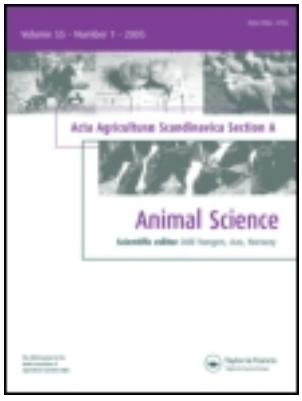

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Different patterns of volatile compounds and fatty acid profiles in the adipose tissues of male and female Hu sheep

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SHORT COMMUNICATION

Different patterns of volatile compounds and fatty acid profiles in the adipose tissues of male and female Hu sheep

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Abstract
This study was conducted to compare the volatile and fatty acid (FA) components between rams and ewes and between perirenal (PF) and subcutaneous adipose tissues (SF). The SF and PF were sampled from 24 Hu sheep. Volatile compounds and FAs were evaluated by gas chromatography–mass spectrometry. Data analysis was performed by principal component analysis. A total of 55 volatiles and 19 fatty acids were identified. Ewes had more aldehydes and less total unsaturated and monounsaturated FAs than did rams, and more aldehydes, total unsaturated and monounsaturated FAs were found in SF than in PF. Aldehydes and unsaturated FAs were closely related with the distinction between ram SF and PF. It is inferred that volatile compound profiles are different between genders and fat sites, with aldehydes as the main contributor, and that aldehydes are related to unsaturated FA contents in Hu sheep.

Keywords: *Volatiles, fatty acid, gender, adipose tissue, Hu sheep.*

1. Introduction

Hu sheep with high fertility are one of the most commonly raised sheep in southern China (Chu & Wang, 2001). The flavor profile of sheep meat is a major determinant for consumer acceptance.

Volatile compounds contribute to the flavor of meat, and lipids, as a solvent for volatiles, play essential roles in aroma evaluation (Calkins & Hodgen, 2007). Many studies have been conducted to detect the volatile compounds, and several relationships have been established between these compounds and lamb flavor category (Resconi et al., 2010; Bueno et al., 2011). In addition to volatile components, the fatty acids in animal products are also related to flavor (Nute et al., 2007), and the flavor of lamb meat can be changed by manipulating the fatty acid profiles, which could further affect the volatile composition (Elmore et al., 2005; de Campos et al., 2007). Therefore, the evaluation of volatile compounds and fatty acid profiles could provide indirect insight into meat flavor. However, no studies have been conducted to compare the volatile compound and fatty acid profiles in different adipose tissues between male and female Hu sheep.

Gas chromatography (GC) and mass spectrometry (MS) is an efficient approach that is widely used to identify volatile compounds in food chemical analyzes (Sivadier et al., 2009; Vasta et al., 2011; Reboredo-Rodríguez et al., 2012). Based on instrumental data, principal component analysis (PCA), a useful multivariate statistical tool (Kozak & Scaman 2008), is commonly used to establish relationships between flavor descriptions and volatile compositions (Holm et al., 2012).

The objectives of this study were to characterize the volatile compounds in perirenal (PF) and subcutaneous fat tissue (SF) from male and female Hu sheep by solid-phase microextraction (SPME)–GC-MS, to characterize the fatty acid profile of the adipose tissues and to provide references to Hu sheep flavor feature.

2. Materials and methods

2.1. Sampling

This experiment was carried out in accordance with the Zhejiang University Guidelines for the Care and Use of Experimental Animals. Twenty-four Hu sheep from the same farm, 12 males and 12 females,

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were slaughtered at the age of 2 years. Approximately 20 g of the PF and SF were sampled and vacuum-packed immediately following collection. After aging at 4°C for 24 hours, the samples were stored at −80°C for the subsequent determination of volatiles and fatty acid compounds.

2.2. Determination of volatile compounds and fatty acids

SPME technique was used to extract the volatile compounds from 1 g of sample with 50/30 μm divinylbenzene/carboxen/ polydimethylsiloxane fibre at 120°C. A GC-MS (GC 3800-MS Saturn 2000, Varian, USA), equipped with a J&W DB-5 capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies Inc., CA, USA), was used to analyze the volatile compounds. The SPME fiber was desorbed in an injection port at 250°C for 5 minutes. The chromatographic conditions were as follows: helium was the carrier gas (flow rate: 0.8 mL/min), and the GC oven was increased from 40°C to 250°C at a rate of 3°C/min, and then held at 250°C for 5 minutes. The mass spectrometer was connected with the GC column. The temperature of the transfer line was set at 280°C. The electron-impact energy was set at 70 eV, and data were collected in the range of m/z 40–650. Compound identification was performed via comparison of the obtained mass spectra with the compounds in the Wiley library and mass spectral database (NIST 2002, Washington, DC, USA), and the Kovats retention indices from series of standards (C6-C25 n-alkanes) were used to further confirm the identities of the compounds.

Fatty acid profiles were determined by GC in comparison to the FAMEs, which were prepared by one-step transesterification according to Rule (1997). The fatty acids were analyzed by GC with a DB-23 column (30 m × 0.32 mm × 0.25 μm). The injector and detector temperatures were kept at 220°C and 260°C, respectively. The temperature program was as follows: an initial temperature of 70°C, increasing at a rate of 5°C/min to 240°C, and held at 240°C for 5 minutes. Fatty acids were identified by comparison to known external standards.

The relative amounts of both volatile and fatty acid compounds were estimated by the area normalization method. Briefly, the peak area of each identified compound was integrated, and all the peak areas were summed up. The content of each compound was calculated from the ratio of its peak area to the sum area of all volatiles or fatty acids. The formula was as follows:

$$\text{Relative content of x} = (A_x / \sum A) \times 100$$

where A_x is the peak area of compound x and ΣA is the total area of all the identified volatiles or fatty acids.

2.3. Statistical analysis

Relative content of compounds was analyzed using the GLM procedure of SAS software system (version 9.1). The model included gender effect, fat location, and interaction of gender × fat location. Mean comparisons were evaluated when the interaction terms of the model were significant ($p < 0.05$) using LAMEANS and PDIFF separation of the entire group. The statistical model was as below:

$$Y_{ij} = \mu + G_i + L_j + GL_{ij} + e_{ij}$$

where Y_{ij} represents dependent variable; μ, average; G_i, gender effect; L_j, fat location effect; GL_{ij}, interaction of gender and fat location and e_{ij}, error.

The PCA was performed on the abundances of the volatiles and the fatty acid data. The original data were normalized by $\log_{10}$ transformation, and only the data for volatiles that were significantly affected by gender or location factor were introduced into the PCA.

3. Results

3.1. Volatile compounds in the adipose tissues

A total of 55 volatile compounds were identified by GC-MS analysis of the adipose tissues. There were seven groups of volatiles, namely, aldehydes (20 kinds), alcohols (9), ketones (11), hydrocarbons (8), esters (2), phenols (1), and others (4). A significantly higher amount of total aldehydes was found in SF compared with PF, while the total hydrocarbon content was higher in the PF than in the SF (Table I).

3.2. Fatty acid profiles of the adipose tissues

Nineteen fatty acids were identified in all adipose tissues, including 10 saturated fatty acids (SFA), 4 monounsaturated fatty acids (MUFA), and 5 poly-unsaturated fatty acids (PUFA). Rams had higher percentage of total unsaturated fatty acids (UFA) and MUFA than ewes, regardless of the fat locations ($p < 0.05$, Table II). The SF contained higher amounts of total UFA and MUFA and less SFA, total n6 fatty acids, and n6/n3 than the PF ($p < 0.05$).

Table I. Different contents of volatile group in adipose tissue from rams and ewes (% of total area).

	Ram[a]		Ewe[a]			Subject effects[b]		
Component	PF	SF	PF	SF	SEM	G	L	G ×L
Aldehydes	17.8	33.6	24.0	38.8	2.37	NS	**	NS
Alcohol	43.0	26.8	34.8	24.4	1.98	NS	NS	NS
Ketone	33.4	27.3	26.5	28.1	1.47	NS	NS	NS
Hydrocarbon	27.8	18.3	20.1	16.8	1.26	NS	**	NS
Esters	0.2	0.1	0.2	6.6	0.71	NS	NS	NS
Phenols	0.2	0.2	0.3	0.9	0.08	*	NS	*
Others	5.2	8.7	6.0	3.8	0.81	NS	NS	NS

[a]The data are expressed as the mean ($n = 12$); [b]The effects of gender (G), adipose tissue location (L) and the interaction between gender and location (G ×L) were analyzed by ANOVA; **$p \leq 0.01$; *$p \leq 0.05$; NS, not significant.

3.3. Principal component analysis of volatile compounds

The PCA results for volatile components are shown in Figure 1. Potential relationships between adipose tissues and volatiles groups could be summarized by comparing the similar spot locations in both scatter plots (Figure 1a and 1b). The aldehyde and ketone groups were associated with ram SF. Meanwhile, phenols and esters seemed to be related with ewe SF.

3.4. Principal component analysis of fatty acids profile

PCA plot of the relative amounts of fatty acids detected in different adipose tissues of Hu sheep is shown in Figure 2. The PCA was processed based on grouped fatty acids data. The SF of ram was separated from other three fat samples (Figure 2a), and the total UFA and total MUFA seemed to play a key role when combined with Figure 2b. On the other hand, the PFs of rams and ewes were not distinguished from each other, and SFA was the essential group (Figure 2b).

4. Discussion

The difference in volatile compounds in SF and PF of ram and ewe

The distribution of volatile compounds in the adipose tissues varied between the two genders. Ewe adipose tissue had higher contents of aldehyde and ketone, but lower alcohol and hydrocarbon contents. It has been reported that aldehydes were derived from oxidation processes of C18:2 (including heptanal, E-2-nonenal and E,E-2,4-decadienal) and C18:3 (E-2-pentenal and benzaldehyde) (Elmore et al., 2005). Furthermore, lower heptanal and hexanal values were detected in cheese with high proteolytic activity, as lipid oxidation was reduced by increased free peptides generated from proteolysis (Dalsgaard et al., 2012). It can be suggested that aldehyde content is closely related to oxidation. Therefore, the higher aldehyde contents in ewe adipose tissue may be attributed to higher UFA and different oxidation levels between the two genders.

Table II. Fatty acid profiles (percentage of total fatty acids) of adipose tissues (% of total area).

	Ram[b]		Ewe[b]			Subject effects[c]		
Component[a]	PF	SF	PF	SF	SEM	G	L	G ×L
SFA	65.2	41.4	66.6	49.3	2.12	*	**	NS
UFA	34.8	58.6	33.4	50.7	2.12	*	**	NS
MUFA	30.3	54.2	29.2	46.7	2.18	*	**	NS
PUFA	4.6	4.4	4.2	4.0	0.29	NS	NS	NS
n3FA	0.8	1.0	0.8	0.9	0.09	NS	NS	NS
n6FA	3.7	3.5	3.5	3.1	0.31	NS	*	NS
p/s	0.1	0.1	0.1	0.1	0.01	NS	NS	NS
n6/n3	5.3	4.0	4.8	3.7	0.57	NS	*	NS

[a]SFA = sum of C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C22:0 and C24:0; UFA = sum of C14:1, C16:1, C18:1 n9c, C18:2 n6c, C20:2 n6, C20:4 n6, C20:3 n3 and C18:3 n3; MUFA = sum of C14:1, C16:1 and C18:1 n9c; PUFA = sum of C18:2 n6c, C20:2 n6, C20:4 n6, C20:3 n3 and C18:3 n3; n3FA = sum of C20:3 n3 and C18:3 n3; and n6FA = sum of C18:2 n6c, C20:2 n6 and C20:4 n6; [b]The data are expressed as the mean ± standard error of mean (SE) ($n = 12$); [c]The effects of gender (G), adipose tissue location (L) and the interaction between gender and location (G ×L) were analyzed by ANOVA; **$p \leq 0.01$; *$p \leq 0.05$; NS, not significant.

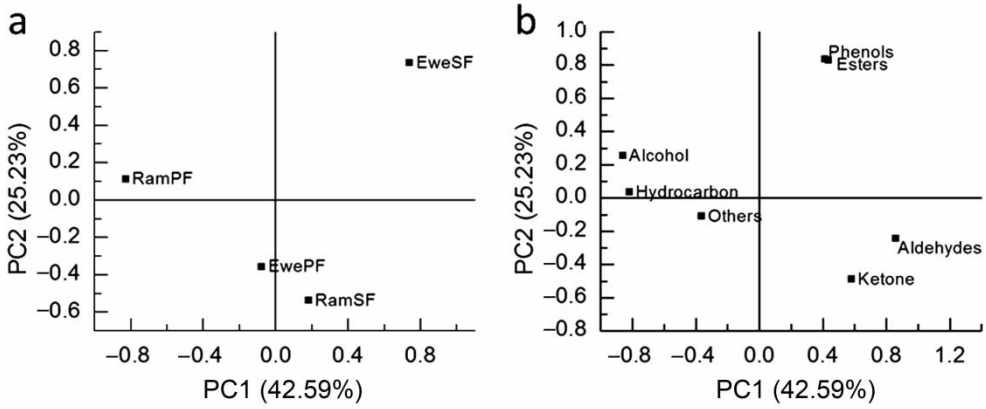

Figure 1. Principal component analysis (PCA) plot of the concentrations of volatiles detected in different adipose tissues of Hu sheep. The first two principal component factor scores of four adipose tissues (perirenal fat tissues [PF] and caudal subcutaneous fat tissues [SF]) are plotted in (a); and The scores of each volatile group (b).

Compared with the PF, the SF contained more aldehydes. The differences in volatiles between SF and PF were consistent with other reports that different volatile fractions can be used as specific feeding diet tracer for different adipose tissues (Sivadier et al., 2008, 2010). It is suggested that different metabolic mechanisms between SF and PF could be considered when volatile character is determined.

From the PCA results, it can be inferred that aldehydes and ketones play an essential role in estimating the aroma of ram SF. However, the threshold of detection for volatiles should be considered when flavor character is predicted; for example, this threshold is 4 ng/g for E-2-octenal (Morales et al., 2005), but 1000 ng/g for nonanal (Olivares et al., 2009). In this study, although the nonenal content was higher than the E-2-octenal content, the nonanal might contribute less to the flavor of the adipose tissue. Meanwhile, the concentration of benzaldehyde was higher in ram SF, but PCA analysis did not consider it to be a key contributor to the differences between these adipose tissues. Considering its low odor threshold (0.06 ng/g; García-González et al., 2008), benzaldehyde may contribute appreciably to the flavor of ram SF despite its low concentration of approximately 1%. Therefore, deep insight and careful consideration should be used when evaluating the aroma of Hu sheep meat.

The differentiation of fatty acids in the SF and PF of rams and ewes

Reports on the effect of gender on adipose fatty acid content have been inconsistent. A higher SFA content has been observed in male lamb meat (Lind et al., 2011) and higher PUFA content in female SF than in male lamb (Díaz et al., 2003). Because quality traits vary with the increasing body weight after the animal reaches adulthood (Goetsch et al., 2011), the conflicting results between our work and the above previous studies might be due to the different ages of the sheep in the studies.

Similar to the volatile compounds, the fatty acid profile was also influenced by adipose tissue site.

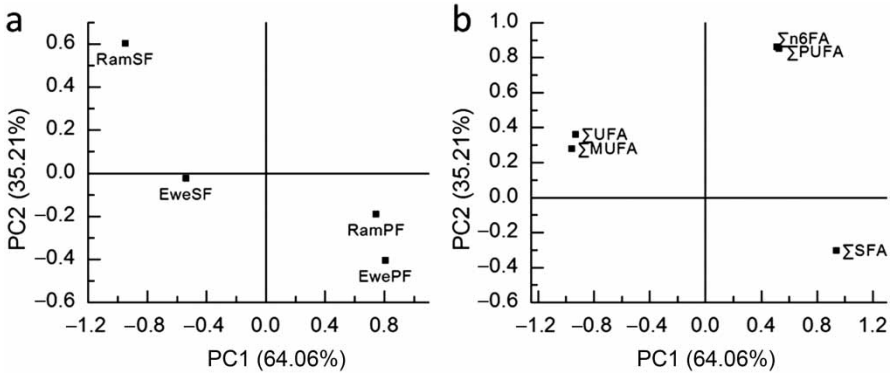

Figure 2. Principal component analysis (PCA) plot of the concentration of fatty acids detected in different adipose tissues of Hu sheep. The PCA was based on fatty acid group content. (a) The average scores of the two principal components were based on subcutaneous and perirenal fat tissues of ram and ewe (RamSF, RamPF, EweSF and EwePF). (b) The scores of each fatty acid group.

It has been reported that the addition of fish oil to the diet changed the fatty acid profile of steer in a fat location–dependent manner, and total SFA in the SF, but not in the PF, was changed by adding fish oil to the diet (Wistuba et al., 2007). The different responses of SF and PF to changes in nutrition imply that there are differences in the function and fat metabolism of the SF and PF, which could be one reason why different fatty acid profiles were observed between SF and PF in our study.

The PCA results also indicated the role of UFA in distributions of ewe SF and ram SF. Since ram SF was closely related with aldehydes, it can be suggested that potential relationship existed between aldehydes and UFA. Therefore, it seems that the UFA might positively contribute to the flavor of these aldehydes. On the other hand, the data of SFA, UFA, PUFA, MUFA, n3FA, and n6FA were all performed with the PCA, while UFA included PUFA and MUFA. From Figure 2, it is indicated that UFA and MUFA were located in the same quadrant with Ram SF, suggesting that MUFA might play the key role in the relationship between UFA and aldehydes. In breeding industry, breeders aim to increase the UFA level of animal products by nutritional methods; for Hu sheep breeding, it has been reported that both corn oil– and soybean oil–supplemented diets increased meat PUFA level (Chen et al., 2008, 2010). However, it should be noted that more aldehydes may accumulate simultaneously when this approach is used, which might cause the development of unappreciated flavor profiles in the animal products.

Conclusion

The volatile chemical profiles, especially aldehydes, varied between the SF and PF of rams and ewes. Ewes had more aldehydes and less UFA and MUFA than did rams, while SF contained more aldehydes, UFA, and MUFA than PF. The results shown here represent a comprehensive characterization of the volatile and fatty acid profiles in Hu sheep.

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