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Authentication of Meat Products: Determination of Animal Feeding by Parallel GC-MS Analysis of Three Adipose Tissues

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Authentication of farm animal rearing conditions, especially the type of feeding, is a key issue in certification of meat quality and meat products. The purpose of this article was to analyze in parallel the volatile fraction of three adipose tissues excised from 16 lambs in order to authenticate two animal diets: pasture ($n = 8$) and concentrate ($n = 8$). On the basis of growth rate and anatomical location, three different lamb adipose tissues were analyzed: perirenal fat (PRF), caudal subcutaneous fat (CSCF), and heart fat (HF). An initial experiment was used to optimize the extraction of volatile compounds from the adipose tissues. Using a lipid liquid phase extraction, heating the ground tissue to 70 °C, was shown to be the best sample preparation mode before dynamic headspace−gas chromatography−mass spectrometry (DH-GC-MS) analysis to achieve a good representation of the starting material, while getting a good extraction and reproducibility. Next, the application of an instrumental drifts correction procedure to DH-GC-MS data enabled the identification of 130 volatile compounds that discriminate the two diets in one or several of the three tissues: 104 were found in PRF, 75 in CSCF, and 70 in HF. Forty-eight of these diet tracers, including 2,3-octanedione, toluene, terpenes, alkanes, alkenes, and ketones, had previously been identified as ruminant pasture-diet tracers and can be considered generic of this type of animal feeding. Moreover, 49 of the 130 compounds could identify diets in only one tissue, suggesting that complementary analysis of several tissues is superior for diet identification. Finally, multivariate discriminant analyses confirmed that the discrimination was improved when PRF, CSCF, and HF were considered simultaneously, even if HF contributed minimal information.

KEYWORDS: Volatile compounds; adipose tissues; food authentication; animal diet; instrumental drift correction; GC-MS

INTRODUCTION

There is an increasing consumer demand for information about ruminant production, in particular diet types (*1, 2*). Indeed, the consumer is aware of the influence of animal feeds on the quality of animal products in terms of organoleptic (*3*), nutritional (*4*), and health (*5*) values. Additionally, the green image of the animal products has caused some consumer concerns (*6*), especially for ovine (*7*) and bovine products (*8, 9*). The BSE outbreak has increased public interest in these issues, resulting in a demand for analytical tools capable of guaranteeing that quality commitments are met (*10*).

Diet strongly influences the composition of animal products, especially volatile compound content. These compounds are transferred directly from the feed to the final product (*11*) or result from the metabolism of the feed (*12*). Dynamic headspace−gas chromatography−mass spectrometry (DH-GC-MS) is suitable for identifying volatile compounds that can discriminate different types of animal feed (*13*−*16*). Additionally, the resolving power of this approach can be increased by correcting the GC-MS signal for instrumental drifts (*17, 18*). Similarly, in order to limit analytical artifacts from degradation of the volatile compounds, the extraction conditions must be optimized. For example, in a study of lamb muscle it was shown that the reliability of GC-MS could be improved by reducing the extraction temperature (*19*).

Volatile compounds are lipophilic, and therefore, most authentication analyses are carried out on adipose tissues (*14, 15, 18, 20*). Nevertheless, the discriminating compounds vary significantly (*21*), which can be attributed to methodological differences including rearing practice, volatile compound analysis, and the tissue chosen for analysis. Differences have mainly been

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identified in fatty acid profiles (*22, 23*) but are predicted to occur in volatile compounds as well because some of these are end-products of fatty acid metabolism. However, these differences between adipose tissues can be used to increase the robustness of feed authentication based on volatile compound analysis.

This three-step study was designed to compare feed authentication data from the simultaneous analysis of three different adipose tissues. The first step aimed at optimizing volatile compound analysis of three lamb adipose tissues, perirenal (PRF), caudal subcutaneous (CSCF), and heart fat (HF). The second step aimed at identifying for each of the three tissues the volatile compounds that could discriminate pasture- and concentrate-based diet feeding. The last step aimed at assessing the relevance of parallel analysis of several adipose tissues for improving feed authentication.

MATERIAL AND METHODS

Animal Products. The lambs used for the experiments were reared at the "La sapinière" INRA research center, under controlled conditions. To determine the best adipose tissue sampling procedure for volatile fraction analysis, and to tentatively identify the compounds characteristic of each adipose tissue, a male lamb reared with an alternate pasture and concentrate diet was slaughtered at the target age of 220 days. For authentication of animal feeding, a herd of 16 male lambs born during a 2 week period were divided into two groups of 8 after weaning. The first group was exclusively pasture fed (90% rye grass) with no additional feed. The second group was kept indoors and fed a pelleted concentrate mixed with 30% hay. The concentrate was a complete lamb diet (GUYOMARC'H Nutrition Animale, Tours, France). The animals were all slaughtered according to conventional EU procedures, at the target age of 220 days.

Perirenal and subcutaneous fat were chosen for this study because these have been used for diet authentication in previous studies (*14, 18, 21*), and they have different growth rates. Perirenal fat develops early in lamb development, whereas subcutaneous fat builds later postnatally (*24*). Heart fat was included as well because of its internal position, near an organ, which provides different diet information.

Preparation of Samples. At one hour postmortem, a sample of each adipose tissue was excised from the lamb carcass: perirenal fat (PRF) from around the left kidney; caudal subcutaneous fat (CSCF) collected 10 cm from the tail; and heart fat (HF) removed from the surface of the heart at the auricle level. The adipose samples were trimmed of any trace of muscle and immediately immersed in liquid nitrogen, wrapped in aluminum foil, vacuum packed, and stored at −80 °C.

For the first set of experiments, the adipose tissue was cut into small cubes (less than 0.1 g). Four different processing schemes were tested: (i) the tissue sample (raw tissue, RT) was cut into small cubes; (ii) the tissue sample was powdered (ground tissue, GT) by grinding the frozen tissue pieces in liquid nitrogen with a crushing machine (model Dangoumeau, Prolabo, Nogent-Sur-Marne, France) obtaining a fine homogeneous powder; lipid liquid extracts were obtained from 3 g of tissue ground under nitrogen flow then heated for 15 min at either (iii) 70 °C (LE70) or (iv) 90 °C (LE90) in a 100−800 oven (Memmert, Schwabach, Germany).

Each sampling procedure consisted of four (PRF and CSCF) or three (HF) replicates, due to the amount of available HF tissue. Samples were put in glass vials (Wheaton Science Products), which were sealed under nitrogen flow and stored at −20 °C. Immediately before analysis, the frozen LE70 and LE90 extracts were melted for 6.5 min at 70 and 90 °C, respectively.

Addition of Standards. Standards were used only in experiments for diet authentication (second and third step of the work) and included in the comprehensive combinatory standard correction (CCSC) of instrumental drifts. As previously described in Engel and Ratel (*18*), six standards were chosen according to several criteria such as (i) boiling point compatible with the experimental conditions, (ii) stability, (iii) absence from samples before analysis, (iv) purity of the available commercial solutions, (v) relative specificity of their mass spectrometry fragmentation, and (vi) innocuousness. The standards were 2-methyl-

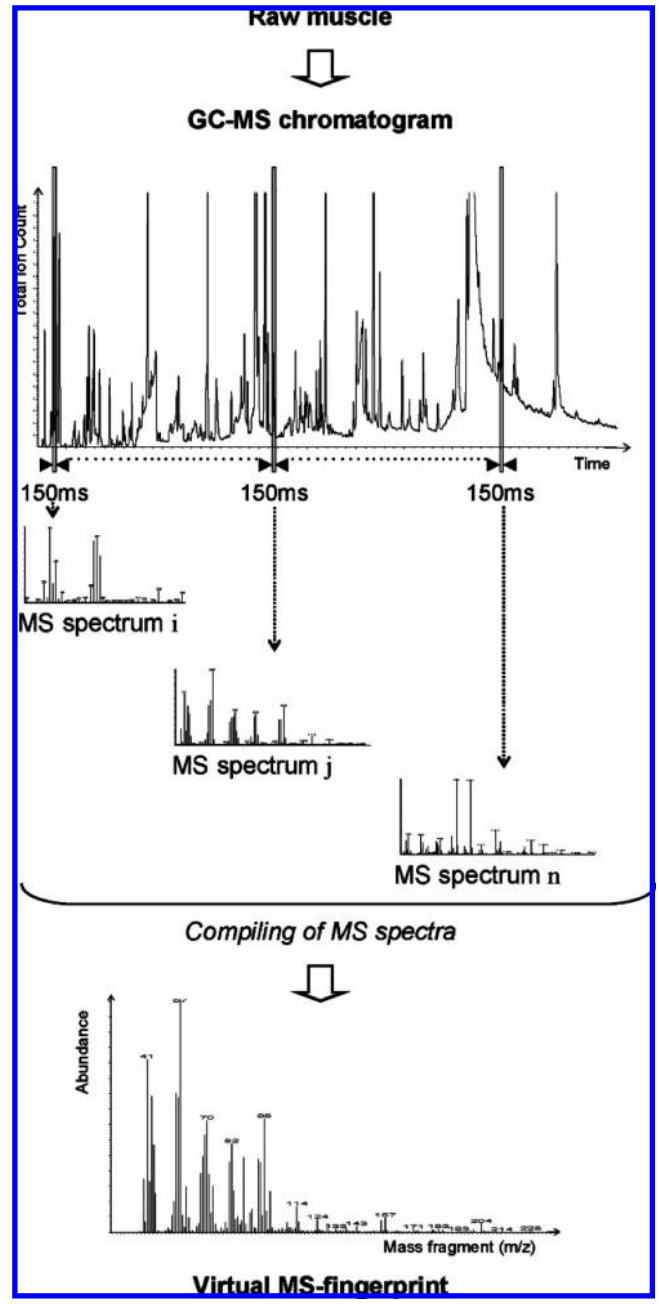

Figure 1. Schematic showing the construction of a virtual MS fingerprint of the volatile fraction of a raw muscle sample from a GC-MS chromatogram of this tissue. The mass spectra were acquired every 150 ms, summed, and then converted in a virtual MS spectral fingerprint characterized by the abundance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

pentane (S1; purity 99.5%), 1-bromo-butane (S2; purity 99.7%), fluoro-benzene (S3; purity 99.7%), bromo-benzene (S4; purity 99.5%), 1-fluoro-naphtalene (S5; purity 99.0%), and 1-phenyl-nonane (S6; purity 99.8%) (Sigma-Aldrich Chimie, St.-Quentin-Fallavier, France). A mix of the six standards was coanalyzed with the LE70 at a final concentration of approximately 50 ppm for each standard (w/w).

Dynamic Headspace−Gas Chromatography−Mass Spectrometry (DH-GC-MS) Analysis. In the initial experiment, the mass of sample analyzed in each replicate was 3 g of native tissue or powder and 1.2 g of liquid lipid extracts, which was the mean weight of the liquid phase extracted from 3 g of powder at either 70 or 90 °C. This enabled the comparison of the volatile compounds from each of the four sampling procedures.

Glass wool (0.2 g) (VWR International, Fontenay-sous-Bois, France) was introduced into a glass extraction cartridge (diameter 28 mm, length

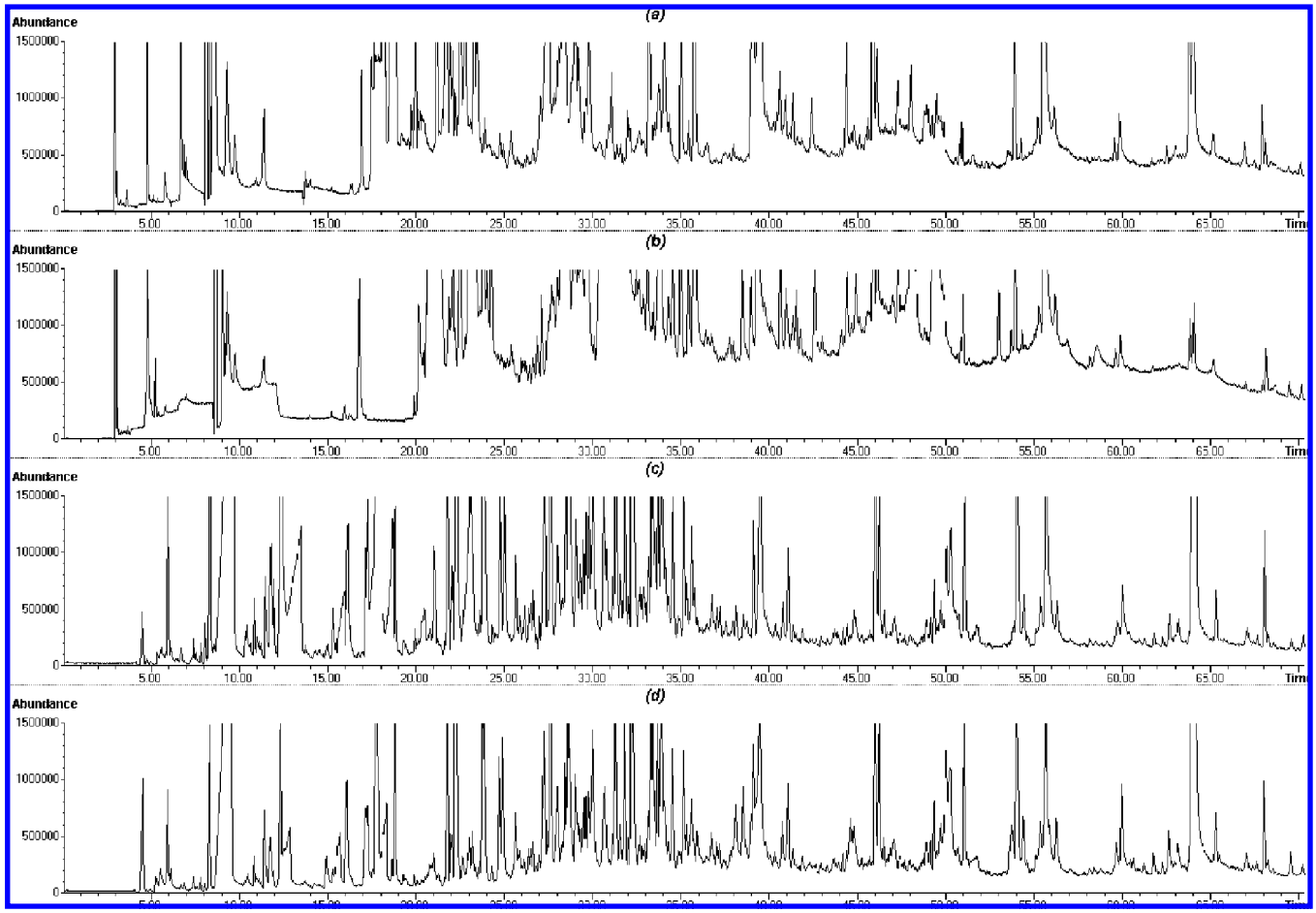

Figure 2. DH-GC-MS profile of lamb perirenal fat (PRF) processed in 4 different ways: RT: raw tissue (**a**), GT: ground tissue (**b**), LE90: 90 °C lipid-extracted tissue (**c**) and LE70: 70 °C lipid-extracted tissue (**d**).

100 mm, Ets. Maillière, Aubière, France). One gram of sample was put onto the glass wool, and 10 μL of the standard mix was added on a second layer of 0.1 g of glass wool placed at the top of the sample. The volatile fraction was extracted by dynamic headspace using a purge-and-trap device (model 3100 Sample Concentrator, Tekmar, Cincinnati, OH, USA). After a 5 min prepurge and a 15 min preheat, the headspace of the sample was purged for 30 min under a 65 mL·min^{-1} helium flow (He U quality, purity 99.995%, Messer, St.-Georges-d'Espéranche, France). The temperature of the sample during the DH extraction step was set at 90 °C for RT and GT, and at 70 or 90 °C for LE70 and LE90, respectively. The volatiles were trapped by adsorption on a porous-polymer adsorbent Tenax trap column (Tenax TA, straight, 12" × 30.5 cm, 24 cm of adsorbent, Supelco, Bellefonte, PA, USA) maintained at 36 °C. After a 5 min dry purge at 36 °C, the volatile compounds were desorbed for 10 min at 230 °C under helium flow (He N55, purity 99.9995%, Messer). Extracted compounds were then transferred to the head of a capillary column after cryoconcentration at −150 °C. After desorption, the Tenax trap was further heated for 30 min at 230 °C.

The compounds condensed at the head of the column were analyzed by GC (model 6890, Hewlett-Packard, PA, USA), the interface being heated to 225 °C for 2 min followed by the injection of the compounds in the splitless mode into the nonpolar phase of the capillary column (SPB5, 60 m × 0.32 mm × 1 μm, Sigma-Aldrich, St. Louis, MO, USA). The oven temperature was maintained at 40 °C for 5 min, then increased to 230 at 3 °C·min^{-1}, and maintained at this temperature for 10 min. The GC column was connected to a mass spectrometer (model 5973A, Hewlett-Packard). The temperature of the transfer line was set to 230 °C. The temperature was fixed at 180 °C in the MS source and at 150 °C in the MS quadrupole. The electron impact energy was set to 70 eV, and data were collected in the range of *m*/*z* 33 to 230 at a scan range of 6.85 scan·s^{-1}. Tentative identification of volatiles was based on (i) mass spectra by comparison to MS spectra databases including NBS 75K, Wiley 275 L, or Masslib (MSP Kofel, Zollikofen, Switzerland) and (ii) comparison of retention indices (RI) with published RIs (*25*) or with those of our internal data bank. The peak area of the tentatively identified compounds was integrated from a specific ion for each of the molecules to avoid coelution problems. The integrations were performed with the Enhanced ChemStation software (version D.01.02.16, Hewlett-Packard).

The principle of the construction of virtual DH-MS fingerprints for the volatile fractions is shown in **Figure 1**: the mass spectra were acquired every 150 ms from the DH-GC-MS chromatogram and were summed, resulting in a virtual DH-MS spectral fingerprint characterized by the abundance of 198 summed mass fragments ranging from 33 to 230 amu (*18*).

Data Treatment. Data were processed using the Statistica Software package (release 7.0, Statsoft, Maisons-Alfort, France) and the R software version 2.1.4.

To compare the sampling procedures of the adipose tissues, the virtual DH-MS data were used (**Figure 1**), and each abundance of the mass fragment was normalized to the sum of all (i.e., internal normalization). A one-way analysis of variance (ANOVA) was then carried out according to the model: abundance of DH-MS mass fragment = factor ($p < 0.05$), with the factor equal to the sampling procedure (raw tissue, ground tissue, and liquid lipid phase extracted at either 70 or 90 °C). The selected mass fragments were again corrected by internal normalization and a one-way ANOVA (with same factor) followed by a leave-one-out cross validation performed according to Engel and Ratel (*18*). Principal component analysis (PCA) was performed on the abundances of the discriminant mass fragments to visualize the structure of the data. To assess the significance of each principal component in the discrimination between the different procedures of sampling, one-way ANOVA (model: first principal component: sampling procedure, $p < 0.05$) and Newman−Keuls mean comparison tests were performed. To identify the qualitative differences

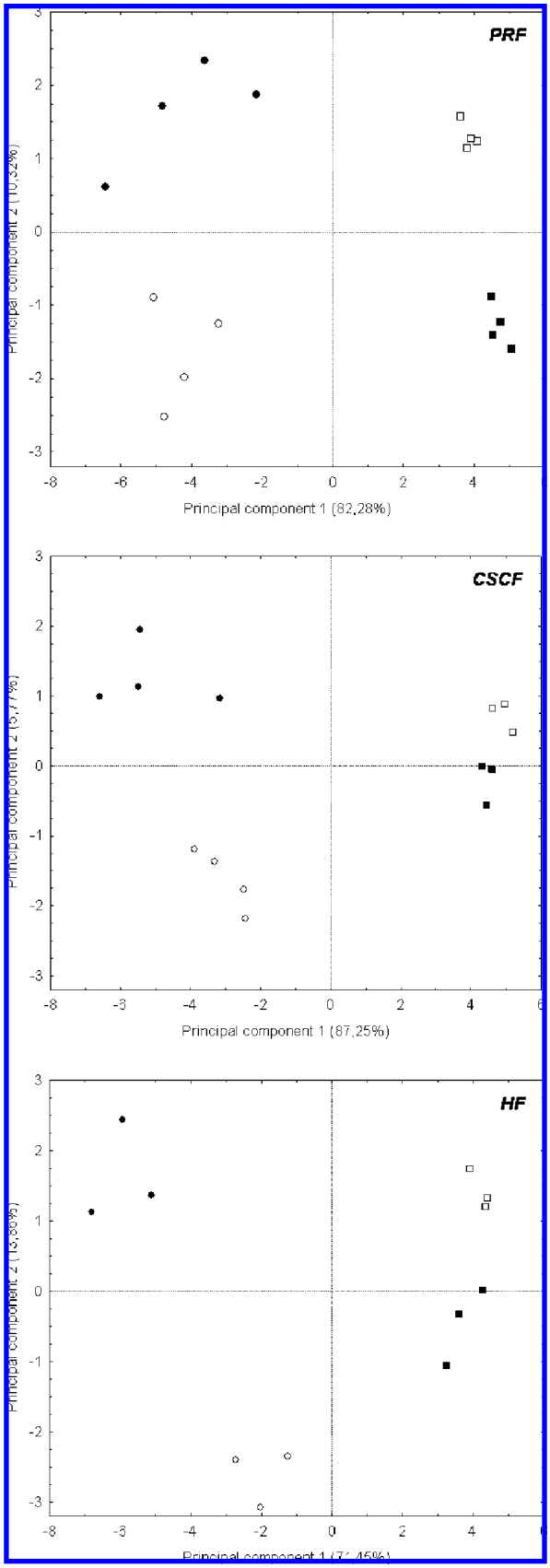

Figure 3. Discrimination of lamb adipose tissues according to sampling procedure, on the basis of virtual MS fingerprints of the volatile compound compositions: RT: raw tissue (●), GT: ground tissue (○), LE70: 70 °C lipid-extracted tissue (□), LE90: 90 °C lipid-extracted tissue (■). PCAs were carried out on significant mass fragments of the perirenal fat (PRF), the caudal subcutaneous fat (CSCF), and the heart fat (HF) samples, processed in the 4 different ways.

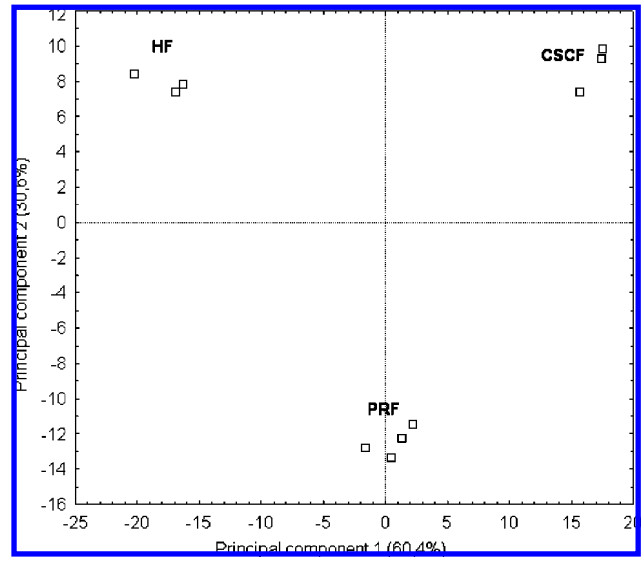

Figure 4. Discrimination of the three adipose tissues on the basis of the GC-MS profile of the volatile fraction of their LE70s. PCA was carried out on the abundances of the volatile compounds affected by the type of adipose tissue in one way ANOVA (136 significant compounds, 11 samples). PRF, perirenal fat; CSCF, caudal subcutaneous fat; HF, heart fat.

between the different extraction modes and between the three tissues, the abundances of the semiquantified targeted compounds were filtered by a one-way ANOVA (model: abundance of compound specific ion: sampling procedure or type of tissues, $p < 0.05$) followed by a leave-one-out cross validation procedure, as in Engel and Ratel (*18*). A Student test was then performed on the abundances of the discriminant compounds to identify significant variations.

For the discrimination of animal feed type, DH-GC-MS raw data were processed with CCSC as described in Engel and Ratel (*18*). Briefly, the mixture of the six selected standards being analyzed along with the sample, the abundance of each compound specific ion was normalized by the sum of the abundances of specific ion of standards, selected among the $\sum_{p=1}^{6} C_6^p$ possible sums, where p represents the number of standards involved in a given sum, that enabled the best product discrimination. According to Engel and Ratel (*18*), a one-way ANOVA followed by a leave-one-out validation procedure (model: CCSC pretreated abundance of compound specific ion = type of feeding, $p < 0.05$) was performed for each specific ion in order to select the discriminant-corrected abundances. When several corrected abundances were significant for a compound-specific ion, the combination of standards with the highest F-Fisher value was selected. Principle component analyses (PCA) were performed on the abundances of the discriminant compound-specific ion to visualize the structure of the data. To assess the significance of each principal component in the discrimination, one-way ANOVA (model: first principal component: type of feeding, $p < 0.05$) was used. To assess the gain of discriminative information by considering the three tissues together, a discriminant analysis (DA) was carried-out on the abundances of the volatile diet tracers selected by a one-way ANOVA followed by leave-one-out cross validation procedure (model: CCSC pretreated abundance of compound specific ion = type of feeding, $p < 0.05$) among the three adipose tissues. The Wilk's λ values give the quality of discrimination between the two diets, and these were determined for the combinations of three tracers (triplets) built up according to the best subset algorithm.

RESULTS AND DISCUSSION

1. Sampling Procedure for Extraction of Volatile Compounds from Adipose Tissues. First, we established a sampling procedure for extraction and DH-GC-MS analysis of the volatile fraction of lamb adipose tissues. The challenge was to achieve a good representation of the starting material, while getting good

extraction and reproducibility. Direct extraction of the volatile compounds from the raw tissue led to a poor quality signal, characterized by a high baseline (**Figure 2a**). Grinding the raw tissue into a fine powder did not improve the signal quality (**Figure 2b**). However, using a lipid liquid phase extraction, heating the ground tissue to 90 °C, resulted in an improved analytic signal with a lower baseline level (**Figure 2c** (*18*)). Next, it was determined if a lower extraction temperature would further improve the signal quality. However, decreasing the extraction temperature from 90 to 70 °C had no effect (**Figure 2d**).

A principal component analysis (PCA) was performed on the DH-MS virtual fingerprints of the PRF, CSCF, and HF, processed using the four sampling procedures, to assess how well these represented the raw tissue. For the three adipose tissues, the first map of the PCA (**Figure 3**) shows the differences in the volatile profile between raw tissue (RT), ground tissue (GT), 90 °C (LE90) and 70 °C lipid-extracted tissue (LE70). Newman−Keuls mean comparison tests of the coordinates of the observations on factorial axes show that these differences between RT, GT, LE90, and LE70 are significant ($p < 0.05$). Moreover, the first PCA map shows a higher dispersion of RT and GT fingerprint clusters, compared to the lipid extracts, consistent with lower reproducibility. This can partially be explained by the poor signal quality observed for RT and GT in **Figure 2**.

Next, it was determined which sampling procedure yielded the best data for identification of animal feed types. Focusing on 66 molecules identified in previous studies as ruminant feeding tracers (*15, 18, 21*), the composition of the volatile fractions from the different extraction modes were evaluated and compared with PRF tissue. Forty of the 66 volatile compounds were significantly influenced by the mode of extraction (data not shown). Student's *t* tests showed that grinding of the RT into GT significantly decreased the amounts of 2,3-butanediol, propanoic and hexanoic acids, but these compounds remained quantifiable. Conversion of GT into LE90 decreased the amount of γ-dodecalactone, ethylester-octanoic and hexanoic acids, although these compounds remained quantifiable, and it significantly increased the amount of 27 other volatile compounds. In a direct comparison of the RT and LE90 chromatograms, the amount of 28 different compounds increased. This relative increase most likely is due to the lower amount of water in the lipid extracts (*19*), consistent with work by Canac-Arteaga et al. (*26*), who showed that water in the sample headspace significantly reduced the extraction of volatile compounds and signal quality.

There were no significant decreases in any of the 40 compounds when the LE90 and LE70 were compared. However, 13 compounds were more abundant in the LE70 volatile fraction, which makes the LE70 procedure preferable to the LE90. Conversion of the RT into LE70 via GT led to a decrease in 5 compounds (hexanoic acid, 2,3-butanediol, γ-dodecalactone, dimethylsulfone, and ethyl-ester-octanoic acid), although these remained quantifiable, and to an increase in the amount of 34 compounds. The similarity of the PCA maps from the PRF, CSCF, and HF fingerprints (**Figure 3**) indicates that similar conclusions can be drawn for all three adipose tissues.

2. Identification of Diet Tracers in Three Lamb Adipose Tissues. Next, LE70 lipid extracts were used to identify volatile feed-specific tracers in three adipose tissues of lambs fed either a pasture ($n = 8$) or a concentrate-based ($n = 8$) diet. A list of 170 potential tracers was constituted. It includes 60 diet tracers previously identified in lamb fat tissues (*15, 18, 21*) and 43 compounds presumed to be relevant because of their belonging to tracer chemical families (*21*). The second part of the list includes 67 additional compounds that can be used to discriminate between perirenal (PRF), caudal subcutaneous (CSCF), and heart fat (HF). These compounds were identified from a total of 454 volatile compounds that were semiquantified in lipid

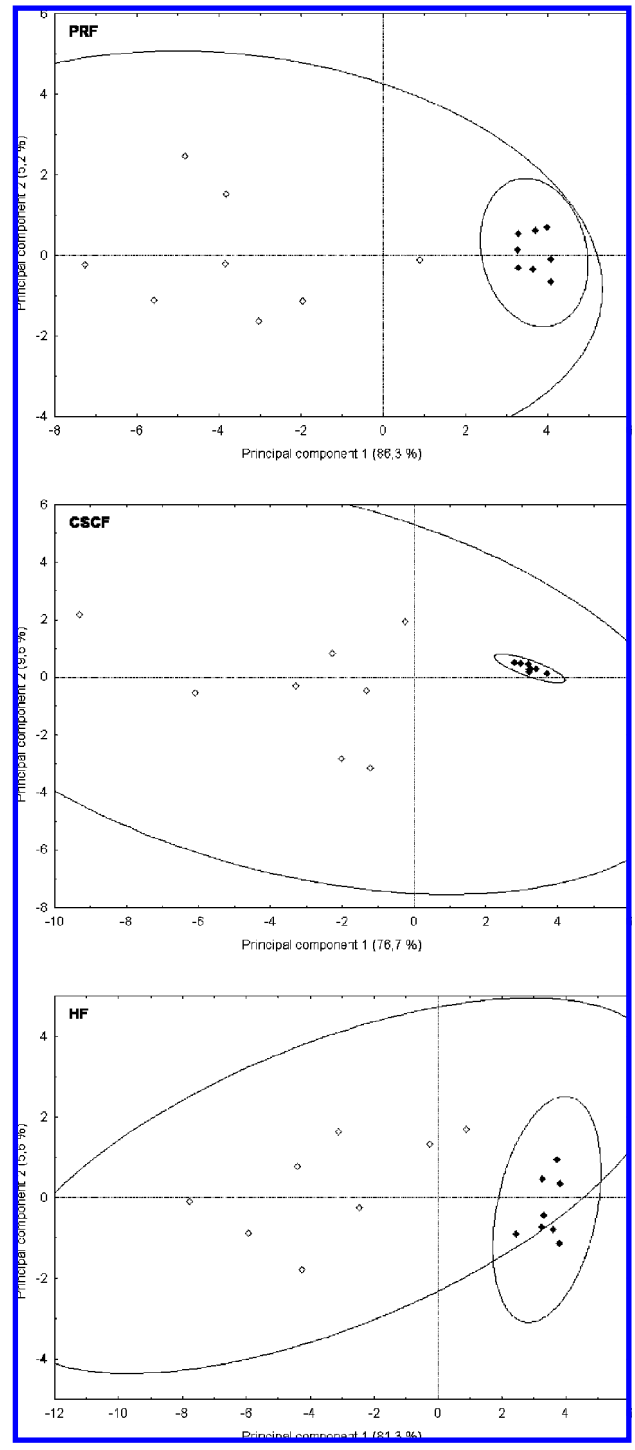

Figure 5. Discrimination of lamb adipose tissues according to feed type: pasture (◊) and concentrate (♦). PCAs were carried out on the abundances of the 20 best discriminant volatile compounds (lowest *p* value) among those stated affected by the type of diet in one way ANOVA in each type of tissue: 104 compounds were identified as diet tracers in perirenal fat (PRF), 75 in caudal subcutaneous fat (CSCF), and 70 in heart fat (HF). The observations have been clustered into confidence ellipses at $\alpha = 5\%$.

Table 1. List of Diet Tracers Determined in Perirenal Fat (PRF), Caudal Subcutaneous Fat (CSCF), and Heart Fat (HF) of 16 Pasture or Concentrate Fed Lambs

compounds[a] ($n = 130$)	ref [b]	diet[d]		
		PRF [c]	CSCF[c]	HF[c]
Alkanes				
heptane, 2,3-dimethyl-		*P*		
hexane	*15*		*P*	
cyclohexane, 1-methyl-	*18*	*P*		
heptane, 3-ethyl-		*P*		
cyclohexane, propyl-		*P*		
undecane		*P*		*P*
heptane, 3-methyl-	*18*			*P*
tridecane	*15,18*		*P*	*P*
pentane		*P*	*P*	
hexadecane	*15*	*P*		*P*
nonane, 3-methyl-	*18*	*P*	*P*	*P*
octane, 2-methyl-		*P*	*P*	*P*
nonane		*P*	*P*	*P*
undecane, 2,10-dimethyl-		*P*	*P*	*P*
tridecane, 2-methyl-		*P*	*P*	*P*
dodecane, 2,6,10-trimethyl-		*P*	*P*	*P*
tetradecane	*15*	*P*	*P*	*P*
decane, 2,3,7-trimethyl-	*15*	*P*	*P*	*P*
tetradecane, 3-methyl-		*P*	*P*	*P*
pentadecane	*15*	*P*	*P*	*P*
cyclododecane, 1-ethyl-2-methyl-		*P*	*P*	*P*
Alkenes				
(*E*)-1-octene			*P*	
1-pentene		*P*	*P*	*P*
butene, dimethyl-		*P*	*P*	*P*
(*Z*)-2-octene	*18,31*	*P*	*P*	*P*
(*E*)-2-octene	*18,31*	*P*	*P*	*P*
(*Z*)-4-octene	*18*	*P*	*P*	*P*
(*E*)-4-octene	*18*	*P*	*P*	*P*
1-pentadecene		*P*	*P*	*P*
Alcohols				
1-octen-3-ol		*P*		
1-hexanol		*P*		
1-hexanol, 2-ethyl-		*P*		
1-octanol		*P*		
1-butanol			*P*	
phenol, 2,6-bis (1,1-dimethyl-ethyl)-4-methyl-			*P*	
2,3-butanediol	*18*		*P*	
1,3-butanediol	*18*		*P*	*P*
1-pentanol		*P*	*P*	
1-penten-3-ol		*P*	*P*	*P*
Aldehydes				
(*Z*)-2-heptenal	*15*			*C*
hexanal	*21*			*C*
heptanal	*15,21*	*P*		
(*E*)-2-Hexenal		*P*	*P*	
2,4-heptadienal	*15,18*	*P*		*P*
pentanal	*18*	*P*	*P*	
(*Z*)-4-heptenal	*15,18*	*P*		*P*
(*E*)-2-pentenal	*18*	*P*	*P*	*P*
2-butenal, 2-methyl-		*P*	*P*	*P*
butanal		*P*	*P*	*P*
propanal, 2-methyl-		*P*	*P*	*P*
butanal, 3-methyl-		*P*	*P*	*P*
2-butenal	*18*	*P*	*P*	*P*
Benzenic Compounds				
o-xylene			*P*	
benzaldehyde, 2-hydroxy-				*P*
styrene		*P*		
benzene, 1,3,5-trimethyl-	*18*	*P*		
benzene, 2-ethyl-1,3-dimethyl-	*18*	*P*		
benzene, 2-ethyl-1,4-dimethyl-		*P*		
p-xylene		*P*	*P*	
benzene, 1-ethyl-2,3-dimethyl-		*P*		*P*
benzene, 1,2,4,5-tetramethyl-	*18*	*P*		*P*
benzene, 1,2,3,4-tetramethyl-	*18*	*P*		*P*
benzaldehyde	*18,31*	*P*		*P*
benzene		*P*	*P*	*P*

Table 1 Continued

compounds[a] ($n = 130$)	ref[b]	diet[d] PRF[c]	diet[d] CSCF[c]	diet[d] HF[c]
toluene	*18*	*P*	*P*	*P*
Ketones				
4-heptanone	*15*	*P*		
2-undecanone	*15, 18*	*P*		
acetone			*P*	
2,3-butanedione			*P*	
ethanone, 1-(1-cyclohexen-1-yl)-		*P*		
2-decanone		*P*		
1-octen-3-one	*18*	*P*		
5-hepten-2-one, 6-methyl-		*P*		
2-nonanone	*15, 18*	*P*		*P*
2-pentanone, 4-methyl-		*P*		*P*
2-butanone, 3-methyl-		*P*	*P*	
2-heptanone	*15*	*P*	*P*	
2-heptanone, 6-methyl-		*P*	*P*	
2-octanone	*15*	*P*	*P*	
acetophenone	*18*	*P*		*P*
3-penten-2-one		*P*	*P*	
2-cyclopenten-1-one, 3-methyl-		*P*	*P*	
2-hexanone		*P*	*P*	
cyclopentanone		*P*	*P*	
(*E*,*E*)-3,5-octadien-2-one	*18*	*P*		*P*
2-cyclopenten-1-one, 2-methyl-		*P*	*P*	
2-pentanone		*P*	*P*	
2,3-octanedione	*15,18,21*	*P*	*P*	
2-tridecanone	*15*	*P*	*P*	*P*
3-cyclohepten-2-one		*P*	*P*	*P*
Chlorines				
ethane, trichloro-				*P*
trichloromethane			*P*	
heptane, 1-chloro-		*P*		
octane, 1-chloro-			*P*	*P*
Esters				
methanoic acid, ethyl-ester-				*P*
acetic acid, ethyl-ester-		*P*		
propanoic acid, ethyl-ester-			*P*	
Nitrogen Compounds				
indole	*15, 21*			*P*
pyrazine		*C*		
1*H*-pyrrole, 2-methyl-		*P*		
1*H*-pyrrole, 1-methyl-		*P*		
pyridine		*P*	*P*	
propanenenitrile, 2-methyl-		*P*	*P*	
1*H*-pyrrole		*P*	*P*	*P*
Lactones				
γ-butyrolactone	*18*		*P*	
5-hydroxydecanoic acid δ-lactone	*21*	*P*	*P*	
Oxygen Compounds				
furan, 2-pentyl-				*C*
2-propanol, 1-methoxy-		*P*		
ethanol, 2-butoxy-		*P*		*P*
furan, 2,3-dihydro-5-methyl-			*P*	*P*
1,4-dioxane		*P*		*P*
furan, 2-ethyl-		*P*	*P*	*P*
furan, 2-methyl-	*18*	*P*	*P*	*P*
Siliceous Compounds				
silicic acid, tetraethyl ester		*P*		
silane, ethoxytrimethyl-		*P*		*P*
Sulfur Compounds				
disulfide, dimethyl-	*21*		*P*	*P*
methional	*21*	*P*		*P*
thiophene, 3-methyl-		*P*	*P*	*P*
sulfone, dimethyl-	*18, 21*	*P*	*P*	*P*
Terpens				
naphtalene		*P*		
1,4-cineol			*P*	
1,8-cineol			*P*	
p-cymen	*18, 21*	*P*		
α-pinene		*P*		*P*

Table 1 Continued

compounds[a] ($n = 130$)	ref[b]	diet[d] PRF[c]	CSCF[c]	HF[c]
m-cymen	*18*	*P*		*P*
limonen		*P*		*P*
β-caryophyllene	*18,21*	*P*	*P*	*P*
geranylacetone		*P*	*P*	*P*
α-copaene	*18*	*P*	*P*	*P*
trans-cadina-1(6),4-diene	*18,21*	*P*	*P*	*P*

[a] Compounds foud significant of the type of diet in the three adipose tissues ($n = 104$ for perirenal fat; $n = 75$ for caudal subcutaneous fat; $n = 70$ heart fat). [b] Literature references where the considered compound has already been stated as a diet tracer. [c] Adipose tissues: PRF, perirenal fat; CSCF, subcutaneous caudal fat; HF, heart fat. [d] Type of diet related to the considered compound in this study.

extracts of the three adipose tissues excised from one lamb. The abundance of 136 compounds differed significantly ($p < 0.05$) between the tissues. A PCA map based on these 136 variables (**Figure 4**) clearly discriminates the 3 adipose tissues and confirms our working hypothesis. The different compositions of these three tissues may in part be due to different metabolic functions, the proximity of other organs, and different growth rates. Of the 136 compounds, 99 were identified in the LE70 of 16 lambs, including 32 diet tracers from the first part of the list.

Correcting for instrumental drifts, the relative amount of the 170 volatile compounds was determined in the LE70 of three adipose tissues from the 16 lambs. One hundred and four compounds were found as feed-specific diet tracers in PRF, 75 in CSCF, and 70 in HF. PCAs were performed on the 20 most discriminative compounds (exhibiting the lowest p value) of the data matrices of each tissue. The differences between pasture- and concentrate-fed lambs were consistent with previous reports and document that volatile fraction analysis is well-suited for lamb diet discrimination (**Figure 5**). One-way ANOVA of the first principal component shows the feed-type differences ($p < 0.05$) were significant for all three tissues. The diet specific tracers are listed in **Table 1**, and of these, 49 were significant in just one tissue, 43 in two, and 38 in three tissues. The fact that some of these 130 diet tracers are tissue-specific confirms the differences in diet information obtained from the selected adipose tissues.

Of the 130 identified diet tracers, only 51 were previously reported in the literature. The increased number of tracers identified in this study can be explained by gentle sample processing, the use of three tissues in parallel, and the use of lambs raised under controlled laboratory conditions. Twenty-five of the tracers were identified in only one previous work where the CCSC method was also processed to correct for instrumental drifts and to unmask some relevant compounds (*18*). Most of these twenty-five compounds were probably revealed in the two studies by the use of this preprocessing method.

Of 51 commonly identified diet tracers, 3 were identified as concentrate-diet tracers, whereas 48 were identified as pasture-specific tracers. On the basis of these results, we propose that these compounds can serve as generic tracers for diet authentication.

2,3-Octanedione. Our data confirm that 2,3-octanedione is a reliable pasture diet tracer. This tracer is 2 to 10 times more abundant in adipose tissues of animals fed a pasture diet when compared to animals fed a concentrate diet (*14, 15, 18*). In this study, the difference between pasture and concentrate feeding varied from 7- to 23-fold. Young et al. (*20*) have suggested

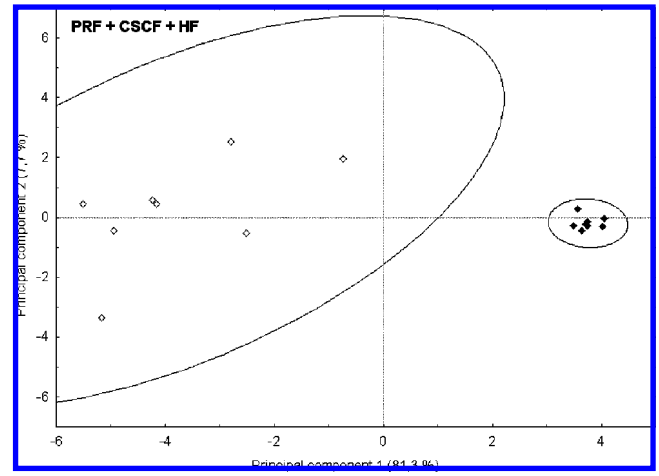

Figure 6. Discrimination of lamb adipose tissues according to feed type: pasture (◊) and concentrate (♦). PCA was carried out on the abundances of the 20 best discriminant diet tracers (lowest *p* value) of the whole data set composed by the diet tracers of the three adipose tissues ($n = 249$): PRF (perirenal fat), CSCF (caudal subcutaneous fat), and HF (heart fat). The observations have been clustered into confidence ellipses at $\alpha = 5\%$.

that 2,3-octanedione originates from oxidation of linoleic acid, catalyzed by the lipoxygenase that is present in leafy plants and absent in seeds except for soybeans (*27*).

Toluene. This compound was found in higher amounts (~70 times more) in adipose tissues of the pasture-fed animals. It may originate from the carotenoids provided by pasture feeding (*28*) or in part from pollutants retained in green plants (*29, 30*). Engel and Ratel (*18*) have proposed that this retention phenomenon of atmosphere pollutants in live green plants may explain the presence of others alkyl-benzenes in adipose tissues.

Terpenes. Eleven terpenes were characteristic of the pasture diet. This is consistent with numerous reports showing that these compounds generally originate from dicotyledons (*21*). Terpenes are generally considered too plant-specific to be used as generic tracers in the authentication of feed type. However, *m*- and *p*-cymen, α-copaene, β-caryophyllene, and *trans*-cadina-1(6),4-diene were identified as pasture diet tracers in this and previous studies (*18, 21*), and these can be considered generic tracers.

Aldehydes. Eleven aldehydes could discriminate the pasture diet, whereas hexanal and (*Z*)-2-heptenal were found as concentrate-diet tracers. This is consistent with previous reports (*15, 18, 20*), although it has been pointed out that these compounds are not easily correlated with diet as they originate from lipid oxidation (*21*).

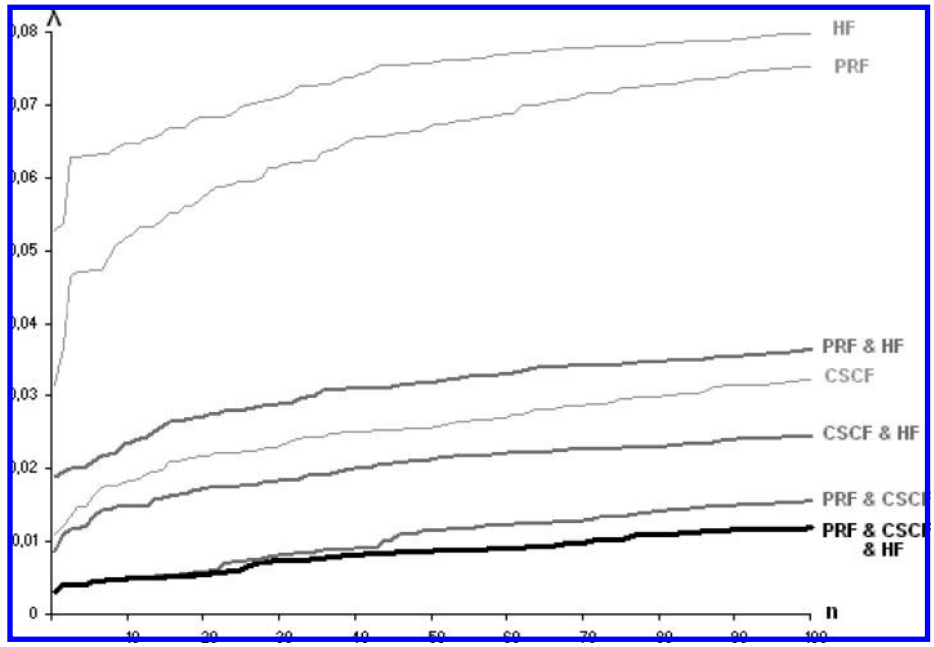

Figure 7. Comparison of diet discrimination quality by different parallel analyses of adipose tissues. Diagram of Wilk's λ values (λ) of triplets ($n = 100$) composed by diet tracers from one, two, or the three adipose tissues (PRF, perirenal fat; CSCF, subcutaneous caudal fat; HF, heart fat) after discriminant analysis (DA).

Alkanes. Several alkanes (hexane, tetradecane, pentadecane, hexadecane, 3-methyl-nonane, 1-methyl-cyclohexane, and 2,3,7-trimethyl-decane) were found in significantly higher amounts in samples from pasture-fed animals, which is consistent with other reports (*15, 18*), and these can be considered robust diet tracers.

Interestingly, several of the identified volatile compounds have not previously been identified as diet tracers: 12 alkanes, 15 ketones, 8 alcohols including 1-octen-3-ol, chlorines (trichloromethane, 1-chloro-heptane, and 1-chloro-octane), esters (ethyl-esters of methanoic, acetic, and propanoic acid), sulfur heterocycles (3-methyl-thiophene), and nitrogen heterocycles (pyridine, pyrazine, and methyls of 1*H*-pyrrole). At this point, the reliability of these compounds remains uncertain. Moreover, 11 compounds, including 5 methyl-ketones (2-heptanone, 2-octanone, 2-nonanone, 2-undecanone, and 2-tridecanone), tridecane, 4-heptanone, 2 lactones (δ-decalatone and γ-butyrolactone), 1,3-, and 2,3-butanediol, which were previously proposed as concentrate diet tracers, were found as pasture-diet tracers in our work. In contrast, no influence of the type of feeding was found for 9 compounds (2-decenal, 2-undecenal, γ-pentalactone, methyl-cyclopentane, 3-methyl-pentane, heptadecane, 3-methyl-indole, 3-methyl-1-ethyl-1-pentene, and undecanal), although they were previously reported as diet tracers (*18, 21*). These discrepancies with previous results may be explained by the composition of the concentrate given to the animals, and finally, the robustness of these compounds as feeding tracers is questionable.

3. Relevance of Parallel Analysis of Three Tissues for Lamb Diet Authentication. A PCA was performed on the 20 most discriminative tracers considering the three tissues. Compared to **Figure 5**, the first map (**Figure 6**) shows a better segregation between the two diets and enables a demonstration of the gain of the parallel GC-MS analysis of the three tissues. A one-way ANOVA performed on the 249 tracers shows that 10 tracers exhibit higher F-Fischer values: α-copaen and toluene from CSCF, (*Z*)-4-heptenal, 2,3,7-trimethyl-decane, pentanal, geranylacetone, 2-tridecanone, and 2,3-octanedione from PRF, and pentadecane and toluene from HF. These compounds had previously been identified as pasture-specific diet tracers, except for geranylacetone, which appears specific to the pasture-fed lambs in this study.

A discriminant analysis was performed on the 249 tracers from the three tissues. Next, they were combined into triplets, and the Wilk's λ values of the corresponding models were determined in oder to assess their ability to discriminate lamb diets. **Figure 7** shows the 100 most discriminative combinations (i.e., showing the lowest Wilk's λ values) formed by triplets of tracers from one, two, or three fat tissues. Triplets comprising one tracer of each of the three adipose tissues were more discriminant than those comprising tracers from two or one adipose tissue. This confirms that parallel use of three adipose tissues increases the robustness of feed authentication However, **Figure 7** also demonstrates that the performances of the models comprising the 20 best triplets from three adipose tissues (PRF, CSCF, and HF) were similar to the performances of models based on the 20 first triplets from PRF and CSCF. Finally, it appears that a systematic parallel analysis of three adipose tissues is not essential for optimal discrimination between two diets. In our model, HF did not offer relevant discriminant information about the two diets in comparison to the information from the parallel analysis of PRF and CSCF. Additionally, most of the best triplets comprised 2,3-octanedione in PRF and toluene in CSCF confirming the relevance and the robustness of these two pasture-diet tracers.

In this study, the DH-GC-MS technique and instrumental drift correction were used to analyze adipose tissues and discriminate pasture- or concentrate-fed ruminants. Individual analysis of the composition of the volatile fraction of perirenal (PRF), caudal subcutaneous (CSCF), and heart fat (HF) from lambs fed either pasture or concentrate provided sufficient information to discriminate the two diets. Nevertheless, the discrimination was improved when the three tissues were considered simultaneously, even if the HF contributed minimal information. Ruminants are typically fed with different types of diets during their breeding, and therefore, robust diet authentication requires consideration of the feed changes that have occurred during an animal's rearing. It may thus be necessary to certify the feeding history of animals by monitoring the persistence and the latency

of pasture- and concentrate-diet tracers in their tissues. The parallel analysis of PRF and CSCF appears to increase the resolving power of the authentication diagnosis, suggesting that the informative content of both tissues regarding animal diet is different possibly because PRF is formed earlier than CSCF in animal life and also because the two tissues can retain feed tracers differently. Finally, the use DH-GCxGC-MS/TOF in further work will give access to a large amount of additional tracers, which coelute in one-dimensional GC-MS, and will make further research easier.

LITERATURE CITED

(1) Bernùes, A.; Olaizola., A.; Corcoran, K. Labelling information demanded by European consumers and relationships with purchasing motives, quality and safety of meat. *Meat Sci.* **2003**, *65* (3), 1095–1106.

(2) Ward, C. E.; Trent, C. A.; Hildebrand, J. L. Consumer perceptions of lamb compared with other meats. *Sheep Goat Res. J.* **1995**, *11*, 64–70.

(3) Rousset-Akrim, S.; Young, O. A.; Berdagué, J. L. Diet and growth effects in panel assesment of sheapmeat odour and flavour. *Meat Sci.* **1997**, *45*, 169–181.

(4) Enser, M.; Hallet, K. G; Hewett, B.; Fursay, G. A. J.; Wood, J. D.; Harrington, G. Fatty acid content and composition of UK beef and lamb muscle in relation to production system and implications for human nutrition. *Meat Sci.* **1998**, *49* (3), 329–341.

(5) Nurnberg, K.; Grumbach, S.; Zupp, W.; Hartung, M.; Nurnberg, G.; Ender, K. Enhancing of n-3 fatty acids and conjugated fatty acid in lamb meat by keeping on pasture. *Fleishwirdschaft* **2001**, *81*, 120–122.

(6) Athanasios, K.; Arvanitoyannis, I. S.; Chryssochoidis, G. Is There a real difference between conventional and organic meat? investigating consumers attitudes towards both meat types as an indicator of organic meat's market potential. *J. Food Prod. Marketing* **2006**, *12* (2), 49–80.

(7) Bernabeù, R; Tendero., A. Preference structure for lamb consumers. A spanish case study. *Meat Sci.* **2005**, *71* (3), 464–470.

(8) Mennecke, B. E.; Townsend, A. M.; Hayes, D. J.; Lonergan, S. M. A study of the factors that influence consumer attitudes toward beef products using the conjoint market analysis tool. *J. Animal Sci.* **2007**, *85* (10), 2639–2659.

(9) Verbeke, W; Wardb, R. W. Consumer interest in information cues denoting quality, traceability and origin: An application of ordered probit models to beef labels. *Food Quality Preference* **2005**, *17* (6), 453–467.

(10) Douzain, N. 1996. A cultural revolution in beef and lamb. *Viandes et Produits carnés* **1998**, *19* (1), 21–24.

(11) Larick, D. K.; Hedrick, H. B.; Bailey, M. E.; Williams, J. E.; Hancock, D. L.; Garner, G. B.; Morrow, R. E. Flavor constituents of beff as influenced by forage - and grain - feeding. *J. Food Sci.* **1987**, *52* (2), 245–251.

(12) Suzuki, J.; Bailey, M. E. Direct sampling capillary GLC analysis of flavor volatiles from ovine fat. *J. Agric. Food Chem.* **1985**, *33* (3), 343–347.

(13) Ha, J. K.; Lindsay, R. C. Volatile alkylphenols and thiophenol in species-related characterizing flavors of red meats. *J. Food Sci.* **1991**, *56* (5), 1197–1202.

(14) Priolo, A.; Cornu, A.; Prache, S.; Krogmann, M.; Kondjoyan, N.; Micol, D.; Berdagué, J. L. Fat volatile tracers of grass feeding in sheep. *Meat Sci.* **2004**, *66*, 475–481.

(15) Sebastian, I.; Viallon-Fernandez., C.; Berge, P.; Berdagué, J. L. Analysis of the volatile fraction of lamb fat tissue: influence of the type of feeding. *Sciences des aliments* **2003**, *23*, 497–511.

(16) Arvanitoyannis, I. S.; Van Houwelingen-Koukaliaroglou, M. Implementation of chemometrics for quality control and authentication of meat and meat products. *Crit. Rev. Food Sci. Nutr.* **2003**, *43* (2), 173–218.

(17) Deport, C.; Ratel, J.; Berdagué, J. L.; Engel, E. Comprehensive combinatory standard correction: A calibration method for handling instrumental drifts if gas chromatography - mass spectrometry systems. *J. Chromatogr., A* **2006**, *1116* (2), 248–258.

(18) Engel, E.; Ratel, J. Correction of the data generated by mass spectrometry analyses of biological tissues: Application to food authentication. *J. Chromatogr., A* **2007**, *1154*, 331−341.

(19) Vasta, V.; Ratel, J.; Engel, E. Mass spectrometry analysis of volatile compounds in raw meat for the authentication of the feeding background of farm animals. *J. Agric. Food Chem.* **2007**, *55* (12), 4630–4639.

(20) Young, O. A.; Berdagué, J. L.; Viallon, C.; Rousset-Akrim, R.; Theriez, M. Fat-borne volatiles and sheepmeat odour. *Meat Sci.* **1997**, *45* (2), 183–200.

(21) Vasta, V.; Priolo, A. Ruminant fat volatiles as affected by diet. A review. *Meat Sci.* **2006**, *73*, 218–228.

(22) Rowe, A.; Macedo, F. A. F.; Visentainer, J. V.; Souza, N. E.; Matsushita, N. Muscle composition and fatty acid profile in lambs fattened in drylot or pasture. *Meat Sci.* **1999**, *51*, 283–288.

(23) Gatellier, P.; Mercier, Y.; Juin, H.; Renerre, M. Effect of finishing mode (pasture- of mixed-diet) on lipid composition, colour stability, and lipid composition, colour stability and lipid oxidation in meat from Charolais cattle. *Meat Sci.* **2005**, *69* (1), 175–186.

(24) Vézinhet, A.; Prud'Hon, M.; Bénévent, M.,. Evolution des differents types de dépôts adipeux après la naissance chez des agneaux Mérinos d'Arles Normaux ou Hypophysectomisés. *Annales de Biologie Animale, Biochimie et Biophysique* **1974**, *14*, 117–129.

(25) Kondjoyan, N.; Berdagué, J. L. *A Compilation of Relative Retention Indices for the Analysis of Aromatic Compounds*; Laboratoire Flaveur: Clermont Ferrand, France, 1996.

(26) Canac-Arteaga, D.; Viallon, C.; Berdagué, J. L. Analytical artefacts caused by the presence of water vapour in the headspace of food products. *Analusis* **2000**, *28*, 550–556.

(27) Keen, A. R.; Wilson, R. D. Pasture feeding - a contribution of additional flavour nuances to milk fat and meat flavour. In *Milkfat Flavour Forum*. New Zealand Diary Research Insitute.: Palmerston North, New Zealand, 1992; pp 24−31.

(28) Rios, J. J.; Fernandez-Garcia, E.; Minguez-Mosquera, M. I.; Oerez-Galvez, A. Description of volatile compounds generated by the degradation of carotenoids in paprika, tomato and marigold oleoresins. *Food Chem.* **2008**, *106*, 1145–1153.

(29) Binnie, J.; Cape, J. N.; Mackie, N.; Leith, I. D. Exchange of organic solvents between the atmosphere and grass - the use of open top chambers. *Sci. Total Environ.* **2002**, *285*, 53–67.

(30) Smith, K. E. C.; Jones, K. C. Particles and vegetation: implications for the transfer of particle-bound organic contaminants to vegetation. *Sci. Total Environ.* **2000**, *246*, 207–236.

(31) Maruri, J. L.; Larick, D. K. Volatile concentration and flavor of beef as influenced by diet. *J. Food Sci.* **1992**, *57* (6), 1275–1281.

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