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Comparative characterization of flavor precursors and volatiles in Chongming white goat of different ages by UPLC-MS/MS and GC–MS

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<i>Keywords</i> : Chongming white goat Lipidomics GC–MS	Age has a significant impact on goat meat flavor. However, reporting the effects of age on free amino acid (FAAs), lipid profiles and aromas of goat meat is limited. Here, the FAAs, lipid profiles and aromas in the Chongming white goat with 12 months and 24 months were investigated in this study. A total 1164 lipids were identified using lipidomics, including 31 subclasses. Multivariate statistical analysis showed that 201 lipids had significant changes, FFA, TG and DG increased with goat age. Furthermore, the pathway analysis indicated that glycerophospholipid metabolism and glycerolipid metabolism were the key pathways that relate to lipid profile

by age, which provides a basis for improving goat meat flavor.

1. Introduction

Goat meat is one of the vital source of protein in the China daily diet, which has low-fat content, high-protein content and no religious taboos (Qi et al., 2022). The meat flavor is the primary reason for the consumer acceptance when purchasing and consuming mutton (Prache et al., 2022). However, the flavor of meat is greatly influenced by age (F. Wang et al., 2021). Hence, the exploration of the influence of age on the flavor of goat meat will be of utmost significance for the development of meat products that meet consumer expectations and demands.

Chongming white goat, also referred to as Yangtze River Delta white goat, is garnering increasing popularity in Shanghai and its surrounding areas due to its "unique species meat flavor" and abundant nutritional content. In 2011, the Ministry of Agriculture and Rural Affairs of China approved the Chongming white goat as a Geographical Indication agricultural product. Chongming white goats are usually slaughtered at 12 months duo to balance the taste and feeding cost. However, some consumers prefer older goats for better flavor (Veiseth et al., 2004). This phenomenon may be account for the accumulation of intermuscular fat (IMF) with advancing age. The flavor of meat is closely related to the composition and distribution of intramuscular fat (IMF) (Q. Wang et al.,

2019). As the goat ages, the solubility of the connective tissue in the muscle decrease, and the IMF accumulates, making the flavor of the goat meat richer (Zhang et al., 2018). Consumers The recent research reported that the meat with high IMF content has rich taste characteristics (Cao et al., 2023). Sensory tests have shown that when the IMF content is between 2.5 % and 7 %, consumer satisfaction with the flavor of lamb increases as the IMF content increases (Realini et al., 2021). On the other hand, lipids were the most important precursors of the formation of meat aroma. Lipids produce most of the volatiles in cooked meat through oxidative degradation or reaction with Maillard products (Sohail et al., 2022). Particularly, aldehydes are the most common aromas found in cooked meat. The characteristic species-specific flavor is imparted by lipids, and the water-soluble fraction contains components that produce basic meat flavors (Khan et al., 2015). In addition to being influenced by the thermal oxidation capacity of lipids, these volatile compounds are also contingent upon the binding sites of individual lipids and lipid classes (Li, Yang, et al., 2022). The double bonds position in unsaturated fatty acids affect the properties and composition ratio of volatile compounds, as shown in a study (Z. Yang, Chen, et al., 2023). Therefore, the flavor of goat meat can be improved by adjusting the feeding years of Chongming white goat. However, describing the

changes during goat growth. Thirty-five volatile compounds were identified, among them, 14 aromas with odor activity value greater than 1 were considered as potential characteristic aroma compounds of Chongming white goat meat. These findings reveal the FAAs, lipids, and aromas profiles in Chongming white goat meat are affected

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formation of goat meat flavor becomes a huge challenge due to the unclear mechanism of lipid molecules conversion related to aromas (Jia et al., 2023). There are few reports detailing the lipids and FAAs and their influence on aroma formation during goat growth. Therefore, it is important to identify the lipid composition of goat meat.

Lipidomics, as an emerging discipline, mainly investigates the classification, abundances, distribution and biological roles of lipids. With the development of mass spectrometry (MS), ultrahigh performance liquid chromatography-triple quadrupole-ion trap MS (UPLC-QTRAP-MS/MS) have been widely used. Bandarra et al. (2018) have found that the meat quality is affected by the lipid molecules composition of meat. Goat meat products with low fat and rich protein will become more popular as consumers have more spending power and health awareness. By elucidating the metabolic pathways of the aroma components of meat, it is possible to effectively monitor and regulate quality changes in the actual breeding process (Han et al., 2024).

This study investigated the lipid profiles and volatile compounds of Chongming white goat using lipidomics and GC–MS and while considering the age of goat as the main element. The aim of this study was to (1) evaluate the composition of lipids, FAAs and volatile compounds in Chongming white goat meat, (2) identify potential key aroma compounds in Chongming white goat, (3) reveal the relationship between flavor precursors and volatiles in goat meat. These data provided the basis for the flavor control of goat meat.

2. Material and methods

2.1. Sample collection

All animal experimental protocol in this study was approved by the Animal Ethics Committee of Shanghai Academy of Agricultural Sciences, China (Permit ID: SAASPZ0522048). Chongming white goats were purchased from Chongming White Goat Experimental Station, Shanghai Academy of Agricultural Sciences. This study selected ten of castrated male Chongming white goats with the same genetic background, and divided into two age groups (12 months and 24 months), with 5 goats in each group. All goats were fed the same commercial diet (Silage Corn, corn stalk, alfalfa and corn husk). As required by the China council on animal care, each goat was fasted for 16 h overnight with free access to water, immobilised by electroshock, then bled, skinned and split down the midline. The longissimus thoracis were collected from the left side of each goat carcass. These goat meat samples were stored at -80 °C.

2.2. Free amino acids (FAAs) measurement

FAAs were performed using Amino Acid Analyzer (L-8900, JAPAN). The goat meat was minced and 3.0 g of sample was weighed and placed into tubes. Subsequently, a hydrochloride solution (6.0 mol/L, 15 mL) was added to the tubes. Then, to prevent oxidation, the tube was vacuumed and filled it with nitrogen. The tubes were hydrolyzed at 110 °C for 22 h. The hydrolyzed samples were volumed to 50 mL with ultrapure water and filtered into the beakers. The filtrate (0.50 mL) was added to the tubes and blew with nitrogen to dry. Finally, the hydrochloride solution (2.0 mL) added to the tubes, then, the solution was collected into an injection bottle for Amino Acid Analyzer analysis.

2.3. Lipidomics analysis by UPLC-MS/MS

Lipidomic profiling was performed by UPLC-QTRAP-MS/MS (AB SCIEX, Framingham, USA). The UPLC system coupled with Thermo AccucoreTM C30 column (2.6 μ m, 2.1 mm \times 100 mm). In brief, the samples were evenly in a liquid nitrogen environment. Then, 50 μ L of pure methanol was added to 20 mg samples, vortexed well and centrifuged (12,000 rpm, 4 °C) for 30 s. The sample containing 1 mL of extraction solvent was vortexed for 15 min and added 200uL of

ultrapure water. Finally, the 200 μ L of the dry extract was dissolved in reconstituted solution and stored in sample flask before UPLC–MS/MS analysis.

The UPLC conditions were as follows: The mobile phase was constituted of phase A and phase B. The mobile phase A was as follows: acetonitrile/water (60/40, ν/ν) with 10 mM ammonium formate and 0.1 % formic acid. The mobile phase B was as follows: acetonitrile/ isopropanol (10/90, ν/ν). The column temperature was set at 45 °C. The gradient elution program was performed as follows: 0 min-2 min, 80 % A; 2 min-4 min, 70 % A; 4 min-9 min, 40 % A; 9 min-14 min, 15 % A; 14 min-15.5 min, 10 % A; 15.5 min-17.3 min, 5 % A; 17.3 min-20 min, 80 % A. Flow rate was 0.35 mL/min. Injection volume was 2 μL . The effluent was alternatively connected to QTRAP-ESI-MS/MS. Mass spectrometric analysis was operated in positive and negative ion mode. All other instrumental parameters applied were as follows: source temperature 500 °C, ion spray voltage 5500 V (positive) and - 4500 V (negative). Collision-activated dissociation parameter was set to Medium. Finally, based on the self-built metware database, the lipids were qualitatively and quantitatively analyzed using UPLC-ESI-QTRAP-MS/MS with multi-reaction monitoring mode (MRM). The chromatographic peaks are integrated and corrected by MultiQuant 3.0 software. Lipids identification was conducted by the self-built database according to Retention time (RT), molecular weight of parent ion and characteristic fragment ion. The peak area of each lipid was represented the relative content.

2.4. GC-MS analysis

The GC–MS (7890 A-5975C, Agilent, USA) was used to analysis the volatiles of Chongming white goat meat according to the method (Gu et al., 2023). In brief, 3.0 g of samples and 5 μ L of 1,2-dichlorobenzene (100 μ g/mL in acetone) were transferred into headspace bottle (20 mL). The samples were heated at 85 °C for 25 min. Then, the SPME fiber (50/30 μ m DVB/CAR/PDMS, Stableflex, Supelco, 57,329-U, USA) was inserted into the headspace bottle and adsorbed in equilibrium for 30 min. Finally, the fiber was put into the GC and desorbed in splitless mode at 250 °C for 300 s.

The GC parameters applied were as follows: A HP-INNOWAX column (60 m × 250 μ m × 0.25 μ m, Agilent) was used for separation. The carrier gas was high-purity helium (99.999 %). Flow rate was 1.0 mL/min. The column initial temperature was maintained at 40 °C for 2 min, then increased to 180 °C at a rate of 3 °C/min for 3 min and rised to 230 °C at a rate of 20 °C/min for 3 min. The MS parameters applied were as follows: the electron ionization energy was 70 eV, the breadth range of detecting quality fragments was 35–400 *m*/z, the ion source temperature was set at 230 °C. The volatiles were identified according to NIST 11 library and retention indices (RIs). The RI values were calculated by n-alkanes (C₆ ~ C₂₄). In addition, quantitative analysis was performed by comparing the peak areas of 1,2-dichlorobenzene and target volatile compounds. OAV was Calculated based on odor threshold of compounds in water. The OAV of aromas were over 1, meaning the volatiles contribute more to overall aromas (Schoenauer & Schieberle, 2019).

2.5. Statistical analysis

In this study, 12-LT and 24-LT represented the longissimus thoracis of Chongming white goat at 12 month and 24 months, respectively. All data are presented as the mean \pm standard deviation (SD) of the mean (n = 5). *t*-test was conducted by IBM SPSS Statistic 27 (Chicago, USA). Heatmap and Volcano plot were drawn by the Metware platform (https: //cloud.metware.cn/). Differential lipids were obtained by variable importance in projection (VIP > 1) and *P*-value (P < 0.05). KEGG database was used to annotate and map the differential lipids. Subsequently, lipids were subjected to enrichment analysis with the significance of the results determined by the hypergeometric test's *p*-values. SIMCA 13.0 (Umetrics, Sweden) was used to conduct partial least

squares regression (PLS-R), PCA, and OPLS-DA analysis. The Pearson correlation network was conducted using metware cloud platform.

3. Results

3.1. Changes in FAAs of Chongming white goat with different ages

FAAs are not only an important nutritional indicator of the eating quality, but also an important flavor precursor for the formation of aroma, and the composition of FAAs has an important role on the aroma of meat (Gu et al., 2023). As shown in Fig. 1, 16 types of target FAA were detected in the 12-LT and 24-LT group, with a total amino acids (TAA) content of 17.16 \pm 0.40 g/100 g and 18.12 \pm 0.61 g/100 g (wet weight), respectively. The dominant essential amino acids (EAAs) in Chongming goat were Lysine (Lys) and Leucine (Leu) and among the nonessential amino acids (NEAAs), relatively high amounts of Glutamic acid (Glu), Aspartic acid (Asp) and Arginine (Arg) were observed. The content of EAAs and TAA in 24-LT group was significantly higher than 12-LT group (P < 0.05). Similarly, the concentration of NEAAs in 24-LT group was higher than 12-LT group. The 24-LT had the higher content of FAA in most classes. The contents of Leucine, Serine, Glutamic acid, Isoleucine and Histidine in the 24-LT group were significantly (P < 0.05) higher than 12-LT group, the other 11 FAAs was not significantly different. Taken together, these results indicated that the concentration of TAA increased in 24-LT group.

3.2. Lipid molecular identification in Chongming white goat

In this study, the lipid molecular in Chongming white goat was identified by UPLC-ESI-QTRAP-MS/MS. The TIC superposition analysis of the QC samples showed that the lipidomics data in this study had a good level of repeatability and reliability (Fig. S1). A total of 1164 lipid molecules in Chongming goat meat were identified (Fig. 2A). These lipid molecules were further divided into 6 classes, among which glycerophospholipids (GPs) contains the most species of lipid molecules, followed by glycerolipids (GLs), fatty acids (FAs), sphingolipids (SPs), sterol lipids (STs) and prenol lipids (PR). Similarly, these six classes were divided into 31 subclasses, the major subclasses being phosphatidylethanolamine (PE), triglyceride (TG), phosphatidylserine (PS), phosphatidylcholine (PC) and phosphatidylglycerol (PG). To further investigate the changes of lipid composition in the two age groups, the sum of peak intensity of the signal lipid molecules in the same class or subclass in goat meat is detected as shown in Fig. 2 B-E. In parallel, the peak areas of individual lipids within a class or subclass were summed in order to analyze and compare the different lipids in goat meat. Compared to 12-month-old goats, the lipid content in 24-month-old goats was significantly increased (Fig. 2B). The lipid profile was dynamically changed between 12-LT and 24-LT. In Fig. 2C, the thickness of two ends of different color bands reflected changes in the relative lipid contents. The content of GL showed an increasing trend in the 24-LT group. Meanwhile, the content of GP, FA, SP, ST, and PR all showed a decreasing trend in the 24-LT group. In Fig. 2C, the overall abundance of lipid molecules in the 24-LT group was significantly higher than that in the 12-LT group (P < 0.01). The level of TG DG and FFA were significantly increased in 24-LT compared to 12-LT (P < 0.05). PC, PE, PS, PA, PG and PI were the main primary forms of GPs, which did not significantly differ between 12-LT and 24-LT. These results suggested that GLs were the primary type of lipid deposition in 24-month-old goats.

3.3. Identification of differential lipids in Chongming white goat

To further investigate the lipid characteristics of the 24-LT group and 12-LT group, the PCA and OPLS-DA was conducted exploring the differences in goat meat samples. The data was firstly undergone log transformation and pareto scaling to obtain the normal distribution dataset. The PCA score plot showed that the 12-LT group and the 24-LT group were separated, indicating that two groups of white goats had different lipid compositions (Fig. 3A). PC1 and PC2 explained 43.9 % and 14.5 % of the variation in lipids, respectively, with a cumulative variance of 58.4 %. Then, the OPLS-DA was used to distinguish the samples from different ages. The R²Y and Q² values were 0.978, 0.788, respectively (Fig. 3B). The OPLS-DA score plot demonstrated that the samples were clearly separated, indicating a notable distinction between the two groups. However, the OPLS-DA model must be tested for reliability as it is susceptible to overfitting. The OPLS-DA model exhibited good predictive performance based on 200 permutations of experimental results (Fig. 3C). Then, the VIP values were obtained from the OPLS-DA model. To gain further insight into the lipid differences between 12-LT and 24-LT, according to the criterion (VIP >1 and P < 0.05), a total of 201 differential lipids were screened out in this study (Table S1). A total of 159 lipids and 42 lipids were significantly downregulated and significantly up-regulated, respectively, in the 12-LT group (Fig. 3D). The differential lipids were distributed in a dynamic manner, as illustrated in Fig. 3E. In addition, the top five differential lipids with highest VIP-value were marked on the plot (Fig. 3D), including TG $(16:0\ 18:1\ 18:1),$ TG (18:1 17:2 18:2) TG (18:1 18:1 18:1) TG (18:1 18:1 20:1) and TG (18:1 18:2 20:0). These lipids were considered as the potential biomarker to distinguish the goat meat from 12-LT and 24-LT groups. This figure (Fig. 3E) showed the changes in the relative levels of the differentially expressed lipids. The differential lipids were grouped into five classes, among these which GL contain the most numbers of lipids, followed by GP, SP, FA and PR (Fig. 3E). The GL, which had two subclasses, including 102 TGs and 10 DGs (Table S1). GP, which has eight subclasses, including 9 PAs, 15 PCs, 14 PEs, 13 PGs, 4 PIs, 19 PSs, 1 LNAPE and 1 LPS. The SP had three lipid subclasses, including 4 SMs, 5 Cer-NS and CerP. Compared to 12-LT

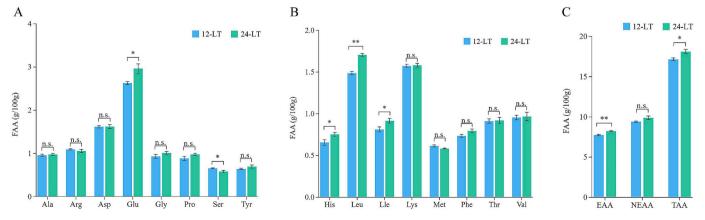


Fig. 1. Concentrations (g/100 g wet weight) of FAAs in goat meats with different ages.

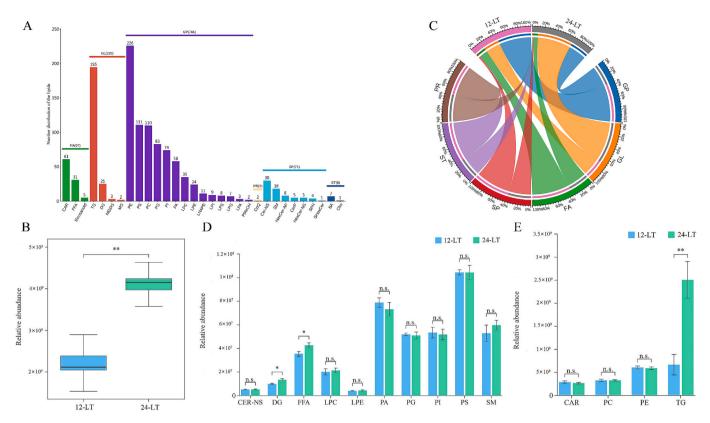


Fig. 2. Lipid molecule composition in Chongming white goat. (A) number of lipid classes and subclasses identified in goat meat, (B) changes of lipid class contents between the 12-LT and 24-LT groups, (C) comparison of lipid content between the 12-LT and 24-LT groups; (D-E) Comparison of lipid subclass contents between the 12-LT and 24-LT groups; "*", P < 0.05; "*", P < 0.01; n. s., not significant.

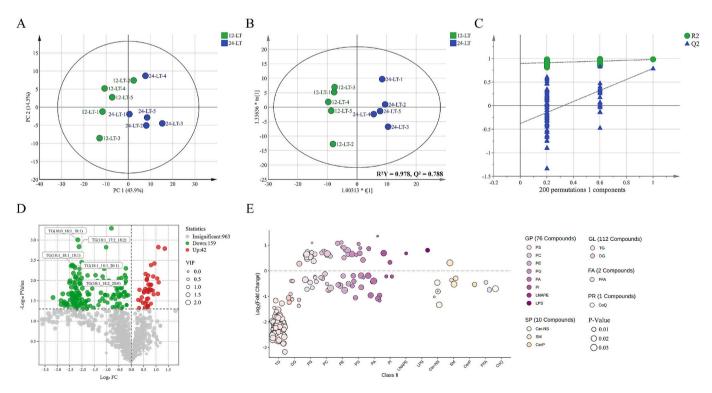


Fig. 3. (A) The PCA plot of lipids between the 12-LT and 24-LT groups. (B) The OPLS-DA score plot of lipids between the 12-LT and 24-LT groups. (C) The plot of the permutations test of OPLS-DA model. (D) The volcano plot of differential lipids. (E) The dynamic distribution plot of differential lipids.

group, the level of 102 TG and 9 DG molecules in 24-LT group were promoted. Thirty-six GP molecules were increased in 24-LT group, compared to 12-LT group. The level of FFA (16:0) and FFA (18:1) were decreased in 12-LT group, compared to 24-LT group. In terms of SP, a total of nine lipid molecules were upregulated in 24-LT group, while only one lipid (SM(d18:1/18:0)) was upregulated in 12-LT group.

3.4. Lipid metabolic pathway analysis

In order to gain further the potential metabolic pathways underlying the effect of age on the lipids of goat meat, KEGG and MESA were used to analyze metabolic pathways of differential lipids. A total of 173 differential lipids were enriched into 102 metabolic pathways (Fig. 4A). These metabolic pathways were divided into five classes, including organismal systems (46 pathways), metabolism (15 pathways), human diseases (23 pathways), environmental information processing (13 pathways) and cellular processes (5 pathways). These pathways included in glycerophospholipid metabolism, glycerolipid metabolism, linoleic acid metabolism. To further investigate the key pathways of differential lipid metabolism, the MetaboAnalystR 6.0 platform was employed to conduct perform topology analysis on these lipids, with the calculation of both the *P*-value and pathway impact (Fig. 4B). In the topology analysis, the horizontal coordinate value and bubble size were used to indicate the degree of impact. Two pathways had lower *p*-value and higher pathway impact between 12-LT and 24-LT, including glycerophospholipid metabolism and glycerolipid metabolism (Fig. 4B). These pathways may be potential impact on the lipid composition of the goat meat. The key lipid pathways of the different lipid profile of goat meat at different months are shown in the Fig. 4C. PA, PC, PE, PS and DG were involved in glycerophospholipid metabolism. PA, TG and DG were involved in glycerolipid metabolism. In these two important lipid metabolisms of Chongming white goat, PA and DG were the key differential lipids. Hence, the above results indicated that there are differences in lipid

metabolism between two age groups, which affect the composition lipid profile.

3.5. Aroma compounds in Chongming white goat

A total of 35 volatiles in samples were detected and semi-quantified by HS-SPME-FC-MS (Table 1). These compounds consisted of 19 aldehydes, 7 alcohols, 3 furans, 4 ketones and 2 acids. The aldehydes and alcohols were mainly volatile compounds in the two groups. The major volatiles in goat meat samples are lipid degradation compounds and Maillard reaction products. The contents of aldehydes in the 24-LT group were higher than 12-LT group (Fig. 5A). It was clear that the aroma compounds in goat meat are generally aldehydes and alcohols which were mostly produced by lipid oxidation. A semi-quantitation was applied to 33 aroma compounds, and the OAVs were calculated (Table 1). In samples, a total of 32 aromas had OAV, including 18 aromas with OAV > 1 (Fig. 5B). Fourteen aromas with OAV > 1 were contained in both the 12-LT and 24-LT groups. It suggested that the 14 aromas could considered the characteristic aroma in Chongming white goat. (E, E)-2,4-decadienal (1454.22) had the largest OAV in 24-LT group. Similarly, (E)-2-Octenal (197.06) had the largest OAV value in the 12-LT group. Additionally, the content of hexanal, heptanal, octanal, nonanal were decreased in 24-LT group. Although these aroma compounds had the same odor thresholds, their OAVs were different due to variation in the concentration of different groups (Fig. 5B). From a holistic point of view, there were differences in volatiles between in the 12-LT and 24-LT groups. Then, we analyzed the sensory features of volatiles in two goat meat samples based on the aroma odors description (Table 1). The correlation network was conducted to describe the relationship between the aroma compounds and their sensory odors description (Fig. 5C). It was observed that aroma characterization of white goats was fatty, green, sweet, floral, and fruity. Additionally, the radar plot was conducted to determine the differences in sensory odors

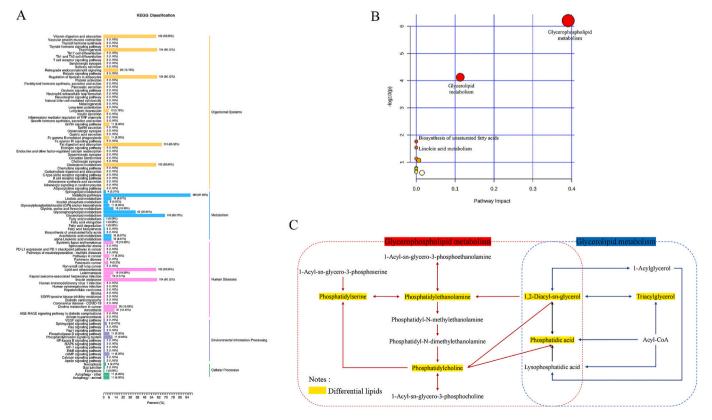


Fig. 4. KEGG pathway analysis of differential lipids. (A) The KEGG pathway annotations plot of differential lipids. (B) KEGG enrichment and topological analysis of differential lipids. (C) key pathways for the lipid profiles difference in Chongming white goat with different ages.

Table 1

Aroma compounds of goat meat between 12-LT group and 24-LT group.

Compounds ^a	RI		Concentration (ug/kg)		Odor threshold ^d (ug/	OAV		Odors ^e
	Experimental ^b	Referenced ^c	12-LT	24-LT	kg)	12-LT	24-LT	
Pentanal	992	993	$2.65 \pm 1.52^{\mathrm{b}}$	$\textbf{5.88} \pm \textbf{1.28}^{a}$	12	0.22	0.49	green
Hexanal	1091	1083	39.82 ± 18.03^{a}	115.45 ± 79.15^{a}	5	7.96	23.09	green, fresh, grass
Heptanal	1181	1186	38.19 ± 19.44^{b}	74.75 ± 20.2^{a}	2.8	13.64	26.7	green, aldehyde
Octanal	1279	1287	76.17 ± 35.61^{a}	$114.7\pm52.16^{\text{a}}$	0.59	129.1	194.41	green, citrus, lemon
(E)-2-Heptenal	1331	1323	$\textbf{4.04} \pm \textbf{1.99}^{a}$	$\textbf{6.89} \pm \textbf{3.74}^{a}$	40	0.1	0.17	fatty
Nonanal	1387	1390	216.77 ± 99.9^{a}	373.22 ± 139.73^{a}	1.1	197.06	339.29	citrus, green, citronella grass
(E)-2-Octenal	1436	1434	12.39 ± 4.57^b	$53.98\pm31.08^{\text{a}}$	3	4.13	17.99	green, floral
(E)-2-Nonenal	1542	1544	34.48 ± 13.17^{a}	56.26 ± 39.96^a	0.19	181.45	296.13	fatty, paper
Benzaldehyde	1548	1530	$\textbf{73.5} \pm \textbf{16.69}^{a}$	$93.29\pm30.82^{\text{a}}$	750	0.1	0.12	almond
Undecanal	1611	1622	$^{-}$ 32.78 \pm	$\textbf{7.75} \pm \textbf{3.08}$	0.12	-	64.57	floral, green, mild
(E)-2-Decenal	1652	1643	23.42 ^a	48.41 ± 22.32^a	0.4	91.52	121.02	green, fat
Dodecanal	1711	1709	$1.54\pm0.82^{\text{a}}$	$6.09 \pm 4.64^{\text{a}}$	0.29	5.3	20.99	lily, fat, citrus
4-Ethylbenzaldehyde	1732	1730	4.52 ± 2.01^{a}	$4.25\pm3.05^{\rm a}$	120	0.04	0.04	caramel-like
Tridecanal	1819	1824	$3.05\pm1.25^{\rm a}$	7.36 ± 4.36^{a}	10	0.31	0.74	fatty, sweet
2-Undecenal	1769	1758	$\begin{array}{c} 20.26 \ \pm \\ 20.14^{\rm b} \end{array}$	93.02 ± 32.19^a	0.78	25.97	119.25	sweet
(E, E)-2,4-decadienal	1826	1805	$3.62 \pm 1.74^{\text{b}}$	$39.26\pm31.53^{\text{a}}$	0.027	134.15	1454.22	plastic, tailing odor
(E, E)-2,4- undecadienal	1935	1931	-	$\textbf{6.47} \pm \textbf{6.32}$	1	-	6.47	green
Tetradecanal	1932	1924	3.99 ± 2.07^{a}	$3.92\pm3.78^{\rm b}$	110	0.04	0.04	roasted, fried
hexadecanal	2145	2390	$35.72\pm9.08^{\rm a}$	$10.28\pm3.55^{\rm b}$	-	-	-	fatty
Methanethiol	688	692	1.21 ± 0.45	-	0.2	6.05	-	cabbage, foot
1-Pentanol	1274	1271	2.95 ± 1.46	-	150.2	0.02	-	green, fruity
Hexanol	1364	1360	2.91 ± 1.21^{a}	$6.04 \pm 4.51^{\text{a}}$	5.6	0.52	1.08	green
1-Octen-3-ol	1455	1458	32.93 ± 11.04^{a}	$\textbf{28.98} \pm \textbf{13.42}^{a}$	1.5	21.95	19.32	mushroom
1-heptanol	1464	1462	$11.83\pm7.96^{\text{a}}$	$18.68 \pm 14.17^{\text{a}}$	5.4	2.19	3.46	floral
1-Octanol	1563	1564	34.51 ± 26.03^{a}	80.82 ± 51.28^a	120	0.29	0.67	fatty
1-Dodecanol	1978	1964	$4.94 \pm 1.16^{\text{a}}$	$1.62\pm0.72^{\rm b}$	16	0.31	0.1	wax, sweet
2-Pentylfuran	1211	1230	23.24 ± 11.25^{a}	$\textbf{8.14} \pm \textbf{4.81}^b$	5.8	4.01	1.4	green bean, butter
2-heptyl-Furan	1417	1429	1.17 ± 0.69^{b}	3.37 ± 2.01^{a}	-	-	-	fatty, green
2-Octylfura	1522	NO	1.55 ± 0.76	-	-	-	-	_
ethanoic acid	1476	1465	$2.88 \pm 1.69^{\text{a}}$	$\textbf{2.94} \pm \textbf{1.84}^{a}$	99	0.03	0.03	sour
Octanoic acid	2081	2067	9.1 ± 3.75^{a}	$5.32\pm2.31^{\texttt{a}}$	3	3.03	1.77	vomit, cheese, rotten meat
3-Octanone	1244	1253	1.41 ± 0.59	-	21	0.07	-	fruity, nutty
2-Nonanone	1395	1402	$0.95\pm0.35^{\text{a}}$	$1.15\pm0.46^{\text{a}}$	41	0.02	0.03	flower petal, floral
2-Decanone	1491	1495	$\textbf{2.84} \pm \textbf{1.2}^{\textbf{a}}$	1.14 ± 0.75^{b}	41	0.07	0.03	heavy, sweet
2-Undecanone	1598	1608	1.16 ± 0.47	_	5.5	0.21	-	fruity, fatty

^a Aroma compounds identified in Chongming white goat. ^b RI calculated from non-isothermal Kovats method; ^c RI Referenced obtained from the online database: https://webbook.nist.gov, http://www.flavornet.org/; ^d The threshold was cited from the book "Odor thresholds Compilations of odor threshold values in air, water and other media", Second enlarged and revised edition; Different letters of the same row represent significant differences; "-", not detected. ^e Odors were obtained from the references (Sohail et al., 2022).

between 12-LT and 24-LT groups based on the odor description of each aroma and concentrations involved (Fig. 5D). The fruity of 12-LT was better than 24-LT, while the rest of odors (green, fatty, sweet, floral, citrus, fat, fresh) in 24-LT was surpassed the 12-LT. It indicated that the overall flavor of 24-LT might be better than 12-LT.

3.6. Correlation analysis of flavor precursors and aromas

The concentration and release of aromas are significantly influenced by lipids and FAAs. Therefore, lipids and FAAs with significantly different levels were evaluated by PLS-R analysis to determine their impact on aromas difference between 12-LT and 24-LT. The PLS-R model was used to detect the lipids and FAAs connected to the aromas with OAV >1 (Fig. 6A). The R²X, R²Y and Q² values were 0.925, 0.957, 0.434, respectively. As shown in Fig. 6A, the 12-LT group and 24-LT group were separated from each other. To elucidate the aroma difference in 12-LT and 24-LT groups, the VIP scores were calculated by the PLS-R former. Seventy-seven flavor precursors with VIP >1 was identified, highlighting their potential role in the differential aromas of goat meat between the 12-LT and 24-LT groups (Fig. 6 B). Therefore, lipids and FAAs with VIP > 1 might play an significant role on aroma differences of goat meat at different ages. Subsequently, a correlation network between flavor precursors and aromas was conducted to illustrate the potential formation relationship (Fig. 6C). Only those correlations that were statistically significant (P < 0.05, r > 0.8 or < -0.8) was plotted between flavor precursors and aromas in this network plot. Thirty-three lipids and one FAA exhibited significant correlation with 11 of aromas. For example, hexanal, heptanal, nonanal, as the saturated fatty aldehyde, exhibited significantly positive correlation with PC (17:0_20:5). 2-Pentylfuran was significantly negative correlation with PC (12:0 14:1). (E, E)-2,4-undecadienal, which has typical green odor, had significant negative correlation with PG (21:0 18:2), LPS (18:2/0:0) and PS (18:2_20:2). Overall, these results indicated that phospholipids and TGs containing unsaturated fatty acid branched chain might contribute to the difference of aromas between 12-LT and 24-LT groups.

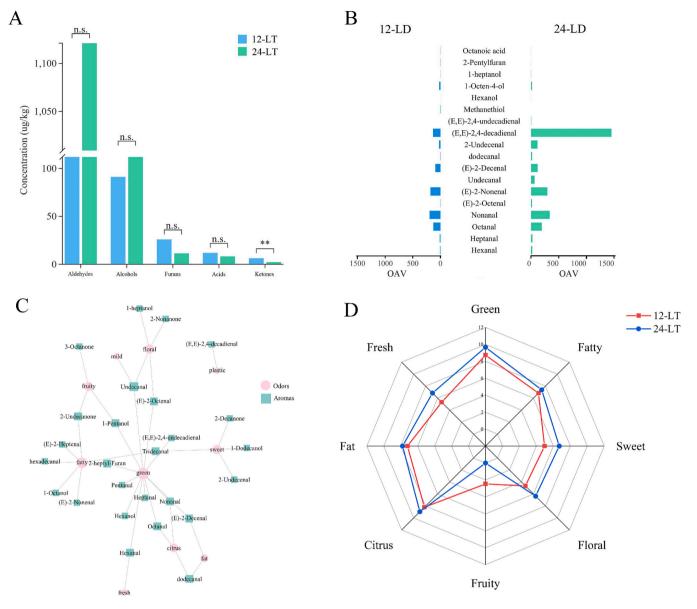


Fig. 5. (A) Comparison of volatile compounds classes content between the 12-LT and 24-LT groups. (B) The Odor active values (OAV > 1) of aroma compounds in the 12-LT and 24-LT groups. "**", P < 0.01; n. s., not significant. (C) Correlation network between the aroma compounds and sensory odors description. (D) The sensory odors characterization between 12-LT and 24-LT groups.

4. Discussion

Meat flavor is an important factors that influence consumer purchases (Sohail et al., 2022). Feeding age is an important factor in the meat flavor. Hence, we investigated the FAAs, lipid profiles and volatiles in the Chongming white goat with 12 months and 24 months. Compared to 12-LT group, the FAAs content was significantly enhanced in 24-LT group (P < 0.05). This could be due to the age factor of the animal. Previous study has shown differences in FAAs composition between different ages of goat meat (Gawat et al., 2023). As animals age, the activity ratio of µ-calpain to calpastatin increases, resulting in greater protein hydrolysis ability in 24-LT compared to 12-LT, increasing the content of FAAs (Veiseth et al., 2004). FAAs were the important flavor precursors. Strecker aldehyde, formed by amino acid reactions, is important for the formation of meat flavor (Lund & Ray, 2017). Sulfurcontaining amino acids react with reducing sugars to produce sulfurcontaining compounds with meat flavor characteristics, which have low olfactory thresholds and are often the key aromas of meat (Sohail et al., 2022). Leucine (leu) and isoleucine produce the aldehydes,

alcohols and acids by transamination and decarboxylation, which produce the malty and pungent odors (Flores, 2018). According to previous study, the Strecker degradation of leucine can produce the 3-methylbutanal that is malty and chocolate odors (Sohail et al., 2022). Glutamic acid is the typical umami amino acids; the Histidine is the typical bitter amino acid that exasperate the flavor of meat (Yin et al., 2022). It is suggested that the flavor of goat meat in the 24-LT group was richer than that in 12-LT group because of the higher amino acid content in 24-LT group.

The lipid content and composition have an important effect on the meat aromas. A total of 1164 lipids were identified, and these lipids can be further divided into 6 classes or 31 subclasses. GPs and GLs were the primary constituents of lipid in Chongming white goat meat. (Li, Yang, et al., 2022) reached similar conclusions for Hu sheep meat. Compared to 12-month-old goats, the lipid content in 24-month-old goats was significantly increased (Fig. 2B). According to research reported that as goat age, the size and number of fat cells also increase, which increases the lipid content (Auqui et al., 2019). On the other hand, goat do not firstly store fat in the early stage of growth (Pophiwa et al., 2020). The

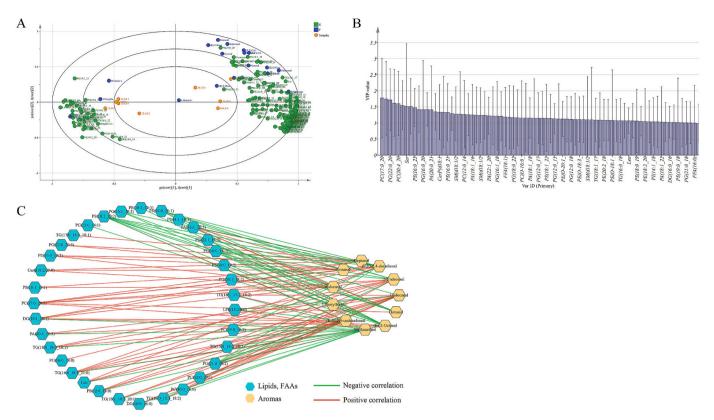


Fig. 6. (A) The plot of partial least squares regression analysis (PLS-R) between differential lipids, and FAAs (X) and aromas (Y). (B) Potentially important lipids and FAAs with variable importance in projection (VIP) scores >1 based on PLS-R model. (C) Correlation network plot among important lipids, FAAs, and aromas. The pearson correlations retained only strong ($|\mathbf{r}| > 0.8$) and statistically significant (p < 0.05) associations.

fat reached superior level when they are mature. The composition lipids were significant difference between in the 12-LT and 24-LT groups (Fig. 2C, D and E). GPs were not significantly difference between the 12-LT and 24-LT groups. We observed the level of TG and DG were significantly increased in 24-LT group compared to12-LT group. Generally, IMF increased as animals aged, but TG are the most abundant lipids in animal muscle fat (Cao et al., 2023). GP is the primary component of cell membranes, and its changes remain fairly constant, or increase little, as the IMF increases (Y. Yang et al., 2010). These results suggested that GLs were the primary type of lipid deposition in 24-month-old goats. The result was similar to that of a recent study, in which a decrease in PC and PE in meat was found with an increase in lipid content (Hou et al., 2023). The level of FFA in 24-LT group were significantly higher than 12-LT group. The upregulation of FFA (16:0) and FFA (18:1) in 24-LT might contribute for the FFA difference. FFA was mainly produced through the hydrolysis of GLs and GPs. It suggested that the level of FFA was related to the content of GLs and GPs. According to the standard of VIP > 1 and *P*-value<0.05, 201 differential lipids were screened out (Table S1), belonging to GL, GP, SP, FFA and PR. Then, the KEGG database were investigated the information and functions of differential lipids. The results indicated that glycerolipid metabolism and glycerophospholipid metabolism were key lipid metabolism pathways. In a previous study, the age was the responsible for the difference of metabolic pathways (Li, Zhang, et al., 2022). Similarly, Chung (2021) reported that the increase or decrease of specific lipid molecules was associated with age and influenced biological processes. Therefore, these results indicated that age had an impact on the glycerolipid metabolism and glycerophospholipid metabolism, resulting in difference of lipid profiles.

In this study, a total of 35 aromas were observed. Overall, the content of aldehydes and alcohols in the 24-LT group was higher than 12-LT group (Fig. 5A). Based on the OAVs of volatile compounds, 18 aromas

with an OAV greater than one was identified in goat meat (Fig. 5B). Among them, 14 aromas were contained in both the 24-LT and 12-LT groups, including hexanal, heptanal, octanal, nonanal, (E)-2-octenal, (E)-2-nonenal, (E)-2-decenal, dodecanal, 2-undecenal, (E,E)-2,4-decadienal, 1-octen-3-ol, 1-heptanol, 2-pentylfuran, octanoic acid, and these aromas have been described as the potential compounds in Chongming white goat meat. Of these aromas were mainly derived from lipid degradation. These results indicated that the volatile compounds form lipid oxidization and degradation were the mainly components of goat meat aroma. We observed that these aldehydes content were higher in 24-LT group. This finding agreed with previous studies (W. Yang, Yang, et al., 2023). And the green and fatty odors in the 24-LT group were stronger than 12-LT group in Fig. 5D, which could be attributed to the high aldehyde content of 24-LT. Due to the high thermal oxidative stability of the unsaturated fatty acid branches of PC and PE, these aldehydes might originate from the oxidation of FFA (Zhou et al., 2014). Tatiyaborworntham et al. (2022a) reported that FFA produced from lipids were the primary precursors of volatile compounds. On the other hand, the lipid in meat only prolongs the release of lipophilic aromas (D.-W. Chen et al., 2019). This result may also be related to the reason that the goat meat is raw meat rather than cooked meat (Benet et al., 2016). Of these odorants, heptanal, octanal and nonanal are saturated fatty aldehydes that generally describe green odor and contribute to the green and fruit odors in cooked meats (Fan et al., 2018). Hexanal was a special n-aldehyde that has the green odor at lower concentrations and the rancid odor at higher concentrations. (E)-2-octenal, (E)-2-decenal, 2undecenal, (E,E)-2,4-decadienal and (E)-2-nonenal are unsaturated fatty aldehydes that were reported to be crucial aromas in cooked goat meat (Qi et al., 2022). 1-octen-3-ol has mushroom odor that are the reason for mushroom-like characteristics in goat meat (Li, Yang, et al., 2022). 2pentylfuran has the musty and floral odors in cooked goat meat. Octanoic acid was described sweaty, vomit and cheese odors. Therefore, the

lipids difference between 12-LT and 24-LT group might be a critical factor that contributed to the differences in their aroma compounds.

The PLS-R result indicated that 77 of flavor precursors could contribute to the aroma differences of goat meat at different ages. We further explored the formation mechanism between these flavor precursors and aromas using correlation networks (Fig. 6C). GPs contributed more to the differences in aroma of goat meat at different age (Fig. 6B). GPs significantly increased the aromas of chicken meat compared to neutral lipids (D.-W. Chen et al., 2019). Compared to GLs, GPs contained more unsaturated fatty acids and were easily oxidized (Y. Chen et al., 2018). We noticed that these lipid molecules contained UFA branch chains, such as oleic acid (18: 1), linoleic acid (18: 2), Linolenic acid (18: 3) and arachidonic acid (20: 4). Oleic acid is a prevalent monounsaturated fatty acid, while octanal, nonanal, and 2-decanal are typically synthesized through the oxidation of oleic acid (Du et al., 2021). Hexanal, (E,E)-2,4-decadienal, and 2-pentylfuran were produced by oxidation of linoleic acid and linolenic acids (Du et al., 2020). 1-Octene-3-ol was mainly oxidized by arachidonic acid, which contributed to the mushroom odor of goat meat (Al-Dalali et al., 2022). Leucine affects the formation of aldehydes and alcohols through decarboxylation and Strecker degradation. Besides, It has been reported that low ratio of UFAs was beneficial to the formation of products from the Maillard reaction in the aroma of meat (Navarro et al., 2021). Therefore, the variation in GP, GL and FAAs might be the cause for the differences on aroma of goat meat of two ages.

Taken together, these results showed that great differences in FAAs and lipid profile between the 12-month and 24-month Chongming white goat. These flavor precursors differences might contribute to the aroma variability. However, further validation was needed to identify the lipid molecules and their pathways that contribute to aroma differences.

5. Conclusions

In this study, change in FAAs, lipids and aromas revealed the effect of age on flavor of goat meat. The content of TAA, FFA and GL significantly increased with the age of Chongming white goat. TG and DG was the main class of lipid deposition in 24 months Chongming white goat meat. Furthermore, The pathway analysis indicated that glycerolipid metabolism and glycerophospholipid metabolism were the key pathways in lipids difference between 12-LT and 24-LT. A total of 18 aromas with OAV > 1 were selected from 35 volatile compounds that identified by GC-MS. Among them, 14 aromas were both in the 12-LT and 24-LT groups, which could be considered potential key aromas in Chongming white goat meat. The correlation analysis indicated that the differences in lipid-derived aroma of goat meat at different ages might be related to FAAs difference and the distribution of unsaturated fatty acids in GP and GL. This study offers scientific basis for comprehensive understanding of the flavor precursors and aroma of Chongming white goat meat at different ages.

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CRediT authorship contribution statement

Lei Chen: Writing – review & editing, Methodology, Conceptualization. Miaoqiang Zhang: Writing – original draft, Methodology, Formal analysis. Tao Feng: Writing – review & editing. Haiyan Liu: Investigation. Yuexia Lin: Investigation. Bing Bai: Writing – review & editing, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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