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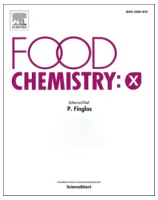

Comprehensive analysis of key aroma compounds enhanced by *Tamarix ramosissima* Ledeb in mutton roasted by air-frying roast technology by means of SAFE-GC-O-MS and lipidomics

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A R T I C L E I N F O

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TG 16:0_18:1_18:1

A B S T R A C T

Little information is known about the increased aroma compounds and possible mechanism in *Tamarix ramosissima* Ledeb roasted mutton (TRM). A comprehensive analysis of aroma compounds and lipids were firstly performed by lipidomics and sensomics approach. The results indicated that 9 out of 53 aroma compounds were considered as key odorants, including 5-methyl-2,3-diethylpyrazine. The roasted mutton contained highest levels of phosphatidylcholine (PC, 13.95%), triglyceride (TG, 13.50%), and phosphatidylethanolamine (PE, 12.25%). TG 18:0_18:0_18:1 and nine odorants were the potential biomarkers for discriminating differential samples due to variable importance in projection (VIP) > 1 and $p < 0.05$. PCs and TGs, including PC 21:0_13:1 and TG 16:0_18:1_18:1, might be predominantly responsible for the formation and retention of aroma compounds, respectively. This will clarify the enhanced effect of *Tamarix ramosissima* Ledeb on the presence of aroma compounds via lipid pathways in roasted mutton.

1. Introduction

Roasted mutton is the most attractive meat products worldwide due to rich aroma compounds (Liu, Hui, Fang, et al., 2022). Our previous study has clarified that eight odorants are considered as the key aroma compounds based on aroma recombination experiments, among which the roasted postrigor *back strap* presented higher concentrations of key odorants in comparison with the prerigor sample (Liu, Hui, et al., 2021). The roasted mutton from electrically heated air presents the most similar aroma profile with that from traditional burning charcoal, indicating the electrically heated air method is a potential technology replacing the traditional method (Liu, Ma, et al., 2021). The air-frying roast technique has the advantages of electrically heated air and hot air circulation, which causes high concentrations of (non)volatile compounds in roasted mutton (Liu, Li, Zhang, et al., 2023). The up-regulated hexanal, heptanol, and 1-octen-3-ol may be attributed to the differential lipids, such as phospholipids, sphingolipids, and glycerolipids in the postmortem lamb and castrated sheep (Li et al., 2020; Xu et al., 2023). However, most roasted mutton shashlik is skewered with *Tamarix ramosissima* Ledeb, especially in the northern region of China, which gives the mutton rich aroma. The enhancement effect of *Tamarix ramosissima* Ledeb on aroma compounds in roasted mutton has not been confirmed. Little study has been reported to clarify the key lipids responsible for the presence of aroma compounds in *Tamarix ramosissima* Ledeb roasted mutton (TRM) from the perspective of lipidomic.

Several technologies have been performed to discriminate the different meat and meat products, including the polymerase chain reaction (Al-Kahtani et al., 2017), spectroscopy methodologies (Prieto et al., 2015), inductively coupled plasma (Park et al., 2018), gas chromatography–mass spectrometry (GC–MS) (Liu, Hui, Fang, et al., 2022), and high performance liquid chromatography-mass spectrometry (UPLC-MS) technologies (Mi et al., 2019). Recently, the combination of GC–MS and UPLC-MS has been regarded as a useful method to distinguish the meat products with different storage stages (Xu et al., 2023).

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The hexanal (3.54) and 1-octen-3-ol (1.98) with high variable importance in projection (VIP) scores from OPLS-DA are potential biomarkers for identifying the mutton at varying roasting times (Liu, Li, Zhang, et al., 2023). But until now, there is little study about the identification of TRM and control group.

The Maillard reaction, lipid pyrolysis, and their synergistic effect may predominantly contribute to the formation of aroma compounds, such as 2,5-dimethylpyrazine and hexanal (Liu et al., 2020). The aroma compounds, including aldehydes, ketones, and alcohols, can be generated from the scission products of lipids, including the hydroperoxide isomers, free fatty acids, and radical (Ho & Hartman, 1994a). The heating parameters and exogenous additives are variables which regulate the thermal oxidation of lipids (Liu et al., 2020). Meanwhile, several studies with model systems have identified a large number of aroma compounds, which originates from the interactions of lipid and other reactions. For instance, the long-chain alkyl-substituted pyrazines are formed in the reaction of carbonyl compounds from lipid oxidation and acetol from Maillard reaction (Ho & Hartman, 1994b). The lipid molecules can be classed into the phospholipids, sphingolipids, glycoglycerolipids, neutral lipids, fatty acyl and others according to their various structures (Fahy et al., 2005). The lipidomic is an effective tool to elucidate the molecular structure of lipids and factors interacting with lipids by using advanced detection technologies and multivariate statistical strategies (Pellegrino et al., 2022). Lipidomics can not only monitor the quality changes and the differential lipids during the cooking process but also elucidate the key lipids involved in the generation of aroma compounds (Wang et al., 2024). Our latest research indicates that phosphatidylcholine and triglyceride are responsible for the generation and retention of aroma compounds, respectively, among which the key lipids contained PC 66:17, PC 37:3, and TG (16:0_18:1_18:1) in roasted chicken and roasted pork (Liu, Liu, Suleman, et al., 2023; Liu, Ma, et al., 2024). Therefore, it's viable that the lipid profile changes and aroma formation in TRM are revealed by lipidomics.

To date, there has been no deep study of TRM to reveal the formation mechanism of differential aroma compounds. Therefore, this study aimed to: (i) discriminate the TRM and control group, and screen the potential biomarkers of aroma compounds and lipids, (ii) clarify the enhanced effect of *Tamarix ramosissima* Ledeb on the aroma profile and key odorants in roasted mutton, (iii) illustrate the key lipids regulating by *Tamarix ramosissima* Ledeb for binding and generating key aroma compounds. This will provide insights into the enhanced mechanism of odorants in roasted mutton by *Tamarix ramosissima* Ledeb, which will guide the industrial production of roasted mutton and the development of new products.

2. Materials and methods

2.1. Samples collection and grouping

A total of six tan sheep aged of six months were obtained from a large farmer in Ningxia Province, China. Each group contained 3 sheep. The slaughter procedure of sheep met the principles and guidelines established by the Animal Care and Use Committee of Ludong University (LDU-IRB202402006). Briefly, the tan sheep were slaughtered using a captive bolt. Immediately, the jugular veins and carotid arteries of sheep were cut off for the exsanguination, causing rapid heart failure and death. The left *back strap* was cut in accordance with the cutting technical specification of mutton and Handbook of Australian Meat (Anonymous, 2005; Zhang et al., 2007). After 72 h of maturation at 4 °C (pH: 5.52 ± 0.12), the samples were transported to our lab at −35 °C. Before use, the *back strap* was thawed to a central temperature of −4 °C at 4 °C incubator. The *back strap* was cut into a cube (3 × 1.5 × 1.5 cm^3). The cubes of the first three tan sheep were skewered with *Tamarix ramosissima* Ledeb and the remaining ones were not punctured as a control. The cubes were roasted at 240 °C for 15 min by using a CKY-298 based on the sensory evaluation. The cubes of each sheep were roasted individually. The core and surface temperature of cubes reached at 79–83 °C and 88–95 °C, respectively.

The following authentic flavor standards were obtained from Sigma-Aldrich (Shanghai, China): nonanal (99.5), benzaldehyde (99.5%), benzeneacetaldehyde (95%), 1-octen-3-ol (98%), 2-methylpyrazine (99%), trimethylpyrazine (99%), 5-methyl-2,3-diethylpyrazine (99%), methional (97%), and 2,3-butanediol (98%). The standards were obtained from TCI (Shanghai, China): hexanal (98%), 2-ethyl-3,5-dimethylpyrazine (98%), propanoic acid ethyl ester (99%), and butanoic acid (99%). The 2-ethyl-6-methylpyrazine (95%), 2-ethyl-5-methylpyrazine (99%), and 5-ethyl-2,3-dimethylpyrazine (97%) were purchased from CATO (Beijing, China). The n-alkanes (C_7-C_{40}, 97%) and 2-methyl-3-heptanone (99%) were obtained from o2si Smart Solutions (Shanghai, China) and Dr. Ehrenstorfer (Beijing, China), respectively.

2.2. Sensory evaluation

We obtained the informed consent of panelists about the sensory evaluation of roasted mutton, among which the consent to participate was mandatory in all human sensory studies. The appropriate protocols for protecting the rights and privacy of all panelists were applied from the institutional review board (IRB) of Ludong University (LDU-IRB202305001) during the execution of the research. The panelists were trained according to ISO 4121:2003 and GB/T 29604–2013. The sensory evaluation of roasted mutton was conducted based on our previous study (Liu, Hui, Fang, et al., 2022). After the discussion, nine panelists selected five aroma attributes of roasted mutton, including roasty, meaty, sweet, fatty, and grassy odors. Briefly, a total of 2 sessions were used for this experiment, namely, TRM group and the control group. Eighteen blocks from two groups of sheep were presented to the panelists, among which each group of sheep had nine roasted mutton blocks. The blocks were coded with a 3-digit number and their scores were recorded on a five-point linear scale.

2.3. Aroma compound analysis

2.3.1. Solvent-assisted flavor evaporation (SAFE) extraction

For each 50 g of roasted mutton, 50 mL dichloromethane and 25 μL 2-methyl-3-heptanone (2 μg/μL) were added. Volatiles were extracted for 3 h and repeated for 3 times. The distillation using SAFE was conducted at the pressure of 10^{-4} Pa. After the distillation, the obtained extract was concentrated by using a Vigreux column (50 cm × 1 cm). Finally, the resulting extracts were further concentrated to 200 μL (Liu, Li, Hamid, et al., 2023).

2.3.2. GC-O-MS analysis

The Thermo Scientific™ TRACE™ 1310 gas chromatography was equipped with an olfactory port (OP275 Pro II, GL Sciences Inc., Japan) and TSQ 9000 mass spectrometer. The experiment was performed at helium constant flow (1.50 mL/min, purity of 99.99%) and oven temperature of 40 °C (3 min), which was then increased to 70 °C at 2 °C/min, ramped to 130 °C at 3 °C/min, ramped to 230 °C at 10 °C/min, and kept for 10 min. A polar DB-Wax column (30 m × 0.25 mm i.d., 0.25 μm film thickness) were applied to separate the aroma compounds. The mass spectra of roasted mutton were acquired at 70 eV by electron impact mode (EI) ranging from 40 to 500 *m/z*.

2.3.3. Identification and quantitation analysis

The obtained peaks were matched with the NIST 2.0 mass-spectrometry database. The results further compared with their linear retention indices (LRI) based on date obtained by GC–MS and those retrieved from a reference database. Meanwhile, the odor attributes and intensities of aroma compounds from the GC-O analysis were recorded. Finally, the aroma compounds were further identified by comparing the peak time of each odorant in samples and flavor standards. For the quantitation analysis, the aroma compounds were initially

semiquantitated based on the peak area and concentration of 2-methyl-3-heptanone. Sixteen odorants (OAVs >1) were quantitated using calibration curves (Liu, Li, Hamid, et al., 2023).

2.4. Confirmation of key aroma compounds

The odor activity values (OAVs) were obtained as ratios of the concentration to their medium thresholds (Schieberle, 1995). The odorant with OAVs higher than 1 might predominantly contributed to the aroma expression of roasted mutton. Meanwhile, the key aroma compounds of roasted mutton were confirmed based on the aroma recombination and omission experiments. The odorless matrix of roasted mutton was made by using a mixture of diethyl ether and pentane (w: w = 2: 1) according to the previous study (Bressanello et al., 2018; Warner et al., 2023). The roasted mutton was still deodorized by the above-mentioned solvents until no odorants were detected. The recombination model 1 comprised 16 flavor standards (OAVs >1) and odorless matrix. The recombination model 1 removed one flavor compound to construct a series of models 2. The recombination model 3 was composed of aroma compounds that caused the odor changes in recombination models 2.

2.5. Lipidomic analysis

2.5.1. Lipid extraction

Briefly, a mixture of 100 mg roasted mutton and 750 μL $CHCl_3$: CH_3OH (v: v = 2: 1) were ground for 60 s at 60 Hz. The mixture was mixed with double distilled water (ddH_2O), vortexed for 30 s, and centrifuged at 10000 ×*g* for 5 min. The 300 μL subnatant were operated repeatedly complying with the above procedures. After the concentration, the sample was mixed with 200 μL isopropanol and filtered using a 0.22 μm membrane (Liu, Liu, Suleman, et al., 2023).

2.5.2. Ultra-high performance liquid chromatography high-resolution mass spectrometry (UHPLC-HRMS) analysis

The Vanquish Flex UHPLC system equipped with a Orbitrap Exploris 240 HRMS (Thermo Fisher Scientific) was applied to detect the lipid molecules. The ACQUITY UPLC HSS T_3 column was applied to separate the lipids. The gradient elution program and instrument parameters were the same as those in our previous study (Liu, Ma, et al., 2024). The Compound Discoverer software was used to process raw data. The chemical formula of each lipid molecule was identified by a Xcalibur software. The deviation of the lipid mass to charge ratio detected by the instrument was <5 ppm. The lipid molecules were determined by comparing their fragments with those in the database. The identification algorithm mzLogic was further applied to verify the results to increase the credibility.

2.6. Statistical analysis

The data of roasted mutton were analyzed by independent samples *t*-test in SPSS 19.0 software (IBM Corporation, USA). The use of *Tamarix ramosissima* Ledeb was the fixed effect, and the sheep and *backstrap* muscles were random effects. For the sensory evaluation, the fixed term for full models was the use of *Tamarix ramosissima* Ledeb. The experimental design factors of carcase, mutton cubes, panelist, and session were regarded as random effects. The data were presented as means and standard errors of 3 measurements. The significant differences ($p < 0.05$) between samples were applied in this study. The orthogonal partial least squares-discriminant analysis (OPLS-DA) was utilized to discriminate the roasted mutton skewered with/without *Tamarix ramosissima* Ledeb. The potential biomarkers were determined by using variable importance in projection (VIP) > 1 and $p < 0.05$.

3. Results and discussion

3.1. Identification and quantitation analysis of aroma compounds in TRM and control group roasted by air-frying roast technology

To compare the difference of aroma compounds responsible for the aroma profile, the dichloromethane was applied to extract the volatiles by using the SAFE technology. To preliminarily determine the similarity in aroma between the extract and the sample, a small the distillate was dropped on a paper, indicating a great effect. As showed in Fig. 1, the TRM and control group had strong roasty, meaty, sweet, fatty, and grassy aromas. As presented in Table 1 and supplemental information (Table 1 and Table 2), a total of 53 and 52 odorants were identified in the TRM and the control group, among which the TRM contained 16 nitrogen-containing compounds, 10 acids, 6 furans, 5 aldehydes, 5 ketones, 5 alcohols, 3 easters, 2 phenols, and 1 sulfur-containing compound. The benzyl alcohol was not detected in the control group. This result is in accordance with the previous study, in which a large amount of pyrazines, aldehydes, alcohols, and acids are generated in the mutton during roasting process (Liu, Hui, Fang, et al., 2022). No pyrazine is detected in the fresh, marinated, and Nang roasted oyster cuts, whereas the aldehydes and alcohols are the predominant odorants, including hexanal and 1-octen-3-ol (Xu et al., 2021). Several aroma compounds were firstly observed in the roasted mutton that contained 5-ethyl-2,3-dimethylpyrazine, 5-methyl-2,3-diethylpyrazine, and (*E*)-2-methyl-5-(1-propenyl)pyrazine.

Meanwhile, the 16 aroma compounds were accurately quantitated based on the standard calibration curves, clarified that these odorants exhibit a good linear fit ($R^2 > 0.99$). Notably, the compound 2,3-butanediol showed the highest concentration by far, with a value of 6056.08–6969.88 ng/g, followed by 1-hydroxy-2-propanone (2782.00–3585.29 ng/g). In contrast, the pyrazines had a lower concentration, ranging from 15.75 to 556.42 ng/g. Obviously, the most aroma compounds in the TRM were pronouncedly higher ($p > 0.05$) those in the control group, except for 13 odorants, such as propanoic acid ethyl ester, hexanal, 1-octen-3-ol, 2,3-butanediol, acetic acid, butanoic acid, pentanoic acid, hexanoic acid, nonanoic acid, tetradecanoic acid, 1-(2-furanyl) ethenone, 1-(1H-pyrrol-2-yl)ethenone, and 5-acetyldihydro-2(3H)-furanone. This result is accordance with the previous results from Kosowska, who determine a prevalence of roasty aroma profile and low concentrations of pyrazines in cooked loin and duck (Kosowska et al., 2018; Liu, Li, Hamid, et al., 2023). This confirmed a fact that *Tamarix ramosissima* Ledeb could enhance the concentrations of most odorants.

3.2. Higher concentrations of pyrazines and aldehydes predominantly caused better aroma profile of TRM than control group

As illustrated in Fig. 2, 16 odorants were regarded as important aroma compounds by OAVs and GC-O. Particularly, the 5-methyl-2,3-diethylpyrazine presented the highest OAVs (930.73–1819.42), followed by methional (1140.89–1705.09). The 5-ethyl-2,3-dimethylpyrazine (291.13–517.54), nonanal (84.03–118.82), benzaldehyde (78.85–120.43), and benzeneacetaldehyde (55.04–107.03) also had high OAVs. Nine aroma compounds were confirmed as key odorants by aroma recombination experiment, including 5-ethyl-2,3-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, 5-methyl-2,3-diethylpyrazine, hexanal, nonanal, benzeneacetaldehyde, 1-octen-3-ol, methional, and 2,3-butanediol. The similarity score of recombination model 3 was evaluated to be 4.6 out of 5 (Fig. 1).

This is in accordance with the previous result, in which the pyrazines, including 2-ethyl-3,5-dimethylpyrazine, 5-ethyl-2,3-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine, are confirmed as key roasty odorants in the roasted goose and smoked duck by using GC-O and flavor dilution factor analysis (Gasior et al., 2021; Liu, Li, Hamid, et al., 2023). The most aldehydes and alcohols mainly originate from the

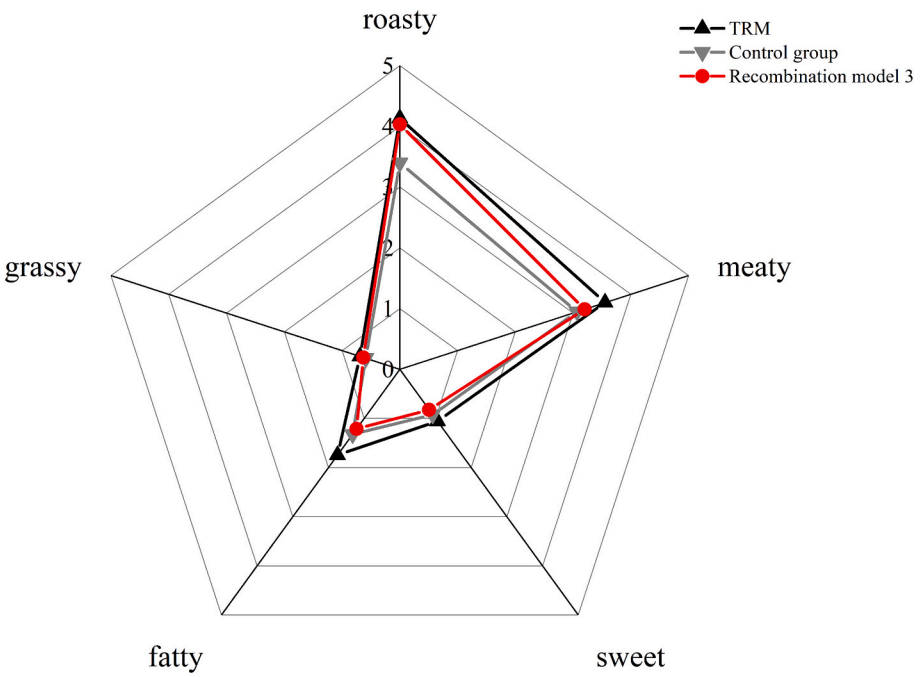


Fig. 1. Aroma profiles in TRM, control group, and recombination model.
TRM represented roasted mutton skewered with *Tamarix ramosissima* Ledeb. The control group represented roasted mutton without *Tamarix ramosissima* Ledeb.

Table 1
Identification analysis of aroma compounds in TRM and control group.

Compounds [a]	LRIs		Identification [d]	Compounds	LRIs		Identification
	Literature [b]	Calculated [c]			Literature	Calculated	
acetic acid methyl ester	864	860	MS, LRI	1-octanol	1546	1539	MS, LRI, S
propanoic acid ethyl ester	946	947	MS, LRI, O, S	dihydro-3-methyl-2(3H)-furanone	1555	1554	MS, LRI
hexanal	1078	1078	MS, LRI, O, S	2,3-butanediol	1556	1558	MS, LRI, S
dihydro-2-methyl-3(2H)-furanone	1242	1241	MS, LRI	butyrolactone	1595	1590	MS, LRI
2-methylpyrazine	1247	1244	MS, LRI, O, S	butanoic acid	1600	1602	MS, LRI, O, S
1-hydroxy-2-propanone	1277	1275	MS, LRI	benzeneacetaldehyde	1619	1617	MS, LRI, O, S
2,5-dimethylpyrazine	1302	1299	MS, LRI, S	2-furanmethanol	1635	1636	MS, LRI
ethylpyrazine	1311	1310	MS, LRI	1-methyl-2-pyrrolidinone	1652	1649	MS, LRI
2,3-dimethylpyrazine	1326	1323	MS, LRI	(*Z*)-3,5-dimethyl-2-(1-propenyl)pyrazine	1629	1649	MS, LRI
2-ethyl-6-methylpyrazine	1363	1363	MS, LRI, O, S	(*E*)-2-methyl-5-(1-propenyl)pyrazine	1635	1687	MS, LRI
2-ethyl-5-methylpyrazine	1376	1368	MS, LRI, O, S	pentanoic acid	1713	1712	MS, LRI
nonanal	1374	1378	MS, LRI, O, S	2(5H)-furanone	1716	1717	MS, LRI
trimethylpyrazine	1381	1381	MS, LRI, O, S	hexanoic acid	1810	1818	MS, LRI
5-ethyl-2,3-dimethylpyrazine	1416	1422	MS, LRI, O, S	benzyl alcohol	1857	1849	MS, LRI
acetic acid	1429	1427	MS, LRI	α-ethylidene-benzeneacetaldehyde	1907	1898	MS, LRI
1-octen-3-ol	1430	1432	MS, LRI, O, S	heptanoic acid	1923	1924	MS, LRI
2-furaldehyde	1437	1435	MS, LRI	1-(1H-pyrrol-2-yl)ethanone	1949	1942	MS, LRI
2-ethyl-3,5-dimethylpyrazine	1438	1438	MS, LRI, O, S	phenol	1978	1977	MS, LRI
methional	1450	1441	MS, LRI, O, S	2-pyrrolidinone	2020	2014	MS, LRI
tetramethylpyrazine	1457	1453	MS, LRI	octanoic acid	2030	2031	MS, LRI
2-ethenyl-6-methylpyrazine	1463	1462	MS, LRI	5-acetyldihydro-2(3H)-furanone	2026	2032	MS, LRI
5-methyl-2,3-diethylpyrazine	1474	1471	MS, LRI, O, S	p-cresol	2050	2055	MS, LRI
2-ethyl-1-hexanol	1480	1472	MS, LRI	nonanoic acid	2144	2138	MS, LRI
1-(2-furanyl) ethanone	1483	1475	MS, LRI	decanoic acid	2246	2244	MS, LRI
pyrrole	1490	1487	MS, LRI	nimethyl phthalate	2276	2278	MS, LRI
benzaldehyde	1495	1489	MS, LRI, O, S	tetradecanoic acid	1546	1539	MS, LRI
propanoic acid	1523	1513	MS, LRI				

MS, mass spectrometry; LRI, linear retention indices; O, odor attributes; S, authentic flavor standards.
[a] The aroma compounds in TRM.
[b] Data in literatures.
[c] Data determined according to the retention time of n-alkanes.
[d] Identification analysis.

lipid oxidation, such as hexanal, nonanal, and 1-octen-3-ol (Resconi et al., 2010). In detail, the breakdown of linoleic acid and α-linolenic acid may predominantly contribute to the generation of lipid-derived carbonyl odorants, including hexanal and benzaldehyde (Elmore et al., 2005). The interactions among lipids, proteins and carbohydrates contribute to the formation of pyrazines, among which the methylpyrazines are determined as important aroma compounds in the glycerine and triglyceride reaction (Ho & Hartman, 1994b). The presence of long-

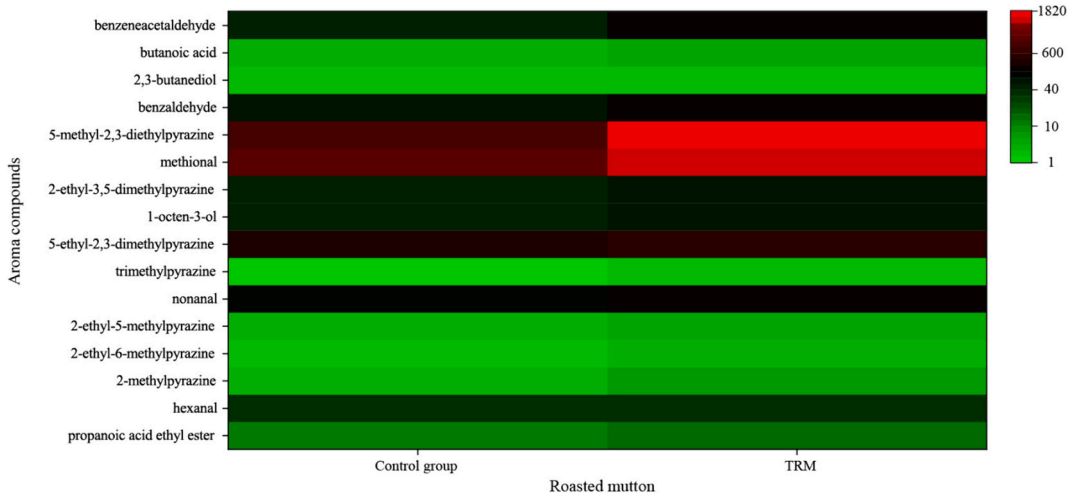


Fig. 2. OAVs of aroma compounds (OAVs >1) in TRM and control group.
TRM represented roasted mutton skewered with *Tamarix ramosissima* Ledeb. The control group represented roasted mutton without *Tamarix ramosissima* Ledeb.

chain alkyl-substituted pyrazines may be attributed to the lipid-Maillard reaction (Ho & Hartman, 1994b). This revealed that *Tamarix ramosissima* Ledeb promoted the generation of aroma compounds related to lipids.

3.3. TG 18:0_18:0_18:1 and nine aroma compounds might be potential biomarkers for distinguishing the TRM and control group

As exhibited in Fig. 3, the lipid molecules in TRM and control group were identified by UHPLC-HRMS, among which 3516 and 2751 lipids

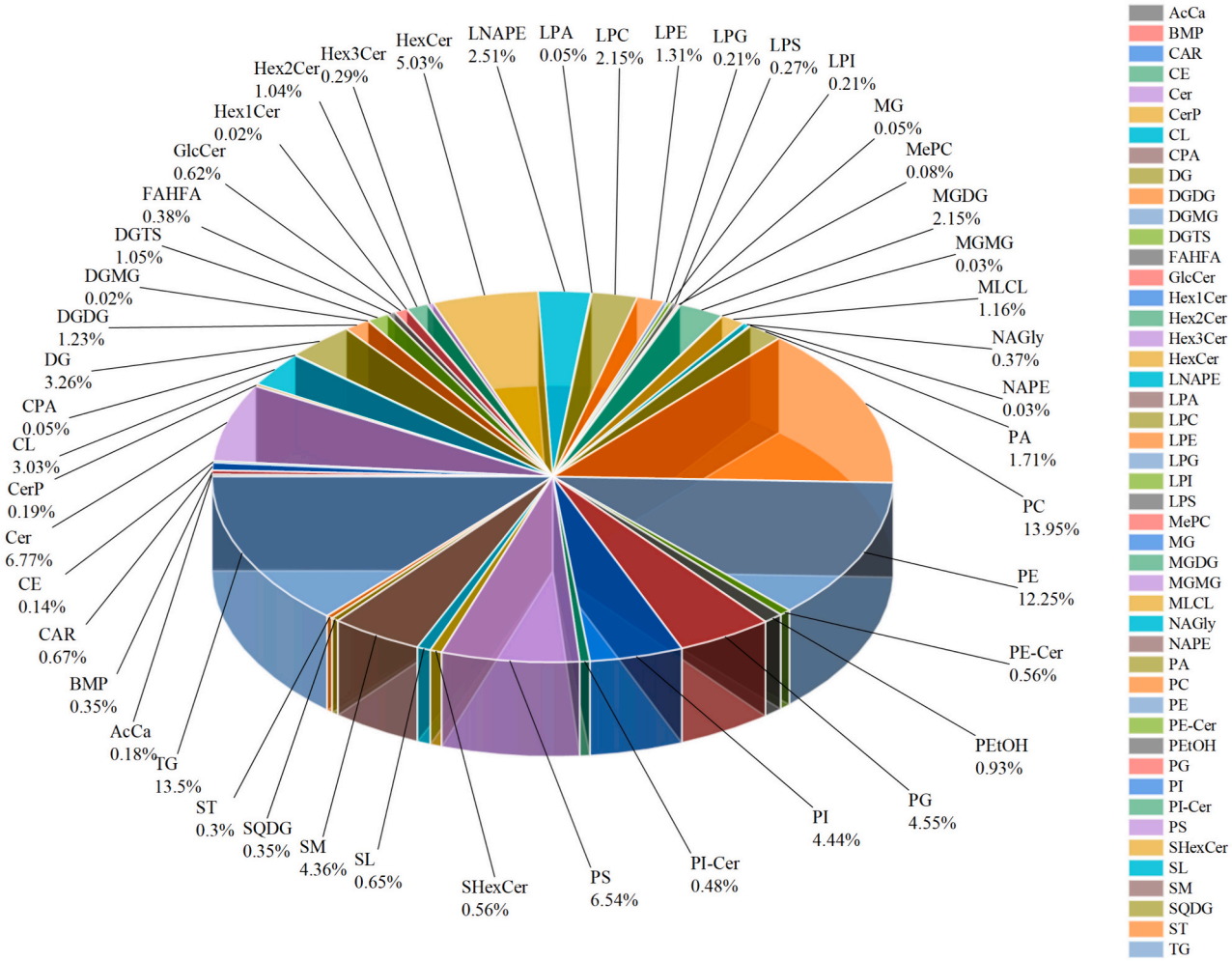
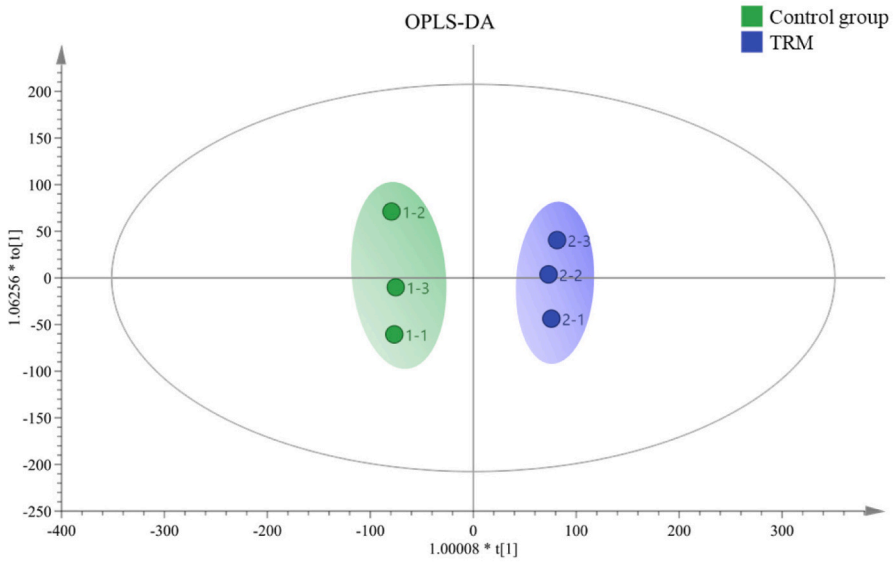


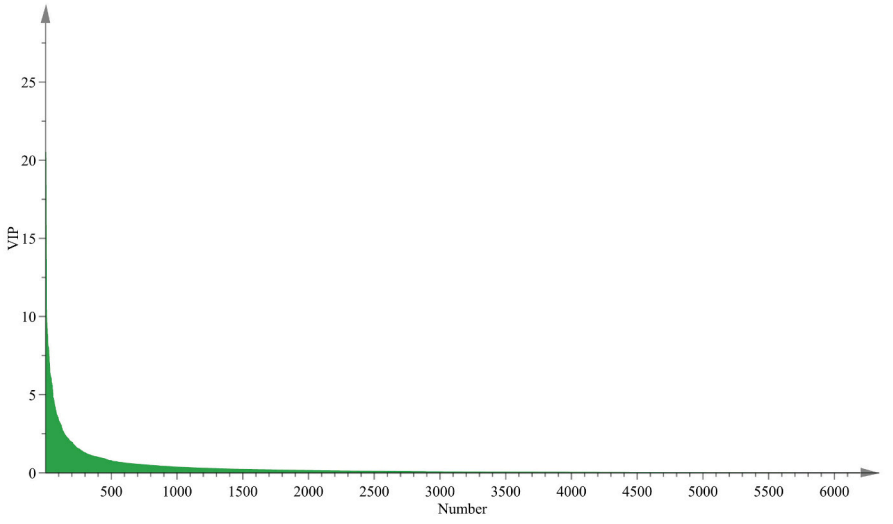
Fig. 3. Lipid class composition of TRM and control group.

were detected in the positive and negative ion mode, respectively. A total of 6267 lipid species were identified, belonging to 47 lipids subclasses, which contained 874 species of phosphatidylcholine (PC), 846 species of triglyceride (TG), 768 species of phosphatidylethanolamine (PE), 424 species of ceramides (Cer), 410 species of phosphatidylserine (PS), and 315 species of simple glcseries (HexCer). This result is accordance with the previous studies, in which PC, PE, and TG present the highest percentages in all lipids in lamb and roasted mutton (Jia et al., 2021; Liu, Hui, Zheng, et al., 2022). To gain the potential biomarkers of lipid and aroma compounds for discriminating the TRM and control group, OPLS-DA model was applied. The R^2 and Q^2 were utilized to evaluate the model reliability. The score plot of the OPLS-DA mode indicated a great interpretation rate ($R^2X = 0.80$, $R^2Y = 1$) and good predictability ($Q^2 = 0.99$), elucidating the different samples were well discriminated (Fig. 4). The TRM samples had positive scores along component one (t_1), Which was differentiated from the control group with negative scores.

The VIP value of OPLS-DA indicated the molecule importance, which might serve as an important parameter for screening the biomarkers. As presented in Fig. 4, a total of 408 biomarkers of lipids and aroma compounds were observed, among which the total contribution of 121 TGs, 85 PCs, and 31 PEs reached >50%. To further accurately screen biomarkers, the variables responsible for the classification were determined based on and highest VIP values and $p < 0.05$ (Liu, Ma, et al., 2024). One TG lipid, namely TG 18:0_18:0_18:1 (VIP = 9.93), could significantly the samples, among which its peak area of TRM was pronouncedly higher ($p < 0.05$) than the control group (Fig. 5). Meanwhile, nine odorants were also the important aroma biomarkers that could distinguish the differential samples, namely nimethyl phthalate (VIP = 20.53), 1-hydroxy-2-propanone (18.42), 2-pyrrolidinone (15.86),



(a)



(b)

Fig. 4. Fig. 4. OPLS-DA of lipids and aroma compounds in TRM and control group.
(a) OPLS-DA. (b) VIP. TRM represented roasted mutton skewered with *Tamarix ramosissima* Ledeb. The control group represented roasted mutton without *Tamarix ramosissima* Ledeb.

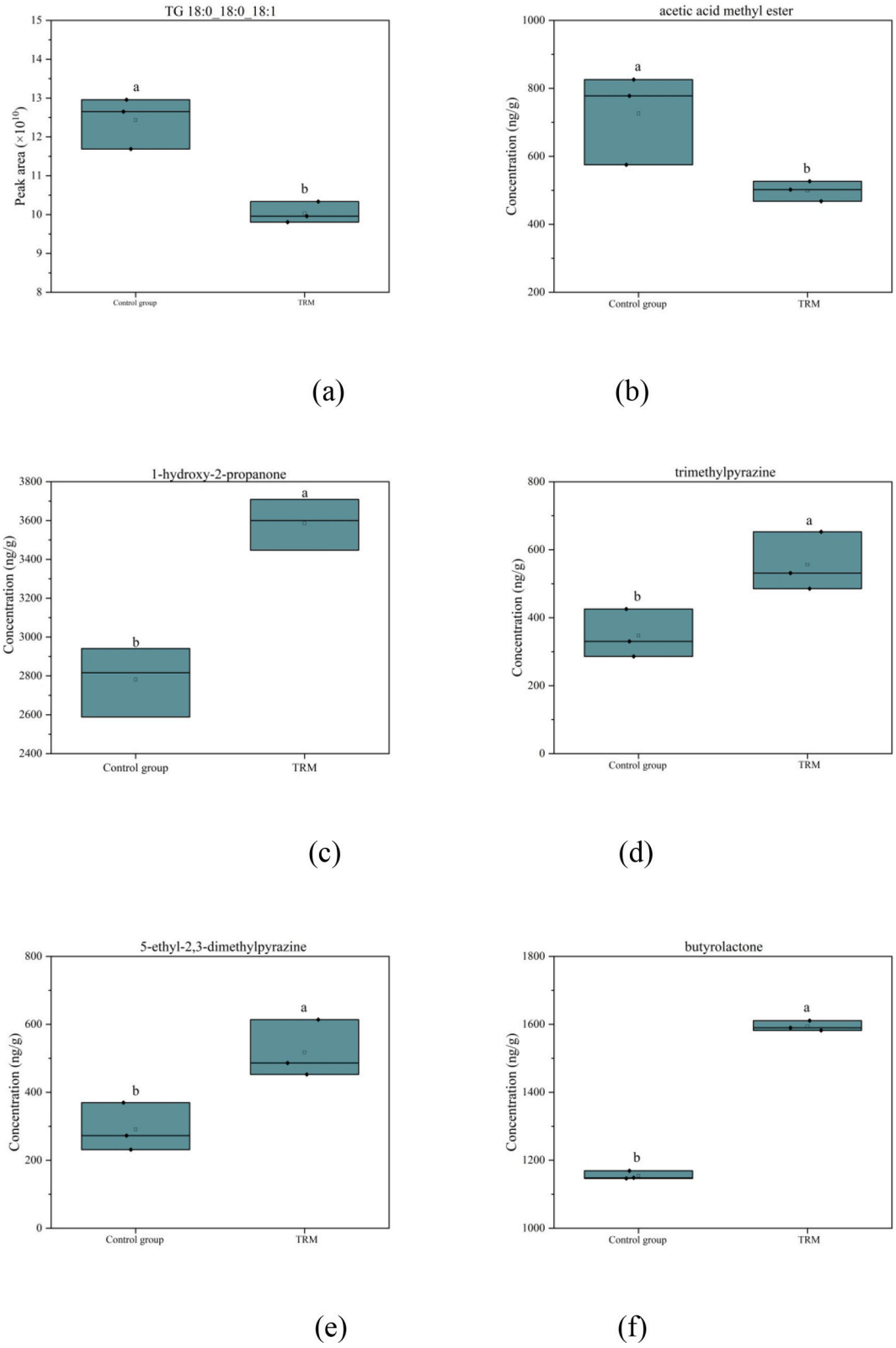


Fig. 5. Box plots of potential aroma compound and lipid biomarkers for distinguishing TRM and control group.
(a) TG 18:0_18:0_18:1. (b) acetic acid methyl ester. (c) 1-hydroxy-2-propanone. (d) trimethylpyrazine. (e) 5-ethyl-2,3-dimethylpyrazine. (f) butyrolactone. (g) benzeneacetaldehyde. (h) 2(5H)-furanone. (i) 2-pyrrolidinone. (j) nimethyl phthalate. TRM represented roasted mutton skewered with *Tamarix ramosissima* Ledeb. The control group represented roasted mutton without *Tamarix ramosissima* Ledeb.

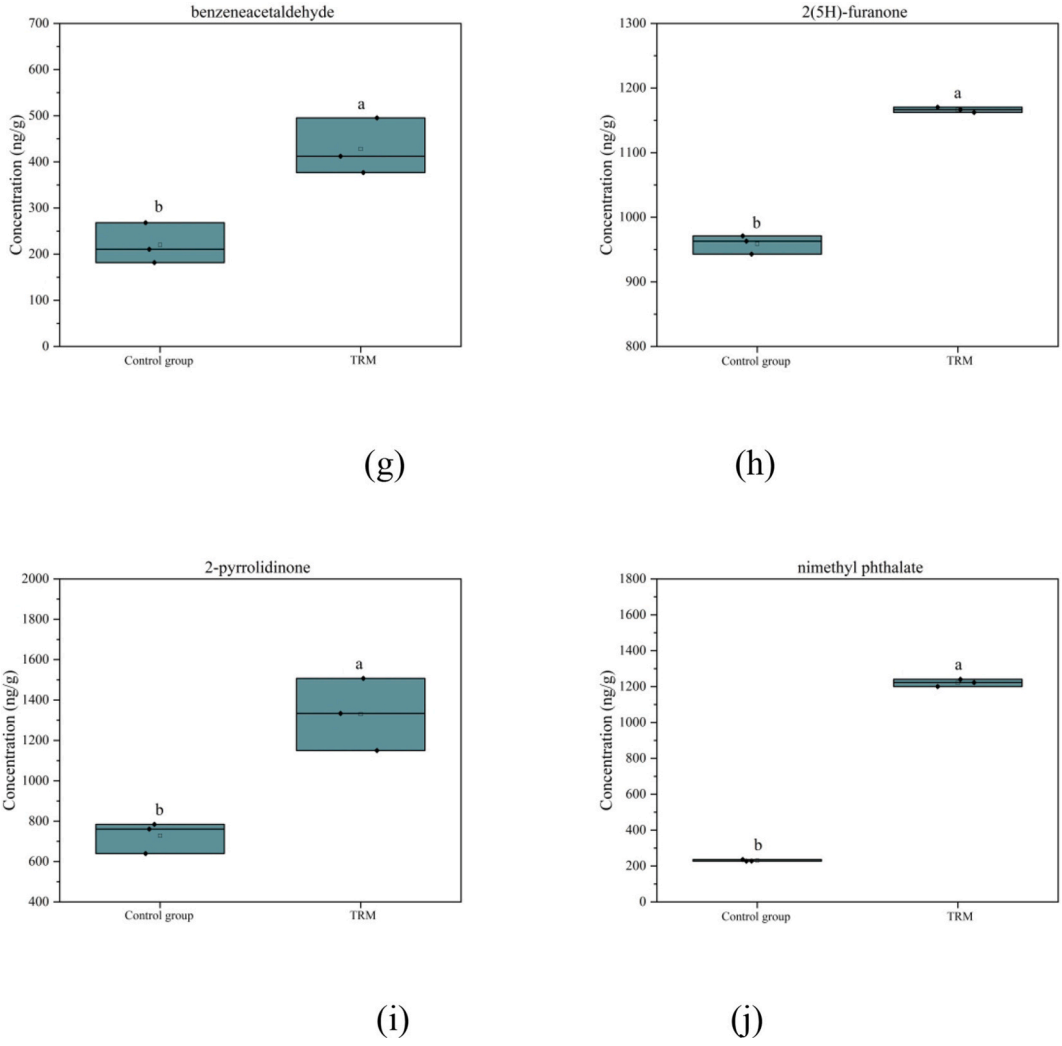


Fig. 5. (*continued*).

butyrolactone (13.68), 5-ethyl-2,3-dimethylpyrazine (9.62), acetic acid methyl ester (9.57), 2(5H)-furanone (9.39), benzeneacetaldehyde (9.27), and trimethylpyrazine (9.23). The concentrations of aroma biomarkers in TRM were all dramatically higher ($p < 0.05$) than those in the control group, except for acetic acid methyl ester. Only trimethylpyrazine, 5-ethyl-2,3-dimethylpyrazine, and benzeneacetaldehyde presented OAVs higher than 1, which might be key aroma compounds in roasted mutton. However, other six aroma compounds presented low OAVs due to high thresholds. The phenomenon indicated that there was a significant difference in lipid molecules and aroma compounds between TRM and control group, which verified that TRM presented a more intense aroma. This result contradicts our study, in which PC (30:6), and PC (28:3) rather than TGs were considered as biomarkers that distinguish the roasted mutton with various heating times (Liu, Hui, Zheng, et al., 2022). TG 20:5_10:3_22:5 is a potential lipid biomarker discriminating Tan sheep and Bahan crossbreed sheep (Liu, Zhang, et al., 2024). Our previous study also clarifies a similar fact that pyrazines and aldehydes are the important biomarkers, which can distinguish the roasted pork from four roasting methods and roasted mutton at different roasting stages, including trimethylpyrazine, 2,3-dimethylpyrazine, and hexanal (Liu, Li, Zhang, et al., 2023; Liu, Ma, et al., 2024).

3.4. Differential TGs and PCs might result in higher concentrations of key aroma compounds in TRM

As illustrated in Fig. 3 and Table 3 (supplemental information), TG, PC, and PE were found to be the major lipids in roasted mutton, representing 13.50%, 13.95, and 12.25%, respectively. Among the 100 lipids with the highest concentration, TGs (51) accounted for more than half. It was worth noting that TG 16:0_18:1_18:1 had the highest peak areas of (185.79–191.37) $\times$ 10^9, followed by TG (17:0/17:2(9Z,12Z)/18:0) (185.51–191.19) $\times$ 10^9, PC 21:0_13:1 (126.04–152.55) $\times$ 10^9, TG 16:0_16:0_18:0 (139.34–150.10) $\times$ 10^9, and TG (17:0/17:1(9Z)/18:0) (136.01–140.09) $\times$ 10^9. TG 18:0_18:0_18:1 (100.33–124.32) $\times$ 10^9, TG 18:0_18:1_18:1 (95.86–107.22) $\times$ 10^9, and TG 16:0_18:0_18:0 (97.26–111.20) $\times$ 10^9 also had high peak areas. Among the above lipid molecules, the peak areas of PC 21:0_13:1, TG 18:0_18:0_18:1, and TG 16:0_18:0_18:0 in TRM sample were pronouncedly lower than those in the control group, whereas peak areas of other lipid molecules presented no significant difference. These TGs had acyl carbon numbers ranging from 22 to 60, revealing that long-chain lipids predominantly contributed to the lipid molecules in meat (Mi et al., 2019). This result also illustrates that TGs, especially TG 16:0_18:1_18:1, may play an important role in the presence of aroma compounds (Liu, Liu, Suleman, et al., 2023). The lipid is regarded as the best retainability of aroma compound because of the lipophilic nature of odorants (Sireswar et al., 2021). The higher concentrations of TGs account for the retention of aroma

compounds due to the increased partition coefficients of aroma components (Ammari & Schroen, 2018; Ma et al., 2023). Therefore, TGs, especially TG 16:0_18:1_18:1 and TG (17:0/17:2(9Z,12Z)/18:0) are the important lipids, which bound the aroma compounds. The control group had higher concentrations of lipid molecules (TGs) and lower aroma compounds, revealing lipids were more involved in the generation of aroma compounds in comparison with the retention effect.

Compared to the neutral lipids, the phospholipids are considered as the key lipids in the generation of aroma compounds (Liu, Ma, et al., 2024). A total of 40 phospholipids, containing PC (28), PE (5), LPC (6), and LPE (1), were observed in the 100 lipids with highest peak areas. Among which, PC 21:0_13:1 had the highest abundance of (126.04–152.55) $\times$ 10^9, followed by PC 17:1_17:1 of (73.57–89.12) $\times$ 10^9, and PC 16:0_20:1 of (71.11–87.64) $\times$ 10^9. Their concentrations in TRM sample were pronouncedly lower ($p < 0.05$) those in the control group. The changes of these lipids were opposite to the aroma compounds, revealing they might mainly participate in the generation of odorants. PC was the dominant phospholipid subclass, which was in agreement with the previous results on Tan sheep in cold storage (Jia et al., 2021). The interconversions among phospholipids and triglycerides will result in the lower of PC concentration (Ecker & Liebisch, 2014). The *Tamarix ramosissima* Ledeb might have accelerated the conversion of PC to PA due to its thermal instability, resulting in a significantly lower in PC concentration in the TRM sample compared to the control group (Fang et al., 2022; Pokotylo et al., 2018). Meanwhile, the downregulated TGs, including TG 18:0_18:0_18:1, TG 16:0_18:0_18:0, and TG 16:1_17:0_18:1, are hydrolyzed to produce relevant free fatty acids in the TRM sample, which can further generate aroma compounds. Particularly, the stearic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) were important fatty acyl chains of TGs observed in roasted mutton, which can be directly degraded or interact with Maillard reaction to form aroma compounds, including pyrazines and aldehydes (Amanpour et al., 2019; Ho & Hartman, 1994b). Therefore, the higher amounts of TGs and lower concentrations of PCs were conducive to the generation of aroma compounds. PC 21:0_13:1 might be a key lipid that promoted the generation of aroma compounds in TRM sample, accompanied by the auxiliary effect of TGs.

4. Conclusion

In this work, the roasted mutton skewered with *Tamarix ramosissima* Ledeb presented higher concentrations of aroma compounds and aroma intensity. A total of nine odorants, containing 5-ethyl-2,3-dimethylpyrazine and 5-methyl-2,3-diethylpyrazine, were determined as key aroma compounds. The OPLS-DA analysis identified one lipid and nine aroma biomarkers, including TG 18:0_18:0_18:1, 5-ethyl-2,3-dimethylpyrazine, and acetic acid methyl ester, that distinguished the TRM and control group. TGs and PCs were postulated to be the predominant lipids, contributing to the retention and formation of key aroma compounds, respectively. For the industries, we can adjust the feed composition and ratio to increase the concentrations of TGs and PCs in lamb muscle. We can further apply *Tamarix ramosissima* Ledeb to skewer lamb meat to enhance its aroma intensity. In the next study, we will concentrate on the effects of different types and positions of lipids on aroma retention.

CRediT authorship contribution statement

Bin Liang: Writing – original draft, Methodology, Funding acquisition, Formal analysis. **Jingyu Li:** Writing – original draft, Visualization, Validation, Resources, Investigation, Data curation. **Shuqi Zhao:** Visualization, Resources, Investigation. **Xiaoming Pan:** Resources, Methodology. **Yanfang Zhang:** Writing – review & editing, Methodology. **Peng Gao:** Software, Methodology. **Pi Li:** Software, Methodology. **Jiangtao Xing:** Software, Methodology. **Raheel Suleman:** Writing – review & editing. **Hansheng Gong:** Visualization, Funding acquisition. **Huan Liu:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

All authors declare no competing financial interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101593.

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