OPTIMIZATION OF CONDITIONS FOR VOLATILE COMPOUNDS ANALYSIS BY SOLID PHASE MICROEXTRACTION (SPME) AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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Background

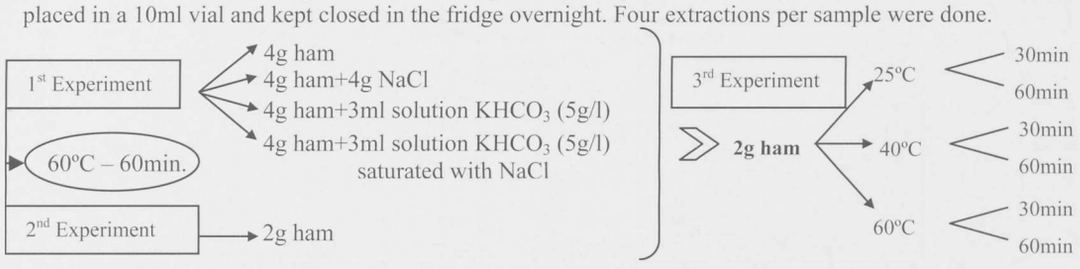
The overall acceptance of dry-cured hams highly depends on their odour compounds, being the solid phase microextraction (SPME) one of the current techniques used to analyse them.
Different extraction conditions of SPME have been applied to dry cured ham in the different researches. Kataoka et al. (2000) studied the application of microextraction in food analysis concluding that extraction of volatile compounds can be optimised by using different conditions of temperature, pH and agitation. They also concluded that extraction is improved by adding soluble salts to the sample such as sodium chloride or potassium carbonate.
Time and temperature are important factors to be taken into account to get the most representative volatile profile in a reasonable period of time. Ruiz et al. (1998) stated that in solid samples, temperature is the main factor in reducing equilibrium time and time analysis, considering that high temperatures applied for a long time may result in changes in the volatile profile.

Objectives

The aim of this study was to conclude about the best extraction conditions for volatile compounds analysis by SPME in ham in relation to the amount of sample, the addition or not of salts and the conditions of temperature and time of extraction.

Methods

Sampling
1.5 cm thick slices were cut, grounded, vacuum packed and frozen until the day before the analysis. 4 or 2g of grounded dry-cured ham were placed in a 10ml vial and kept closed in the fridge overnight. Four extractions per sample were done.



SPME characteristics
A 100 µm thickness SPME polydimethylsiloxane fiber was used for this study. The fiber was conditioned for one hour at 250ºC before the analysis and exposed to the sample, previously conditioned for 15 minutes at the assay temperature. The vial was immersed in a water bath at the temperature studied.
Gas Chromatography/Mass Spectrometry (GC/MS)
The fiber was desorbed at 250ºC in the injection port of a gas chromatograph HP 6890 GC System (Hewlett-Packard) coupled with a mass detector 5973 Mass Selective Detector (Hewlett-Packard). Column: 5% phenyl-95%methyl siloxane (HP-5MS; 30m x 250 µm x 0.25 µm). Initial oven temperature was isothermal at 40ºC for 10 min., increased to 120ºC at 3ºC/min., and to 250ºC at 10ºC/min maintaining this temperature for 5 min. Carrier gas was Helium (1ml/min). Identification of peaks was based on the comparison of the retention times to those of standard compounds, of the mass spectra to those of the Wiley library and of the Kovats indices to those from the bibliography (Kondjoyan and Berdagué, 1996). Electron impact at 70 eV; multiplier voltage at 1500; mass range 30-350 amu.

Results and discussion

The first experiment was carried out with 4g of ham in different conditions, as shown in the figure. Samples where extraction was made with salts showed much lower total areas than those without salts. From a total number of 24 compounds considered to be important for aroma development, between 17 and 21 decreased in area when salts were used. Furthermore, 2 and 3-methylbutanoic acids, which are known for being characteristic aged flavour compounds, were not detected in those extraction conditions.
In the second experiment, the volatile profile of extraction with 4g was compared to extraction with 2g, to analyse the influence of different ratios sample / headspace. Results showed that total area was quite similar and approximately half of the compounds increased using 2g and half of them decreased. Considering the coefficient of variation, 12 compounds showed less value using 2g, whereas with 4g only 9 compounds showed lower CV than with 2g. Important aged flavour compounds (2 and 3-methylbutanal), oxidation compound (hexanal) and some aroma precursors (tetradecanal, hexadecanal, octadecanal) increased using 2g.
Another experiment, using different samples from experiments 1 and 2, was carried out with 2g ham without salts, at different combinations temperature/time. Results showed that both temperature and time contributed to increase total area. At 60ºC, octanal, hexanal and nonanal increased with time probably because of their generation during the time of analysis (Ruiz et al. 1998). The lowest total areas corresponded to the extractions at 25ºC, with hardly any compound with a K.I. higher than 1200. At 40ºC, close to body temperature, and 60 minutes of extraction time, almost all considered compounds were detected.

Conclusion

Extraction with 2g at 40ºC/1h could be the most appropriate condition, among those assayed, for the application of SPME to obtain the aroma profile in dry-cured ham.

Pertinent literature

Ruiz, J., Cava, R., Ventanas, J., y Jensen, M. T. (1998). J. Agric. Food Chem. 46, 4688-4694.

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Kataoka, H., Lord, H. L. and Pawliszyn, J. (2000). Journal of Chromatography A, 880, 35-62.

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Table1. Results for the 1st and 2nd experiments, expressed in area counts (x1000).

K.I.	COMPOUND	4g Jam Mean	4g Jam c.v.	4g Jam + NaCl Mean	4g Jam + NaCl c.v.	4g Jam + 3ml $KHCO_3$ Mean	4g Jam + 3ml $KHCO_3$ c.v.	4g Jam + 3ml $KHCO_3$ SAT NaCl Mean	4g Jam + 3ml $KHCO_3$ SAT NaCl c.v.	2g Jam Mean	2g Jam c.v.
650	Acetic acid	5179	188	170	137	0	-	0	-	2207	184
657	3-methylbutanal	190	126	285	48	161	28	167	59	282	94
665	2-methylbutanal	131	134	199	57	113	22	112	65	174	107
804	Hexanal	2019	77	2410	44	2093	25	2319	29	2512	60
877	3-methyl butanoic acid	1319	200	0	-	0	-	0	-	516	200
887	2- methyl butanoic acid	743	200	0	-	0	-	0	-	324	200
892	2-heptanone	341	116	577	25	380	19	353	29	323	70
903	Heptanal	384	58	364	30	372	19	419	23	386	71
998	Hexanoic acid	1505	128	1801	38	243	200	727	20	640	85
1004	Octanal	815	97	702	39	464	10	786	9	616	73
1043	Phenylacetaldehyde	396	19	366	35	149	86	365	17	489	29
1080	2-butyl-1-octanol	374	200	0	-	0	-	387	200	283	200
1104	Nonanal	2445	22	1777	13	1641	21	2136	6	2471	23
1183	Octanoic acid	321	131	264	117	0	-	77	200	141	200
1196	1-Tetradecanol	0	-	0	-	0	-	175	121	77	200
1367	dihydro-5-pentyl -2(3H)-furanone	1606	32	1524	11	1498	20	1379	11	1403	8
1379	Decanoic acid	988	21	989	38	582	40	748	32	781	22
1568	Dodecanoic acid	455	36	336	48	194	81	249	31	420	26
1612	Tetradecanal	1796	17	953	30	1321	18	1254	17	2230	5
1695	2-pentadecanone	1791	5	1450	30	1482	17	1480	22	2276	9
1711	Hexadecanal	2952	6	2019	29	2479	18	2350	20	4075	10
1761	Tetradecanoic acid	4771	27	4075	50	3505	14	3192	27	6347	18
	Octadecanal	3140	14	1349	79	2489	11	2449	15	3745	15
	9-Octadecenal	8679	18	3145	71	7776	9	6909	4	9879	15
	TOTAL	**42336**		**24757**		**26941**		**28033**		**42594**	

Table 2. Results for the 3rd experiment, expressed in area counts (x1000).

K.I.	COMPOUND	25°C 30min Mean	25°C 30min c.v.	40°C 30min Mean	40°C 30min c.v.	60°C 30min Mean	60°C 30min c.v.	25°C 1h Mean	25°C 1h c.v.	40°C 1h Mean	40°C 1h c.v.	60°C 1h Mean	60°C 1h c.v.
650	Acetic acid	0	-	260	200	0	-	120	67	356	53	202	173
657	3-methylbutanal	472	16	627	24	499	27	436	16	529	24	589	53
665	2-methylbutanal	63	12	121	32	158	28	73	30	108	29	168	55
804	Hexanal	110	14	220	11	232	26	105	70	212	14	468	21
877	3-methyl butanoic acid	0	-	0	-	0	-	0	-	0	-	140	173
887	2- methyl butanoic acid	0	0	0	0	0	0	0	0	0	0	0	0
892	2-heptanone	296	8	190	18	94	38	322	7	187	21	131	16
903	Heptanal	0	-	47	116	110	7	32	129	55	34	203	5
998	Hexanoic acid	45	200	47	200	0	-	319	16	122	120	567	173
1004	Octanal	27	134	121	14	170	40	51	116	182	5	261	20
1043	Phenylacetaldehyde	126	6	189	21	205	23	221	16	234	13	567	26
1080	2-butyl-1-octanol	225	67	378	120	485	15	454	68	507	6	656	44
1104	Nonanal	681	15	892	16	1185	12	1118	14	871	11	1759	24
1183	Octanoic acid	0	0	0	-	0	-	7	200	22	116	302	173
1196	1-tetradecanol	0	-	57	119	55	9	79	67	94	22	95	94
1367	dihydro-5-pentyl 2(3H)-furanone	0	-	216	32	174	22	136	17	217	7	246	22
1379	Decanoic acid	0	-	0	-	496	42	0	-	359	12	1792	56
1568	Dodecanoic acid	0	-	0	-	97	77	0	-	0		893	64
1612	Tetradecanal	0	-	0	-	319	32	0	-	76	117	830	11
1695	2-pentadecanone	0	-	0	-	493	22	0	-	42	200	1032	6
1711	Hexadecanal	0	-	0	-	620	31	0	-	136	73	1474	3
1761	Tetradecanoic acid	0	-	71	125	465	45	0	-	0	-	3546	22
	Octadecanal	0	-	0	-	547	22	0	-	32	120	1588	12
	9-Octadecenal	0	-	20	200	1336	17	0	-	0	-	3610	10
	TOTAL	**2045**		**3455**		**7739**		**3471**		**4339**		**21120**	

Cv: coefficient of variation

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