Research Article

Received: 20 November 2024 Revised: 12 March 2025 Published online in Wiley Online Library:

(wileyonlinelibrary.com) DOI 10.1002/jsfa.14319

Identification of volatile flavor substances in four key muscle portions of five Tibetan sheep breeds

Yuqian Xu,[a,b] Qingfeng Yang,[a] Xiaochun Zheng,[a] Huiguo Yang,[c] Martine Schroyen,[b] Dequan Zhang[a] and Chengli Hou[a*]

Abstract

BACKGROUD: To clarify the variations in flavor characteristics among various Tibetan sheep breeds (Gangba, Huoerba, Sewa, Duoma and Awang) across different mutton portions (*M. longissimus thoracis et lumborum*, topside, thick flank and shoulder fillet), HS-SPME-GC-MS and HS-GC-IMS were performed with multivariate statistical analysis to characterize the volatile organic compounds (VOCs) in Tibetan sheep mutton.

RESULTS: The results identified 89 and 75 VOCs using HS-SPME-GC-MS and HS-GC-IMS, respectively, with aldehydes, ketones and alcohols as the primary compound classes. The main flavor characteristic substances were 1-octen-3-ol, 3-hydroxy-2-butanone, 2,3-pentanedione, acetone, octanal, 2-pentylfuran, nonanal, hexanal, 1-hexanol, 2-butanone, 1-pentanol, heptanal, 2-pentanone, 1-penten-3-ol and benzaldehyde, contributing notes of fruity, floral, grassy, caramel, baking and creamy aromas. The contributing compounds of odor activity value > 1 were successfully determined, enabling the characterization of Tibetan sheep mutton flavor profiles. Fingerprints were applied to distinguish common and unique VOCs across different mutton portions from the studied breeds. Samples were effectively categorized by principal component analysis and partial least squares discriminant analysis (PLS-DA) according to their aroma characteristics, and a stable model (variable importance in projection (VIP)) was established through PLS-DA, highlighting hexanal, 1-hexanol, 2-butanone, 1-pentanol, heptanal, 1-octen-3-ol, 3-hydroxy-2-butanone, 2-pentanone, octanal, ethanol, acetone, 1-penten-3-ol, 2,3-pentanedione and acetic acid with VIP > 1.

CONCLUSION: The findings reveal the VOC profiles of various mutton portions of Tibetan sheep breeds, offering insights for product development and enhancing the understanding of flavor in Tibetan sheep meat products.

Keywords: Tibetan sheep; HS-SPME-GC-MS; HS-GC-IMS; VOCs

INTRODUCTIONS

An increasing number of consumers are becoming more conscious of sustainability, diversity and health within the framework of a balanced diet. Consequently, mutton has gained popularity as a meat option due to its low cholesterol and fat content, high protein and vitamin levels, tender texture and easy digestibility.[1] The Qinghai-Tibet Plateau and surrounding regions serve as primary producing areas representing unique local traits, such as Gangba, Huoerba, Duoma, Sewa and Awang sheep.[2] Tibetan mutton, recognized as a high-quality, healthy and environmentally friendly organic product, is prized for its freshness, tenderness and distinct aroma, owing to year-round grazing of sheep on natural pastures and diet consisting solely of forage.[3]

Recent studies have carried out quality assessments of Tibetan sheep to evaluate the unique qualities of their mutton.[4,5] Research suggests that the taste, texture and composition of volatile organic compounds (VOCs) might serve as crucial criteria for assessing the mutton quality across different geographical regions.[6] The composition of VOCs in mutton is complicated, with diverse generation pathways.[7] The diversity and content of short-chain fatty acids, aldehydes, ketones and alcohols contribute to the distinct aroma profiles of mutton from various regions.[8] Headspace–gas chromatography–ion mobility spectrometry (HS-GC-IMS), a novel VOC detection technology, has been effectively applied to the identification of flavor compounds in meat.[9] With high sensitivity, GC-IMS allows for visual analysis and characterization, clearly highlighting VOC variations between samples, making it widely utilized in meat classification, identification and other fields.[10-12]

* *Correspondence to: C Hou, Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, 2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China. E-mail: houchengli@caas.cn*

a *Institute of Food Science and Technology, Chinese Academy of Agricultural Sciences, Key Laboratory of Agro-Products Quality and Safety Control in Storage and Transport Process, Ministry of Agriculture and Rural Affairs, Beijing, China*

b *Precision Livestock and Nutrition Unit, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium*

c *Xinjiang Uyghur Autonomous Region Academy of Animal Science, Urumqi, China*

Currently, the broad variety of Tibetan sheep breeds results in little distinction among Tibetan sheep products, making it challenging to distinguish them based on unique flavors, processing methods or innovative ingredients. This similarity in Tibetan mutton offerings has limited consumer appeal for unique experiences. The convergence of various Tibetan mutton localizations and lack of individual distinctions reduce the differentiation and added value of products, hindering their market competitiveness.[13] Most current research focuses on geographic locations and breeding modes, with limited studies on VOCs across different Tibetan sheep breeds and muscle portions.[3,14] Therefore, headspace–solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS) and GC-IMS were used to analyze and compare VOC variations across four popular mutton portions (*M. longissimus thoracis et lumborum*, topside, thick flank and shoulder fillet) in five Tibetan sheep breeds (Gangba, Huoerba, Duoma, Sewa and Awang). By identifying the characteristic flavor compounds in these portions of five breeds, developing flavor fingerprints and labeling target compounds, this study enhances understanding of quality evaluation and breed identification among Tibetan sheep products.

MATERIALS AND METHODS

Sample collection

All samples were collected from a local enterprise in Sa'gya County, China, in 2024. A total of 20 female Tibetan sheep, aged 24 months, were included in the study. These sheep were divided into five groups based on their breed: Gangba Tibetan sheep (G), Huoerba Tibetan sheep (H), Sewa Tibetan sheep (S), Duoma Tibetan sheep (D) and Awang Tibetan sheep (A). Within each group, four sheep were randomly selected for analysis. All Tibetan sheep were raised under identical environmental conditions controlled by the enterprise. Once the sheep reached the slaughter standard, they were slaughtered by professionals following prescribed ethical procedures.

Four specific muscle types were sampled from each sheep: *M. longissimus thoracis et lumborum* (B), topside (M), thick flank (L) and shoulder fillet (ML). Visible external fat and connective tissues were removed from the muscle samples, which were then transferred to 2 mL cryotubes. The samples were snap-frozen in liquid nitrogen for 30 min and stored at −80 °C until further analysis. All analyses were performed in the same laboratory. First group (B): GB, HB, DB, SB and AB. Second group (M): GM, HM, DM, SM and AM. Third group (L): GL, HL, DL, SL and AL. Fourth group (ML): GML, HML, DML, SML and AML. This systematic approach ensured consistency and reliability in sample collection and subsequent analysis.

HS-SPME-GC-MS analysis

The GC-MS analysis procedure was carried out as described by Sun *et al.*[15] and Nie *et al.*[16]

Sample processing: Samples (3.0 ± 0.02 g) were accurately taken and put into 20 mL headspace vials and the concentration of 2-methyl-3-heptanone was set at 1.68 μg μL^{-1} as an internal standard (2 μL).

SPME: The conditions were with reference to the procedure described by Zhu *et al.*[17] and Wang *et al.*[6] with slight modifications. PDMS/DVB-coated fiber (75 μm, Supelco, Inc., Bellefonte, PA, USA) was used for extraction. The conditions were as follows: preheating temperature was 60 °C, preheating time was 10 min, extraction temperature was 60 °C, extraction time was 40 min, desorption temperature was 250 °C, desorption time was 3 min.

GC conditions: The GC system (Agilent 8890 gas chromatography, Agilent Technologies, Palo Alto, CA, USA and 5977B GC/MCD) was then operated. The temperature at the forward sample port was set to 250 °C and the flow rate of carrier gas (He) was 1.0 mL min^{-1} in splitless injection mode. A polar capillary column (DB-wax; 60 m × 0.25 mm × 0.25 μm) was used for separation. Heating procedure: the initial column temperature was 40 °C, held for 3 min, rose to 70 °C at 2 °C min^{-1}, rose to 130 °C at 3 °C min^{-1}, rose to 230 °C at 10 °C min^{-1}, held for 10 min.

MS conditions: The electron ionization is set to 70 eV. A full scan (*m/z* 40–550) was performed. The NIST standard reference database and literature reports were compared and linear retention index was determined. The volatile compounds were quantified (semi-quantitative analysis) by dividing the peak areas of the compounds of interest by the peak area of 2-methyl-3-heptanone as internal standard.[18] The measurements were made in three batches.

Odor activity value (OAV)

OAV value is the ratio of the concentration of VOCs to the threshold value. OAV > 1 indicates that the substance has an important contribution to the overall flavor of the samples; OAV < 1 indicates that the substance has an auxiliary contribution to the sample flavor.

HS-GC-IMS analysis

A GC-IMS flavor analyzer (FlavourSpec®, G.A.S, Dortmund, Germany) was used to analyze VOCs in the samples. A 1.0 ± 0.02 g sample was incubated in a 20 mL HS bottle for 15 min before injection, with three tests for each sample. Injection conditions: incubation temperature was 60 °C; injection volume was 500 μL; incubation speed was 500 rpm; injection needle temperature was 85 °C.

The GC column was maintained at a temperature of 60 °C. High-purity nitrogen (N_2) (purity ≥ 99.999%) was used as the carrier gas, with flow rates set at 2.0, 10.0 and 100.0 mL min^{-1} for 2, 10 and 20 min, respectively. The total chromatographic run time was 20 min, with an inlet temperature of 80 °C.

IMS detection was conducted in positive ion scan mode, utilizing a tritium (^3H) ionization source. The migration tube was 53 mm in length, with an electric field strength of 500 V cm^{-1}, and maintained at a temperature of 45 °C. High-purity N_2 (purity ≥ 99.999%) was used as the drift gas, with a flow rate of 150 mL min^{-1}.

The retention index of each substance was calculated from its retention time using VOCal software (VOCal data processing software, v0.4.03, G.A.S, Dortmund, Germany). The built-in GC retention index database (NIST 2020) and the IMS migration time database were used for the qualitative analysis of target compounds through a search-and-comparison process.

Statistical analysis

The Reporter and Gallery Plot in the VOCal data processing software were used to generate the topography plots and comparison topography plots of VOCs respectively. Fingerprint diagrams were used to compare VOCs between samples. SPSS 26 software was used to process and analyze the test data, and the results were expressed as mean ± standard error.

RESULTS AND DISCUSSION

VOCs analyzed by HS-SPME-GC-MS

A key sensory attribute that affects consumer preference and acceptance of Tibetan sheep mutton is its profile of VOCs. After collecting samples from four different muscles across five Tibetan sheep breeds, variations in VOC composition were observed. To analyze these differences, VOCs in each sample were identified using HS-SPME-GC-MS, resulting in the identification of 89 VOCs across all samples. The results showed that those of Gangba sheep were the most abundant, with four portions containing 13 (GB), 30 (GL), 29 (GM) and 24 (GML), respectively. The VOC contents of the Huoerba and Awang Tibetan sheep were 16 (HB), 27 (HL), 21 (HM), 23 (HML) and 20 (AB), 16 (AL), 27 (AM), 11 (AML), respectively. VOC contents in the four portions of Duoma and Sewa sheep samples were 19 (DB), 20 (DL), 15 (DM), 9 (DML) and 16 (SB), 7 (SL), 6 (SM), 9 (SML), respectively (Fig. 1(a)). These VOCs were divided into 10 classes, based on their chemical properties: 16 ketones, 21 aldehydes, 22 alcohols, 6 furans, 11 esters, 3 acids, 1 pyrazine, 7 alkanes, 1 phenol and 1 amine.

Figure 1(b) presents a heat map analysis used to evaluate the degree of similarity in VOCs across different samples. The samples were subsequently clustered into 20 subclasses, demonstrating a high correlation of similar properties. The AML, AM, DM, HM, SML and GB groups formed a major category with a high ketone content, while the AL, DL, GL and SL groups showed elevated levels of aldehydes. The DML, GM, GML and HL groups were characterized by higher alcohol content, indicating a relative similarity in their composition. In contrast, the AB, HB and SB groups were distinct, as they contained higher amounts of other compounds such as acids, esters and alkanes, leading to their separate classification. Additionally, the VOC content in the GB, AML, DML and SM groups was particularly low, whereas the AM group exhibited a more abundant content of various substances.

OAV, the ratio of the concentration of a VOC to its threshold value, was used to assess the contribution of specific components to the aroma of mutton.[10] Figure 1(c) provides detailed information on the VOCs identified in the four portions of mutton from the five breeds. The GB, GL, GM and GML samples contained 7, 17, 19 and 14 VOCs with OAV > 1, respectively, each exhibiting distinct flavors. Aldehydes exhibit the highest contribution rate to GL (35.29%), followed by alcohols and ketones (23.52%). Key compounds contributing to aroma expression include (*E*)-2-octenal, 1-heptanol, 1-octen-3-ol, 1-penten-3-ol, 2-butylfuran, 2-heptanone, 2-hexanone, 2-nonenal, (*E*)-heptanal and *n*-pentanal. Substances with OAV > 1 in GB primarily consist of 1-penten-3-one, 4-mercaptophenol, 6-nonenoic acid, methyl ester, acetic acid and benzaldehyde. Compounds with OAV > 1 in GM are predominantly alcohols. Notable VOCs in GM, characterized by high OAV, include 1-nonanol, 2,3-pentandione, 3-furanmethanol, 3-octanone, (*E*)-4-nonenal, ethyl hexanoate, 2-pentylfuran, hexanal, nonanal and propanal. Aldehydes represent the VOCs with the highest contribution rate in ML. Additionally, 2-butylfuran and 2-pentylfuran provide meat flavor and fat flavor.

Similarly, the AB, AL, AM and AML samples contained 12, 9, 16 and 11 VOCs with OAV > 1, respectively. The characteristic compounds identified in AM include 1-butanol, 1-octen-3-ol, 1-penten-3-ol, 2,3-pentandione, 2-butanone, 2-heptanone and 2-hexanone. Ketones contribute more significantly to the aroma profile of mutton compared to others, imparting caramelized, creamy and fruity aromas. Aldehydes such as benzaldehyde, heptanal, nonanal and octanal dominate in AB. Similarly, the primary aroma components in AL are aldehydes, including (*E*)-2-nonenal, 2-methylbutanal, nonanal and propanal, which enhance the grassy and slightly sweet flavor of mutton. The contribution rates of 1-butanol, 2-butanone, octanal and propanal in ML are particularly notable, adding green, floral and fruity attributes to the mutton aroma.

The DB, DL, DM and DML samples had 11, 13, 8 and 4 VOCs with OAV > 1, respectively. DL contains a diverse range of odor compounds, with aldehydes comprising the highest proportion, including (*E*)-2-pentenal, benzaldehyde, heptanal, nonanal, octanal and propanal. Additionally, 1-octen-3-ol, 1-penten-3-ol, 2-butylfuran and 2-pentylfuran exhibit high contribution rates. 1-Nonanol, 1-penten-3-one, 2-methylpropyl butanoate, 4-octanol and nonanal provide the rose, fruit and wine aromas of DB. The VOCs of ketones in DM were at a relatively high level, including 2,3-pentandione, 2-butanone, 2-heptanone and 2-hexanone. 1-Nonanol is the dominant flavor compound in ML, imparting a slightly fruity and floral aroma to mutton.

The HB, HL, HM and HML samples contained 9, 14, 11 and 12 VOCs with OAV > 1, respectively. Alcohols and aldehydes are the VOCs with the highest contribution rates in HL, including 1-octen-3-ol, 2-heptanone, 2-butylfuran, 2-pentylfuran, benzaldehyde, heptanal and propanal. Ketones dominate the VOC profile in HM, contributing 45.45% and primarily comprising 1-penten-3-one, 2,3-pentandione, 2-butanone, 2-heptanone and 2-hexanone. Acetic acid, γ-butyrolactone, 1-penten-3-one, 4-mercaptophenol and 4-octanol are present at high levels in HB. The highest contribution rate of ML was from ketones and alcohols, accounting for 33.33%. 1-Butanol, 1-butanol 3-methyl, 2,3-pentandione, 2-butanone, 2-heptanone, 2-hexanone and ethyl hexanoate are present, giving mutton a wine, fruity and caramel flavor.

Finally, the SB, SL, SM and SML samples contained the fewest VOCs with OAV > 1, at 8, 5, 4 and 6, respectively. Compounds such as 4-mercaptophenol, 4-octanol and 6-nonenoic acid were detected in SB, with methyl ester, 9-decenoic acid, benzaldehyde, γ-butyrolactone and heptanal exhibiting higher contribution rates to the overall flavor profile. 2-Butylfuran, 4-octanol and nonanal are more easily perceived in SL. 2-Butylfuran imparts a mildly fruity and sweet aroma, while nonanal provides a fresh, fruity smell. The representative VOCs in SM include 1-penten-3-one, 3-methylfuran, methyl acetate and propanal, all of which are characterized by strong pungent odors. Acetic acid, butanal, ethanol, 2,3-pentandione, 2-butanone and acetic acid are the key VOCs of ML. Acetic acid contributes caramel and nutty aromas to ML with an irritating spicy taste.

VOCs analyzed by HS-GC-IMS

HS-GC-IMS was employed to quantitatively analyze VOC content across the different samples, utilizing topographic plots, comparison topographic plots and fingerprint diagrams. According to the GC-IMS database, a total of 75 signal peaks were detected. Among them, 67 compounds were successfully identified by database retrieval software. However, due to the limited scope of the database, the results also revealed 8 VOCs that were not included. Therefore, they were named 1, 2, 3, 4, 5, 6, 7 and 8. According to the characteristics of different chemical substances, they can be categorized into the following seven groups: 1 furan, 1 ether, 1 acid, 6 esters, 10 aldehydes, 12 alcohols and 12 ketones. Monomers, dimers and polymers were observed, likely due to differences in the concentrations of adducts as they passed through

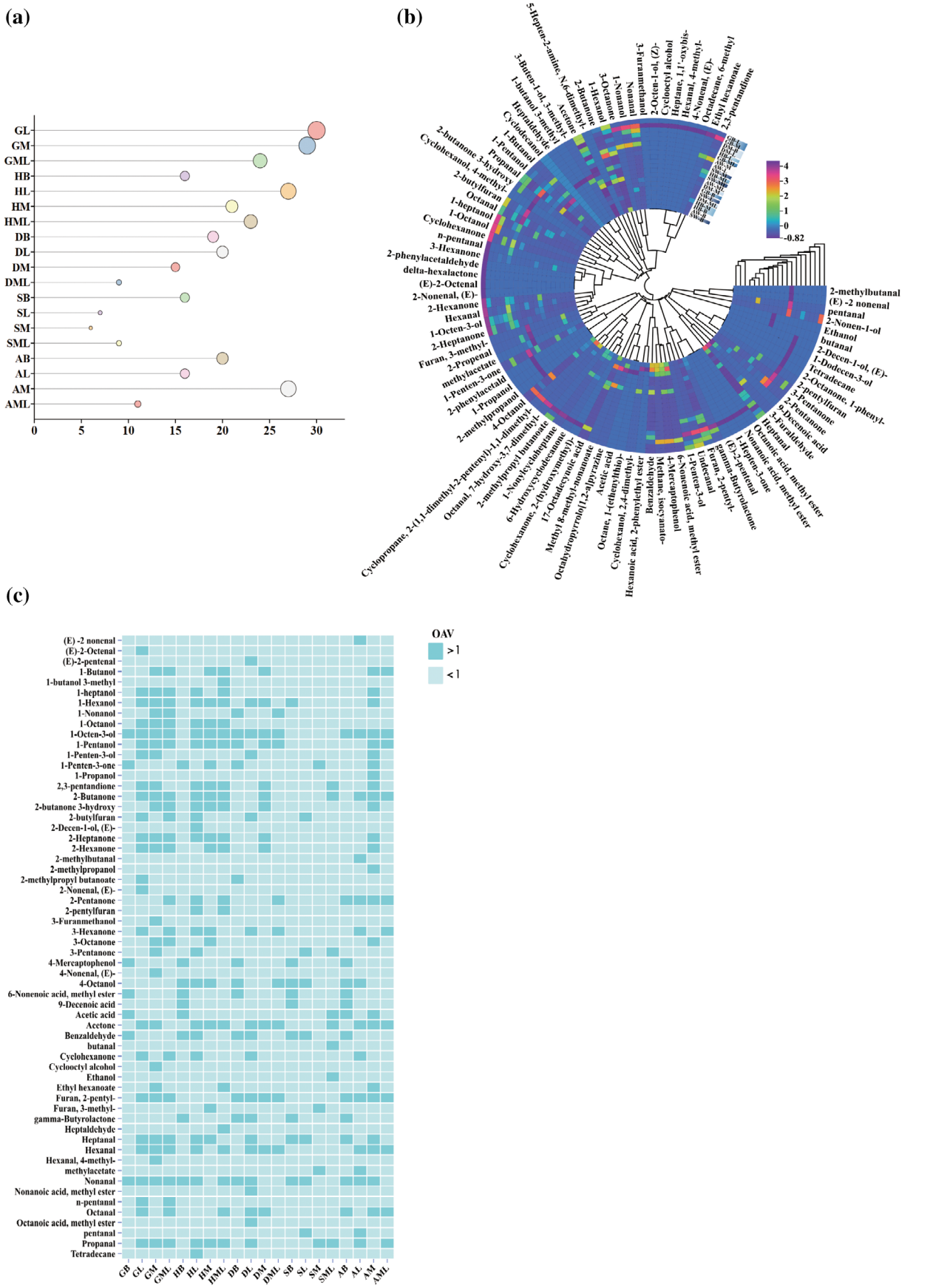


Figure 1. VOC concentration changes in four muscle portions of five Tibetan sheep breeds. (a) Number of compounds. (b) Annular heatmap visualization of VOCs. (c) Heat map of OAV.

the drift region, causing individual compounds to generate multiple signals.

Topography plots, comparison topography plots and fingerprint diagrams of B muscles of the five species were obtained, as shown in Fig. 2. As shown in Fig. 2(a), the HB and AB groups exhibit more red dots, indicating a relative increase in VOC content, while the blue dots for the DB and SB groups reflect a decrease in VOC content. Additionally, the presence of more white dots for the HB group suggests greater similarity with the GB group. The same pattern was also evident in the fingerprint spectrum (Fig. 2(b)). The color, brightness and size of each signal in the fingerprint spectrum represent the relative content of each VOC in the respective groups. Spot brightness and red color of VOC spectra revealed that ketones, alcohols and aldehydes were the most abundant substances detected in B muscle of the various sheep breeds.

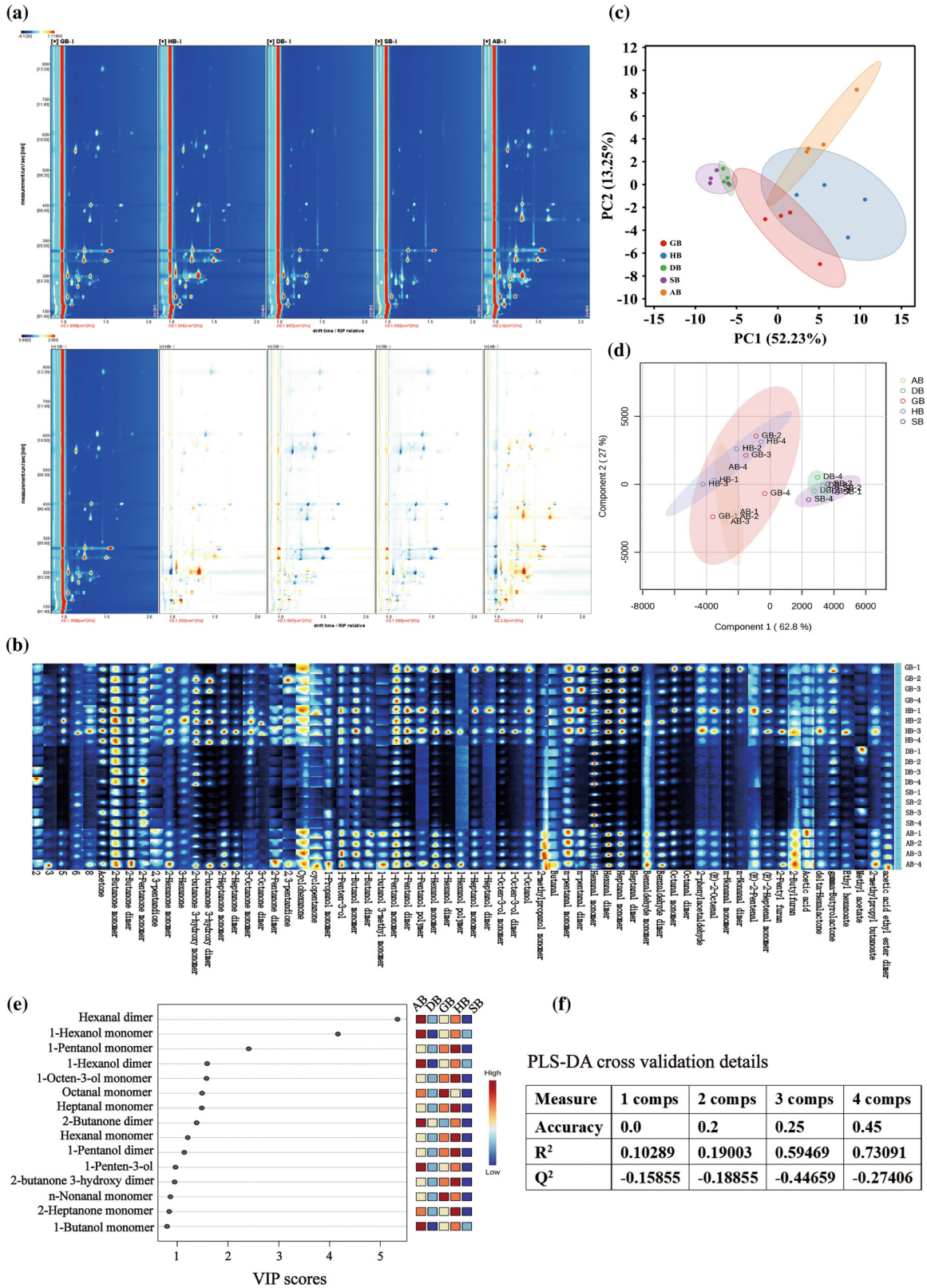
The compounds shared by the B muscles of the five breeds were 2-butanone, 2-pentanone, 3-hydroxy-2-butanone, *n*-pentanal, γ-butyrolactone, benzaldehyde and acetic acid. It is obvious that these compounds have the highest proportion in the GB, HB and AB groups, being relatively lower in the DB and SB groups. 2-Butanone exhibited the highest concentration in the HB group, and studies have shown that it could be generated through fat oxidation and Maillard reaction, presenting creamy and mushroom flavors, which may be more linked to the fatty and meaty flavors of fresh meat. The highest levels of 3-hydroxy-2-butanone were found in the GB and HB groups, contributing a pleasant milky flavor to the meat. *n*-Pentanal, a saturated fatty acid aldehyde, is commonly present in fresh mutton and was observed at high peak intensities in the GB, HB and AB groups. Known for its light fruity and floral flavors, *n*-pentanal serves as an important marker of fat oxidation and esterification reactions during the cooking process.[17] The AB group showed a high content of benzaldehyde, which imparts a bitter almond flavor. Benzaldehyde is a secondary oxidation product resulting from the degradation of fatty acids, amino acids or thiamine, effectively enhancing the flavor of the meat and contributing to a rich fatty aroma during cooking. γ-Butyrolactone was detected in all groups, with the highest expression in the GB group, adding a creamy and milky flavor to the mutton.[19] Additionally, acetic acid displayed the strongest signal intensity in the AB group. As a product of aldehyde and ketone oxidation or fatty acid degradation, acetic acid plays a significant role in flavor modification, primarily contributing a sour and vinegar-like taste to fresh meat.[20]

Among the B muscles of the five groups, it was found that there were more unique substances in the AB group, including 1-butanol, 1-butanol 3-methyl, 1-hexanol, 1-heptanol, 2-methylpropanol, benzaldehyde and 2-methylpropyl butanoate. Most of them were alcohols, which mainly came from conjugated linoleic acid catalyzed by lipoxygenase and hydrogen peroxidase in muscles. A higher concentration of 1-butanol typically indicated further formation of ketones, which result from the continued oxidation of alcohols. 1-Hexanol, which imparts a grassy taste to fresh meat, was associated with lipid oxidation and produces a caramel-like sweet flavor upon heating, contributing to the sweet aroma observed in the AB group. 1-Heptanol is formed by glucose metabolism, lipid oxidation and amino acid decarboxylation dehydrogenation, and has a pleasant green odor.[21] Additionally, 1-butanol-3-methyl imparts an apple brandy aroma with a spicy note, while 2-methylpropanol releases a volatile alcohol-like odor. Benzaldehyde is a VOC with branched or benzene rings, which imparts the taste of bitter almonds. 2-Methylpropyl butanoate, known for its fruity, green and sweet apple flavor, results from the esterification of aldehydes, short-chain acids and alcohols.[22] The content of these compounds in the AB group was significantly higher than in the other groups, suggesting they were the main characteristic flavor compounds that distinguish Awang Tibetan sheep from the other types. In addition, 3-hydroxy-2-butanone, 3-octanone and 2,3-pentanedione were found in the HB group, which were independent of other groups and exhibited distinct spot brightness, and could be considered as characteristic flavor compounds of the HB group. 3-Hydroxy-2-butanone has a milky flavor and is a precursor to butanedione. 3-Octanone provides a fruity aroma. According to studies, 2,3-pentanedione improves the creamy and buttery flavor of mutton, possibly due to a combination of photooxidation, autooxidation and enzyme oxidation that promotes its accumulation.

Figure 3 illustrates the VOC expression levels of L muscles across the five groups (Fig. 3(a)). Most of the VOCs detected in L muscles were alcohols, ketones, aldehydes and some furan compounds. Hexanal and 2-butylfuran exhibited significantly higher signal strength in the GL group compared to the DL, SL and AL groups. Hexanal has been shown to occur at high levels in meat, which is the main compound responsible for mutton flavor.[23] In the HL group, 2-pentanone and 2-butylfuran were particularly prominent. Although the overall VOC content in the GL group was relatively high, certain compounds, such as 2-pentanone and 2-butylfuran in the HL group, were the most abundant among the five groups, highlighting the distinctive characteristics of the L muscles from Huoerba Tibetan sheep. In contrast, no VOCs were detected in the SL group, indicating a very low concentration of flavor compounds. Different from B and M muscles, 2-butylfuran was present in L muscles of all five groups, with the strongest signals in the GL and HL groups, suggesting its highest concentration and its role in providing a pleasant, ethereal fruit aroma to the mutton.

In addition, three VOCs were detected in the GL group (Fig. 3(b)). Heptanal is characterized by an unpleasant fatty odor with notes of rancid fat and a penetrating fruity aroma due to fat oxidation and Maillard reaction in mutton. The GL group also contained (*E*)-2-heptenal, a compound contributing soapy, almond and grassy flavors to the mutton. Besides, δ-hexalactone was present only in the GL group. Volatile fatty acid derivatives, such as δ-hexalactone, are key components of important VOCs and contribute to the complexity of aroma. Ethanol, butanal and acetic acid ethyl ester, which were exclusively detected in the HL group, showed significant differences compared to other groups. Ethanol likely originates from the reduction of methyl ketones, amino acid metabolism and the degradation of linoleic and linolenic acids. Alcohols typically represent a large proportion in VOCs, imparting distinct flavors to mutton and are easily detectable by machines.[23]

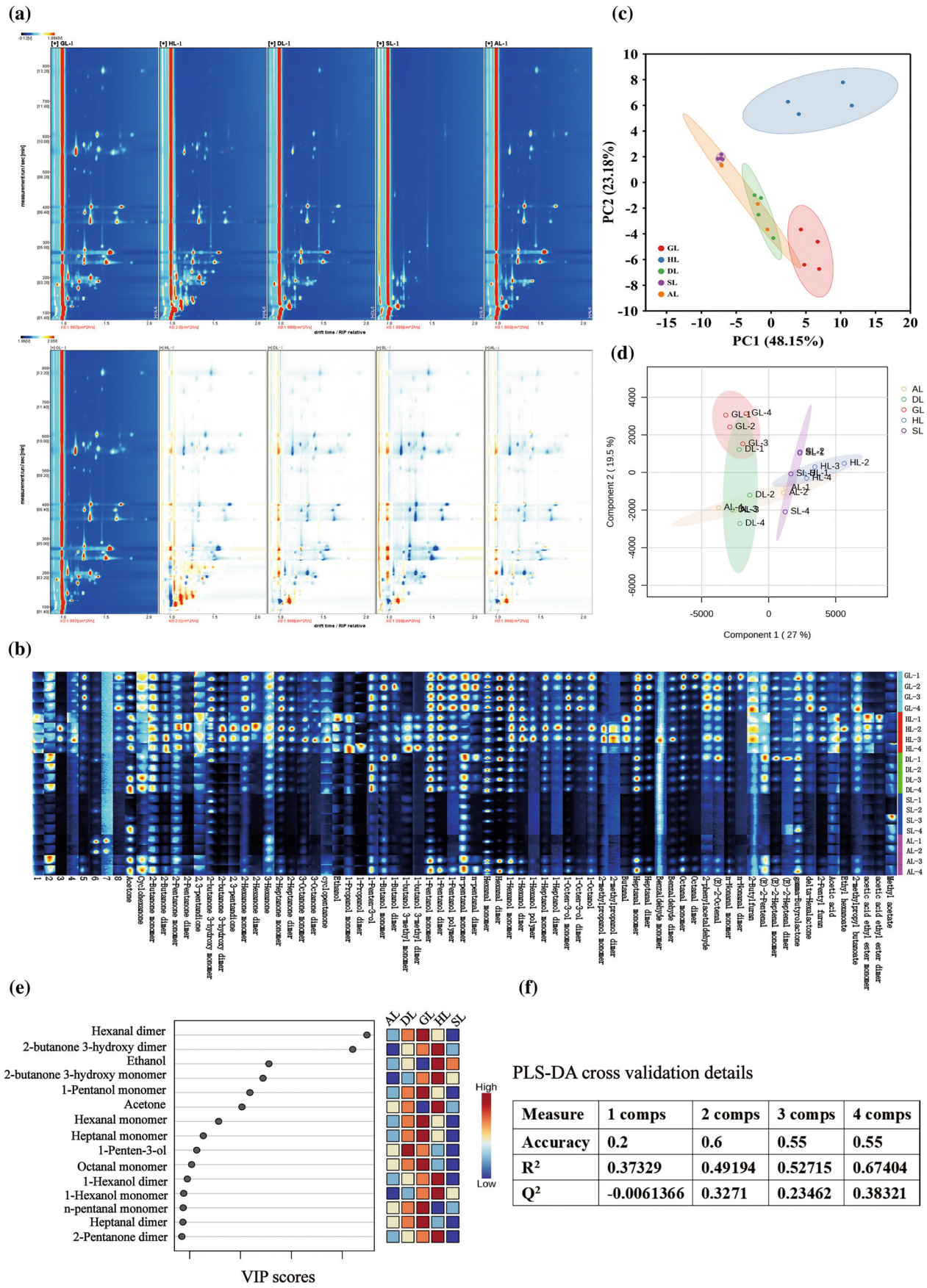
The production of aldehydes results from fat oxidation and decomposition and could also occur as a byproduct of Strecker degradation of amino acids. The high content of butanal in the HL group typically indicated the presence of rancid and irritating odors in the mutton. The study also found a higher concentration of acetic acid ethyl ester in the HL group compared to the other groups, which may contribute to the strong odor observed in Huoerba Tibetan sheep mutton. Additionally, the prominent presence of methyl acetate in the AL group suggests it creates distinct flavors like fruity aromas. Esters are an essential component of mutton aroma, working in conjunction with other ketones and alcohols to give mutton floral and fruity aromas, thereby reducing the impact of undesirable odors and helping to balance the overall flavor profile.[24]



Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.0	0.2	0.25	0.45
R^2	0.10289	0.19003	0.59469	0.73091
Q^2	-0.15855	-0.18855	-0.44659	-0.27406

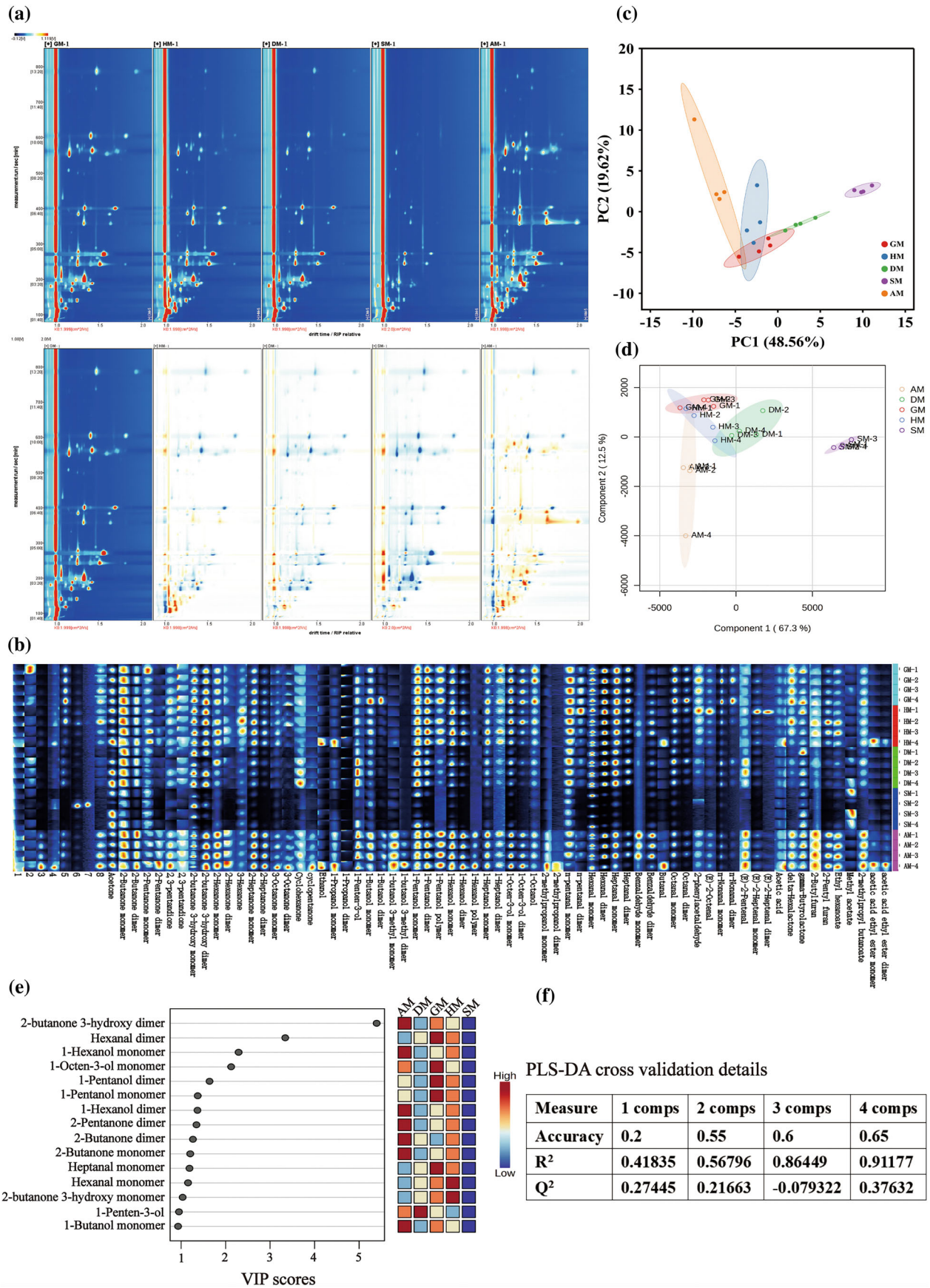
Figure 2. VOC profiles of B muscle across the five breeds determined by HS-GC-IMS. (a) Topographic plots and comparison topographic plots. (b) Fingerprint diagrams. (c) PCA. (d): PLS-DA. (e) VIP score. (e) PLS-DA cross-validation details.

The VOC expression levels across the M muscles of the five groups are presented in Fig. 4. The topography and comparison plots show more bright spots for the AM, GM and HM groups, while the DM and SM groups displayed very few bright spots. It is worth noting that the SM group exhibited almost no bright spots, indicating a low VOC content in this group. In the M



Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.2	0.6	0.55	0.55
R^2	0.37329	0.49194	0.52715	0.67404
Q^2	-0.0061366	0.3271	0.23462	0.38321

Figure 3. VOC profiles of L muscle across the five breeds determined by HS-GC-IMS. (a) Topographic plots and comparison topographic plots. (b) Fingerprint diagrams. (c) PCA. (d) PLS-DA. (e) VIP score. (e): PLS-DA cross-validation details.



Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.2	0.55	0.6	0.65
R^2	0.41835	0.56796	0.86449	0.91177
Q^2	0.27445	0.21663	-0.079322	0.37632

Figure 4. VOC profiles of M muscle across the five breeds determined by HS-GC-IMS. (a) Topographic plots and comparison topographic plots. (b) Fingerprint diagrams. (c) PCA. (d) PLS-DA. (e) VIP score. (e) PLS-DA cross-validation details.

muscles, the detected VOCs were dominated by ketones, alcohols and aldehydes, with some esters and furan compounds also identified. The contents of δ-hexalactone, ethyl hexanoate and 2-methylpropyl butanoate were prominent as esters, which impart fruity aromas such as caramel and pineapple to the meat. Characteristic furan compounds, including 2-butylfuran and 2-pentylfuran, were relatively abundant in the M muscles. Acetone, 2-pentanone, 2-butanone, 3-hydroxy-2-butanone, *n*-pentanal, hexanal and γ-butyrolactone were identified as common VOCs with strong signals across M muscles of the five different breeds. Except for acetone, the remaining five compounds were also commonly found in B muscles. Ketones, which have a much higher threshold than their isomeric aldehydes, are typically derived from lipid degradation and are known for their stable structure and long-lasting aroma.[25] Acetone, a representative ketone, is primarily produced through glycolysis and the Maillard reaction, and it significantly contributes to the aroma of meat by emitting creamy and fruity notes.[26]

The fingerprint effectively illustrated the variation trends among different samples, allowing for an accurate assessment of the relationships between groups. Notably, many characteristic VOCs were found in the AM group, drawing increased attention to the M muscles of Awang Tibetan sheep among consumers. These substances have a high proportion of ketones and aldehydes, including 2-pentanone, 2-hexanone, cyclopentanone, 1-propanol, 1-butanol, 1-butanol 3-methyl, 1-hexanol, benzaldehyde and acetic acid. 2-Hexanone, classified as a 2-ketone, was abundant and contributes a distinctive sweet, creamy and fruity aroma. The production of cyclopentanone could enhance the minty scent characteristic of the AM group. 1-Propanol has a smell similar to that of ethanol.[24] High concentrations of other aldehydes, octanal and *n*-nonanal, were also found in the GM group. Octanal, derived from lipid degradation, imparts fruity and green notes, while *n*-nonanal provides floral, citrus, fatty and grassy aromas. The production of ketones is mostly a result of oxidation, thermal degradation or microbial metabolism of unsaturated fats, which generate smooth, creamy flavors similar to those of cheese. In the HM group, the concentration of 3-hexanone is relatively high, contributing to a rich and pleasant aroma due to its lipid content. Additionally, the presence of methyl acetate differs from that of the other four groups, being particularly prominent in the SM group. Esters, known for their sweet and fruity aromas, are typically produced through the esterification of acids and alcohols.[19] Methyl acetate has the highest relative content in the SM group, which may be the main reason for the milky aroma of that group.

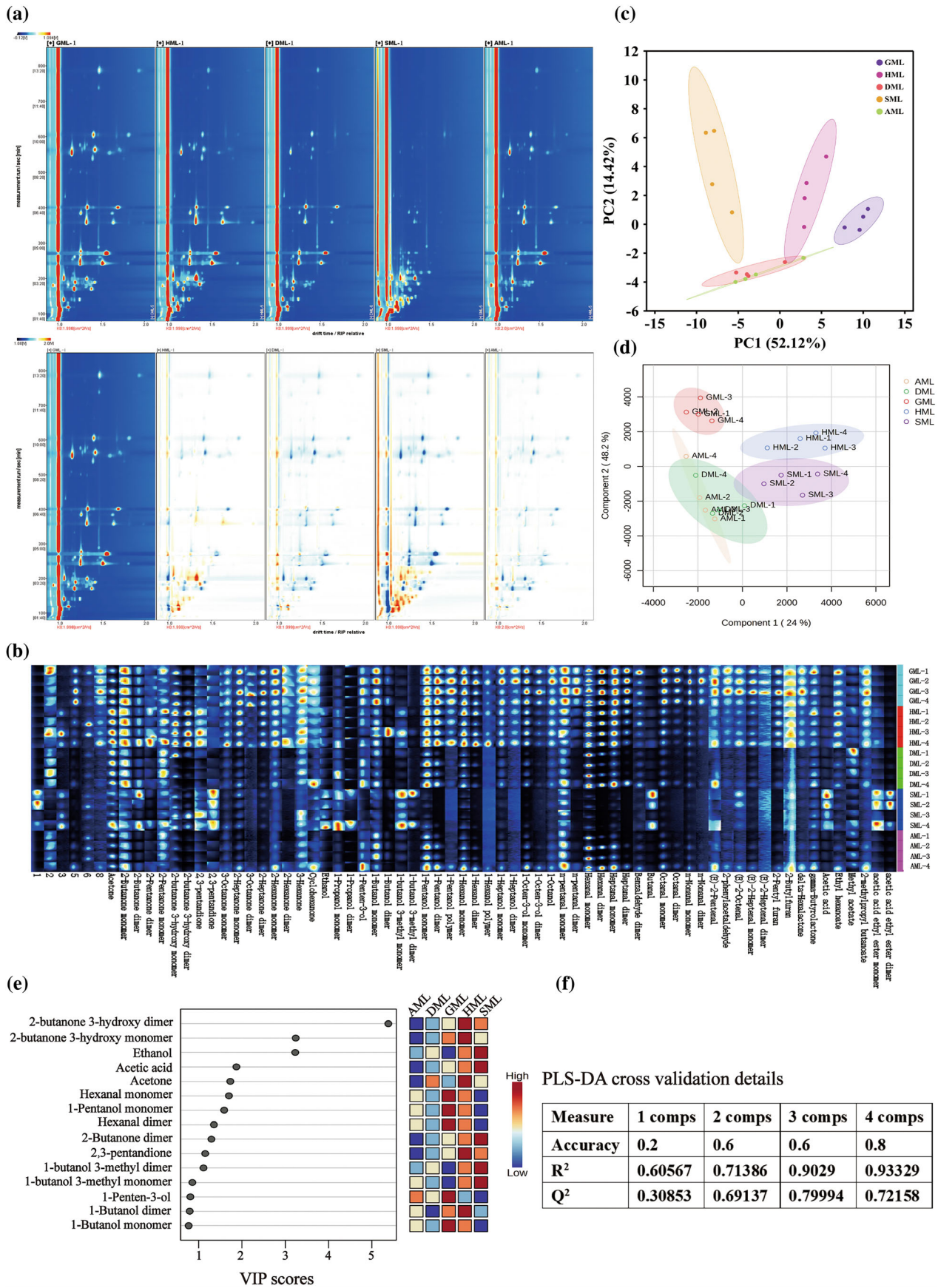
Figure 5(a) presents the VOC content of ML muscles in the various groups. All groups exhibited reduced levels of compounds such as acetone, 2-butanone, *n*-pentanal and 2-butylfuran. Notably, *n*-pentanal was significantly higher in all groups, indicating its presence in each part of the five varieties. In this study, linear aldehydes were predominantly identified, mainly produced through the oxidation of fatty acids.[24] Pentanal contributes positively to the formation of favorable aroma characteristics in mutton, with increased aldehyde levels potentially enhancing the overall flavor profile. 1-Penten-3-ol, 1-octen-3-ol, 1-penten-3-ol, 2-phenylacetaldehyde and (*E*)-2-octenal were identified as characteristic flavoring compounds in the GML group. 1-Penten-3-ol, with its tomato, grass, fruit and mint aromas, enhanced the flavor of mutton. High levels of 1-octen-3-ol are found, contributing mushroom, lavender and green-like flavors to mutton. As a main representative of alcohol VOCs, studies suggested that 1-octen-3-ol is frequently expressed in mutton.[27] In this study, 1-octen-3-ol was detected in all four muscle portions of Gangba Tibetan sheep, the B, M and L muscles of Huoerba Tibetan sheep, the M muscles of Duoma Tibetan sheep and the B and M muscles of Awang Tibetan sheep, which aligned with previous research.[28] Produced through the oxidation of linoleic and arachidonic acids, 1-octen-3-ol has a low threshold and significantly contributes to the flavor of mutton.[29] Similarly, 2-pentylfuran followed a comparable trend to 1-octen-3-ol, exhibiting higher concentrations in the GML and HML groups and emitting floral, fruity and beany aromas.[30] In addition, the GML group displayed the highest levels of 2-phenylacetaldehyde and (*E*)-2-octenal, enhancing the elegant daffodil-like, fleshy and nutty aromas of mutton.

Principal component analysis (PCA) of HS-GC-IMS results

Figure 2(c) presents the PCA score and loading plots for B muscles of the different breeds using the HS-GC-IMS VOC dataset. PC1 (13.25%) and PC2 (52.23%) together accounted for 65.48% of the total variance, indicating that the VOCs from the same muscle part in different breeds were significantly separated. The processed samples of SB and DB were closely positioned, while GB and HB showed some overlap, and AB displayed the greatest dispersion. For L muscles, it is observed that PC1 (48.15%) and PC2 (23.18%) account for 71.33% of the total variance (Fig. 3(c)). The SL group was included within the AL group, which also intersected with the DL group, while the GL and HL groups were more distant from the other three groups, indicating that these samples were markedly different. For M muscles, PC1 (48.56%) and PC2 (19.62%) accounted for 68.18% of the total variance (Fig. 4(c)). The GM group was in close proximity to the HM and DM groups, with no significant difference in aroma detected. The figure also shows that the AM and SM groups were farther from the other three groups, suggesting a stronger correlation of VOCs within the AM and SM groups. The distance of GML, HML, DML and SML in Fig. 5(c) is far, indicating that the VOCs of these groups were better distinguished. The AML group only had some crossover with the DML group. PC1 and PC2 were 52.12% and 14.42%, respectively, and accounted for 66.54% of the total variance. Overall, the results of PCA revealed that HS-GC-IMS effectively captured and classified the VOCs in different portions of mutton and breeds of sheep.

Partial least squares discriminant analysis (PLS-DA) of HS-GC-IMS results

The PLS-DA model was applied to identify key candidate odors. In the PLS-DA plot for B muscles (Fig. 2(d)), the first two components accounted for 62.80% and 27.00% of the total variance, respectively. Samples from the GB, AB and HB groups overlapped in the center of the score plot, while those from the DB and SB groups were positioned to the right. These findings suggested that VOCs in the B muscles of the GB, AB and HB groups share similarities, whereas the B muscles from the DB and SB groups exhibited unique aromatic characteristics. It is generally believed that VOCs with variable importance in projection (VIP) scores ≥1 have strong explanatory power for sample classification and discrimination, and are considered to be significant discrimination factors (Fig. 2(e)).[31] A total of 10 VOCs were identified as key discriminant factors (Fig. 2(e)). The results showed that hexanal, 1-hexanol and 2-butanone were the primary discriminant compounds in the AB group, while higher levels of octanal were detected in the GB group. Differences in the content of 1-pentanol, heptanal, 1-octen-3-ol and other volatiles may be critical in explaining the prominent flavor characteristics of the HB samples.



Measure	1 comps	2 comps	3 comps	4 comps
Accuracy	0.2	0.6	0.6	0.8
R^2	0.60567	0.71386	0.9029	0.93329
Q^2	0.30853	0.69137	0.79994	0.72158

Figure 5. VOC profiles of ML muscle across the five breeds determined by HS-GC-IMS. (a) Topographic plots and comparison topographic plots. (b) Fingerprint diagrams. (c) PCA. (d) PLS-DA. (e) VIP score. (e): PLS-DA cross-validation details.

The similarities and differences in VOCs among various L muscle varieties are presented in Fig. 3(d). The PCs account for 27.00% and 19.5% of the total variance, respectively. The GL group was positioned further from the other groups and located above the score plot, while the SL, HL, AL and DL groups intersect near the center, suggesting lower variation. Hexanal, 1-pentanol, heptanal and octanal were present at higher concentrations in the GL group, with some dimers and polymers identified due to the formation of accessory compounds from monomer ions and neutral molecules (Fig. 3(e)). 3-Hydroxy-2-butanone, ethanol and acetone were more strongly associated with the HL group, while a high level of 1-penten-3-ol was exclusively detected in the DL group.

Similarly, Fig. 4(d) shows that the first two PCs represent 67.30% and 12.50% of the total variance, respectively. The AM group was positioned on the left and the SM group on the right, farther from the other groups. This suggested that the VOCs in the M muscles of the AM and SM groups exhibited unique aromatic characteristics. By observing the VIP plot for M muscles in each group, it was found that a total of 12 VOCs met the criteria (Fig. 4(e)). The AM group was primarily characterized by 3-hydroxy-2-butanone, 1-hexanol, 2-pentanone and 2-butanone. In the GM group, hexanal, 1-octen-3-ol, 1-pentanol and heptanal were detected. Hexanal and 2-butanone-3-hydroxy were key discriminants in the MB group. These compounds have been recognized as potential key biomarkers in the M muscles of the five breeds.

Figure 5(d) shows that the AML and DML groups intersect near the center of the score plot, suggesting that the VOCs of these two groups were relatively similar. The PCs explain 24.00% and 48.20% of the total variance, respectively. In contrast, the GML, HML and SML groups were positioned at the top right of the score plot, indicating significant differences between these three groups and the others. These findings were further supported by the VIP score graph, where 11 key discriminant factors were identified in the ML group (Fig. 5(e)). Notably, the HML group exhibited higher levels of 3-hydroxy-2-butanone, acetone and 2,3-pentanedione. Hexanal and 1-pentanol were key discriminant factors in the GML group, while ethanol, acetic acid and 2-butanone were prominent in the SML group, indicating their critical role in differentiating the ML muscles of various Tibetan sheep breeds.

CONCLUSION

In this study, HS-SPME-GC-MS and HS-GC-IMS, combined with multivariate statistical analysis, were employed to investigate the composition of VOCs across four muscle portions in five breeds of Tibetan sheep. The results revealed that alcohols, aldehydes and ketones were the dominant VOCs in Tibetan mutton. Gangba sheep exhibited the highest signal intensity, followed by Huoerba and Awang sheep, while Duoma and Sewa sheep showed the lowest levels. The OAV results further provided the proportion of key compounds in the muscles of various portions of the five breeds. It was determined that the M and L muscles of Gangba sheep contained the highest levels of effective VOCs, whereas the L, M and ML muscles of Duoma and Sewa sheep contained the lowest. Additionally, by analyzing the VOC fingerprint profiles, the special aroma compounds of five breeds and four muscle portions of Tibetan sheep mutton were identified, allowing for the development of a potential marker model for discriminant analysis. PCA effectively segregated and identified the VOCs in the samples. PLS-DA further highlighted both the differences and similarities in VOC profiles between samples, identifying 14 compounds with VIP scores >1, including hexanal, 1-hexanol, 2-butanone, 1-pentanol, heptanal, 1-octen-3-ol, 3-hydroxy-2-butanone, 2-pentanone, octanal, ethanol, acetone, 1-penten-3-ol, 2,3-pentanedione and acetic acid, and others. In this study, the combination of HS-SPME-GC-MS and HS-GC-IMS effectively distinguished the characteristic VOCs of various breeds and muscle portions of Tibetan mutton, as well as being used to assess the aroma similarities and differences between samples. The diverse Tibetan sheep mutton samples and distinct muscle portions exhibited unique flavors. The method applied in this research enabled rapid analysis of VOC composition across different samples, while the use of more representative samples provided a scientific basis for exploring flavor characteristics. These findings contribute to quality identification for Tibetan sheep mutton and support the industrial development of high-quality Tibetan sheep products.

ACKNOWLEDGEMENTS

This work was supported by the National Key R&D Program of China (2021YFD1600700).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Yuqian Xu performed the investigation of all indexes. Also carried out data analysis, prepared figures and original manuscript preparation; Qingfeng Yang carried out work conceptualization and manuscript preparation. Xiaochun Zheng carried out manuscript review & editing and language revision. Huiguo Yang carried out manuscript review & editing and language revision. Martine Schroyen carried out manuscript review & editing and language revision. Dequan Zhang carried out manuscript review & editing. Chengli Hou carried out funding acquisition, conceptualization, formal analysis, writing – review & editing, project administration and supervision. All authors reviewed the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1 Fan Y, Liang Y, Deng K, Zhang Z, Zhang G, Zhang Y *et al*., Analysis of DNA methylation profiles during sheep skeletal muscle development using whole-genome bisulfite sequencing. *BMC Genomics* **21**:327 (2020).
2 Zhang Q, Que M, Li W, Gao S, Tan X and Bu D, Gangba sheep in the Tibetan plateau: validating their unique meat quality and grazing factor analysis. *J Environ Sci* **101**:117–122 (2021).
3 Zhang X, Han L, Hou S, Raza SHA, Wang Z, Yang B *et al*., Effects of different feeding regimes on muscle metabolism and its association with meat quality of Tibetan sheep. *Food Chem* **374**:131611 (2022).
4 Hastie M, Ha M, Jacob RH, Hepworth G, Torrico DD and Warner RD, High consumer acceptance of mutton and the influence of ageing method on eating quality. *Meat Sci* **189**:108813 (2022).
5 Jia W, Di C and Shi L, Applications of lipidomics in goat meat products: biomarkers, structure, nutrition interface and future perspectives. *J Proteomics* **270**:104753 (2023).
6 Wang Q, Li L, Ding W, Zhang D, Wang J, Reed K *et al*., Adulterant identification in mutton by electronic nose and gas chromatography-mass spectrometer. *Food Control* **98**:431–438 (2019).

7 Liu H, Hui T, Zheng X, Li S, Wei X, Li P *et al*., Characterization of key lipids for binding and generating aroma compounds in roasted mutton by UPLC-ESI-MS/MS and orbitrap Exploris GC. *Food Chem* **374**:131723 (2022).
8 Li J, Yang Y, Tang C, Yue S, Zhao Q, Li F *et al*., Changes in lipids and aroma compounds in intramuscular fat from Hu sheep. *Food Chem* **383**:132611 (2022).
9 Pereira PFM, de Sousa Picciani PH, Calado V and Tonon RV, Electrical gas sensors for meat freshness assessment and quality monitoring: a review. *Trends Food Sci Technol* **118**:36–44 (2021).
10 Yao W, Ma S, Wu H, Liu D, Liu J and Zhang M, Flavor profile analysis of grilled lamb seasoned with classic salt, chili pepper, and cumin (*Cuminum cyminum*) through HS-SPME-GC-MS, HS-GC-IMS, *E*-nose techniques, and sensory evaluation on Sonit sheep. *Food Chem* **454**:139514 (2024).
11 Yao W, Wu H, Cai Y, Chen Y, Liu D and Zhang M, Comprehensive analysis of geographical impact on flavor profiles of braised chicken across eastern, central, and western China using GC-IMS, E-nose techniques and sensory evaluation. *Food Chem Adv* **5**:100808 (2024).
12 Zhou B, Zhao X, Laghi L, Jiang X, Tang J, Du X *et al*., Insights into the flavor profile of yak jerky from different muscles based on electronic nose, electronic tongue, gas chromatography–mass spectrometry and gas chromatography–ion mobility spectrometry. *Foods* **13**:2911 (2024).
13 Wang J, Xu Z, Zhang H, Wang Y, Liu X, Wang Q *et al*., Meat differentiation between pasture-fed and concentrate-fed sheep/goats by liquid chromatography quadrupole time-of-flight mass spectrometry combined with metabolomic and lipidomic profiling. *Meat Sci* **173**:108374 (2021).
14 Zhang C, Zhang J, Tuersuntuoheti M, Zhou W, Han Z, Li X *et al*., Landscape genomics reveals adaptive divergence of indigenous sheep in different ecological environments of Xinjiang, China. *Sci Total Environ* **904**:166698 (2023).
15 Sun X, Yu Y, Wang Z, Akhtar KH, Saleh ASM, Li W *et al*., Insights into flavor formation of braised chicken: based on E-nose, GC–MS, GC-IMS, and UPLC-Q-Exactive-MS/MS. *Food Chem* **448**:138972 (2024).
16 Nie R, Wang Z, Liu H, Wei X, Zhang C and Zhang D, Investigating the impact of lipid molecules and heat transfer on aroma compound formation and binding in roasted chicken skin: a UHPLC-HRMS and GC-O-MS study. *Food Chem* **447**:138877 (2024).
17 Li C, Al-Dalali S, Wang Z, Xu B and Zhou H, Investigation of volatile flavor compounds and characterization of aroma-active compounds of water-boiled salted duck using GC–MS–O, GC–IMS, and E-nose. *Food Chem* **386**:132728 (2022).
18 Zhu X, Healy LE, Sevindik O, Sun D-W, Selli S, Kelebek H *et al*., Impacts of novel blanching treatments combined with commercial drying methods on the physicochemical properties of Irish brown seaweed *Alaria esculenta*. *Food Chem* **369**:130949 (2022).
19 Zhao J, Wang M, Xie J, Zhao M, Hou L, Liang J *et al*., Volatile flavor constituents in the pork broth of black-pig. *Food Chem* **226**:51–60 (2017).
20 Hao Y, Kang J, Guo Y, Meng L, Li Z and Qin X, Bacterial interactions mediated by acetic acid and their impact on flavor profile during acetic acid fermentation stage of Shanxi aged vinegar. *Food Biosci* **64**:105996 (2025).
21 Huang Q, Dong K, Wang Q, Huang X, Wang G, An F *et al*., Changes in volatile flavor of yak meat during oxidation based on multi-omics. *Food Chem* **371**:131103 (2022).
22 Wen R, Hu Y, Zhang L, Wang Y, Chen Q and Kong B, Effect of NaCl substitutes on lipid and protein oxidation and flavor development of Harbin dry sausage. *Meat Sci* **156**:33–43 (2019).
23 Schuster L, Franke C, Silcock P, Beauchamp J and Bremer PJ, Development of a novel sample reuse approach to measure the impact of lean meat, bone and adipose tissue on the development of volatiles in vacuum-packed chilled lamb stored at 2 °C for 15 days. *Meat Sci* **145**:31–39 (2018).
24 Jiang H, Dai A, Yan L, Zhang Z, Ding B, Bai J *et al*., Analysis of volatile organic compounds (VOCs) in yak ghee from different pastoral areas of China based on GC-IMS. *Int Dairy J* **160**:106098 (2024).
25 Wang F, Gao Y, Wang H, Xi B, He X, Yang X *et al*., Analysis of volatile compounds and flavor fingerprint in Jingyuan lamb of different ages using gas chromatography–ion mobility spectrometry (GC–IMS). *Meat Sci* **175**:108449 (2021).
26 Sun A, Wu W, Soladoye OP, Aluko RE, Bak KH, Fu Y *et al*., Maillard reaction of food-derived peptides as a potential route to generate meat flavor compounds: a review. *Food Res Int* **151**:110823 (2022).
27 Mariutti LRB and Bragagnolo N, Influence of salt on lipid oxidation in meat and seafood products: a review. *Food Res Int* **94**:90–100 (2017).
28 Chen X, Luo J, Lou A, Wang Y, Yang D and Shen QW, Duck breast muscle proteins, free fatty acids and volatile compounds as affected by curing methods. *Food Chem* **338**:128138 (2021).
29 Shen C, Cai Y, Wu X, Gai S, Wang B and Liu D, Characterization of selected commercially available grilled lamb shashliks based on flavor profiles using GC-MS, GC × GC-TOF-MS, GC-IMS, E-nose and E-tongue combined with chemometrics. *Food Chem* **423**:136257 (2023).
30 Zhang L, Badar IH, Chen Q, Xia X, Liu Q and Kong B, Changes in flavor, heterocyclic aromatic amines, and quality characteristics of roasted chicken drumsticks at different processing stages. *Food Control* **139**:109104 (2022).
31 Liang M, Zhang Z, Wu Y, Wang R and Liu Y, Comparison of *Amomum tsaoko* Crevost et Lemaire from four regions via headspace solid-phase microextraction: variable optimization and volatile characterization. *Ind Crops Prod* **191**:115924 (2023).