longer time, but also to formation during extraction.

The optimal time for extraction should be the time to reach equilibrium. However, in 60 min equilibrium had not been achieved for the low volatile compounds. Ng et al. (1996) reported a time >2 h to achieve the equilibrium for high MW compounds. Therefore, for practical reasons, we think a shorter time would be enough to analyze ham volatiles, as long as the extraction conditions are kept constant (Louch et al., 1992).

To quantify volatiles, several internal standards were tested. However, the variability of their areas was high. This might be due to the complexity of the system, because there were several small slices in the vial. Variations in the slice size and thickness could produce differences in the time to reach equilibrium between samples. In addition, differences among different muscle areas in fat and lean content could contribute to different times to reach equilibrium for the internal

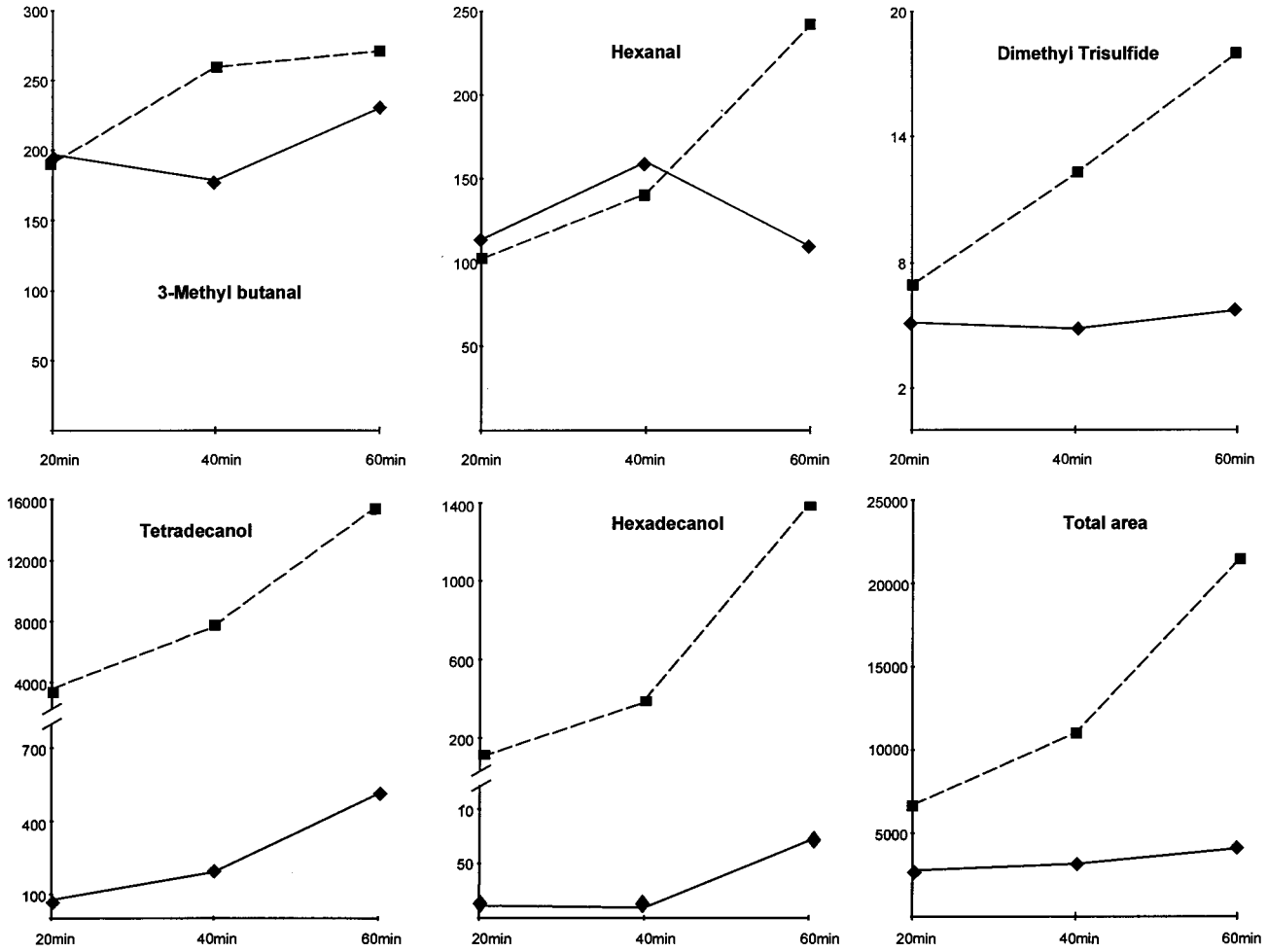

Figure 2. Effect of sampling time and temperature on the uptake of different molecular weight volatiles and on the total chromatographic area: (◆) 40 °C; (■) 60 °C (*y*-axis in area counts × 1000).

standard. Problems for quantitative analysis of volatile compounds using SPME on solid samples (cheese) have been cited by Chin et al. (1996). These authors have pointed out the standard addition method as the best for quantitation using SPME, but further research on quantitative analysis of volatiles using SPME on solid samples is needed.

Variations in area found were higher than those reported in other papers working with liquid samples (Ibañez and Bernhard, 1996; Elmore et al., 1997). Some compounds had coefficients of variance (CV) around 75%, whereas in other compounds CV values were <10%. Elmore et al. (1997) observed high CV values in compounds with small peak areas, but in this study there was no relationship between the area of the peak and the CV. To test whether the high CV values found for some compounds could be related with low homogeneity of muscles, some samples were frozen with liquid nitrogen, immediately ground, and analyzed under the same conditions, but the variations in the area were very similar to those obtained with slices (data not shown). Such variability could be explained by the complexity of the matrix. A portion of some analytes could be trapped in cellss; to be extracted, they must diffuse through the cell wall to the surface of the solid sample. In addition, the variation in the size of fat and lean areas in the sample surface could contribute to the high variability found, because each tissue has a different polarity.

Conclusions. SPME fits with the requirements for both a research tool and a quality control method in the dry-cured ham processing industries, because it has several advantages (short analysis time, solvent-free, cheap compared with the other referred methodologies, and easy to use), provides information about a broader range of dry-cured ham volatile compounds than the methods used previously, and successfully extracts compounds pointed out as related to the sensory characteristics of dry-cured hams in the literature. However, both extraction temperature and time strongly affect the chromatographic area of less volatile compounds, and therefore extraction conditions should be selected depending on the goals of the study.

LITERATURE CITED

Acree, T.; Arn, H. Flavornet. Gas chromatography−olfactometry (GCO) of natural products. Available at http://www.nysaes.cornell.edu/flavornet/, 1997.

Alexandrou, N.; Lawrence, M.; Pawliszyn, J. Cleanup of complex organic mixtures using supercritical fluids and selective absorbents. *Anal. Chem.* **1992**, *64*, 301−311.

Antequera, T.; López-Bote, C.; Córdoba, J. J.; García, C.; Asensio, M. A.; Ventanas, J. Lipid oxidative changes in the processing of Iberian pig hams. *Food Chem.* **1992**, *45*, 105−110.

Arthur, C. L.; Pawliszyn, J. Solid-phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145−2148.

Arthur, C. L.; Potter, D. W.; Buchholz, K. D.; Motlagh, S.; Pawliszyn, J. Solid-phase microextraction for the direct analysis of water: theory and practice. *LC-GC Int*. **1992**, *10*, 656−661.

Badings, H. T. Cold storage defects in butter and their relation to the oxidation of unsaturated fatty acids. *Ned. Melk-Zuiveltijdschr*. **1970**, *24*, 147−257.

Barbieri, G.; Bolzoni, L.; Parolari, G.; Virgili, R. Flavor compounds of dry-cured ham. *J. Agric. Food Chem*. **1992**, *40*, 2389−2394.

Berdagué, J. L.; García, C. Les composants volatils du jambon sec. *Viande. Prod. Carnés*. **1990**, *6*, 319−320.

Berdagué, J. L.; Denoyer, C.; Le Quere, J. L.; Semon, E. Volatile components of dry-cured ham. *J. Agric. Food Chem*. **1991**, *39*, 1257−1261.

Berdagué, J. L.; Bonnaud, N.; Rousset, S.; Touraille, C. Influence of pig crossbreed on the composition volatile compound content and flavour of dry cured ham. *Meat Sci*. **1993**, *34*, 119−129.

Bolzoni, L.; Barbieri, G.; Virgili, R. Changes in volatile compounds of Parma ham during maturation. *Meat Sci*. **1996**, *43*, 301−310.

Buscailhon, S.; Berdagué, J. L.; Monin, G. Time-related changes in volatile compounds of lean tissue during processing of French dry-cured ham. *J. Sci. Food Agric*. **1993**, *63*, 69−75.

Buscailhon, S.; Berdagué, J. L.; Bousset, J.; Cornet, M.; Gandemer, G.; Touraille, C.; Monin, G. Relations between compositional traits and sensory qualities of French dry-cured ham. *Meat Sci*. **1994**, *37*, 229−243.

Careri, M.; Mangia, A.; Barbieri, G.; Bolzoni, L.; Virgili, R.; Parolari, G. Sensory property relationship to chemical data of Italian type dry-cured ham. *J. Food Sci*. **1993**, *58*, 968−972.

Charrier, S. Influence de la nature des ferments sur la biogenèse des composès d′arôme et sur les qualités organoleptiques du saucisson sec. Doctoral Thesis, University of Montpellier, 1992.

Chin, H. W.; Bernhard, R. A.; Rosenberg, M. Solid-phase microextraction for cheese volatile compounds analysis. *J. Food Sci*. **1996**, *61*, 1118−1122.

Córdoba, J. J.; Antequera, T.; García, C.; Ventanas, J.; López-Bote, C.; Asensio, M. A. Evolution of free amino acids and amines during ripening of Iberian cured ham. *J. Agric. Food Chem*. **1994**, *42*, 2296−2301.

Elmore, J. S.; Erbahadir, M. A.; Mottram, D. S. Comparison of dynamic headspace concentration of tenax with solid-phase microextraction for the analysis of aroma volatiles. *J. Agric. Food Chem*. **1997**, *45*, 2638−2641.

Flores, M.; Grimm, C. C.; Toldrá, F. A.; Spanier, A. M. Correlations of sensory and volatile compounds of Spanish Serrano dry-cured ham as a function of two processing times. *J. Agric. Food Chem*. **1997**, *45*, 2178−2186.

García, C.; Berdagué, J. J.; Antequera, T.; López-Bote, C.; Córdoba, J. J.; Ventanas, J. Volatile components of dry cured Iberian ham. *Food Chem*. **1991**, *41***,** 23−32.

García-Regueiro, J. A.; Díaz, I. Volatile compounds in dry-cured ham produced from heavy and light large white pigs. 40th ICoMST, The Hague; 1994; S.-IVA, pp 9−11.

Hinrichsen, L. L.; Pedersen, S. B. Relationship among flavor, volatile compounds, chemical changes, and microflora in Italian-type dry-cured ham during processing. *J. Agric. Food Chem*. **1995**, *43*, 2932−2940.

Ibáñez, E.; Bernhard, R. Solid phase-microextraction (SPME) of pyrazines in model reaction systems. *J. Sci. Food Agric*. **1996**, *72*, 91−96.

Kondjoyan, N.; Berdagué, J. L. *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*; Laboratoire Flaveur, Station de recherches sur la Viande, INRA: Theix, 1996.

Lay-Keow, Ng.; Hupé, M.; Harnois, J.; Moccia, D. Characterisation of commercial vodkas by solid-phase microextraction and gas chromatography/mass spectrometry analysis. *J. Sci. Food Agric*. **1996**, *70*, 380−388.

Lillard, D. A.; Ayres, J. C. Flavour compounds in country cured hams. *Food Technol*. **1969**, *23*, 251−254.

López, M. O.; de la Hoz, L.; Cambero, M. I.; Gallardo, E.; Reglero, G.; Ordóñez, J. A. Volatile compounds of dry hams from Iberian pigs. *Meat Sci*. **1992**, *31*, 267−277.

Louch, D.; Motlang, S.; Pawliszyn, J. Dynamic of organic compound extraction from water using liquid-coated fused silica fibers. *Anal. Chem*. **1992**, *64*, 1187−1199.

Matich, A. J.; Rowan, D. D.; Banks, N. H. Solid-phase microextraction for quantitative headspace sampling of apple volatiles. *Anal. Chem*. **1996**, *68*, 4114−4118.

Miranda-Lopez, R.; Bibbey, L. M.; Watson, B. T.; McDaniel, M. R. Odor analysis of pinot noir wines from grapes of different maturities by a gas chromatography-olfactometry technique (Osme). *J. Food Sci*. **1992**, *57*, 985−993.

Ng, L.-K.; Hupé, M.; Harnois, J.; Moccia, D. Characterisation of commercial vodkas by solid-phase microextraction and gas chromatography/mass spectrometry analysis. *J. Sci. Food Agric*. **1996**, *70*, 380−388.

Nielsen, J. H.; Sørensen, B.; Skibsted, L. H.; Bertelsen, G. Oxidation of pre-cooked minced pork as influenced by chill storage of raw muscle. *Meat Sci*. **1997**, *46*, 191−197.

Nilsson, T.; Larsen, T. O.; Montanarella, L.; Madsen, J. Ø. Application of headspace solid-phase microextraction for the analysis of volatile metabolites emitted by *Penicillium* species. *J. Microbiol. Methods* **1996**, *25*, 245−255.

Pelusio, F.; Nilsson, T.; Montanarella, L.; Tilio, R.; Larsen, B.; Facchetti, S.; Madsen, J. Ø. Headspace solid-phase microextraction analysis of volatile organic sulfur compounds in black and white truffle aroma. *J. Agric. Food Chem*. **1995**, *43*, 2138−2143.

Piotrowski, E. G.; Zaika, L. L.; Wasserman, A. E.. Studies on aroma of cured ham. *J. Food Sci*. **1970**, *35*, 321−325.

Robbat, A.; Liu, T.; Abraham, B. On-site detection of polycyclic aromatic hydrocarbons in contaminated soils by thermal desorption gas chromatography/mass spectrometry. *Anal. Chem*. **1992**, *64*, 1477−1483.

Song, J.; Gardner, B. D.; Holland, J. F.; Beaudry, M. Rapid analysis of volatile flavor compounds in apple fruit using SPME and GC/time-of-flight mass spectrometry. *J. Agric. Food Chem*. **1997**, *45*, 1801−1807.

Statistical Analysis Systems Institute. *SAS User's Guide: Statistics*; SAS Institute Inc.: Cary, NC, 1997.

Steel, R. G. D.; Torrie, J. H. *Principles and Procedures of Statistics, Biometrical Approach*, 2nd ed.; McGraw-Hill Book: New York, 1980.

Steffen, A.; Pawliszyn, J. Analysis of flavor volatiles using headspace solid-phase microextraction. *J. Agric. Food Chem*. **1996**, *44*, 2187−2193.

Ventanas, J.; Córdoba, J. J.; Antequera, T.; García, C.; López-Bote, C.; Asensio, M. A. Hydrolysis and Maillard reactions during ripening of Iberian ham. *J. Food Sci*. **1992**, *57*, 813−815.

Xiaogen, Y.; Peppard, T. Solid-phase microextraction for flavor analysis. *J. Agric. Food Chem*. **1994**, *42*, 1925−1930.

Zhang, Z.; Pawliszyn, J. Headspace solid-phase microextraction. *Anal. Chem*. **1993**, *65*, 1843−1852.

Zhang, Z.; Pawliszyn, J. Quantitative extraction using an internally cooled solid-phase microextraction device. *Anal. Chem*. **1995**, *67*, 34−43.

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