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ARTICLES

Mass Spectrometry Analysis of Volatile Compounds in Raw Meat for the Authentication of the Feeding Background of Farm Animals

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The authentication of the conditions of animal production, based on the analysis of meat commercial cuts, is a major challenge on both societal and analytical grounds. The aim of the present work was to propose a method for the extraction of the volatile compounds from ruminant raw muscles trimmed of fat and to assess by mass spectrometry-based techniques the relevance of these compounds for the authentication of the type of feeding offered to the animals. The first step of the study consisted of validating conditions of dynamic headspace (DH) extraction of volatile compounds that enabled us to minimize the appearance of heat-induced artifacts and to maximize the richness of the DH-gas chromatography−mass spectrometry profile (DH-GC-MS) of raw lamb muscle. An extraction temperature of 35 °C (vs 60 and 90 °C) and a sample mass of 6.25 g (vs 12.5, 25, and 50 g) were shown to be suitable. The second step aimed at identifying volatile compounds enabling us to discriminate muscle samples from 16 experimental lambs fed either concentrate ($n = 8$) or pasture ($n = 8$). Before, to carefully explore the information given by the DH-GC-MS signal, the MS spectra acquired along the chromatogram were summed and then converted in a virtual-DH-MS spectral fingerprint to have a quick overview of the discriminative potential of the volatile fraction. According to univariate (analysis of variance) and to multivariate (principal component analysis) data treatments performed on virtual-DH-MS fingerprints, the meat volatile fraction was relevant to reveal the type of feeding of the living animal. The detailed examination of the information given by the GC dimension showed that 33 volatile compounds among the 204 detected in the muscle by DH-GC-MS enabled us to discriminate the type of feeding of the lambs. The relevance of these results is discussed in light of previous studies performed on adipose tissues.

KEYWORDS: Dynamic headspace; virtual MS fingerprint; GC-MS; raw meat; volatile compounds; feed tracer; authentication

INTRODUCTION

Meat volatile compounds have been widely studied as tracers of animal feeding systems (*1*) and for their influence on cooked meat flavor (*2*, *3*). Because of their lipophilic nature, meat volatiles are stored mainly in animal adipose tissues and, to a lesser extent, in intramuscular fat. Most studies found in the literature on the volatile profile of beef, lamb, chicken, or pork meat have been performed on fatty tissues (*1*, *4*−*6*) or on muscle tissues including external fat (*7*, *8*). In contrast, only few research studies have focused on volatile compounds present in the muscle trimmed of external fat (*9*−*12*). More information about the volatile profile of muscle trimmed of fat is thus needed to construct a generic traceability strategy based on the analysis of commercial meat cuts, as the meat available on the market does not comprise systematically adipose tissues.

A large number of studies have been undertaken by subjecting meat to various cooking procedures before analysis, such as grilling (*13*−*15*), pressure cooking (*7*), or roasting (*16*). It is well-known that many volatile compounds responsible for meat flavor arise from heat-induced reactions during cooking (*17*). Ahn et al. (*18*) reported that increasing the temperature applied during the extraction of volatiles from turkey meat enhanced

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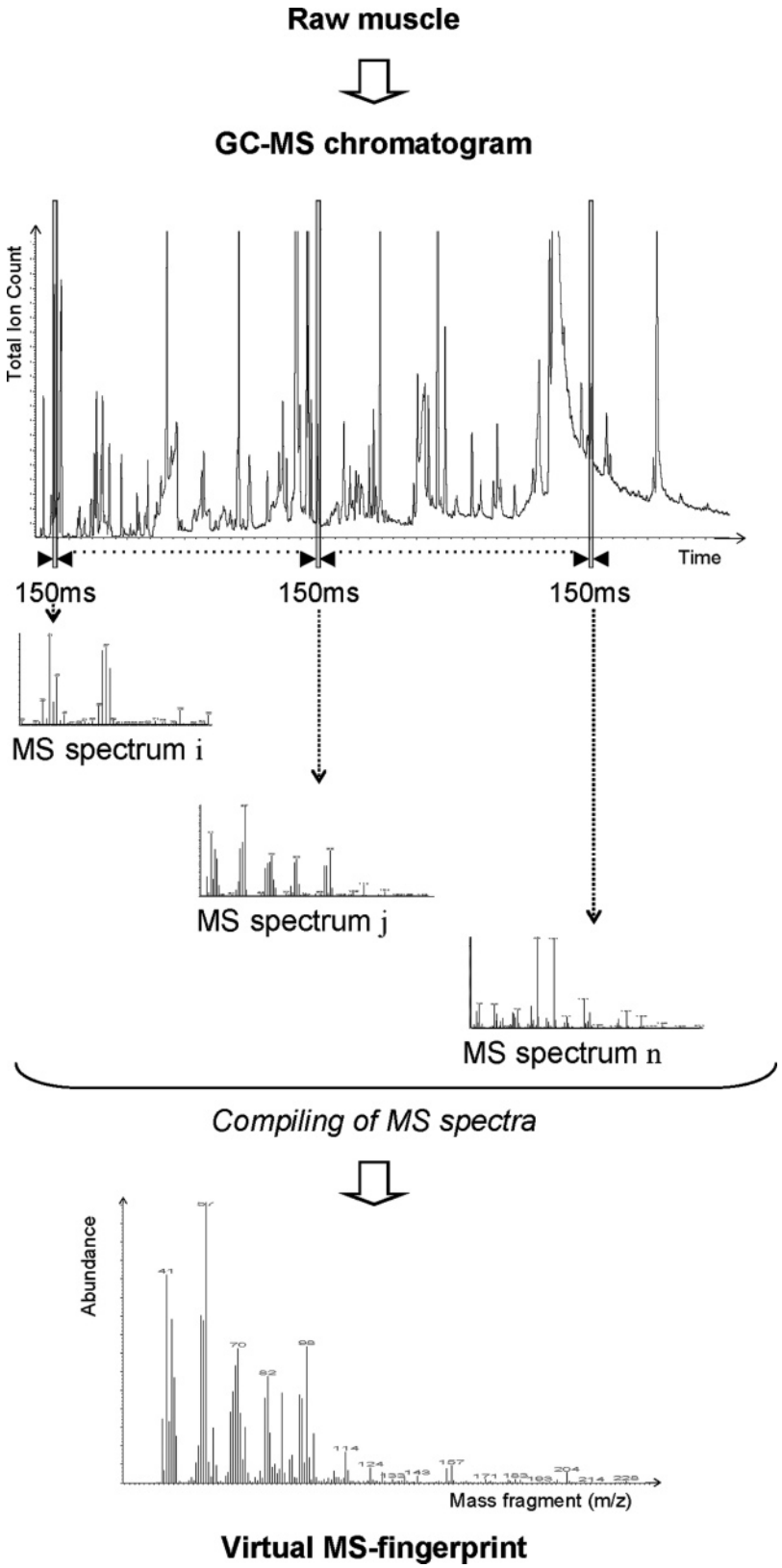

Figure 1. Scheme of the construction of a virtual MS fingerprint of the volatile fraction of a raw muscle sample from a GC-MS chromatogram of this tissue. The mass spectra that were acquired every 150 ms of the GC-MS chromatogram were summed and then converted in a virtual MS spectral fingerprint characterized by the abundance of 198 mass fragments ranging from 33 to 230 amu.

the production of those compounds derived from heat-induced lipid oxidation. Therefore, the results of the previously published works on meat volatile compounds should be considered cautiously, taking into account the possible undesired occurrence of volatiles due to meat heating during process or during analyses (*19*). To our knowledge, only a few studies have been performed on volatile compounds in raw meat by using low-temperature analytical procedures (*18*, *20*, *21*). When the heating of the raw product is limited during analysis, the volatile profile can be considered as original information displayed by raw meat;

Table 1. Volatile Compounds Identified in the Headspace of 50 g Muscle Samples by DH-GC-MS and Which Specific Ion Area Was Affected by Variations of the Temperature of Extraction (35, 60, and 90 °C)

				abundance of the compound[a] ($\times 10^4$ arbitrary area unit)		
compound	RI	MI[b]	specific ion[c]	35 °C	60 °C	90 °C
acetaldehyde		MS	44	62.2 a	140.2 b	173.7 b
methanamine		MS	59	ND[d]	ND	434.3
2-propenal		MS	56	8.7 a	8.8 a	29.7 b
2-propanone		MS	59	413.0 a	818.5 b	1491.7 c
pentane	500	MS	72	57.9 a	57.5 a	177.2 b
2-propanol	506	MS	59	83.6 a	249.9 b	816.7 c
thiobis-methane	518	MS	62	2590.3 a	2661.6 a	14704.4 b
dichloro-methane	531	MS	49	3014.6 a	8004.0 b	12131.4 c
2-methyl-propanal	555	MS	72	7.9 a	86.1 a	10501.3 b
1-propanol	559	MS	60	120.2 a	402.9 a	1632.7 b
2,3-butanedione	594	MS	43	7502.4 a	12116.7 a	61869.0 b
2-butanone	598	MS	72	1129.3 a	4885.7 b	7222.4 c
2-butanol	605	MS, RI	59	105.3 a	344.6 b	1226.1 c
2-methyl-3-buten-2-ol	614	MS, RI	71	84.2 a	255.2 a	1147.1 b
ethyl-acetate	614	MS, RI	61	42.1 a	52.8 a	130.9 b
chloroform	618	MS, RI	83	49.9 a	136.6 a	706.5 b
2-methyl-propanenitrile	623	MS, RI	68	11.9 a	23.5 a	77.6 b
2-methyl-propanol	628	MS, RI	74	131.8 a	443.8 b	1500.8 c
3-methyl-butanal	655	MS, RI	58	11.7 a	168.0 a	15809.0 b
2-methyl-butanal	657	MS, RI	86	15.2 a	35.4 a	2775.3 b
benzene	661	MS, RI	78	474.5 a	349.9 a	756.9 b
1,1-dimethoxy-ethane	662	MS	59	86.4	117.5	ND
1-butanol	664	MS, RI	56	303.3 a	1128.8 b	4836.4 c
1-penten-3-ol	682	MS, RI	84	234.0 a	614.9 a	2261.2 b
2-pentanone	685	MS, RI	59	282.0 a	673.0 b	1904.4 c
3-pentanone	697	MS, RI	86	110.8 a	204.3 b	604.7 c
heptane	700	MS, RI	100	125.1 a	336.4 b	1012.1 c
2-ethyl-furan	702	MS, RI	81	220.1 a	376.3 a	5954.2 b
cyclopentadiene or cyclohexadiene	711	MS	79	49.6 b	57.3 b	2.5 a
methyl-cyclohexane	725	MS, RI	83	12.9 a	17.6 b	44.7 c
2-nitro-benzoic acid	732	MS	137	54.3	3.9	ND
3-methyl-3-buten-1-ol	733	MS, RI	68	141.0 a	510.1 b	2026.7 c
3-methyl-1-butanol	735	MS, RI	70	179.2 a	702.1 b	2795.3 c
4-methyl-2-pentanone	739	MS, RI	100	20.2 a	37.3 b	74.2 c
1-methyl-pyrrole	741	MS, RI	81	24.8 a	36.8 a	88.1 b
dimethyl-disulfide	746	MS, RI	94	65.2 a	127.6 a	422.8 b
2,3-diethyl-oxirane	749	MS	58	40.7 a	67.9 a	242.9 b
1-pentanol	768	MS, RI	70	1113.8 a	3516.7 b	15181.4 c
toluene	770	MS, RI	91	384.2 a	358.6 a	1079.7 b
2-penten-1-ol	773	MS	68	97.6 a	327.4 b	1799.8 c
3-methyl-2-buten-1-ol	778	MS, RI	86	37.2 a	125.3 a	631.1 b
1-fluoro-2-methyl-benzene	783	MS, RI	109	273.3 c	79.4 a	215.9 b
1,3,5-trioxane	785	MS	117	29.3 a	77.9 b	206.2 c
cyclopentanone	791	MS, RI	84	25.8 a	74.6 b	233.0 c
octane	800	MS, RI	114	213.8 a	1317.6 b	2088.2 c
tetrachloro-ethene	814	MS, RI	166	8.8 a	6.5 a	17.8 b
2-methyl-octane	865	MS, RI	85	8.1 a	13.3 a	21.5 b
ethyl-benzene	866	MS, RI	91	98.2 a	101.1 a	287.4 b
1-hexanol	870	MS, RI	84	34.3 a	126.5 a	698.5 b
1,4-dimethyl-benzene (*p*-xylene)	874	MS, RI	91	181.7 a	145.5 a	488.9 b
3-heptanone	886	MS, RI	85	8.8 a	16.0 b	50.2 c
2,4-dithia-pentane	894	MS, RI	108	61.9 a	107.4 b	278.1 c
1,2-dimethyl-benzene (*o*-xylene)	898	MS, RI	91	68.4 a	65.0 a	195.4 b
nonane	900	MS, RI	128	45.4 a	88.1 a	149.1 b
heptanal	901	MS, RI	96	36.1 a	529.2 b	1807.5 c
3-methyl-thio-propanal	907	MS, RI	104	ND	ND	242.2
γ-butyrolactone	916	MS, RI	86	37.2 c	25.5 b	13.2 a°
cumene	929	MS	91	151.5 b	42.8 a	15.2 a°
6-methyl-heptan-2-one	955	MS, RI	58	41.7 a	91.3 b	300.5 c
ethyl-cyclopentanone	962	MS	112	11.7 a	37.5 a	211.8 b
2-methyl-nonane	965	MS, RI	70	ND	9.2	33.3
benzaldehyde	969	MS, RI	106	36.9 a	104.6 b	1746.7 c
1-heptanol	970	MS, RI	70	192.6 a	800.5 a	4121.6 b
3-methyl-nonane	970	MS, RI	112	7.5 a	14.7 b	3.9 a
1-octen-3-ol	979	MS, RI	72	783.6 a	2525.1 b	8177.6 c
2-ethyl-hexanol	980	MS	70	18.0 a	83.6 b	256.7 c
2,3-octanedione	982	MS, RI	142	14.6 a	49.1 a	216.9 b
phenol	983	MS, RI	94	121.4 b	22.4 a	45.6 a

Table 1. (Continued)

compound	RI	MI[b]	specific ion[c]	abundance of the compound[a] (× 10^4 arbitrary area unit) 35 °C	60 °C	90 °C
6-methyl-5-hepten-2-one	986	MS, RI	108	179.9 a	549.5 a	1527.7 b
3-octanone	987	MS, RI	99	32.9 a	73.2 b	197.5 c
2-octanone	991	MS, RI	58	47.0 a	167.7 b	652.0 c
2-pentyl-furan	994	MS, RI	138	21.4 a	55.0 a	781.3 b
decane	1000	MS, RI	142	6.8 a	18.7 a	116.8 b
1,3,5-trimethyl-benzene	1001	MS, RI	120	32.8 a	23.3 a	91.1 b
octanal	1003	MS, RI	84	137.0 a	909.1 b	2561.7 c
dichloro-methyl-benzene	1022	MS, RI	146	12.8 a	21.1 b	57.5 c
phenyl-acetaldehyde	1049	MS, RI	120	ND	ND	334.0
trans-2-octenal	1061	MS, RI	83	9.0 a	26.0 a	356.3 b
2-pyrrolidinone	1069	MS	110	32.9 a	143.2 a	827.6 b
1-octanol	1070	MS, RI	83	88.7 a	537.8 b	2337.1 c
dihydromyrcenol	1073	MS, RI	59	77.3 a	223.2 b	406.0 c
nonanal	1110	MS, RI	98	342.5 a	5014.7 b	8235.3 b
nonanol	1166	MS	98	13.9 a	142.2 b	326.9 c
ethyl-benzaldehyde	1171	MS, RI	142	24.8 a	44.9 a	202.6 b
menthol	1185	MS	71	14.4 a	41.8 b	266.8 c
dodecane	1200	MS, RI	170	5.6 a	7.1 a	14.6 b
2-decenal	1267	MS, RI	110	4.3 a	30.1 b	82.0 c
2-undecanone	1296	MS, RI	59	23.8 a	13.6 a	45.0 b
tridecane	1300	MS, RI	184	13.5 a	16.8 a	34.3 b
2,4-decadienal	1320	MS, RI	99	ND	1.4	6.9
propionic acid, 2-methyl-2,2-dimethyl-1-(2-hydroxy-1-methylethyl)-, propyl ester	1389	MS, RI	98	30.2 a	91.3 a	236.3 b
propionic acid, -3-hydroxy-2-methyl-, 2,4,4-trimethylpentyl ester	1387	MS, RI	89	132.9 a	403.0 b	514.0 b
2,4-dimethyl-quinoline	1472	MS, RI	157	10.4 a	2.3 a	108.8 b
pentadecane	1500	MS, RI	212	10.2 a	12.9 a	35.8 b
heptadecane	1700	MS, RI	85	43.9 a	33.8 a	155.9 b

[a] Values are means of five replicates at 35 and 60 °C and of two replicates at 90 °C. In the same row, means with different letters (a–c) are statistically different ($p < 0.05$). [b] MI, method of identification: MS, mass spectrum comparison using Wiley and NIST libraries; RI, RI in agreement with literature values. [c] MS mass fragment of which area was integrated. [d] Not detected.

therefore, it could be used as a means of authenticating the conditions of meat production.

The analysis of volatile compounds in the muscle is made difficult by the low percentage of intramuscular fat (2% on average on a fresh w/w basis) distributed heterogeneously and the high percentage of water. Moreover, volatiles are stored in the muscle fat only at trace levels. These factors imply that for the analysis of muscle volatile compounds, a mild and sensitive technique is required to avoid quantitative and qualitative losses of volatiles. Several studies performed on beef and sheep meats have shown that a dynamic headspace (DH) extraction technique coupled with gas chromatography−mass spectrometry (GC-MS) is suitable for the extraction and the analysis of fat volatiles (*1*, *22*, *23*). However, the high amount of water present in the muscle could reduce the extraction yield of DH because of a competition between volatile compounds and water for the Tenax trap (*24*). This effect could be limited by using a type of Tenax trap with a low affinity for water but also by reducing the sample mass. Nevertheless, the last proposition requires us to consider the decrease of extractable volatile compounds that is associated with the reduction of mass sample and, finally, its consequences on the representativeness of the sample.

The purpose of the present study was to validate a DH-GC-MS method for investigating the volatile profile of raw muscle trimmed of fat and for assessing its relevance for the authentication of animal feeding. In a first step, this study was dedicated to validate the relevance of using moderate extraction temperature and limiting the muscle sample mass on the recovery of volatile compounds (experiments 1 and 2, respectively). The second step of the work aimed to validate the relevance of our DH-GC-MS method for determining molecular tracers of pasture-fed and concentrate-fed lambs in raw meat samples. Prior to studying the detailed information embedded in DH-GC-MS chromatogramms, the relevance of using a virtual DH-MS spectral fingerprint constructed by summation of MS spectra acquired along the chromatogram will be investigated (experiment 3).

MATERIALS AND METHODS

Nature and Sampling of the Animal Products. For the determination of operative conditions to analyze raw meat trimmed of fat (experiments 1 and 2), one muscle *semimembranosus* was excised from the cold carcass of adult cattle. The muscle was vacuum-packed and aged at 4 °C for 6 days postmortem. Meat was trimmed of any visible external fat, cut into small cubes (about 2 cm^3), frozen in liquid nitrogen, wrapped in aluminum foil, then vacuum-packed, and stored at −80 °C to avoid oxidation. Before analysis, raw meat was ground in liquid nitrogen until a fine and homogeneous powder was obtained. For experiment 1, 15 tablets of meat powder were made, each of them weighing 50 g (±2.5 g). For experiment 2, four series of three tablets were prepared weighing 50 (±2.5 g), 25 (±1.25 g), 12.5 (±0.6 g), and 6.25 g (±0.3 g), respectively. Each tablet was shaped into a small aluminum cup wrapped in aluminum foil, then vacuum-packed, and stored at −80 °C. Twenty-four hours before analysis, samples were transferred to a 4 °C chiller.

For the authentication of the type of animal feeding (experiment 3), 16 weaned male lambs born during the same 1 week period were divided into two groups of eight lambs each: The first group of eight lambs was allowed to graze at pasture without any other additional feed, while the second group was kept indoors and fed concentrate

Table 2. Volatile Compounds in the Headspace of Muscle Samples by DH-GC-MS at 35 °C and Which Specific Ion Area Was Affected by Variation of the Sample Size Analyzed (6.25, 12.5, 25, and 50 g)

				abundance of the compound[a] (10^4 arbitrary area unit)			
compound	RI	MI[b]	specific ion[c]	6.25 g	12.5 g	25.0 g	50.0 g
ethanol	444		45	211.0 ab	239.2 b	134.4 a	216.7 ab
2-methyl-propanal	555	MS, RI	72	8.1 b	5.6 a	4.7 a	5.3 a
1-propanol	559	MS, RI	60	7.6 a	21.6 b	4.7 a	6.0 a
1-butanol	664	MS, RI	56	81.3 b	52.1 a	26.1 a	37.7 a
3-hydroxy-butan-2-one	712	MS, RI	88	2562.3 ab	2230.3 a	3054.7 b	2132.2 a
4-methyl-pentan-2-one	739	MS, RI	100	101.6 b	75.1 ab	34.1 ab	23.6 a
dimethyl-disulfide	747	MS, RI	94	83.9 c	46.4 b	20.8 a	21.7 a
unidentified	757			20.2 a	17.2 a	24.5 a	39.3 b
toluene	770	MS, RI	91	213.2 b	142.8 a	118.0 a	124.8 a
1,4-dimethyl-benzene (*p*-xylene)	876	MS, RI	91	96.4 b	40.4 a	31.5 a	24.5 a
1,2-dimethyl-benzene (*o*-xylene)	901	MS, RI	91	41.2 b	19.0 a	16.7 a	9.6 a
α-pinene	942	MS, RI	93	15.2 b	4.7 a	3.8 a	2.1 a
benzaldehyde	969	MS, RI	106	26.9 b	21.6 b	10.7 ab	5.5 a
1,3,5-trimethyl-benzene	1001	MS, RI	120	49.3 b	24.0 a	10.1 a	5.4 a
δ-3-carene	1019	MS, RI	93	4.7 b	1.9 a	1.1 a	1.4 a
2-ethyl-hexanol	1030	MS, RI	98	14.8 b	12.2 ab	7.2 a	5.5 a
dihydromyrcenol	1076	MS, RI	59	43.4 b	9.5 a	8.8 a	5.5 a
2-nonanone	1090	MS, RI	112	2.4 b	1.2 a	1.6 a	1.0 a
6-methyl-γ-ionone	1502	MS	135	14.8 b	3.2 a	6.4 a	4.4 a
benzophenone	1660	MS	105	63.5 b	19.6 a	22.4 a	9.0 a

[a] Values are means of three analyses. In the same row, means with different letters (a–c) are statistically different ($p < 0.05$). [b] MI, method of identification: MS, mass spectrum comparison using Wiley and NIST libraries; RI, RI in agreement with literature values. [c] MS mass fragment of which area was integrated.

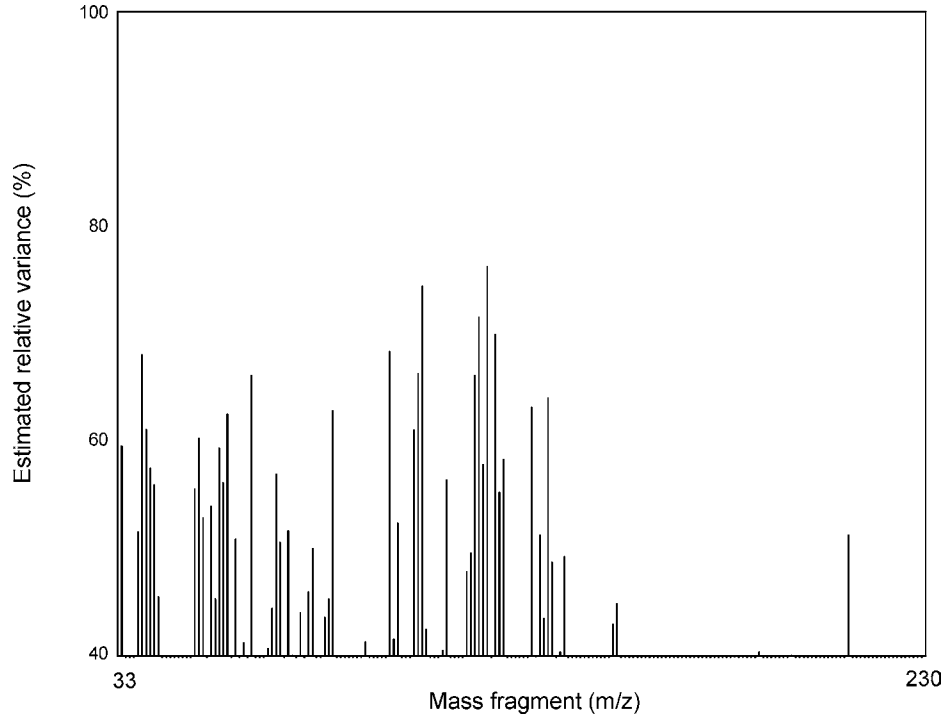

Figure 2. Discriminative power of each of the 198 mass fragment abundances of the virtual MS spectral fingerprints according to the "type of feeding". For each mass fragment, the discriminative power is represented by the estimated relative variance of the type of feeding factor after one-way ANOVA performed on the 16 samples of the data set. The minimum estimated relative variance value reported on the *Y*-axis (39.8%) corresponded to the threshold value above which the "type of feeding" factor is significant ($p < 0.05$) and validated according to the "leave-one-out" procedure.

only. After 85 days of experimental feeding, lambs were slaughtered according to the conventional EU procedures and carcasses were stored at 4 °C. At 24 h postmortem, one *semimembranosus* muscle was excised from each carcass and handled as described above for cattle muscle until a fine meat powder was obtained. Tablets weighing 6.25 g were prepared as described previously. Twenty-four hours before analysis, tablets were taken from −80 °C and stored at 4 °C.

DH-GC-MS. Raw meat volatile compounds were extracted and analyzed by DH-GC-MS (DH: model 3100 Sample Concentrator, Tekmar, Cincinnati, OH; GC: model 6890, Hewlett-Packard, PA; MS: model 5973A, Hewlett-Packard). After thawing, the meat tablet was put into a glass cartridge (Ets. Maillière, Aubière, France) partially filled with glass wool. The meat samples were purged with a 60 mL min^{-1} helium flow (He U quality; purity, 99.995; Messer, St.-Georges d'Espéranche, France) for 30 min. In experiment 1, three different purge temperatures were tested (35, 60, and 90 °C). In experiments 2 and 3, the purge temperature was set at 35 °C. The volatiles were trapped by adsorption on a porous polymer adsorbent Tenax trap column (Tenax TA, straight, 12 in. × 30.5 cm, 24 cm of adsorbent, Supelco, Bellefonte, PA) maintained at 35 °C. After a dry-purge step held for 5 min, the

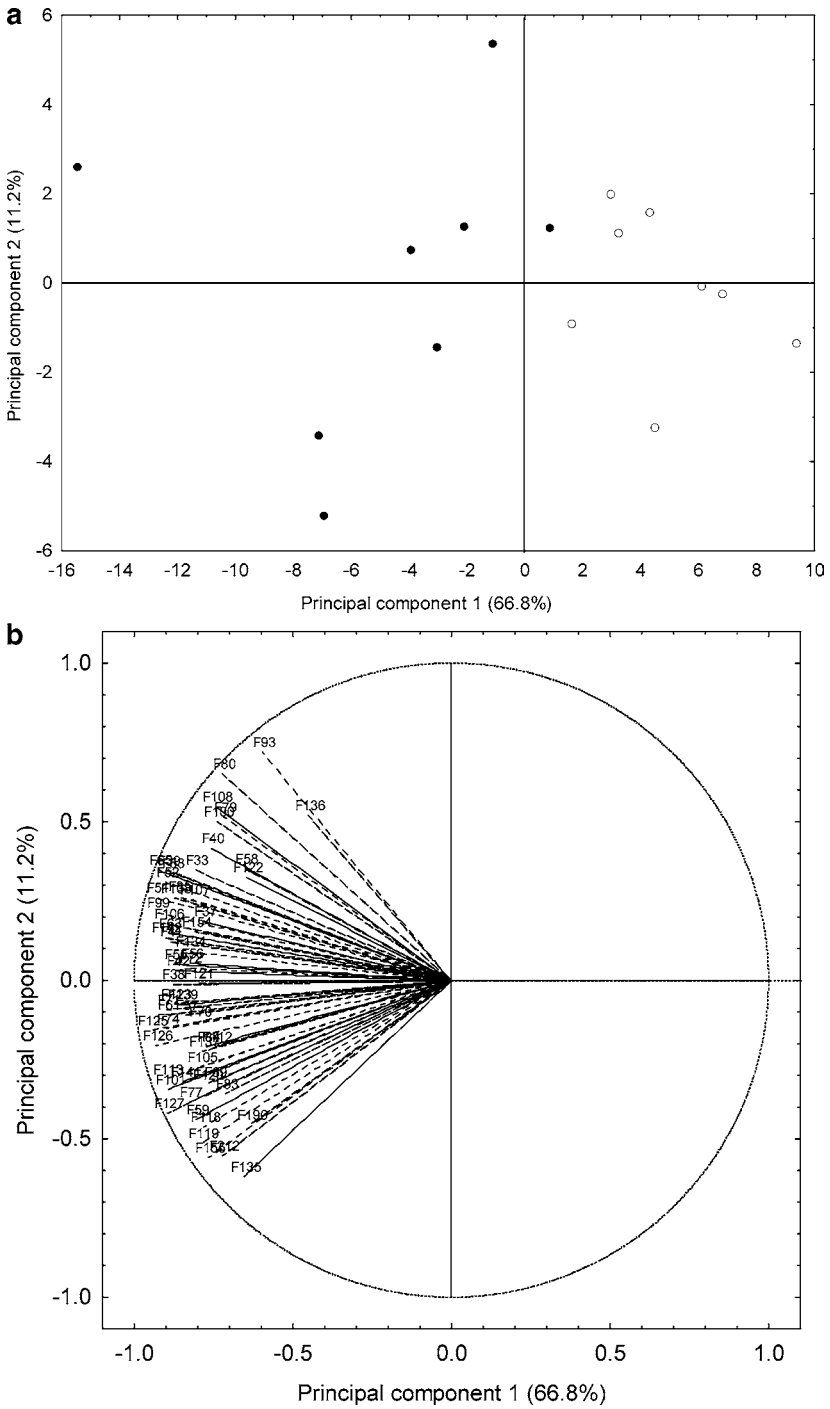

Figure 3. Discrimination of lamb muscle according to the type of feeding based on the virtual MS fingerprint of their volatile fraction. (**a**) First map of the normed PCA carried out from the virtual MS fingerprints of the volatile fractions of the raw muscle of lambs after filtering of mass fragment abundances by a one-way ANOVA (60 significant mass fragments, 16 samples). Symbols related to the two types of lamb feeding are as follows: solid circles, concentrate-fed lambs; open circles, pasture-fed lambs. (**b**) Correlation circle with the projection of significant mass fragments on the first map of the normed PCA.

volatile compounds were desorbed for 10 min at 180 °C under high-quality helium flow (He N55; purity, 99.9995%; Messer). After desorption, the Tenax trap was further heated for 15 min at 180 °C. Extracted volatile compounds were transferred to the head of a capillary column and cryofocused at −150 °C. The condensed low molecular weight compounds were separated by GC after heating the interface for 2 min at 225 °C and injected through an automatic splitless injector onto a nonpolar capillary column (SPB5, 60 m × 0.32 mm × 1 μm, Sigma-Aldrich, St. Louis, MO) with a 1 mL min^{-1} carrier gas flow. The initial oven temperature of 40 °C was held for 5 min followed by an increase to 230 °C at a rate of 3 °C min^{-1}; this temperature was then maintained for 5 min. The GC-MS interface was heated at 280 °C with the temperature reaching 180 °C in MS source and 150 °C in MS quadrupole. The electronic impact ionization energy was set to 70 eV, and the mass range was 33−230 atomic mass units (amu). The acquisition rate was 6.85 scan s^{-1}. Tentative identification of volatiles was achieved by comparing the mass spectral data with those of MS spectra library including NBS 75K, 275L Wiley, and Masslib V8.6-D (MSP Kofel, Zollikofen, Switzerland) and by comparing the experimental retention indices (RI) with those reported in published databases (*25*) and with those of our internal data bank. The peak area of the volatile compounds was integrated from specific ions for each

Table 3. Volatile Compounds Discriminating Animal Feeding System (Concentrate vs Pasture)

compound	RI	MI[b]	specific ion[c]	abundance of the compound[a] (10^4 arbitrary area unit)		significance[d]
				concentrate	pasture	
2-methyl-prop-1-ene		MS	56	15.5	12.2	*
2-propenal		MS	56	7.1	5.9	**
2-propanone		MS	59	115.5	50.2	*
ethanol		MS	45	364.5	204.4	*
2-methyl-propanal	555	MS	72	4.6	3.3	**
2-butanone	598	MS, RI	72	386.7	193.8	*
1-butanol	664	MS, RI	56	99.8	55.9	**
unidentified	741		95	3.5	1.7	*
1-methyl-pyrrole	742	MS, RI	81	8.8	3.9	**
pentan-1-ol	768	MS, RI	70	223.2	90.2	*
cis-2-octene	806	MS, RI	112	10.9	2.6	***
trans-2-octene	816	MS, RI	112	7.8	2.6	*
1-hexanol	870	MS, RI	84	62.9	26.5	*
1,2-dimethyl-benzene (o-xylene)	898	MS, RI	91	9.1	4.5	*
1-octen-3-ol	979	MS, RI	72	265.0	78.5	**
unidentified	1053		81	13.3	6.5	**
unidentified	1086		69	4.7	1.7	**
menthol	1185	MS	71	17.4	6.5	**
dodecane	1200	MS, RI	170	10.5	7.0	*
unidentified	1273		69	13.9	9.1	**
tridecane	1300	MS, RI	71	36.0	14.3	**
unidentified	1379		85	21.1	14.1	**
tetradecane	1400	MS, RI	85	19.9	11.8	**
pentadecane	1500	MS, RI	85	32.2	21.2	***
lilial	1549	MS	189	22.6	9.3	*
unidentified	1642		91	3.7	2.3	*
unidentified	1647		91	7.8	4.1	*
jasmal	1672	MS, RI	129	5.0	2.6	**
unidentified	1694		71	5.7	3.4	**
heptadecane	1700	MS, RI	85	19.9	14.9	*
unidentified			120	9.8	6.4	**
unidentified			91	28.9	7.4	*
unidentified			91	10.6	6.9	*

[a] Values (area units) are means of eight concentrate-fed lambs and eight pasture-fed lambs, respectively. [b] MI, method of identification: MS, mass spectrum comparison using Wiley and NIST libraries; RI, RI in agreement with literature values. [c] MS mass fragment of which area was integrated. [d] ***, $p < 0.001$; **, $p < 0.01$; and *, $p < 0.05$.

molecule to avoid coelution problems. The integrations were performed with Enhanced ChemStation software (version B.01.00, Hewlett-Packard).

The principle of the construction of virtual DH-MS fingerprints of the volatile fraction is shown in **Figure 1**. All mass spectra acquired during one experiment were summed and then converted into a virtual DH-MS spectral fingerprint characterized by the abundance of 198 mass fragments ranging from 33 to 230 amu.

Statistical Analysis. Data were processed using the Statistica Software release 6.1 package (Statsoft, Maisons-Alfort, France). For assessing the effect of the purge temperature and that of the muscle sample mass on the volatiles extraction efficiency, a one-way analysis of variance (ANOVA) was processed on the DH-GC-MS data according to the following model: abundance of specific ion = factor ($p < 0.05$) with the factor equal to the purge temperature for experiment 1 or sample mass for experiment 2. For experiment 3, one-way ANOVA was performed systematically on the virtual DH-MS fingerprint data and the DH-GC-MS data according to the model: abundance of DH-MS fragment or DH-GC-MS compound specific ion = type of feeding ($p < 0.05$). To assess the robustness of the discriminative variables (DH-MS fragments or DH-GC-MS compounds), a leave-one-out cross-validation procedure was undertaken on one-way ANOVA models obtained for each discriminative variable on the complete data set ($n = 16$ tissue samples). A discriminative variable was considered validated when it was significant ($p < 0.05$) for all of the 16 ANOVAs performed on the $n - 1$ data set. Normed principal components analysis (PCA) was performed on the abundances of the discriminative mass fragments or specific ions for the 16 samples to visualize the structure of the data and to assess if lamb feeding background (pasture or concentrate) could be differentiated on the basis of meat volatile composition. To assess the significance of each principal component in the discrimination, one-way ANOVAs (model: principal component = type of feeding, $p < 0.05$) were performed on both DH-MS and DH-GC-MS data sets.

RESULTS AND DISCUSSION

Operative Conditions To Analyze Raw Meat Trimmed of Fat. In experiment 1, dedicated to validate the use of a moderate extraction temperature, five replicates were carried out for each temperature tested. At 90 °C, only two replications gave consistent results in terms of RI and noise level. The number of peaks quantified using the extraction temperatures of 35, 60, and 90 °C was 213, 214, and 222, respectively. Among these peaks, 140 were affected by extraction temperature ($p < 0.05$). The compounds related to 98 of the affected peaks were tentatively identified (**Table 1**). The abundance of most of them gradually increased ($p < 0.05$) by increasing the purge temperature. This "temperature" effect certainly results partly from the modification of partition coefficient between the sample matrix and the extractive gas but also from the formation of compounds originating by thermal degradation or by lipid oxidation, which is known to be enhanced when extraction temperatures are higher than 60 °C (*23*). Nevertheless, when using a purge temperature of 35 °C, the abundances of the 98 tentatively identified compounds were measurable with a signal-to-noise ratio over 10. Consequently, the purge temperature was set at 35 °C for the following trials.

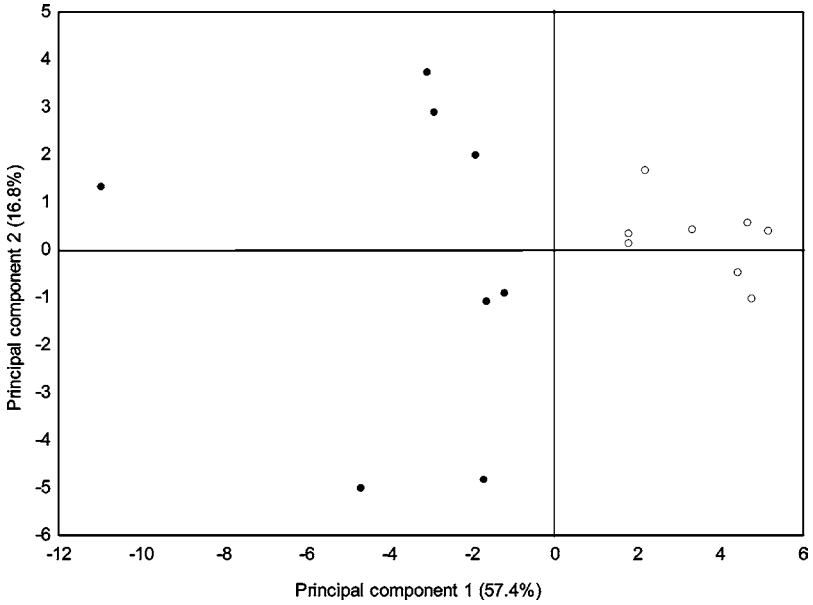

Figure 4. Discrimination of lamb muscle according to the type of feeding based on the GC-MS profile of their volatile fraction. First map of the normed PCA carried out from the abundance of the volatile compounds affected by the type of animal feeding in one-way ANOVA (33 significant compounds, 16 samples). These compounds are listed in **Table 3**. Symbols related to the two types of lamb feeding are as follows: solid circles, concentrate-fed lambs; open circles, pasture-fed lambs.

In experiment 2, the relative abundances of 80 tentatively identified volatile compounds were measured in the chromatograms of the meat samples. The abundance of 20 compounds was significantly affected by sample mass (6.25, 12.5, 25, or 50 g). Among them, 18 were detected at a higher or equal amount in the samples with the smallest mass (**Table 2**). A similar phenomenon was reported by Burbank and Qian (*26*) who found that the recovery yield of volatile compounds extracted by SPME from a cheese matrix decreased as the sample mass increased from 2 to 5 g. It is likely that increasing the sample mass also increased the amount of water to be analyzed by the purge and trap system. According to Canac-Arteaga et al. (*24*), the extraction yield of polar volatile compounds is reduced by the presence of water in the analyzed matrix because of a competition for the Tenax trap between water and polar volatiles. Finally, the smallest sample mass studied was found suitable for the identification and quantification of volatile compounds in raw meat. Because a small sample mass is also advantageous with respect to the reduced quantity of material generally available in practice for analysis, the 6.25 g sample mass was selected for the following trials.

Authentication of Lamb Feeding Based on MS Analyses of Raw Meat. Virtual DH-MS fingerprints were obtained to investigate the potential of meat volatile fraction for discriminating the type of animal feeding (pasture vs concentrate). **Figure 2** points out that 60 of the 198 mass fragments obtained from the virtual DH-MS fingerprint of muscle volatile fraction discriminated meat samples according to the type of animal feeding. The first map of the PCA performed on the abundance of those 60 fragments is shown on **Figure 3a**. One-way ANOVA showed that only the first principal component enabled a significant discrimination of muscle samples according to the type of feeding ($p < 0.05$). **Figure 3a** also reveals that the clustering was better for pasture-fed lambs as compared with concentrate-fed lambs. Moreover, the variable projection on the first map of the PCA (**Figure 3b**) indicates that the abundance of all discriminative fragments is negatively correlated with the first principal component and reveals the systematic higher abundance of fragments in the virtual fingerprint of muscle excised from concentrate-fed lambs. Finally, the examination of virtual DH-MS fingerprints demonstrated that the volatile fraction of raw muscle contained relevant information for the discrimination of the type of animal feeding.

To explain the origin of the differences in the virtual fingerprints of the raw muscle of the pasture-fed and the concentrate-fed lambs, the molecular composition of the volatile fraction was determined by DH-GC-MS. One-hundred fourteen compounds were tentatively identified in the muscle samples, whereas 90 other compounds detected above the detection threshold remained unknown despite their presence in all of the samples analyzed. According to a leave-one-out cross-validation procedure, the abundance of 33 compounds was affected by the type of animal feeding ($p < 0.05$). The tentatively identified compounds comprised two aldehydes, five alcohols, five alkanes, three alkenes, two ketones, three terpenes, one aromatic hydrocarbon, and one nitrogen compound (**Table 3**). Interestingly, all of the discriminating compounds were found in higher amounts in the muscles of the concentrate-fed lambs as compared with those found in the pasture-fed lambs. This is consistent with the systematic higher abundance of discriminative fragments in the virtual fingerprints of muscles from concentrate-fed lambs, as described above.

The higher content in most discriminating compounds in the muscle of concentrate-fed lambs is consistent with the data obtained from adipose tissues published in the literature. First, the higher abundance of 2-propanone, 2-butanone, and 2-octanone in the muscle of concentrate-fed lambs is in agreement with Sebastian et al. (*5*) who reported that these 2-ketones tend to accumulate to a larger extent in the subcutaneous fat tissue of concentrate-fed lambs. Second, the higher alkane abundance found in the muscle of concentrate-fed lambs is consistent with studies reporting that several alkanes were present at higher levels in the fat of concentrate-fed lambs as compared with pasture-fed ones (*5*, *27*). Third, the higher amount of compounds such as *cis*- and *trans*-2-octene and 1,2-dimethylbenzene in the muscle of concentrate-fed animals may have an environmental origin, as was previously shown for adipose tissues (*28*). The

capacity of plants to retain contaminants like volatile organic compounds from soil, air, and water and their contribution to the introduction of these compounds into the food chain through farm animals has been widely reviewed in the literature dealing with phytoremediation (*29*, *30*). According to Young et al. (*2*), the appearance of these volatile organic compounds in meat cannot be related to a particular type of animal feeding probably because both concentrate and pasture may be contaminated. Fourth, discriminative terpenoids (menthol, lilial, and jasmal) were also found at higher amounts in the muscle of the concentrate-fed lambs as compared with the pasture-fed lambs, and it is widely known that terpenoids can be added intentionally to concentrate feeding due to their therapeutic or aromatic properties (*31*).

However, the molecular tracers extracted from muscle differed in many aspects from those previously obtained from adipose tissues. First, numerous studies based on the analysis of adipose tissues of ruminants showed that compounds like terpenoids or 2-3-octanedione are relevant markers of animal grazing (*1*, *2*, *19*, *28*, *32*, *33*). Neither terpenoids nor 2,3-octanedione were found at higher levels in the muscle of pasture-fed lambs, suggesting that probably the information obtained from the muscular tissue differs from that obtained from the adipose tissues. Second, while the first map of the PCA performed on the GC-MS data (**Figure 4**) confirmed the discrimination of animal feeding type based on the analysis of meat volatile compounds it also confirmed the higher dispersion of the plot of the samples from concentrate-fed lambs. This later observation contradicts previous work performed on adipose tissue showing a higher interindividual variability for the lambs allowed to graze (*1*). Finally, the differences in the response of the two tissues to contrasted animal feeding are probably a consequence of the different metabolic activities of adipose tissue and muscle, the first one being an energy-storing tissue and the latter an energy-consuming tissue.

Applied to raw lamb muscles, the conditions of DH-GC-MS, which are proposed above, enabled us to successfully discriminate the type of animal feeding (pasture vs concentrate). The present study evidenced that tracers of animal feeding in lamb raw muscle differ from those previously identified in the adipose tissue (*1*−*3*, *5*). The complementarities of the information provided by both tissues are under study for the authentication of complex information regarding animal history or origin. This investigation will benefit from the recent progress in the field of chromatographic resolution, like comprehensive two-dimensional GC-MS techniques, and from the additional information extracted from MS-based system using the comprehensive combinatory standard correction data preprocessing method (*28*, *34*).

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