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Unraveling gender-specific lipids and flavor volatiles in giant salamander (*Andrias davidianus*) livers via lipidomics and GC-IMS

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ABSTRACT

To uncover the relationships between lipid components and flavor volatiles, distinctness in lipid components and odor substances in giant salamander livers of different genders were comparatively characterized through UPLC-Q Exactive-MS lipidomics and gas chromatography-ion migration spectrometry (GC-IMS). A total of 2171 and 974 lipid metabolites were detected in positive and negative ion modes, respectively. Triglycerides (TG) and phosphatidylcholines (PC) are the most abundant types of lipids. TG level in male livers was higher than that in female livers ($P < 0.05$), whereas PC level showed no marked variation ($P > 0.05$). Additionally, a total of 51 volatile components were detected through GC-IMS. Ketones (42.18 % ~ 45.44 %) and alcohols (24.19 % ~ 26.50 %) were the predominant categories, and their relative contents were higher in female livers. Finally, 30 differential lipid metabolites and 12 differential odor substances were screened and could be used as distinguishing labels in giant salamander livers of different genders. Correlation analysis indicated that PS(36:2e), TG(48:13), ZyE(37:6), and ZyE(33:6) correlated positively with 3-methyl butanal, 3-hydroxy-2-butanone, and 2-methyl-1-propanol ($P < 0.05$), but adversely linked with 1-penten-3-one, and 1-octen-3-one ($P < 0.01$). By three-fold cross-validation, prediction accuracies of these differential lipids and volatile compounds for gender recognition based on random forest model were 100 % and 92 %, respectively. These findings might not only add knowledge on lipid and volatile profiles in giant salamander livers as affected by genders, but also provide clues for their gender recognition.

1. Introduction

Artificial farming of giant salamander (*Andrias davidianus*) has been rapidly industrialized in China, because of its nutritional and medicinal benefits for human body (Jin, Chen, et al., 2021). The tails and livers of giant salamander are internal organs and underutilized during processing due to their high-fat content and fishy odor (Wang et al., 2023). The lipids of giant salamanders are mainly distributed in livers and tails. Giant salamander liver oil has been found containing abundant linoleic acid, docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (AA) (He et al., 2018). Even though the strong fishy odor derived from *Andrias davidianus* feeding with aquatic

Abbreviations: AA, arachidonic acid; CL, cardiolipin; DALs, differentially abundant lipids; DHA, docosahexaenoic acid; DG, diglyceride; EPA, eicosapentaenoic acid; FA, fatty acyls; FC, fold-change; FL, female livers; GC-IMS, gas chromatography-ion mobility spectrometry; GC–MS, gas chromatography mass spectrometry; GC-O, gas chromatography olfactometry; GL, glycerolipids; GP, glycerophospholipids; Hex2Cer, hexosyl ceramide; LC-MS, liquid chromatography-mass spectrometry; LPC, lysophosphatidylcholine; ML, male livers; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PLs, polar lipids; PLS-DA, partial least squares discriminant analysis; PR, prenol lipids; PS, phosphatidylserine; SM, sphingomyelin; SoG1, glucosylsphingosine; SP, sphingolipids; ST, sterol lipids; TG, triglyceride; UFAs, unsaturated fatty acids; VOCs, volatile organic compounds; WE, wax esters; ZyE, zymosterol..

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baits, the lipids in giant salamander livers have great nutritional values and deserve further exploitation. Therefore, it is essential to characterize their lipids and volatile profiles, thus uncovering their mode of interaction.

Many studies have found that there are close relationships between lipids and odorant for aquatic food products (Huang et al., 2024; Zhou et al., 2023), mainly because triacylglycerols and phospholipids could be hydrolyzed, and further oxidized to manufacture odor chemicals (Zheng, Pan, et al., 2024). Currently, lipidomics is an advanced method that can completely analyze whole cellular lipid profiles, elucidating the mechanisms of action of lipids in varied circumstances when combined with stoichiometric tools (Wang et al., 2024). Gas chromatography mass spectrometry (GC–MS) and liquid chromatography-mass spectrometry (LC-MS) are broadly utilized in assay of fat metabolites. LC-MS can offer comprehensive details of fat compositions, thus fostering it extensively utilized for characterization of food lipids (Zhou et al., 2023). Moreover, the diagnosis of odor substances in agricultural products has gained widespread attention. GC–MS and gas chromatography-ion mobility spectrometry (GC-IMS) are broadly used for odor or volatile flavor analysis. Compared to the conventional GC–MS approach, GC-IMS exhibited significant advantages and application potentials, particularly due to its rapidness, subtle distinctness, and gallery mapping capabilities without complex preparation procedures (Jin, Pei, et al., 2021). GC-IMS has been successfully used for characterization of volatile organic compounds (VOCs) in various foods as influenced by processing methods, origin distinction, and grade classification. So, both technologies provide powerful tools for characterization of lipids and odor substances in liver tissues of giant salamanders.

To date, several studies have confirmed the correlation between lipids and odor profiles in food products via lipidomics and volatile flavor assay. For example, Zhou et al. (2023) identified the key lipids involved in odorant formation using lipidomics and volatile flavor analysis. Zheng, Pan, et al. (2024) investigated the impacts of curing methods on fat profiles and odor substances in hams using lipidomics and GC-IMS. These studies demonstrated the potential application of lipidomics and volatile flavor analysis in the integrated analysis of lipids and odor substances in giant salamander livers.

Up to now, giant salamander livers have been mainly used for dishes, functional oils, and protein hydrolysates with potential health benefits. A large number of reports have proved that the majority of animals possess gender-specific odors and lipids (Alves-Oliveira et al., 2018), such as abalones (Tian et al., 2024), discus fish (Zhao et al., 2024), and crabs (Wu et al., 2010). Besides, considering that giant salamanders are dioecious aquatic animals, it is extremely difficult for consumers to accurately distinguish genders in retail markets and artificial farms (Pei et al., 2019; Zhao et al., 2022). The lipid profiles and odor characteristics of giant salamander may vary due to gender differences. Slade et al. (2021) found that lipid biomarkers were associated with age (young and elderly) and gender (male and female). Colón-Crespo et al. (2017) pointed out that marker volatile compounds could be used for the classification of individuals by gender. Therefore, it would be possible to achieve gender recognition through lipid components and volatile compounds.

Our previous studies have characterized quality characteristics and volatile profiles of jerky and meatball products of giant salamander (Jin Chen et al., 2021; Jin Pei et al., 2021). However, little information on lipids and odor profiles of giant salamander livers among genders can be found. Therefore, the objectives of this study are to explore the differences in lipids and odor profiles in female and male giant salamander livers based on lipidomics and GC-IMS, and investigate their relationships between lipids and odor substances. Moreover, the feasibility of differential lipids and odor chemicals for gender prediction of giant salamanders were also performed.

2. Materials and methods

2.1. Material and reagents

Six adult males (weighing of 4.56 ± 0.26 kg) and six female giant salamanders (weighing of 4.25 ± 0.31 kg) were artificial picked out by an experienced technical farmer, and purchased from Hanzhong Weidani Aquaculture Co. Ltd. (Hanzhong, China). These giant salamanders grew and fed in the same conditions and age range. The slaughtering included the fierce hitting on head of giant salamanders, bloodletting, and cutting according to the industrial practice (Jin et al., 2019), then male and female livers were put in self-sealed plastic bags, respectively. Immediately they were transported to the Key Laboratory for Bioscience resources (Hanzhong, China), and stored at ice temperature before analysis.

Acetonitrile, isopropanol, and methanol were purchased from Thermo Fisher (Pittsburgh, PA, USA). Isotopic internal standards were bought from Avanti Polar Lipids, Inc. (Alabaster, AL, USA); formic acid (DIMKA) and ammonium formate (Honeywell Fluka) were obtained from the USA. SPLASH™. Other chemicals used for lipidomic research were of the highest analytical grades, and obtained from Sigma-Aldrich (Shanghai, China). Standard *n*-ketones (2-butanone, 2-pentanone, 2-hex-anone, 2-heptanone, 2-octanone, and 2-nonanone, purity≥99 %) were bought from Guoyao Chemical Co., Ltd. (Shanghai, China).

2.2. Treatment of livers samples

The raw giant salamander livers were first cleaned, sliced, and grounded, respectively. They were labeled as male livers (ML) and female livers (FL), and then quickly kept at −80 °C prior to formal assay (less than 48 h).

2.3. Lipidomics analysis

Referencing the method of Wang et al. (2024), 100 mg liver samples of different genders were separately taken, added with 200 μL water and 20 μL internal lipid standard mixture, vortexed, added with 800 μL methyl tert-butyl ether (MTBE), vortex mixed, added with 240 μL pre-cooled methanol, vortex mixed, sonicated in a water bath (DZKW-S-4, Ke Heng Industrial Co., Ltd. Shanghai, China) for 15 min (4 °C), centrifuged at 14000*g* for 15 min at 10 °C(5430R, Eppendorf AG, Germany), and the upper organic phase was taken. N_2 was used to dry the phase, and during mass spectrometry analysis, 200 μL of 90 % isopropanol/acetonitrile solution was added for reconstitution, thoroughly vortexed, 90 μL of reconstitution solution was taken, centrifuged, and the liquid was taken for injection analysis. Meanwhile, equal amounts of samples were mixed as quality control (QC) to assess the system stability during the whole process.

Referencing the method of Zheng Pan et al. (2024), reverse phase chromatography was selected for separation using CSH C18 column (1.7 μm, 2.1 mm × 100 mm, Waters). Throughout the analysis, samples were placed in an autosampler at 10 °C. Detection was performed using both positive and negative ion modes of electrospray ionization (ESI). After separation by UHPLC (Nexera LC-30 A, Shimadzu Corporation, Japan), samples were detected by Q Exactive series mass spectrometer (Thermo Scientific™).

2.4. GC-IMS quantitation for VOCs

Detection of odor components in liver samples of male and female giant salamanders was carried out using a GC-IMS device (FlavourSpec®, Germany). Liver samples of different genders of were taken, homogenized using a tissue disperser (IKA T25, Germany), and accurately weighed 3.0 g. The weighed samples were placed in 20.0 mL headspace vials and incubated at 60 °C for 15 min. Each sample was analyzed in 6 parallel groups. The detailed methods of instrumental conditions, quantification of volatile substances was as follows. The gas chromatographic prefractionation was done at 60 °C on a MXT-5 column (15 m × 0.53 mm). The 99.99 % nitrogen was as a vehicle air at a programmed speed as follows: 2 mL/min for 2 min, 30 mL/min for 8 min, 100 mL/min for 10 min, and 150 mL/min for 5 min. The n-ketones C4-C9 were as foreign standards to estimate the retention index (RI) of single volatile chemical. Via collations of RI and the drift time (DT) through the instrumental database (FlavourSpec®, Germany), the volatile substances were characterized and identified through comparing DT and RI with those of the standard chemicals (Jin Pei et al., 2021).

2.5. Gender recognition based on random Forest

Random forest is a supervised machine learning algorithm that builds several decision trees based on the dataset to create a highly accurate prediction of classification. Based on this method, a three-fold cross-validation was performed on the ultimately detected differential volatiles and differential lipids (using Python version 3.12) in giant salamander of different genders. After model training, the classification reports are used to calculate precision, recall, and f1 scores to assess the robustness of the model (Wong & Yeh, 2020). Accuracy refers to the proportion of correctly predicted samples to the total number of samples. It measures the overall classification performance of the model. Precision refers to the proportion of samples predicted by the model as positive that are actually positive. It measures the accuracy of the model when predicting positive classes. Recall refers to the proportion of actual positive samples that are correctly predicted as positive by the model. It measures the model's ability to identify positive samples. The F1 score is the harmonic mean of precision and recall, combining both metrics to balance them. Finally, feature importance data of differential lipids and volatile compounds were also performed.

2.6. Statistical analysis

All data were first analyzed in Excel 2018, with all experiments independently conducted at least 6 times. The results were reported as mean values ± standard deviation. Subsequently, *t*-tests and Pearson correlation analyses were performed using SPSS 27.0 software. Characterization of volatile organic compounds (VOCs) utilized the NIST 2014 and IMS databases. Percentage plots and bar charts were created using Origin 2022. The partial least squares-discriminant analysis (PLS-DA), principal component analysis (PCA), correlation heatmap were generated via online platforms such as https://omicsolution.org/wkomics/main/, https://www.metaboanalyst.ca/MetaboAnalyst/, and RStudio with packages like psych, ggplot2, and ggsignif (Zheng Pan et al., 2024).

3. Results and discussion

3.1. Lipid compounds analysis in giant salamander livers of different genders

3.1.1. Lipid composition in giant salamander livers

Fig. S1 shows the original total ion chromatograms of lipidomic metabolites in giant salamander livers of different genders in positive and negative ion modes. A cumulative of 2171 and 974 lipid substances were identified in giant salamander livers of different genders using UPLC-Q Exactive-MS in positive and negative ion modes. As shown in Fig. 1A, the lipid composition is mainly 44.45 % glycerophospholipids (GP), 34.50 % glycerolipids (GL), 15.07 % sphingolipids (SP), 2.98 % sterol lipids (ST), 2.64 % fatty acyls (FA), and 0.45 % other lipids, including glucosylsphingosine (SoG1) and prenol lipids (PR). GP is a major constituent of biological membranes, participating in different cellular functions and transport processes. Because of their high unsaturation, GPs readily turned into materials for the emission of odor substances (Cui & Decker, 2016). Mi et al. (2018) investigated different age and sex groups of chickens through lipidomics based on LC-MS. They found that the identified lipids primarily included GP and GL, which aligned with our results. Fig. 1B demonstrates that more fat molecules were detected in positive ion mode, with some lipids detected exclusively in this mode, such as triglycerides (TG, 840 species), diglyceride (DG, 239 species), and zymosterol (ZyE, 20 species). Zhao et al. (2024) found that PE, TG, PC, and LPC were the main subclasses in discus fish (*Symphysodon* spp) skin mucus of different genders, which is different from present results perhaps due to variances of portions and species.

TG levels are higher in the male group ($P < 0.05$), while phosphatidylcholine (PC) levels are higher in the female group ($P > 0.05$) (Fig. 1C). A previous study examined the blood lipid levels of male and female Meishan pigs, and found that the levels of TG, PL, and other lipids in female Meishan pigs were higher than those in males (Kojima et al., 2008). Wu et al. (2010) demonstrated that TG, PL, and cholesterol were more abundant in female meat and gonad of blue swimmer crab (*Portunus pelagicus*) than in male groups. These results were significantly different from present study which could also be attributed to variances of portions and species. The subsequent breakdown of TG to generate free fatty acids is crucial for flavor development in giant salamander livers. TG constitutes the primary component of fats in both plant and animal sources. The oxidation of PC to yield an abundance of volatile flavor compounds imparts a distinctive taste to giant salamander livers. Previous research indicated that a substantial amount of PC and phosphatidylethanolamine (PE) played a crucial role in quality deterioration and were considered key lipid metabolism products in the storage process of tuna (Wang et al., 2021). In addition, the content and types of PC and PE were regarded as associated with the freshness of Atlantic salmon fillets (Chen et al., 2022). Liu et al. (2024) utilized the HPLC-HRMS system to conduct lipidomic analysis of roasted pork, effectively characterizing the lipid components in roasted pork, with PC and PE being the most predominant. This once again underscores the significance of GP and GL in animal tissues.

3.1.2. Multivariate statistical analysis of lipid compounds in giant salamander livers

PCA is an unsupervised learning approach that employs linear dimensionality reduction techniques for data analysis, visualization, and preprocessing. Its objective is to achieve a linear transformation that minimizes the error while converting data into a lower-dimensional space. The PCA model identified distinct variances among the fat data in giant salamander livers, as illustrated in Fig. 2A and Fig. 2B. The contribution percentages of principal component analysis were 61.5 % in positive mode and 57.3 % in negative mode. The graphical representation indicates that, under both positive and negative modes, the PCA model can discriminate the lipid components in the giant salamander livers as affected by gender.

Unlike PCA, a supervised PLS-DA model can better reduce dimension of data, and it can evaluate variable importance for distinguishing different categories using the VIP scores. This multivariate statistical analysis provides more comprehensive information of the dataset. Fig. 2C and Fig. 2D depict the PLS-DA analysis conducted on lipid components in giant salamander livers. The donation ratios of the PLS-DA simulation were 60.7 % in positive ion mode and 62.5 % in negative ion mode. Moreover, a supervised PLS-DA was executed for both cohorts, revealing a clear tendency towards group differentiation. The cross-validation findings of PLS-DA indicated $R^2 = 0.99087$, $Q^2 = 0.96726$ in the positive ion mode, and $R^2 = 0.97016$, $Q^2 = 0.87093$ in the negative ion mode, suggesting commendable classification predictive capacity and consistency of the model without overfitting. Liu et al. (2023) also performed a lipidomics analysis of the drying and salting process of mackerel using UPLC-Orbitrap/MS technology and PLS-DA to demonstrate differences in lipid species of mackerel at different processing stages.

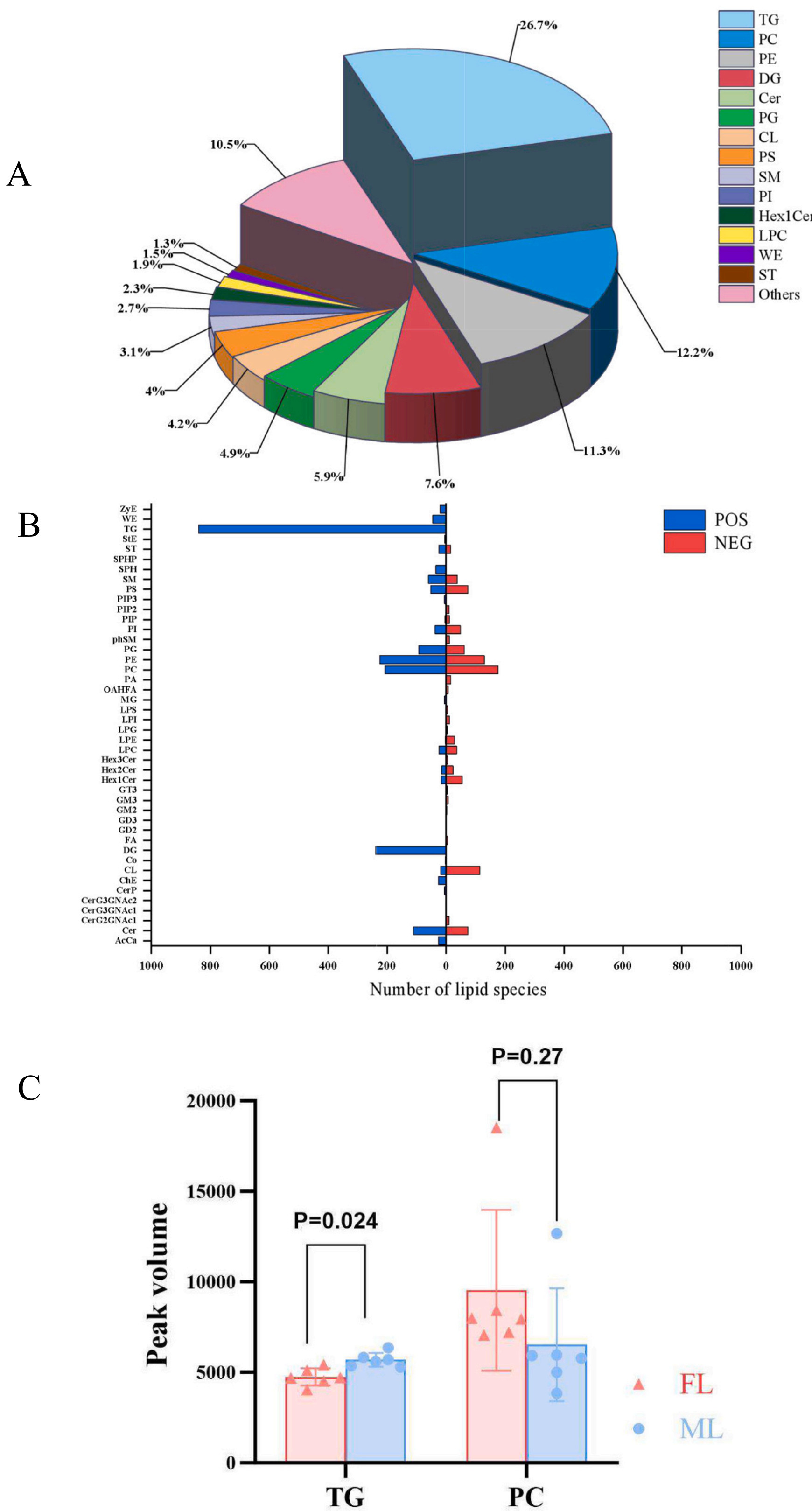

Fig. 1. Comprehensive lipid profiles in giant salamander livers of different genders through lipidomics analysis: (A) the percentage of the number of major and subclasses of lipids, (B) the number of all lipid species identified. (C) Comparison of TG and PC content in giant salamander livers of different genders.

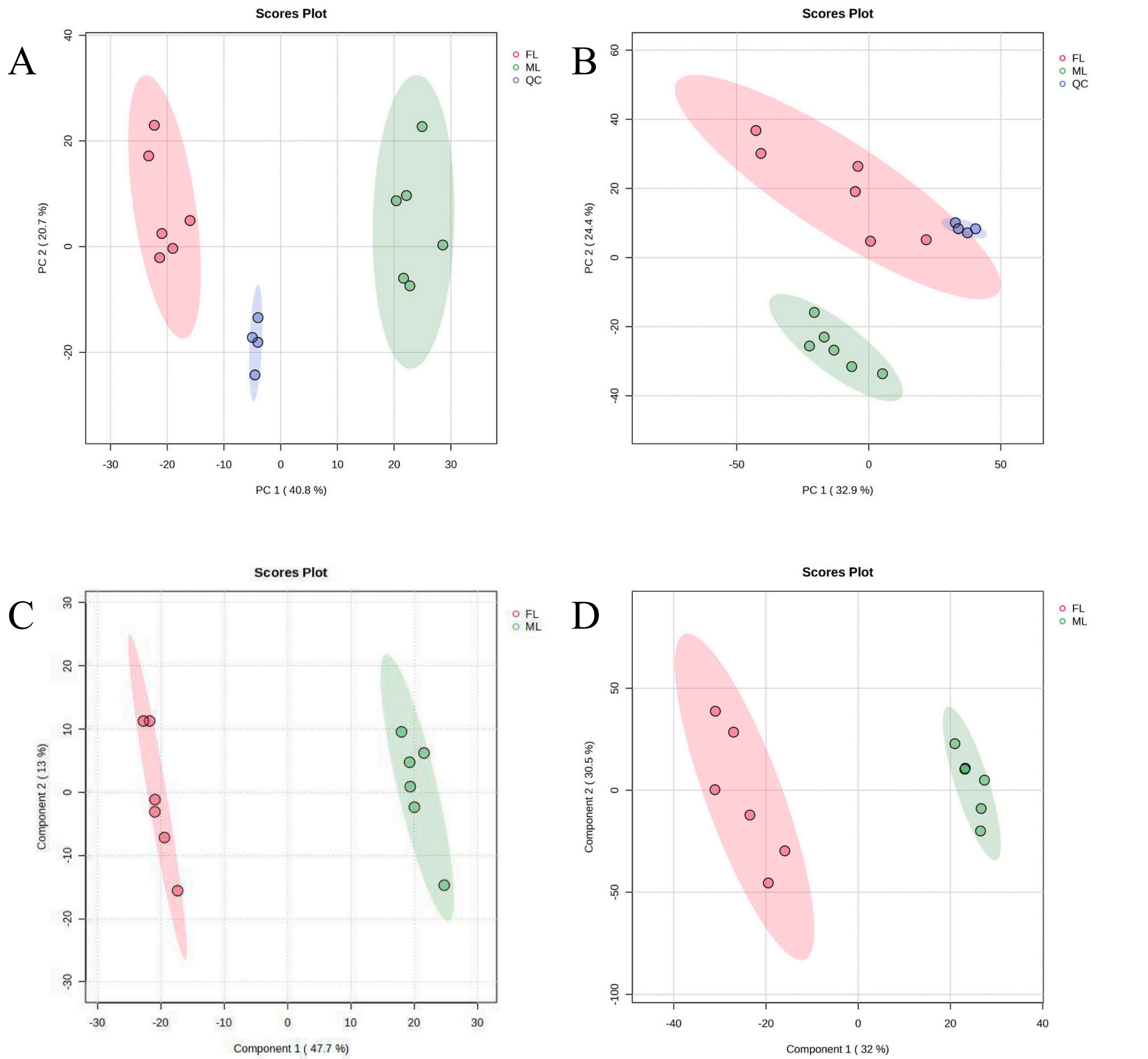

Fig. 2. PCA scores of test and quality control (QC) samples in positive (A) and negative (B) modes, respectively. PLS-DA scores of test samples in positive (C) and negative (D) ion modes in the livers of giant salamanders with different genders.

3.1.3. Identification of key lipids

Differentially abundant lipids (DALs) were picked out on the basis of VIP ≥ 1 and $P < 0.05$, identifying 730 distinct lipids. The top 25 DALs were selected, as illustrated in Fig. 3A. PG(22:6_22:6)-H, PE(18:2_22:6) + Na, PC(22:6_22:6) + HCOO, PG(20:4_22:6)-H, Hex2Cer(d14:1_20:5) + HCOO, PC(20:5_22:6) + HCOO, PG(22:5_22:6)-H, PC(38:9) + H, and PE(20:5_22:6)-H were more abundant in FL group, whereas TG (16:0_12:0_14:0) + NH4, LPC(20:0) + H, PC(8:0e_12:0) + H, LPC(14:0) + HCOO, PE(16:1p_24:1) + H, PS(36:2e) + H, PE(16:0_16:0)-H, LPE (20:1)-H, AcCa(18:0) + H, LPE(22:4)-H, TG(18:3e_17:1_19:1) + H, LPE (20:2)-H, PG(46:1) + H, PG(47:2) + H, and LPE(20:3)-H were more abundant in ML group (Fig. 3A). Overall, PG, lysophosphatidylcholines (LPC), PE, PC, phosphatidylserines (PS), and LPE, belong to the GP category except for TG attributed to GL, while hexosyl ceramides (Hex2Cers) belong to the SP category, GP and SP, known as polar lipids (PLs), are found universally in all tissues as essential components of cell membranes. A study explored the impact of PLs on neurological disorders, cognitive impairment, hepatic pathologies, cancer, as well as the impact of lipid profiles and cholesterol (Castro-Gómez et al., 2015). Most of the dominant GP species contain unsaturated fatty acids (UFAs) especially EPA and arachidonic acid (AA) (Zhou et al., 2018). These results confirmed that GP was the most important unsaturated fatty acids (UFAs) in giant salamander livers, which was consistent with the report of Jin Chen et al. (2021).

UFAs are inherently prone to oxidation, leading to structural changes that often impact the quality characteristics of aquatic products. UFAs belong to the fatty acid group within the acyl chains. The hydrocarbon chain is unsaturated, containing at least one double bond. Lipids containing unsaturated double bonds, such as PG(22:6/22:6), PE(18:2/22:6), PC(22:6/22:6), and PG(20:4/22:6), are closely associated with the flavor of meat products. These lipids belong to GP category. Due to their high UFAs content (more susceptible to lipid oxidation), they foster

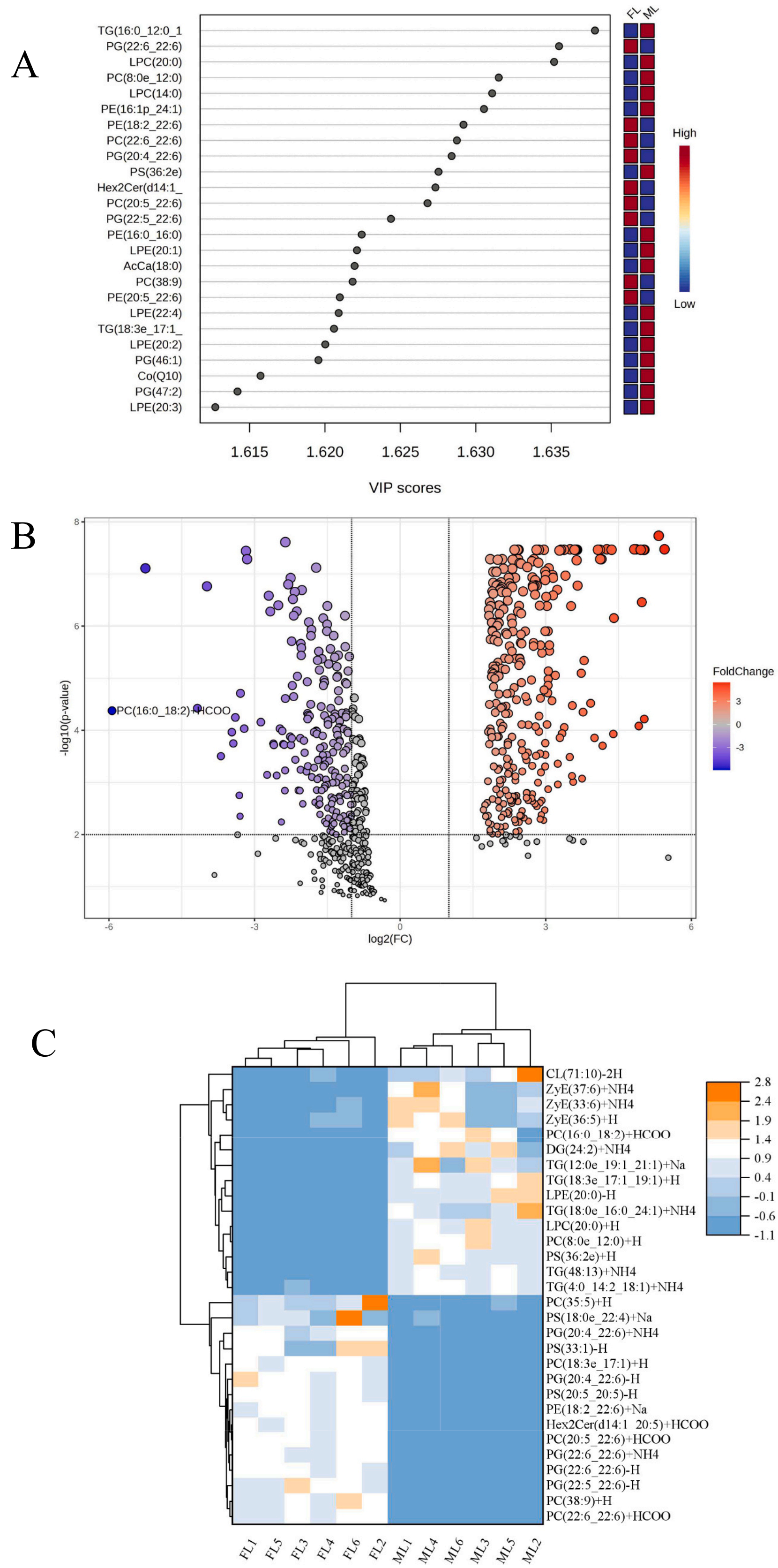

Fig. 3. Lipid screening: (A) The VIP scores plots of PLS-DA in the lipid components detected in the livers of giant salamanders with different genders (top 25 species). (B) Volcano plots of the lipid components detected in the livers of different genders of giant salamanders. (C) Heatmap of lipid correlations (VIP ≥ 1, $p < 0.01$, FC > 2, or FC < 0.5 , FDR < 0.05).

the aroma and flavor of meat products (Li et al., 2021). Some studies suggest that UFAs present in seafood surpass the saturated fatty acids typically found in dairy products (Wang et al., 2019). Compared to the TG form, the PL form of n-3 UFAs has drawn widespread attention ascribable to their great bioavailability, superior tissue penetration ability, and significant biological functions related to cardiovascular and neurodegenerative diseases (Lu et al., 2017). Phospholipids, containing phosphoric acid, can be further classified into GP and SP categories. Thus, giant salamander livers accumulate a substantial amount of GP and PL.

Using criteria of VIP ≥ 1, FDR < 0.01, fold-change (FC) > 2, or < 0.5, DALs were identified and selected, as shown in Table S1. Giant salamander liver oil has been reported abundant in DHA, and EPA (He et al., 2018). Table S1 demonstrated that most of UFAs in male and female livers could be attributed to DHA and EPA, particularly the relative levels of DHA (PC(22:6_22:6) + HCOO, PG(22:6_22:6)-H, PG (22:5_22:6)-H, PG(22:6_22:6) + NH4, PC(20:5_22:6) + HCOO, PE (22:6_22:6)-H, etc.) and EPA (PS(20:5_20:5)-H, PC(20:5_20:5) + Na, PE(20:5_20:4)-H, PI(16:0_20:5) + H, PC(16:0_20:5) + HCOO, PE (16:1_20:5)-H, etc.) were higher in FL group than that in ML group. A similar result of DHA and EPA in male and female gonads of blue swimmer crab (*Portunus pelagicus*) was also reported by Wu et al. (2010).

In Fig. 3B, the volcano plot illustrates upregulated or downregulated differential lipids, where orange represents upregulation, purple represents downregulation, and gray indicates no significant change. Elevated VIP scores and decreased *P* values indicate notable distinctions in the targeted lipids among different groups. Comparing ML group with FL group, a total of 306 up-regulated lipids and 203 down-regulated lipids were detected. Among the 306 upregulated lipids, PC has the highest number of species, up to 79, followed by PE, TG, and PS (Fig. 3B). These data indicated that the relative levels of PC, PE, TG, and PS were higher in female livers than that of male livers, which is consistent with Table S1. Similar studies of upregulated or downregulated differential lipids screened from 40-day stored shrimps and 0-day shrimps (Wang et al., 2024), and chickens of different genders (Mi et al., 2018).

Furthermore, a heatmap illustrating the top 15 up-regulated and top 15 down-regulated substances by FC value was presented, visually contrasting distinct lipid sub-types. As depicted in Fig. 3C, individual color differences within groups could be attributed to experimental errors. Cluster analysis reveals significant differences in various lipids between the FL and ML groups. Lipids in the upper right corner, such as CL (71:10), ZyE(37:6), TG(12:0e/19:1/21:1), and TG (18:0e/16:0/24:1), show strong correlations with the ML group, whereas lipids in the lower left corner, like PC (35:5), PS (18:0e/22:4), and PS (33:1), exhibit strong correlations with the FL group (Fig. 3C). Both male and female livers contain large amounts of DHA and EPA, which could prevent hyperactivity disorders, alleviate eye conditions, and combat inflammation (Zhang et al., 2019). Fig. 3C also showed that DHA (PC (22:6_22:6) + HCOO, PG(22:6_22:6)-H, PG(22:5_22:6)-H, PG (22:6_22:6) + NH4, PC(20:5_22:6) + HCOO) and EPA (PS(20:5_20:5)-H, PC(20:5_22:6) + HCOO) correlated positively with FL group, which is consistent with the results of Fig. 3A and Table S1. Among the 30 differential lipids, 20 belong to GP, 6 belong to GL, 3 belong to ST, and 1 belongs to SP. GP is the most abundant type of phospholipid, a crucial element of the cell membrane's bilayer structure, and is involved in membrane recognition and signal transduction. GLs are polar lipids, the second-largest class of membrane lipids after phospholipids.

Next, we selected the top 5 and bottom 5 differential lipids based on their FC values to construct box plots (Fig. S2), detailed information for the different groups can be found in Table S2. Fig. S2 showed marked variations in lipid contents among FL and ML. The FC values of PC (22:6_22:6), PG(22:6_22:6), and PG(22:5_22:6) are higher, indicating that their abundance is higher in FL. Conversely, the FC values of ZyE (33:6), TG (18:3e_17:1_19:1), and ZyE (37:6) are lower, indicating that their abundance are higher in ML. Huang et al. (2024) utilized Log_2FC values to screen for the top 10 up-regulated and top 10 down-regulated non-volatile substances in various Chinese matcha for further comparative analysis. A similar study also identified significantly different components such as PC, PE, PI, and SM between Laiwu and Yorkshire pigs (Hou et al., 2023). These findings again suggest that the lipid profiles in giant salamander livers differ among genders, potentially influencing the volatile odor profiles of these livers.

3.2. Analysis of volatile compounds qualitatively in giant salamander livers using GC-IMS

GC-IMS was utilized to identify volatile constituents in giant salamander livers. 3D spectra of salamander livers were obtained, and the differential contrast map (Fig. S3) facilitated a comparison of the differences in volatile components between genders. The differences in its 2D planes are shown in Fig.S4. The volatile components were well-separated in the gas phase ion mobility spectra, characterizing variations in the proportion of certain components between FL and ML. Based on the NIST 2014 and instrumental databases, a total of 51 odor substances were identified, including 19 alcohols, 15 aldehydes, 11 ketones, 3 acids, 1 ether, 1 ester, and 1 amine (Table S3). Several odor substances such as hexanal, heptanal, hexanol, octanal, nonanal, (*E*)-hept-2-enal, dimethyl sulfide, and 1-octen-3-ol were reported in aquatic products as the typical fishy odor chemicals. The present results found that 1-nonanal, 1-octanal, and dimethyl sulfide were potential fishy odor chemicals, and the relative content of dimethyl sulfide was the highest of all, compared to 1-hexanol, 1-nonanal and 1-octanal. Besides, higher levels of 1-nonanal and 1-octanal were spotted in ML group ($P < 0.05$), while a higher level of 1-hexanol was found in FL group ($P < 0.05$). However, the relative proportion of dimethyl sulfide showed no marked differences between genders ($P > 0.05$). Other fishy odorants not identified in giant salamander livers might be attributed to the variances of detection methods and raw material traits (Wang et al., 2023).

3.3. Fingerprint profiles of giant salamander livers of different genders

Using GC-IMS analysis to demonstrate variations in odor substances in the liver of giant salamanders, the resulting flavor fingerprint is shown in Fig. 4A. The vertical axis displays the volatile compound components in the liver of giant salamanders, while the horizontal axis displays the liver samples (from top to bottom: female group and male group, each with 6 parallel samples). Differences in the content of various compounds in the FL and ML groups can be observed from the brightness of the fingerprint colors, with brighter colors indicating higher content. For example, the relative contents of 1-pentanol, 1-hexanol, 3-methyl-1-butanol-D, 3-methyl-1-butanol-M, and 1-penten-3-ol were higher in the FL group, while the relative contents of 2-methyl-1-propanol-D, 2-methyl-1-propanol-M, propanoic acid, acetic acid-D, acetic acid-M, and 3-methyl-3-buten-1-ol were higher in ML(Fig. 4A). Some compounds (dimethyl sulfide, acetaldehyde, acetaldehyde, etc.) appear brighter in both groups, indicating that both groups contain these compounds simultaneously and at relatively higher levels (Fig. 4A). These differences might be attributed to variances in free amino acids, lipids, and unique flavor among different genders (Tian et al., 2024; Zhao et al., 2024). A similar fingerprint of VOCs in oysters with different ploidy and gender based on GC-IMS was also established and compared by Sun et al. (2023). Although the fingerprint of VOCs between ML and FL was compared, specific odor characteristics were still unclear and should be revealed by gas chromatography olfactometry (GC-O) and GC–MS in the future.

To visually exhibit the discrepancies in volatile organic compounds in giant salamander livers, the peak intensities of diverse odor substances on the gallery dactylograms were standardized to determine the ratios of different odor chemicals. As shown in Fig. S5, the volatile components in giant salamander livers can be broadly categorized into 7 compound classes (the two least abundant categories were merged into

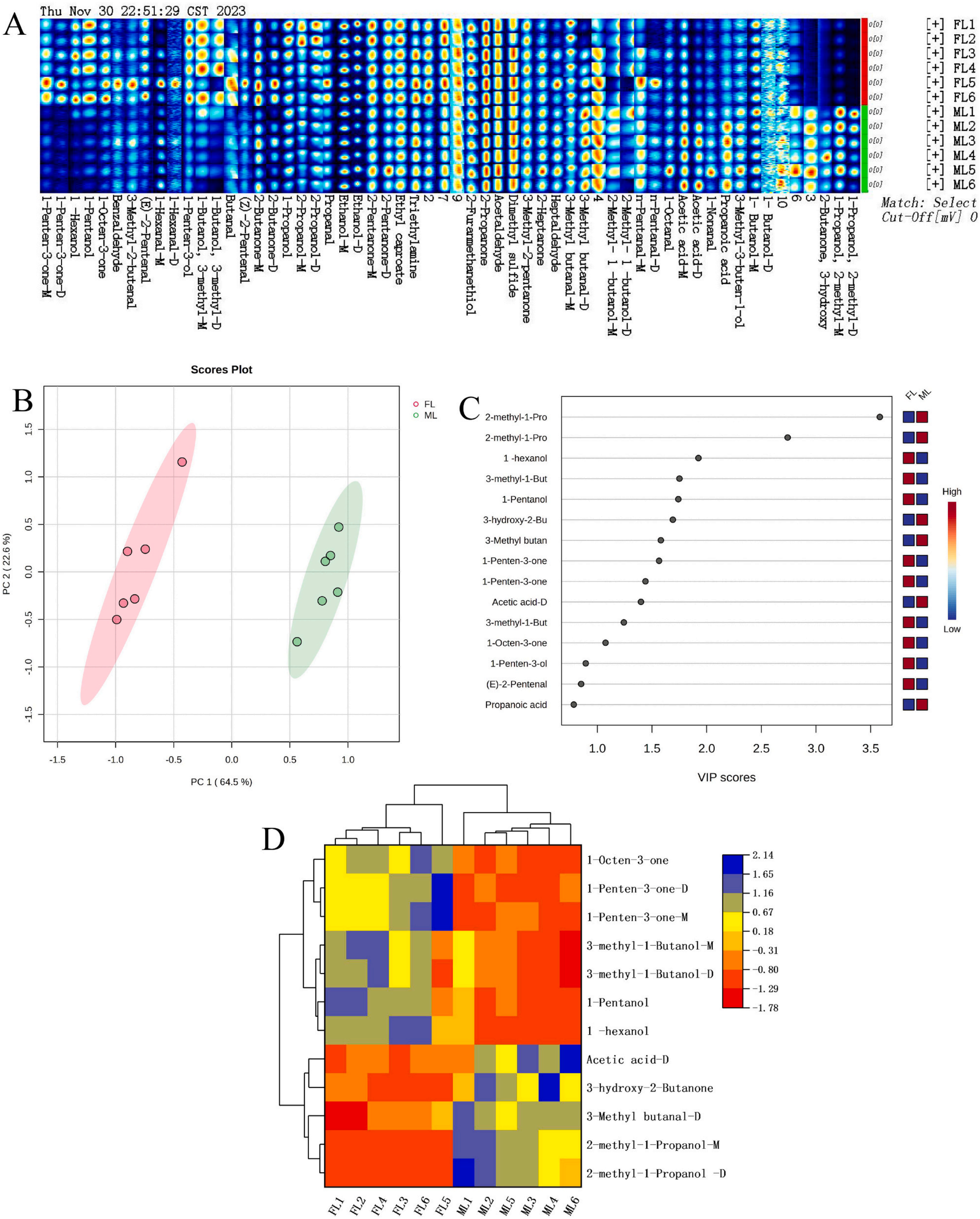

Fig. 4. The GC-IMS spectra of livers of giant salamanders with different genders: (A) Fingerprint map (vertical axis represents volatile compound components in the livers of different genders, horizontal axis displays different genders of salamander livers). (B) PCA score plot. (C) The VIP scores plots of PLS-DA(top 15 species). (D) The correlation heatmap between 12 selected different lipids and different gender groups.

others): alcohol (24.19 % ~ 26.50 %), ether (1.02 % ~ 1.11 %), aldehyde (14.91 % ~ 17.31 %), acid (6.42 % ~ 9.56 %), ketone (42.18 % ~ 45.44 %), ester (0.77 % ~ 0.83 %), and amine (4.884 % ~ 4.886 %). It also can be seen that the ML group has higher levels of aldehydes and acids, while the FL group has higher levels of alcohols and ketones. The other components show only subtle differences between the two groups. These differences in VOC categories of male and female giant salamander livers might be attributable to differences in lipid and protein content between the sexes. Research indicates that variations in amino acid content cause Guangyuan gray chickens of different ages and sexes to exhibit different nutritional values and flavors (Yin et al., 2023). The present differences in VOCs of giant salamander livers were mainly caused by genders, as they were stored and detected in the same conditions. Moreover, variations in some volatile components of oysters and abalones aquatic animals between male and female were also confirmed (Sun et al., 2023; Tian et al., 2024), probably owing to gender differences caused by variations in amino acids, lipids, proteins, etc.

3.4. Multivariate statistics of liver odor substances of different genders

Apart from the above-mentioned fingerprint differences, multivariate statistics are widely used to further emphasize the differences. The present study also performed multivariate statistics of unsupervised PCA and supervised PLS—DA to compare the differences between the sexes in certain volatile compounds. Fig. 4B shows the overall PCA score plot of differences between data points of volatile compounds in giant salamander livers. PC1 contributed 64.5 % of the total variances, PC2 contributed 22.6 %, and the total contribution was 87.1 %. A distinct segregation trend was observed between the two groups. A similar investigation employed the PCA method to distinguish the ploidy and gender of oysters based on volatile organic compounds, and significant characteristic differences were also observed among different ploidy and genders based on the degree of sample clustering and dispersion (Sun et al., 2023). Another study on male and female Hu sheep also indicated that the volatile components differ between sexes (Peng et al., 2012). Therefore, the combination of GC-IMS and PCA can better differentiate volatile organic compounds in giant salamander livers of different genders.

3.5. Selection of characteristic volatile components in giant salamander livers of different genders

Further, a supervised PLS—DA was also performed, as it can not only reduce data dimension, but also evaluates variable importance for distinguishing different categories using the VIP scores. Through PLS—DA analysis, 12 characteristic volatile flavor compounds were selected from 51 compounds using VIP $\geq$1 and $P < 0.05$ as the filtering criteria, as depicted in Table S4. These included 2-methyl-1-propanol, 1-hexanol, 3-methyl-1-butanol, 1-pentanol, 3-hydroxy-2-butanone, 3-methyl butanal, 1-penten-3-one, acetic acid, 1-octen-3-one, etc. Fig. 4C displays the top 15 compounds with the highest VIP. Compounds of various types have distinct aroma characteristics. 3-methyl butanal-D is an aldehyde. Aldehydes are primary products of fat degradation, having low thresholds and making significant contributions to the overall flavor of giant salamander livers. The fishy odor in aquatic products originates from farming, processing, and storage processes. During storage, lipid oxidation produces aldehydes, leading to a fishy smell in giant salamander livers (Wang et al., 2023). Ketones and alcohols mainly derive from fat oxidation and have thresholds higher than aldehydes. Table S4 shows that substances such as 2-methyl-1-propanol, 1-hexanol, 3-methyl-1-butanol-D, and 1-pentanol have relatively high VIP values, and they all belong to the alcohol class. Alcohols possess unique fruity and pleasant flavors. 2-methyl-1-propanol is a colorless liquid with a characteristic odor and, along with 3-methyl-1-butanol, is one of the main components of the aroma of both black and green teas. 1-hexanol is found in citrus fruits, apples, and other fruits, whereas 1-pentanol is a commonly used flavoring agent. Numerous studies have demonstrated a close relationship between lipid composition and the volatile flavor of *Crassostrea gigas* meat (Fu et al., 2023). Fig. 4D depicts the heatmap between 12 selected lipids and different gender groups. It can be observed that 2-methyl-1-propanol-D, 2-methyl-1-propanol-M, 3-methyl butanal-D, 3-hydroxy-2-butanone, and acetic acid-D exhibit strong positive correlations with male livers, indicating their close links with the flavor profile of the ML. Meantime, 1-octen-3-one, 1-penten-3-one-D, 1-penten-3-one-M, 3-methy-1-butanol-M, 3-methy-1-butanol-D, 1-pentanol, and 1-hexanol show stronger positive correlations in female livers, while 2-methyl-1-propanol-D, 2-methyl-1-propanol-M, 3-methyl butanal-D, 3-hydroxy-2-butanone, and acetic acid-D show stronger negative correlations in female livers (Fig. 4D). These differences in volatile compounds between FL and ML groups were almost consistent with those shown in Fig. 4A.

3.6. Correlation examination between fat constituents and odor substances

To gain deeper insights into how lipid degradation and oxidation influence the generation of odor substances, we selected characteristic lipid metabolites and odor substances for correlation analysis. The results, shown in Fig. 5A, display positive correlations in orange and negative correlations in blue. The intensity of color reflects the strength of correlation, and vice versa. Some volatile compounds exhibit a positive correlation with corresponding lipid components. Conversely, some volatile compounds show a negative correlation with corresponding lipids. For example, some flavor compounds (such as 3-methyl butanal, acetic acid, 3-hydroxy-2-butanone, 2-methyl-1-propanol) show a positive correlation with lipids such as PS(36:2e), TG(48:13), ZyE(37:6), and ZyE(33:6), but exhibit a clear negative correlation with lipids such as PC (18:3e/17:1), PC(22:6/22:6), PG(20:4/22:6), and PC(38:9) (Fig. 5A). Conversely, compounds like 1-penten-3-one and 1-octen-3-one show a significant negative correlation with lipids such as PS (36:2e), TG (48:13), ZyE(37:6), and ZyE(33:6), but exhibit a clear positive correlation with lipids such as PC(18:3e/17:1), PC(22:6/22:6), PG(20:4/22:6), and PC(38:9) (Fig. 5A). These UFAs are major contributors to the flavor of meat products and are beneficial in the diet (Liu et al., 2022; Lu et al., 2017). Meantime, UFAs contain double bonds that are easily oxidized under heating conditions, affecting the aroma of meat products (Han et al., 2023). Present results implied that PS(36:2e), TG(48:13), ZyE (37:6), and ZyE(33:6) may be degraded to produce more 3-methyl butanal with lower threshold, and they also produce more 3-hydroxy-2-butanone and 2-methyl-1-propanol with higher threshold, thus leading to the differences in odor profiles of giant salamander livers of different genders. However, the accurate relationships between odor profiles and specific lipids should be further explored. A similar correlation heatmap of distinctive lipids and characteristic aroma molecules in Nuodeng ham was also established by Zheng Pan et al. (2024), indicating that the odor compounds in Nuodeng ham are primarily formed through reactions associated with UFAs.

3.7. Use of differential lipids and volatile compounds for gender prediction based on random forest

Although the present results screened differential lipids and volatile compounds in giant salamander livers of different genders, they deserve further investigation for gender prediction. First, the PCA score plots of differential lipids and differential volatile compounds in giant salamander livers of different genders were performed. As shown in Fig. 5B, the 30 differential lipids can favorably discriminate between female and male livers, with the total of the contributions of the first two principal components being 96.8 %. Meanwhile, the 12 differential volatile compounds can also classify female and male livers clearly, with the total of the contributions of the first two principal components reaching 92.4 % (Fig. 5C). These results suggest that the 30 differential lipids and

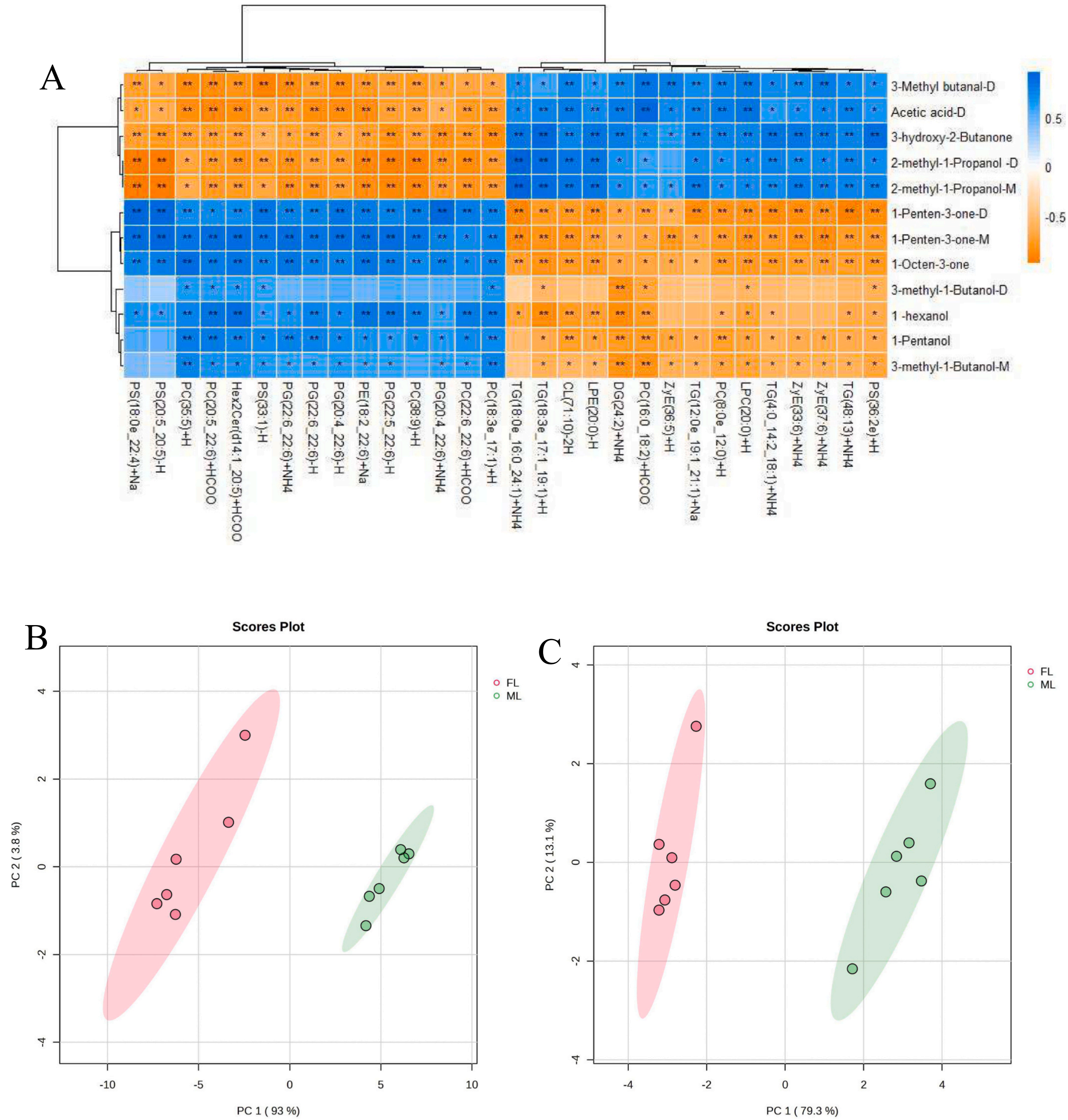

Fig. 5. (A) Correlation heatmap between characteristic volatile organic compounds and differential lipids, and the asterisks denote statistical significance (*$P < 0.05$, **$P < 0.01$). (B) Key differential volatile compounds of PCA score plot. (C) Key differential lipids of PCA score plot.

12 differential volatile compounds represent the majority of the lipid profiles and flavor volatiles in giant salamander livers.

As several studies pointed out that both lipid biomarkers and volatile compounds could be used for the gender classification of animals (Colón-Crespo et al., 2017; Slade et al., 2021), the feasibility of gender prediction of giant salamander was also performed by the 30 differential lipids and 12 characteristic volatile compounds. The performance metrics of the random forest model using the 30 differential lipids and 12 characteristic volatile compounds for gender prediction were demonstrated in Table S5 and Fig. 6. By three-fold cross-validation, the accuracy of the 30 differential lipids based on Random Forest model were 100 % (Fig. 6A), and it is consistent with parameters like precision, recall, and f1-score for gender prediction (Table 5S). Fig. 6B demonstrates feature importances data of differential lipids, and the top 5 features are PC(22:6_22:6) + HCOO, ZyE(33:6) + NH4, PC(8:0e_12:0) + H, CL(71:10)-2H, and PS(18:0e_22:4) + Na. Compared to others, these feature lipids showed relatively significant importance for the prediction model. Likewise, the accuracy of the 12 characteristic volatile compounds (Fig. 6C) were 92 %, implying that they could be used for gender recognition of giant salamanders. Compared to others, volatile

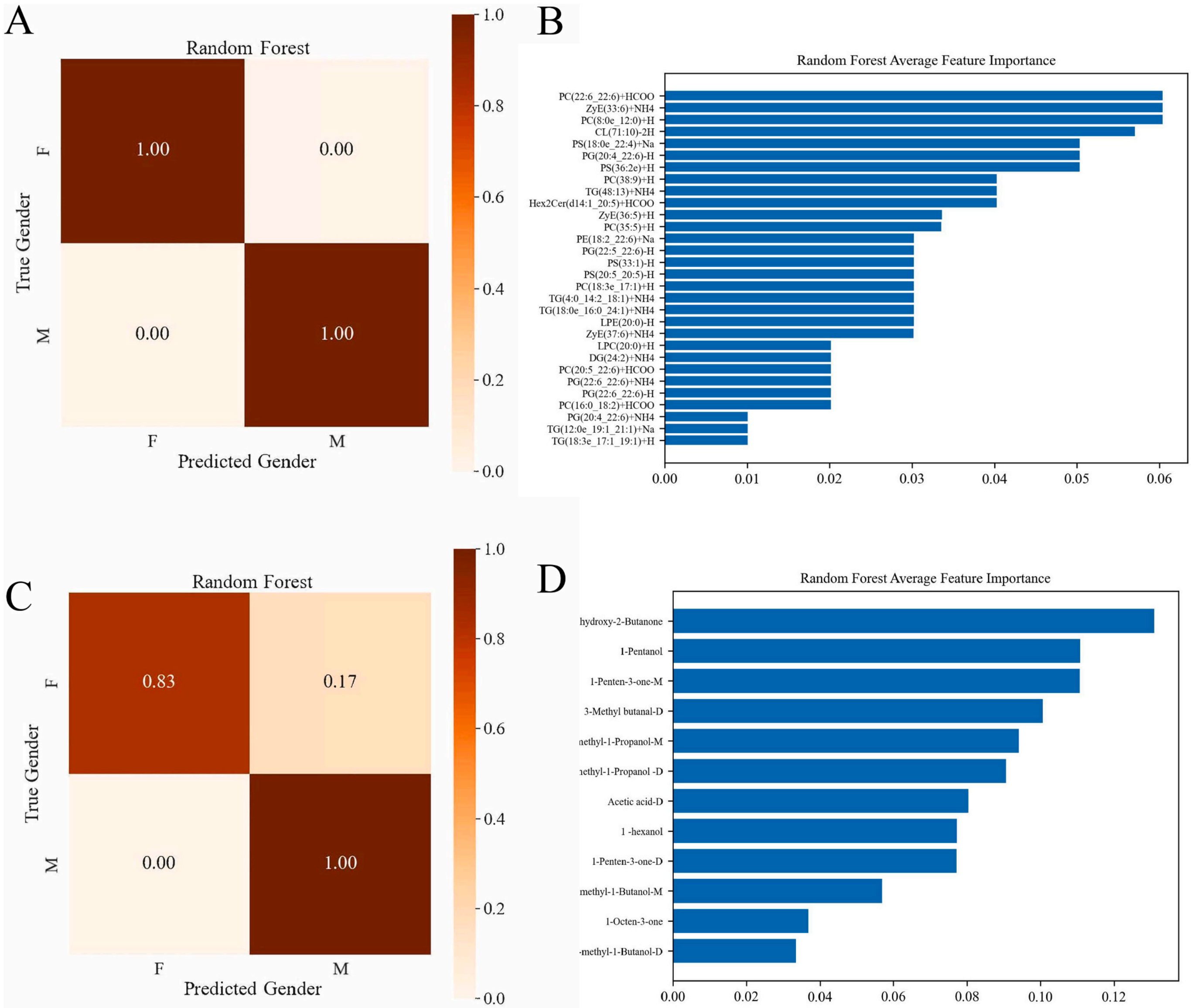

Fig. 6. Confusion matrix and feature importance of differential lipids and volatile compounds for gender prediction by Random Forest: (A) confusion matrix and (B) feature importance based on 30 differential lipids, respectively. (C) confusion matrix and (D) feature importance based on 12 characteristic volatile compounds, respectively.

compounds such as 3-hydroxy-2-butanone, 1-pentanol, 1-penten-3-one-M, 3-methyl butanal-D showed relatively significant importance for the prediction model (Fig. 6D). Zheng, Tian, et al. (2024) reported that the Random Forest could decrease overfitting and thus had the best classifying capacity, as it introduced randomness, possessed good noise "immunity" and was capable of prediction. Even though the present Random Forest approach possessed perfect accuracy and extracted important features for the classification of giant salamander genders, future studies should validate these parameters (precision, recall, accuracy, f1-score, and feature importance) on a huge sample scale.

4. Conclusions

In conclusion, a total of 3145 lipid metabolites were detected in giant salamander livers of different genders. Triglycerides (TG) and phosphatidylcholines (PC) are the predominant types of lipids. TG levels are higher in male livers, while PC levels are higher in female livers. Meantime, a total of 51 volatile components were identified in giant salamander livers based on GC-IMS. Ketones and alcohols were the predominant categories of odor substances, and both were more abundant in female livers. Finally, 30 differential lipid metabolites and 12 differential odor substances were screened based on multivariate statistical analysis and could be used as distinguishing labels. Correlation analysis indicated that PS(36:2e), TG(48:13), ZyE(37:6), and ZyE(33:6) correlated positively with 3-methyl butanal, acetic acid, 3-hydroxy-2-butanone, and 2-methyl-1-propanol, but they negatively correlated with 1-penten-3-one, and 1-octen-3-one. By three-fold cross-validation, both accuracies of these differential lipids and volatile compounds for gender prediction based on Random Forest were 100 % and 92 %, respectively. These results could not only provide insights into understanding lipid and volatile profiles in giant salamander livers as affected by genders, but also contribute to clues for gender prediction in the future.

CRediT authorship contribution statement

Wengang Jin: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Shibo Zhao:** Software, Methodology, Investigation, Formal analysis. **Jiayao Li:** Visualization, Validation, Software, Investigation, Formal analysis. **Kaiqi Cheng:** Visualization, Validation, Software, Data curation. **Linjie Xi:** Visualization, Validation, Software, Methodology. **Jinjin Pei:** Software, Resources, Project administration. **Ruichang Gao:** Writing – review & editing, Supervision, Conceptualization. **Pengfei Jiang:** Writing – review & editing, Supervision, Conceptualization.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101786.

References

Alves-Oliveira, P., Carvalho, J., Ferreira, J., Alho, L., Nobre, P., Olsson, M. J., & Soares, S. C. (2018). Influence of body odors and gender on perceived genital arousal. *Archives of Sexual Behavior, 47*(3), 661–668. https://doi.org/10.1007/s10508-017-1091-5

Castro-Gómez, P., Garcia-Serrano, A., Visioli, F., & Fontecha, J. (2015). Relevance of dietary glycerophospholipids and sphingolipids to human health. *Prostaglandins, Leukotrienes and Essential Fatty Acids, 101*, 41–51. https://doi.org/10.1016/j.plefa.2015.07.004

Chen, J., Kong, Q., Sun, Z., & Liu, J. (2022). Freshness analysis based on lipidomics for farmed Atlantic salmon (Salmo salar L.) stored at different times. *Food Chemistry, 373*, Article 131564. https://doi.org/10.1016/j.foodchem.2021.131564

Colón-Crespo, L. J., Herrera-Hernández, D., Holness, H., & Furton, K. G. (2017). Determination of VOC marker combinations for the classification of individuals by gender and race/ethnicity. *Forensic Science International, 270*, 193–199. https://doi.org/10.1016/j.forsciint.2016.09.011

Cui, L., & Decker, E. A. (2016). Phospholipids in foods: Prooxidants or antioxidants? *Journal of the Science of Food and Agriculture, 96*(1), 18–31. https://doi.org/10.1002/jsfa.7320

Fu, J., Sun, Y., Cui, M., Zhang, E., Dong, L., Wang, Y., Wang, W., Li, Z., & Yang, J. (2023). Analysis of Volatile Compounds and Flavor Fingerprint Using Gas Chromatography–Ion Mobility Spectrometry (GC-IMS) on *Crassostrea gigas* with Different Ploidy and Gender. *Molecules, 28*(11), 4475. https://doi.org/10.3390/molecules28114475

Han, D., Deng, S., Wang, H., Huang, F., Fauconnier, M.-L., Li, H., Zheng, J., Meng, L., Zhang, C., & Li, X. (2023). Lipid oxidation and flavor changes in saturated and unsaturated fat fractions from chicken fat during a thermal process. *Food & Function, 14*(14), 6554–6569. https://doi.org/10.1039/D3FO01061A

He, D., Zhu, W., Zeng, W., Lin, J., Ji, Y., Wang, Y., Zhang, C., Lu, Y., Zhao, D., Su, N., & Xing, X. (2018). Nutritional and medicinal characteristics of Chinese giant salamander (*Andrias davidianus*) for applications in healthcare industry by artificial cultivation: A review. *Food Science and Human Wellness, 7*(1), 1–10. https://doi.org/10.1016/j.fshw.2018.03.001

Hou, X., Zhang, R., Yang, M., Niu, N., Wu, J., Shu, Z., Zhang, P., Shi, L., Zhao, F., Wang, L., Wang, L., & Zhang, L. (2023). Metabolomics and lipidomics profiles related to intramuscular fat content and flavor precursors between Laiwu and Yorkshire pigs. *Food Chemistry, 404*, Article 134699. https://doi.org/10.1016/j.foodchem.2022.134699

Huang, D., Chen, X., Tan, R., Wang, H., Jiao, L., Tang, H., Zong, Q., & Mao, Y. (2024). A comprehensive metabolomics analysis of volatile and non-volatile compounds in matcha processed from different tea varieties. *Food Chemistry: X, 21*, Article 101234. https://doi.org/10.1016/j.fochx.2024.101234

Jin, W., Chen, X., Geng, J., Jin, J., Pei, J., Gao, R., & Chen, D. (2021a). Quality characteristics and moisture mobility of giant salamander (Andrias davidianus) jerky during roasting process. *Journal of Food Quality, 2021*. https://doi.org/10.1155/2021/9970797. Article ID: 9970797.

Jin, W., Pei, J., Chen, X., Geng, J., Chen, D., & Gao, R. (2021b). Influence of frying methods on quality characteristics and volatile flavor compounds of Giant salamander (*Andrias davidianus*) meatballs. *Journal of Food Quality, 2021*, Article e8450072. https://doi.org/10.1155/2021/8450072

Jin, W., Pei, J., Du, Y., Pan, J., Gao, R., Chen, D.-J., Wu, H.-T., & Zhu, B.-W. (2019). Characterization and functional properties of gelatin extracted from Chinese Giant salamander (*Andrias Davidianus*) skin. *Journal of Aquatic Food Product Technology, 28*(8), 861–876. https://doi.org/10.1080/10498850.2019.1652716

Kojima, M., Sekimoto, M., & Degawa, M. (2008). Gender-related differences in the level of serum lipids in Meishan pigs. *Journal of Health Science, 54*(1), 97–100. https://doi.org/10.1248/jhs.54.97

Li, C., Al-Dalali, S., Zhou, H., Wang, Z., & Xu, B. (2021). Influence of mixture of spices on phospholipid molecules during water-boiled salted duck processing based on shotgun lipidomics. *Food Research International, 149*, Article 110651. https://doi.org/10.1016/j.foodres.2021.110651

Liu, H., Ma, Q., Xing, J., Li, P., Gao, P., Hamid, N., Wang, Z., Wang, P., & Gong, H. (2024). Exploring the formation and retention of aroma compounds in ready-to-eat roasted pork from four thermal methods: A lipidomics and heat transfer analysis. *Food Chemistry, 431*, Article 137100. https://doi.org/10.1016/j.foodchem.2023.137100

Liu, Q., Lin, J., Zhao, W., Lei, M., Yang, J., & Bai, W. (2023). The dynamic changes of flavors and UPLC-Q-Exactive-Orbitrap-MS based lipidomics in mackerel (Scomberomorus niphonius) during dry-cured processing. *Food Research International, 163*, Article 112273. https://doi.org/10.1016/j.foodres.2022.112273

Liu, Z., Zhao, M., Wang, X., Li, C., Liu, Z., Shen, X., & Zhou, D. (2022). Investigation of oyster *Crassostrea gigas* lipid profile from three sea areas of China based on non-targeted lipidomics for their geographic region traceability. *Food Chemistry, 386*, Article 132748. https://doi.org/10.1016/j.foodchem.2022.132748

Lu, F. S. H., Nielsen, N. S., Baron, C. P., & Jacobsen, C. (2017). Marine phospholipids: The current understanding of their oxidation mechanisms and potential uses for food fortification. *Critical Reviews in Food Science and Nutrition, 57*(10), 2057–2070. https://doi.org/10.1080/10408398.2014.925422

Mi, S., Shang, K., Jia, W., Zhang, C.-H., Li, X., Fan, Y.-Q., & Wang, H. (2018). Characterization and discrimination of Taihe black-boned silky fowl (*Gallus gallus domesticus Brisson*) muscles using LC/MS-based lipidomics. *Food Research International, 109*, 187–195. https://doi.org/10.1016/j.foodres.2018.04.038

Pei, J., Chen, D., Jin, W., Geng, J., Wang, W., Zhang, S., Yue, T., & Zhang, H. (2019). Structure and mode of action of a novel antibacterial peptide from the blood of *Andrias davidianus*. *Letters in Applied Microbiology, 69*(5), 312–317. https://doi.org/10.1111/lam.13219

Peng, Y., Wang, J., Ren, D., Lin, J., & Liu, J. (2012). Different patterns of volatile compounds and fatty acid profiles in the adipose tissues of male and female Hu sheep. *Acta Agriculturae Scandinavica, Section A - Animal Science, 62*(3), 153–158. https://doi.org/10.1080/09064702.2012.756540

Slade, E., Irvin, M. R., Xie, K., Arnett, D. K., Claas, S. A., Kind, T., … Graf, G. A. (2021). Age and sex are associated with the plasma lipidome: Findings from the GOLDN study. *Lipids in Health and Disease, 20*(1), 30. https://doi.org/10.1186/s12944-021-01456-2

Sun, Y., Fu, J., Zhang, E., Dong, L., Cui, X., Sun, Y., Wang, Z., Feng, Y., Li, B., Xu, X., Luo, Q., Wang, W., & Yang, J. (2023). Fingerprint analysis of volatile flavor compounds in *Crassostrea gigas* of different ploidy and gender under high-temperature incubation. *Molecules, 28*(19), 6857. https://doi.org/10.3390/molecules28196857

Tian, H., Li, Y., Wang, X., Huang, X., Zhang, Y., Zhang, Z., Zhou, D., & Qin, L. (2024). Metabolomic insights into the effects of seasonal shifts on the dynamic variation of non-volatile compounds of abalone (*Haliotis discus hannai*). *Food Chemistry, 449*, Article 139197. https://doi.org/10.1016/j.foodchem.2024.139197

Wang, L., Zang, M., Cheng, X., Wang, S., Zhao, X., Zhao, B., & Li, D. (2024). Evaluation of changes in the lipid profiles of dried shrimps (*Penaeus vannamei*) during accelerated storage based on chemical and lipidomics analysis. *LWT, 191*, Article 115564. https://doi.org/10.1016/j.lwt.2023.115564

Wang, W., Zhao, S., Pei, J., Jiang, P., Jin, W., & Gao, R. (2023). Antioxidative activity and volatile profiles of Maillard reaction products between Giant salamander (*Andrias davidianus*) peptides and glucose during the heating process. *Journal of Food Quality, 2023*, 8804009. https://doi.org/10.1155/2023/8804009

Wang, X., Xie, J., & Chen, X. (2021). Differences in lipid composition of bigeye tuna (Thunnus obesus) during storage at 0 °C and 4 °C. *Food Research International, 143*, Article 110233. https://doi.org/10.1016/j.foodres.2021.110233

Wang, X., Zhang, H., Song, Y., Cong, P., Li, Z., Xu, J., & Xue, C. (2019). Comparative lipid profile analysis of four fish species by Ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. *Journal of Agricultural and Food Chemistry, 67*(33), 9423–9431. https://doi.org/10.1021/acs.jafc.9b03303

Wong, T.-T., & Yeh, P.-Y. (2020). Reliable accuracy estimates from k-fold cross validation. *IEEE Transactions on Knowledge and Data Engineering, 32*(8), 1586–1594. https://doi.org/10.1109/TKDE.2019.2912815

Wu, X., Zhou, B., Cheng, Y., Zeng, C., Wang, C., & Feng, L. (2010). Comparison of gender differences in biochemical composition and nutritional value of various edible parts of the blue swimmer crab. *Journal of Food Composition and Analysis, 23*(2), 154–159. https://doi.org/10.1016/j.jfca.2009.08.007

Yin, L., Xu, M., Huang, Q., Zhang, D., Lin, Z., Wang, Y., & Liu, Y. (2023). Nutrition and flavor evaluation of amino acids in Guangyuan Grey chicken of different ages. *Genders and Meat Cuts. Animals, 13*(7), 1235. https://doi.org/10.3390/ani13071235

Zhang, T., Xu, J., Wang, Y., & Xue, C. (2019). Health benefits of dietary marine DHA/EPA-enriched glycerophospholipids. *Progress in Lipid Research, 75*, Article 100997. https://doi.org/10.1016/j.plipres.2019.100997

Zhao, H., Huang, J., Fang, C., Ma, H., Zhang, H., Deng, J., Jiang, W., Kong, F., Zhang, H., Liu, H., & Wang, Q. (2022). Predicting sex-related transcripts in the Chinese Giant salamander (*Andrias davidianus*): A transcriptomics study, selection gender for preservation. *Breeding and Reintroduction. Fishes, 7*(6), Article 6. https://doi.org/10.3390/fishes7060399

Zhao, S., Wen, B., Liang, H., Gao, J., & Chen, Z. (2024). Sex-dependent lipid profile differences in skin mucus between non-parental and parental Discus fish (*Symphysodon haraldi*) determined by Lipidomics. *Fishes, 9*(1), 27. https://doi.org/10.3390/fishes9010027

Zheng, X., Pan, F., Naumovski, N., Wei, Y., Wu, L., Peng, W., & Wang, K. (2024). Precise prediction of metabolites patterns using machine learning approaches in distinguishing honey and sugar diets fed to mice. *Food Chemistry, 430*, Article 136915. https://doi.org/10.1016/j.foodchem.2023.136915

Zheng, Z., Tian, M., Liao, G., Chen, G., Zhong, Y., Yang, Y., & Wang, G. (2024). Evaluation of the effects of compound curing agents on the lipid profiles and volatile flavors in Nuodeng ham based on lipidomics and GC-IMS analysis. *Food Research International, 176*, Article 113810. https://doi.org/10.1016/j.foodres.2023.113810

Zhou, M., Liu, D., Qiu, W., Wang, C., Yu, W., Xiong, G., Wang, L., Shi, L., Wu, W., Chen, S., & Qiao, Y. (2023). Lipidomics and GC-MS analyses of key lipids for volatile compound formation in crayfish meat under different thermal processing treatments. *LWT, 189*, Article 115522. https://doi.org/10.1016/j.lwt.2023.115522

Zhou, X., Zhou, D., Yin, F., Song, L., Liu, Y., Xie, H., Gang, K., Zhu, B., & Shahidi, F. (2018). Glycerophospholipids in sea cucumber (Stichopus japonicus) and its processing by-products serve as bioactives and functional food ingredients. *Journal of Food Bioactives, 1*, 134–142. https://doi.org/10.31665/JFB.2018.1132