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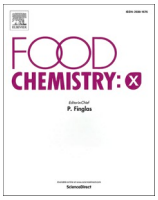

Insights into the molecular mechanisms of lipid transformation in sturgeon fillets: Interplay between specific spoilage and dominant bacteria

Chunming Tan [a,c], Shiqi Zhang [a], Fanglei Zou [b], Boya Gao [a], Yujin Li [d], Pinglan Li [a,*], Nan Shang [b,*]

[a] Beijing Laboratory for Food Quality and Safety, College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, China
[b] College of Engineering, China Agricultural University, Beijing 100083, China
[c] National R&D Center for Freshwater Fish Processing, College of Life Sciences, Jiangxi Normal University, Nanchang, Jiangxi 330022, China
[d] College of Food Science and Engineering, Ocean University of China, Qingdao, Shandong 266003, China

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ABSTRACT

This study investigates spoilage bacteria's impact on lipid metabolism in sturgeon fillets using UHPLC-Q-Orbitrap-MS/MS-based untargeted lipidomic analysis. A total of 4041 lipid molecules across five classes and 42 subclasses were identified, including glycerophospholipids (GPs, 50.88%), glycerolipids (GLs, 36.08%), sphingolipids (SPs, 10.47%), fatty acyls (FAs, 2.45%), and sterol lipids (STs, 0.12%). *Aeromonas sobria*, a specific spoilage bacterium, reduced GPs and FAs while increasing GLs, SPs, and STs via extracellular lipases and esterases. *Acinetobacter albensis*, the dominant bacterium, mainly elevated SPs and FAs. Their interaction promoted lipid metabolism and oxidation while producing volatile organic compounds (VOCs). Ethyl isobutyrate, ethyl propionate, isobutyl formate, pentan-2-one, propan-2-one, 2-butanone, 3-methyl-3-buten-1-ol, and dimethyl sulfide were mainly associated with *Acinetobacter albensis*, while 1-hexanol, 1-pentanol, 1-penten-3-ol, 1-hydroxypropan-2-one, 3-methyl-1-butanol, 2-methylbutanal, 3-hydroxy-2-butanone, and propionaldehyde were mainly related to *Aeromonas sobria*. This work unveils the mechanism of lipid transformation in sturgeon fillets during refrigerated storage, offering insights for aquatic products quality control.

1. Introduction

Sturgeon, an important aquatic product in China, primarily valued for caviar, leaves 40% of its weight as underutilized meat (Chen et al., 2020). Growing sales of refrigerated sturgeon fillets, especially among younger consumers, face challenges of quality degradation and spoilage, particularly microbial activities affecting freshness, texture, odor, and nutritional content (Jia et al., 2019; Li et al., 2020).

Sturgeon meat, high in fat and rich in unsaturated fatty acids (UFA), undergoes lipid transformation during storage, impacting color, flavor, taste, and texture (Wang et al., 2022; Yu et al., 2020). This transformation during storage significantly influences consumer preferences (Jia et al., 2021). Studies across various aquatic products, including white shrimp (Okpala et al., 2016), whelk (Yu et al., 2020), sardine (Pinheiro et al., 2020), red drum fillets (Mi et al., 2016), and sturgeon fillets (Tan et al., 2022), highlight the role of both endogenous enzymatic action and microbial activities in lipid metabolism. Compared with endogenous lipases, microorganisms can produce various types of lipolytic enzymes (e.g., acid lipases, neutral lipases, phospholipases, and esterases) and lipoxygenases (Feng et al., 2021). Microbial lipases can act on triglycerides (TG) and phospholipids (PL) to produce free fatty acid (FFA), and promote UFAs to form fatty acyl hydroperoxides, aldehydes, and carbonyl substances (Wang et al., 2022; Yu et al., 2020). According to Wang et al. (2016), degradation of lipids can produce different levels of FFA that affect the volatile flavor components of meat. In addition, the exogenous lipases produced by microorganisms accelerate lipid oxidation to some extent through enzymatic reactions (Feng et al., 2021). At the action of lipoxygenase, UFAs such as linoleic acid and arachidonic acid can be converted to oxides in fish products, thus altering lipid peroxide values (POV) and thiobarbituric acid reactive substances (TBARS) (Mi et al., 2016). It can be seen that the degradation and metabolism of lipids by microorganisms is one of the most important factors that decrease the quality of fish meat. In the study of Tan et al. (2022), the dominant bacteria *Acinetobacter* showed a significant positive correlation with lipid metabolism and oxidation. However, most researchers have only focused on the effects of specific spoilage

* Corresponding authors.
E-mail addresses: lipinglan@cau.edu.cn (P. Li), nshang@cau.edu.cn (N. Shang).

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bacteria on protein and lipid oxidation (Lou et al., 2021). An increasing number of studies indicated that spoilage microorganisms can alter their metabolic profiles through interactions (Wang & Xie, 2020; Tan, Li, & Shang, 2023).

Despite identifying *Aeromonas sobria* and *Acinetobacter albensis* as dominant bacteria in spoiled sturgeon fillets, both bacteria have the ability to produce lipase and degrade lipids (Tan et al., 2022; Tan, Li, & Shang, 2023). However, the pathways and molecular mechanisms of lipid transformation induced by these microorganisms and their interactions remain unknown. Therefore, studying the lipid changes of sturgeon fillets induced by microorganisms during refrigeration has great significance for controlling the quality of sturgeon fillets.

Recently, lipidomics based on liquid chromatography and mass spectrometry has been widely used in animal food research, which provides a new tool to characterize the molecular mechanisms of lipid metabolism regulation (Jia et al., 2021). Thus, lipidomics can be used to analyze the quality and safety of meat, especially for the study of chemical changes related to lipid oxidation and metabolism. For example, the use of untargeted lipidomics can provide more comprehensive information on the overall lipid composition and abundance signals of lipid metabolism without predetermined screening conditions (Wang et al., 2022). In this study, we explored the biochemical changes and lipid profiles induced by spoilage microorganisms in sturgeon fillets during refrigeration by combining physicochemical indices and ultra-high performance liquid chromatography-Orbitrap high resolution mass spectrometry (UHPLC-Q-Orbitrap MS/MS), including quality characteristics, lipid degradation, exogenous lipase activity and lipid metabolism. And changes in volatile organic compounds (VOCs) associated with lipid decomposition and oxidation in fish fillets were analyzed using gas chromatography-ion mobility spectrometry (GC-IMS). The comprehensive insight gained lay the foundation for precise quality control of sturgeon products in the future.

2. Materials and methods

2.1. Sample preparation

Hybrid sturgeon (age of 5 years, weight of 6582.0 ± 305.9 g, length of 89.3 ± 5.7 cm) were obtained from the Sturgeon Farm of Tianyuan Fishing Port (Huairou, Beijing, China). The sterile fish fillets were prepared according to our previous study (Tan, Hu, et al., 2023). Briefly, life sturgeons were stunned with a wooden club, then gutted, washed, surface cleaned with 75% ethanol wipes. The dorsal flesh of sturgeon was obtained using sterile knives and filleted into cubes (size of 4 × 3 × 2.5 cm), and treated with 0.5% (*v*/v) formalin solution for 30 s. After rinsing with cold sterile water for 3 times, they were drained on sterile gauze for 15 min as sterile fish fillets.

2.2. Bacterial strains and inoculation

Aeromonas sobria LT-101 (As, GenBank accession number OK103778), a specific spoilage organism in refrigerated (4 °C) sturgeon fillets, is considered as the main contributor for sturgeon spoilage. While *Acinetobacter albensis* LT-106 (Aa, GenBank accession number OK481116) used in this study was the dominant bacterial strain isolated from spoiled sturgeon fillet at 4 °C, which has the most abundance and related to lipid metabolism. The strains inoculation was performed according to the method of Lou et al. (2021). Briefly, the prepared sterile fish fillets were randomly divided into four groups and immerged into different bacterial suspension (6 lg CFU/mL) to reach around 4 lg CFU/g, including: (1) Inoculated with sterile saline (Control group, C), (2) Individual inoculation of *A. sobria* (As), (3) Individual inoculation of *A. albensis* (Aa), (4) *A. albensis* and *A. sobria* were co-inoculated at a ratio of 1:1 (AsAa). After that, the inoculated fillets were aerobically packed in sterile bags and stored at 4 °C for 12 days. During storage, three bags in each group were randomly selected every two days for biochemical changes, and 6 parallel samples were randomly collected for lipid metabolism analysis on day 12.

2.3. Determination of bacterial counts and total volatile basic nitrogen (TVB-N)

A plate count method with tryptone soy broth medium was used to determine the bacterial counts of sturgeon fillets during refrigeration. In brief, 5.0 g fillet sample was aseptically collected, added to 45 mL sterile saline and homogenized for 60 s. 1.0 mL of sample homogenized liquid was prepared in 10-fold serial dilution with sterile saline. Two suitable dilutions were selected for microbial enumeration, incubated at 30 °C for 48 h to determine the bacterial counts.

The TVB-N content was extracted and measured as described by Tan et al. (2022). In brief, 5.0 g fillet sample was added 50 mL distilled water and homogenized at high speed for 60 s. The extraction was carried out for 30 min with shaking, then centrifuged at 3000*g* for 3 min. The TVB-N content in the samples was analyzed using a Kjeldahl Apparatus (KDY-9830, Beijing, China), and expressed as mg/100 g sample.

2.4. Analysis of free fatty acid

A 0.1 g sample of fish meat was taken and determined by using the free fatty acid content determination kit (No. BC0595, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Fatty acids can combine with copper ions to form fatty acid copper salts and dissolve in chloroform, and the free fatty acid content can be deduced by measuring copper ions.

2.5. Analysis of POV and TBARS values

The POV values of lipids in sturgeon fillets were measured according to the American Oil Chemists Society (AOCS) official method Cd 8–53 (AOCS, 2003). Briefly, 5.0 g of fish sample was added with chloroform (1:2, m/v), homogenized and centrifuged at 4000*g* for 5 min, and the supernatant was collected. Then 30 mL of chloroform/glacial acetic acid mixture (2:3, *v*/v), 1.0 mL of saturated potassium iodide solution and 1.0 mL of 1% starch indicator were added, and manually shaken well for 30 s, kept in the dark for 3 min, and then 100 mL of distilled water was added and titrated with 0.01 mol/L sodium thiosulfate. While the TBARS values were measured by using the malondialdehyde (MDA) assay kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China). MDA can condense with Thiobarbituric acid (TBA) under acidic and high temperature conditions to produce 3,5,5-trimethyloxazol-2, 4-one, which has a characteristic absorption at 532 nm. The results were expressed as mg MDA Eq./kg sample.

2.6. Evaluation of lipase and esterase activities

Lipase activities in sturgeon fillets were measured using a lipase assay kit (No. BC2340, Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), and results were expressed as U/g sample. While esterase activities were measured as described by Tan, Li, and Shang (2023). Briefly, 2.0 g fillet sample was homogenized with 20 mL 50 mmol/L sodium phosphate buffer (pH 7.5) for 60 s, and centrifuged at 10,000*g*, 4 °C for 20 min. 100 μL of supernatant was mixed with 100 μL of 20 mmol/L *p*-nitrophenol acetate and 800 μL of 50 mmol/L sodium phosphate buffer, then incubated at 37 °C for 10 min. After that, 1.0 mL of 0.2 mol/L sodium phosphate buffer containing 5 mg/L SDS was added to terminate the reaction. The absorbance was measured at 405 nm, and released *p*-nitrophenol was calculated from a standard curve. The enzyme activities were expressed as U/g sample.

2.7. Analysis of VOCs

VOCs analysis of sturgeon fillets was carried out according to the

method as described by Jia et al. (2019). Briefly, 2.0 g fish sample was accurately weighed and taken in a 20 mL headspace vial sealed with a threaded cap, then placed on the sample holder of a GC–IMS spectrometer. After incubation at 40 °C for 20 min, 500 μL of the headspace sample was injected into the GC-IMS instrument by a heated syringe (85 °C). Subsequently, the sample gas was carried through the carrier gas (N_2) into a capillary column SE-54-CB-1 (5% phenyl-1% vinyl-94% methylpolysiloxane, 15 m × 0.53 mm, 1 μm inner diameter) at 60 °C for the separation of VOCs. The gradient elution procedure of gas chromatography is as follows: $t = 0$ min, 2 mL/min N_2; $t = 2$ min, 2 mL/min N_2; $t = 10$ min, 10 mL/min N_2; $t = 20$ min, 100 mL/min N_2; and $t = 30$ min, 150 mL/min N_2. The VOCs were carried by 150 mL/min N_2 and were driven to a 9.8 cm drift tube (5 kV constant voltage, 45 °C) after ionizing in the ionization chamber to be separated again.

2.8. Total lipid extraction

Lipid in sturgeon fillets was extracted according to the method of Wang et al. (2021). In brief, 20 mg sample was mixed with 200 μL water and 20 μL lipid standard mixture (containing 13 isotopic internal standards), vortexed and added 800 μL methyl tert-butyl ether, followed by adding 240 μL pre-cooled methanol and mixing well. After sonication in an ice water bath for 20 min, the samples were left at room temperature for 30 min and centrifuged at 12000*g* and 10 °C for 15 min. The upper organic phase was blown dry with N_2, then re-dissolved with 200 μL 90% isopropanol/acetonitrile solution, vortexed thoroughly and centrifuged at 12000*g* and 10 °C for 15 min. The resulting supernatant was collected and used for further analysis.

2.9. Lipidomic analysis

Lipidome profiling of fish lipid samples were carried out by an UHPLC- Q- Orbitrap-MS/MS system (Thermo Fisher Scientific, MA, USA), which equipped with C18 column (100 mm × 2.1 mm, 1.7 μm). A binary solvent system was consisted of mobile phase A (60% acetonitrile and 40% water) and mobile phase B (10% acetonitrile and 90% isopropanol). The gradient elution program was as follows: 0–2 min, 30% B; 2–25 min, 30% B increasing linearly to 100%; 25–35 min, 30% B. The samples were placed in an autosampler at 10 °C during the entire analysis process, and the injection volume was 2.0 μL at a mobile phase flow rate of 0.3 mL/min.

Sample MS1 and MS2 data were collected by a Q-Exactive series mass spectrometer. The ESI source conditions were as follows: Spray voltage 3.0 kV, heater temperature 300 °C, sheath gas flow rate 45 arb, auxiliary gas flow rate 15 arb, purge gas flow rate 1 arb, capillary temperature 350 °C, S-lens RF level 50%, MS1 scan range: 200–1800 *m/z*. The mass-to-charge ratios of lipid molecules and lipid fragments were acquired as follows: 10 fragment profiles were acquired after each full scan (MS2 scan, HCD). MS1 had a resolution of 70,000 at $M/Z = 200$, and MS2 had a resolution of 17,500 at M/Z = 200.

LipidSearch was used for peak detection, identification, grouping, extraction, and alignment. The main parameters were set as follows: precursor tolerance: 5 ppm, product tolerance: 5 ppm, product ion threshold: 5%. The raw data were quality assessed and values with relative standard deviation >30% were filtered out. The peak area data, with ≤50% null values in a single group or all groups, were retained. Then data were normalized using the internal standard for further statistical analyses, including identification quantity statistics, lipid composition analysis and lipid difference analysis.

2.10. Analysis fatty acids

30 mg sample was mixed with 1.0 mL chloroform-methanol solution (2:1, *v*/v), sonicated in an ice water bath for 30 min. The supernatant was added with 2.0 mL 1% sulfuric acid-methanol solution, and placed in a water bath at 80 °C for 0.5 h, followed by an extraction of 1.0 mL n-hexane. The extract was washed with 5.0 mL purified water. 25 μL methyl nonadecanoate was added to the resulting 500 μL supernatant as an internal standard, mixed well and transferred to the injection vial for gas chromatography–mass spectrometry (GC–MS) analysis.

Analysis of fatty acids was carried out on an Agilent DB-WAX capillary column (30 m × 0.25 mm × 0.25 μm) GC system. The injection volume was 10 μL with a 10:1 splitting ratio. A programmed heating was as follows: 50 °C, held for 3 min; increasing linearly to 220 °C at 10 °C/min, maintained 5 min; Increase to 250 °C at 15 °C/min and hold for 10 min. The carrier gas was helium with a carrier gas flow rate of 1.0 mL/min. The MS program was as follows: sample inlet temperature was 280 °C, the ion source temperature was 230 °C, and the transmission line temperature was 250 °*C. electron* bombardment ionization (EI) source, SIM scan mode, electron energy 70 eV. The GC–MS data were extracted using MSD ChemStation software for peak area and retention time, and the calibration curves were plotted to calculate the content of medium and long chain fatty acids in the samples.

2.11. Statistical analysis

The multivariate statistical analysis was performed using principal component analysis (PCA), partial least squares-discriminant analysis (PLS-DA), and orthogonal partial least squares-discriminant analysis (OPLS-DA). The differential lipids and fatty acids were selected based on the Student's *t*-tests ($P < 0.05$) and VIP >1. One-way analysis of variance (ANOVA) with Duncan's test and significant difference analysis ($P < 0.05$) were performed using SPSS software v 21.0 (SPSS Inc., Chicago, IL, USA). Lipid metabolic pathways were revealed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/).

3. Results and discussion

3.1. Microbial growth and changes in TVB-N

The results of microbial growth indicated that the aseptic processing of the fillets was successful and they were not contaminated during refrigeration, as the bacterial counts of the aseptically treated fillets were consistently below 2.0 lg CFU/g throughout the refrigeration process (Fig. S1). The initial bacterial counts of each inoculated group were 3.5, 3.5, and 3.7 lg CFU/g, which were also close to the initial microbial level of 4.0 lg CFU/g for fresh fish fillets (Tan et al., 2022). With the extension of refrigeration time, the number of bacteria increased significantly ($P < 0.05$), especially in the early stages of refrigeration. The multiplication speed of *A. albensis* was much higher than that of *A. sobria*, and the coexistence of *A. sobria* and *A. albensis* greatly increases the number of microorganisms in the later stage. Finally, the co-cultured group reached 9.7 lg CFU/g, significantly higher than 8.7 lg CFU/g of *A. sobria* and 8.9 lg CFU/g of *A. albensis* alone.

The growth of spoilage bacteria in sturgeon fillets directly affects the changes in TVB-N content, as shown in Fig. 1A. In the early storage period, there was no significant change in the TVB-N content of sturgeon fillets in each treatment group, indicating that the generation of TVB-N requires the microorganisms to multiply to a certain number. Finally, the TVB-N content of *A. sobria* inoculation group showed a significant increase ($P < 0.05$) change, and the content reached 22.62 mg/100 g on the 12th day of refrigeration. It is evident that *A. sobria* has a high capacity to produce spoilage metabolites. However, from the results of the *A. albensis* inoculated group and the co-inoculated group of *A. albensis* and *A. sobria*, the addition of *A. albensis* did not increase the production of TVB-N and even decrease the TVB-N content. This suggested that *A. albensis* has a low capacity to produce spoilage metabolites by protein degradation.

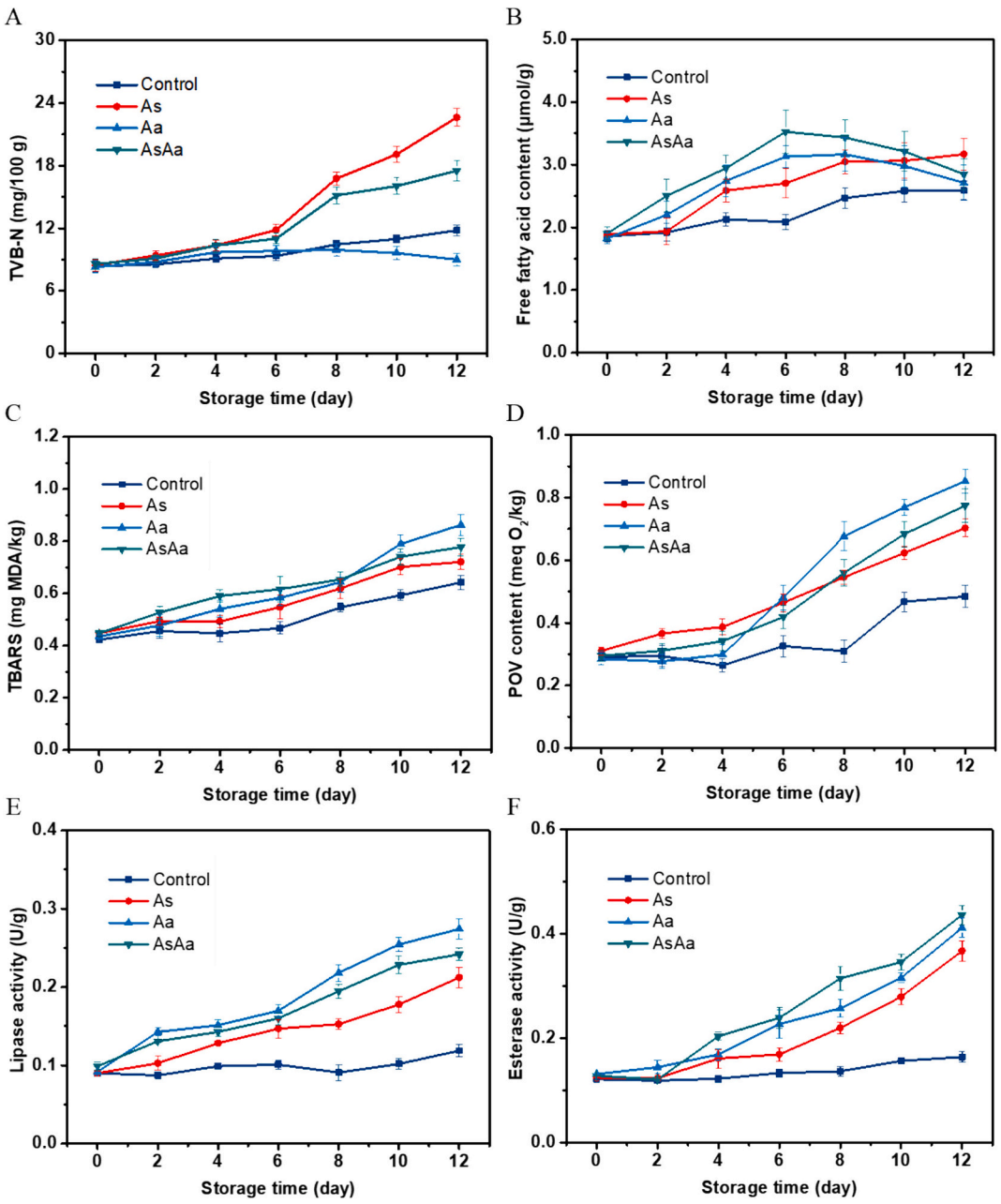


Fig. 1. Changes in total volatile basic nitrogen (A), free fatty acid content (B), TBARS value (C), POV value (D), lipase activities (E), and esterase activities (F) of sturgeon fillets in different inoculated groups during refrigeration at 4 °C. Control, As, Aa, and AsAa represent the uninoculated groups, *A. sobria* inoculated groups, *A. albensis* inoculated groups, and *A. sobria* and *A. albensis* co-cultured groups, respectively.

3.2. Changes in free fatty acid

The changes in free fatty acid content in sturgeon fillets from different inoculation groups were shown in Fig. 1B. The free fatty acid content of the control group and *A. sobria* inoculated group increased gradually with storage time, rising from an initial content of 1.9 μmol/g to 2.6 and 3.2 μmol/g by the end of refrigeration period, respectively. In the *A. albensis* inoculated group and the co-cultured group of *A. sobria* and *A. albensis*, the free fatty acid content increased significantly ($P < 0.05$) during the first 6 days of refrigeration to 3.1 μmol/g and 3.5 μmol/g, respectively, and then gradually decreased. Free fatty acids were first released from lipids and then further utilized and oxidized by microorganisms (Balakrishna et al., 2020). The results indicated that *A. albensis*, the dominant bacterium at the end of refrigeration, has a strong ability to degrade lipids and utilize fatty acids. This is consistent with the previous spoilage metabolome results (Tan, Li, & Shang, 2023), and further demonstrates that *A. albensis* does affect lipid metabolism in sturgeon fillets.

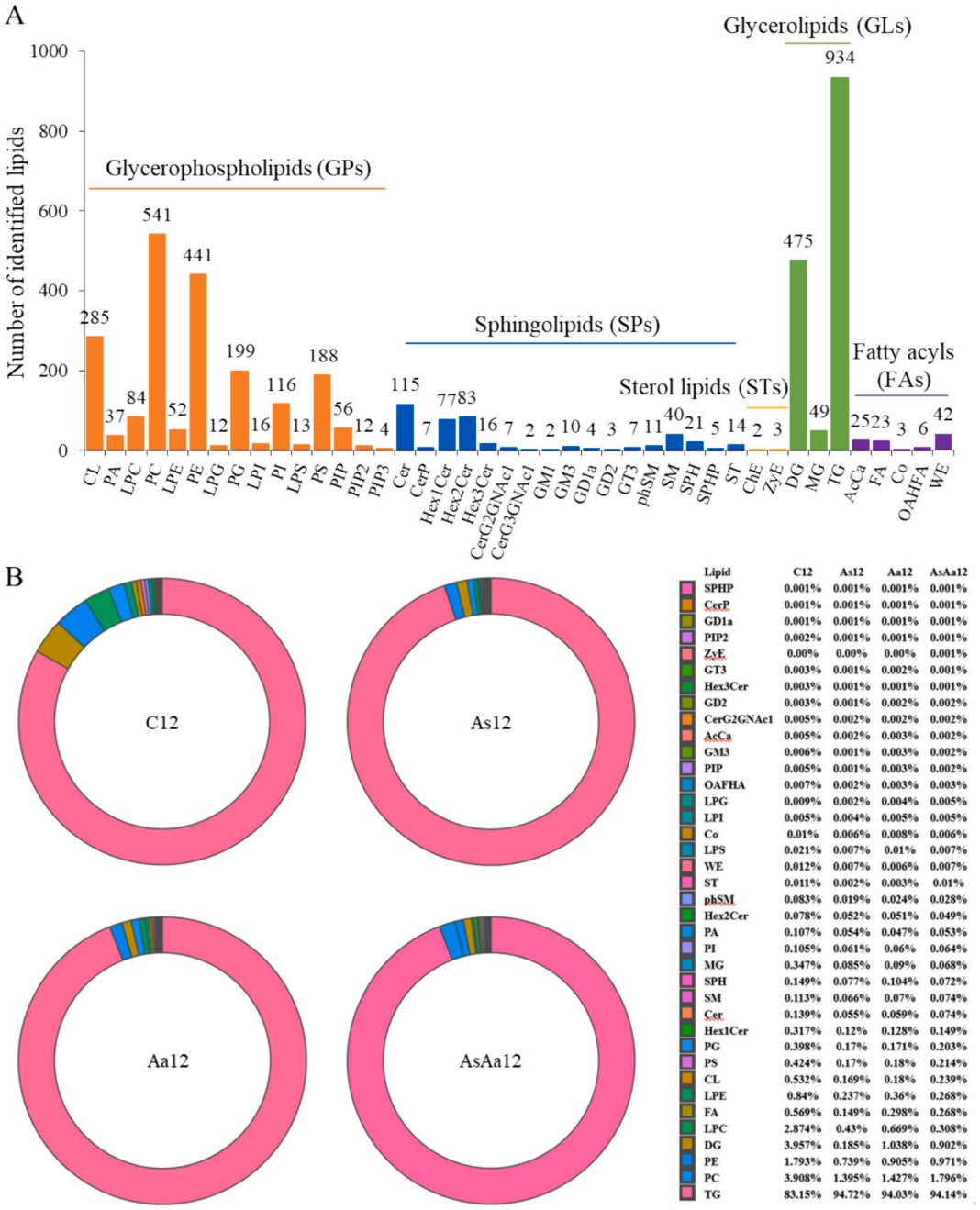
3.3. Changes in TBARS and POV values

Sturgeon fillets are susceptible to lipid oxidation during aerobic storage, and the primary and intermediate products of lipid oxidation can be characterized by the common indicators TBARS and POV. As shown in Fig. 1C and D, the TBARS value and POV value of the inoculated group increased significantly with storage time ($P < 0.05$) and were higher than those of the control group, indicating the dominant bacteria in the sturgeon fillets significantly promoted lipid oxidation. The TBARS value of the co-cultured group was higher than those of the individually inoculated group in the early storage period, but lower than

that of the *A. albensis* inoculated group in the later period. This may be since *A. albensis* can produce more key enzymes for lipid oxidation such as lipoxygenase at the end of storage (Mariutti & Bragagnolo, 2017). The changes in the POV values also indicated that the *A. albensis* inoculated group underwent a more severe oxidation at the end of the storage period than the others, and this oxidation may be related to the lipase production of microorganisms through metabolism. However, the increase of POV was greater than that of TBARS in the later stages of refrigeration, probably because the decomposition of hydroperoxides was more slowly than their generation, and unstable secondary oxidation products such as aldehydes were further oxidized or degraded to form low molecular weight VOCs during long-term refrigeration (Wang et al., 2023). Al-Dalali et al. (2022) pointed out that changes in oxide content are also related to protein-lipid interactions, in which malondialdehyde and hexanal form polymers with lysine residues in meat proteins.

3.4. Lipase and esterase activities

The metabolism of lipids in fish meat is mainly attributed to endogenous enzymes and microbial activities, where microorganisms can produce a variety of lipolytic enzymes to hydrolyze lipids (Feng et al., 2021). For example, microbial lipases can act on triglycerides and phospholipids to produce free fatty acids and promote saturated fatty acids to form fatty acyl hydroperoxides, aldehydes, and carbonyl species (Wang et al., 2022; Yu et al., 2020). To verify whether the changes in fatty acids were related to lipolytic enzymes secreted by microorganisms, lipase and esterase activities were measured. As shown in Fig. 1E,



Lipid	C12	As12	Aa12	AsAa12
SPHP	0.001%	0.001%	0.001%	0.001%
CerP	0.001%	0.001%	0.001%	0.001%
GD1a	0.001%	0.001%	0.001%	0.001%
PIP2	0.002%	0.001%	0.001%	0.001%
ZyE	0.00%	0.00%	0.00%	0.001%
GT3	0.003%	0.001%	0.002%	0.001%
Hex3Cer	0.003%	0.001%	0.001%	0.001%
GD2	0.003%	0.001%	0.002%	0.002%
CerG2GNAc1	0.005%	0.002%	0.002%	0.002%
AcCa	0.005%	0.002%	0.003%	0.002%
GM3	0.006%	0.001%	0.003%	0.002%
PIP	0.005%	0.001%	0.003%	0.002%
OAFHA	0.007%	0.002%	0.003%	0.003%
LPG	0.009%	0.002%	0.004%	0.005%
LPI	0.005%	0.004%	0.005%	0.005%
Co	0.01%	0.006%	0.008%	0.006%
LPS	0.021%	0.007%	0.01%	0.007%
WE	0.012%	0.007%	0.006%	0.007%
ST	0.011%	0.002%	0.003%	0.01%
phSM	0.083%	0.019%	0.024%	0.028%
Hex2Cer	0.078%	0.052%	0.051%	0.049%
PA	0.107%	0.054%	0.047%	0.053%
PI	0.105%	0.061%	0.06%	0.064%
MG	0.347%	0.085%	0.09%	0.068%
SPH	0.149%	0.077%	0.104%	0.072%
SM	0.113%	0.066%	0.07%	0.074%
Cer	0.139%	0.055%	0.059%	0.074%
Hex1Cer	0.317%	0.12%	0.128%	0.149%
PG	0.398%	0.17%	0.171%	0.203%
PS	0.424%	0.17%	0.18%	0.214%
CL	0.532%	0.169%	0.18%	0.239%
LPE	0.84%	0.237%	0.36%	0.268%
FA	0.569%	0.149%	0.298%	0.268%
LPC	2.874%	0.43%	0.669%	0.308%
DG	3.957%	0.185%	1.038%	0.902%
PE	1.793%	0.739%	0.905%	0.971%
PC	3.908%	1.395%	1.427%	1.796%
TG	83.15%	94.72%	94.03%	94.14%

Fig. 2. The lipid subclasses and categories identified in sturgeon fillets (A) and percentages of lipid subclasses and categories in different inoculated group samples (B). C, As, Aa, and AsAa represent the control groups, *A. sobria* inoculated groups, *A. albensis* inoculated groups, and *A. sobria* and *A. albensis* co-cultured groups, respectively. 12 represented the 12th day of storage.

both *A. sobria* and *A. albensis* possessed strong lipase-producing ability, and the lipase activity of their inoculated group of fillets increased significantly with storage time ($P < 0.05$) during refrigeration, and *A. albensis* exhibited a stronger lipase-producing ability than *A. sobria*. Researchers have demonstrated that some lipase-producing bacteria has an ability to utilize fat as their sole carbon source (Li et al., 2014; Sarkar et al., 2012). This may be one of the reasons why they still grow faster during the later stages of refrigeration.

The ability of microorganisms to secrete esterases was shown in Fig. 1F, where *A. albensis* and *A. sobria* similarly showed a high capacity to secrete extracellular esterases. Compared with inoculating only the specific spoilage bacterium *A. sobria*, the addition of the dominant bacterium *A. albensis* significantly increased the esterase activity ($P < 0.05$). This may be the reason why there are more species of differential fatty acids in the co-cultured group than in the single inoculation group. Under the action of microbial extracellular lipase and esterase, lipids were hydrolyzed, causing the fatty acid content to increase. Changes in lipase and esterase activities can well reflect the level of lipid metabolism because they have multiple catalytic abilities and can also be used to express the activities of phospholipases, cholesterol esterases, amidases, etc. (Bora et al., 2013).

3.5. Analysis of lipid metabolism

3.5.1. Lipids in refrigerated sturgeon fillets

Excluding ion features with RSD >30%, a total number of 4041 lipid molecules in samples were identified in both ESI+ and ESI- modes, which were classified into glycerophospholipids (GPs), glycerolipids (GLs), sphingolipids (SPs), fatty acyls (FAs) and sterol lipids (STs) (Fig. 2A and Table S1). Among them, 934 triglycerides (TG) were detected, accounting for 23.11% of the total lipids, followed by 541 phosphatidylcholine (PC) (13.38%), 475 diacylglycerol (DG) (11.75%) and 441 phosphatidylethanolamine (PE) (10.91%). These findings are consistent with what Domínguez et al. (2019) reported that triglycerides (TG) and phospholipids (PL) occupied the largest portion of intramuscular and subcutaneous lipids, while cholesterol and other lipids made smaller contributions.

The main lipid composition and content distribution in the samples were shown in Fig. 2B. The largest proportion in different treatment groups was TG, and the presence of microorganisms affected the lipid composition.

3.5.2. Multivariate statistical analysis of sturgeon fillet samples

To ensure the stability of the experimental apparatus and the reproducibility of the experiments, Pearson correlation analysis was performed on the QC samples (Fig. S2A). The correlation coefficients were all >0.95, indicating that the test instrument is stable, and the repeatability of the experiment is good. The lipidomic changes in sturgeon fillets of each inoculated group and the control group were performed using a PCA model. There were significant differences in lipid metabolite profiles between each inoculated group and the control group, but fewer difference in the metabolic profiles were observed in the *A. sobria* inoculation and co-cultured samples (Fig. S2B). This indicated that *A. sobria* is the most dominant microorganism causing lipid changes in sturgeon fillets.

The OPLS-DA score plots showed that each group of samples for comparison were clearly separated, and the samples from the same group were well aggregated (Fig. 3A, C, E, G). The interactive validation of the OPLS-DA model mainly reflects the interpretability of the model's X-variables and Y-variables (R^2X and R^2Y), as well as the model's degree of predictability (Q^2). The R^2Y and Q^2 values for As12 vs C12 were 0.92 and 0.93 respectively (Fig. 3B), while that for the other three pairs were 0.90–0.97 (Fig. 3D, F and H). These results indicated that the model without overfitting, and the data has high reliability and predictive ability. Therefore, both PCA and OPLS-DA models indicated significant differences between the inoculated group and control group, and the changes of lipids in sturgeon fillets during refrigeration were influenced by microbial composition.

3.5.3. Lipid changes during sturgeon fillets refrigeration

Lipids identified in the fillet samples consisted of five subclasses, and their content changes are shown in Fig. 4A. Compared to the control group, the content of glycerophospholipids and fatty acyls decreased in the *A. sobria* inoculated group, whereas the content of glycerolipids, sphingolipids, and sterol lipids increased; The *A. albensis* inoculated group significantly increased the content of sphingolipids and fatty acyls, while the co-cultured group increased the content of sphingolipids and sterol lipids.

Further analysis of lipid subclass showed that 42 categories were detected, and 18 categories with significant differential changes, as shown in Fig. 4B. The categories whose levels increased in the *A. sobria* inoculated group compared to the control group were glycosylceramide series (including CerG3GNAc1, Hex2Cer and Hex3Cer), phosphoceramide (CerP), cholesteryl ester (ChE), cofactor (Co), ganglioside (GD1a), phosphatidic acid (PA), phosphatidylinositol (PI), phosphatidylethanolamine (PE), sphingomyelin (SM), sphingomyelin (SPH), sphingosine phosphate (SPHP), and wax esters (WE), and the decreasing categories were diglycerides (DG), lysophosphatidylcholine (LPC), monoglycerides (MG), and zymosterol ester (ZyE). The categories in *A. albensis* inoculation group and co-cultured group suggested that *A. sobria* and *A. albensis* exhibit similar lipid metabolism properties, but they differ in their ability to transform different lipids. Compared with *A. sobria*, *A. albensis* was more capable of producing CerP, Co, GD1a, PE and SPH, while it was less capable of producing ChE, Hex2Cer, Hex3Cer, PA, PI and SPHP, and more capable of utilizing DG. These results indicated that the microorganisms in sturgeon fillets mainly utilized DG, LPC, and MG to convert them into other subclasses during refrigeration, and both *A. sobria* and *A. albensis* showed strong lipid metabolic capabilities.

3.5.4. Fatty acids composition

To obtain more comprehensive information on lipid metabolism, medium- and long-chain fatty acids were further examined. Compared to control group, *A. sobria* inoculated group significantly ($P < 0.05$) increased the content of undecanoic acid (C11:0), palmitic acid (C16:0), eicosapentaenoic acid (C20:5 N3) and docosahexaenoic acid (C22:6 N3) (Fig. S3A). And the content of stearic acid (C18:0) was significantly increased in the *A. albensis* inoculated group (Fig. S3B). The *A. albensis* and *A. sobria* co-cultured group significantly increased the content of octanoic acid (C8:0), undecanoic acid, lauric acid (C12:0), tridecanoic acid (C13:0), myristic acid (C14:0), palmitic acid (C16:0), palmitoleic acid (C16:1 N7), trans-oleic acid (C18:1TN9), trans-linoleic acid (C18:2TTN6), linoleic acid (C18:2 N6), γ-linolenic acid (C18:3 N6), arachidonic acid (C20:0), eicosatrienoic acid (C20:3 N3), eicosapentaenoic acid (C20:5 N3), docosadienoic acid (C22:2 N6), docosatetraenoic acid (C22:4 N6), docosapentaenoic acid (C22. 5 N6), and docosahexaenoic acid (Fig. S3C). However, the content of fatty acid fractions was lower in the *A. albensis* inoculated group compared to *A. sobria* inoculated group, and it was the stearic acids that were significantly different ($P < 0.05$) (Fig. S3D). Whereas, the co-cultured group significantly increased the content of undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid (C15:0), pentadecenoic acid (C15:1 N5), palmitic acid, trans-oleic acid, oleic acid (C18:1 N9), trans-linoleic acid, behenic acid (C22:0), docosatetraenoic acid, docosapentaenoic acid (C22:5 N3), and docosahexaenoic acid (C22:6 N3) compared to the *A. sobria* inoculated group alone ($P < 0.05$) (Fig. S3E). It can be seen that *A. sobria* had a greater effect on fatty acids than *A. albensis*, and the co-cultured group significantly altered the composition of medium- and long-chain fatty acids in the sturgeon fillets.

Further, these significantly different fatty acids (VIP >1, $P < 0.05$) of the samples were used to perform a hierarchical cluster analysis (Fig. 5A). The samples with the same treatment were clustered together

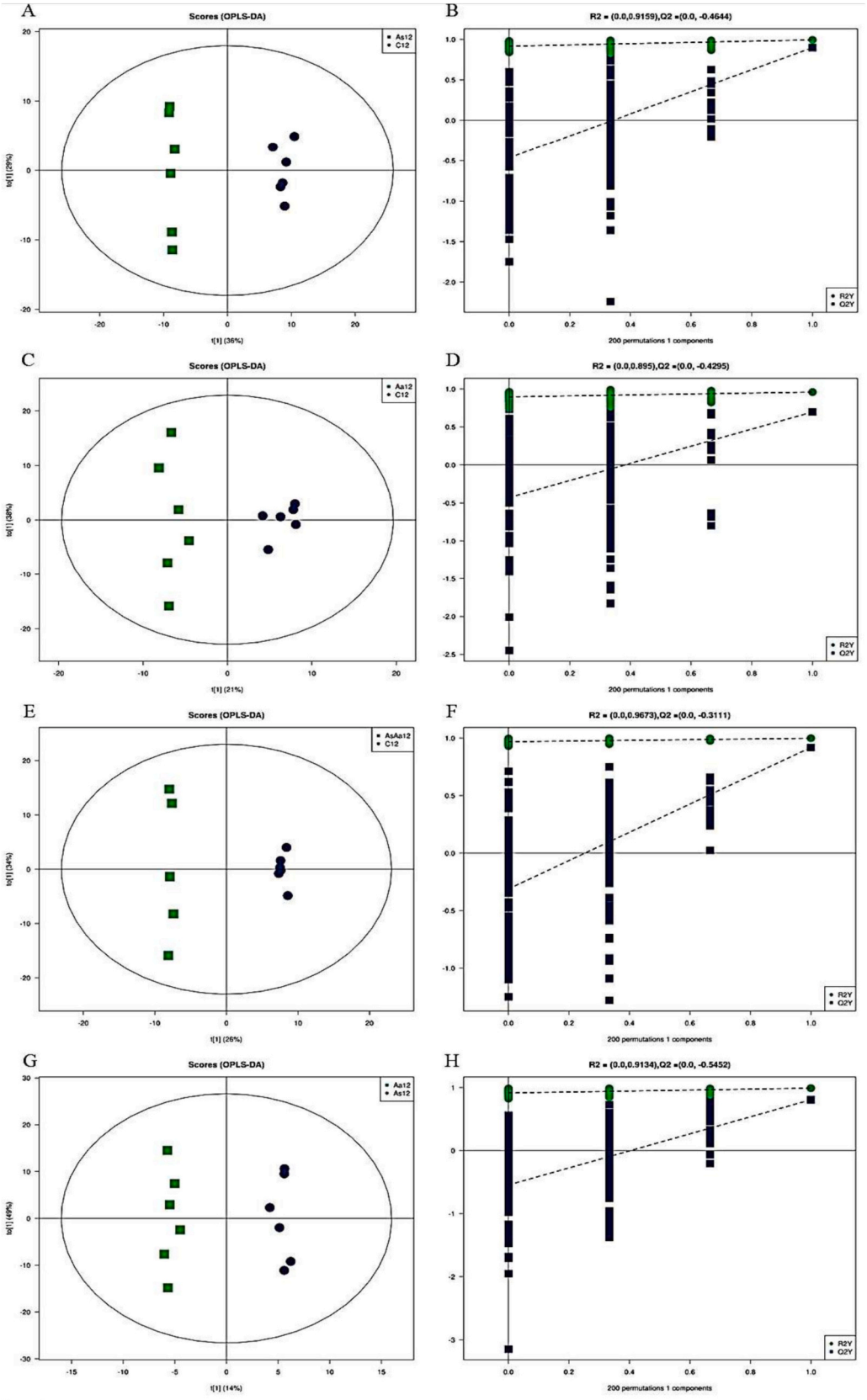


Fig. 3. The orthogonal partial least squares-discriminant analysis (OPLS-DA) score plots and substitution test plot for As vs C (A and B), Aa vs C (C and D), AsAa vs C (E and F), and Aa vs As (G and H).

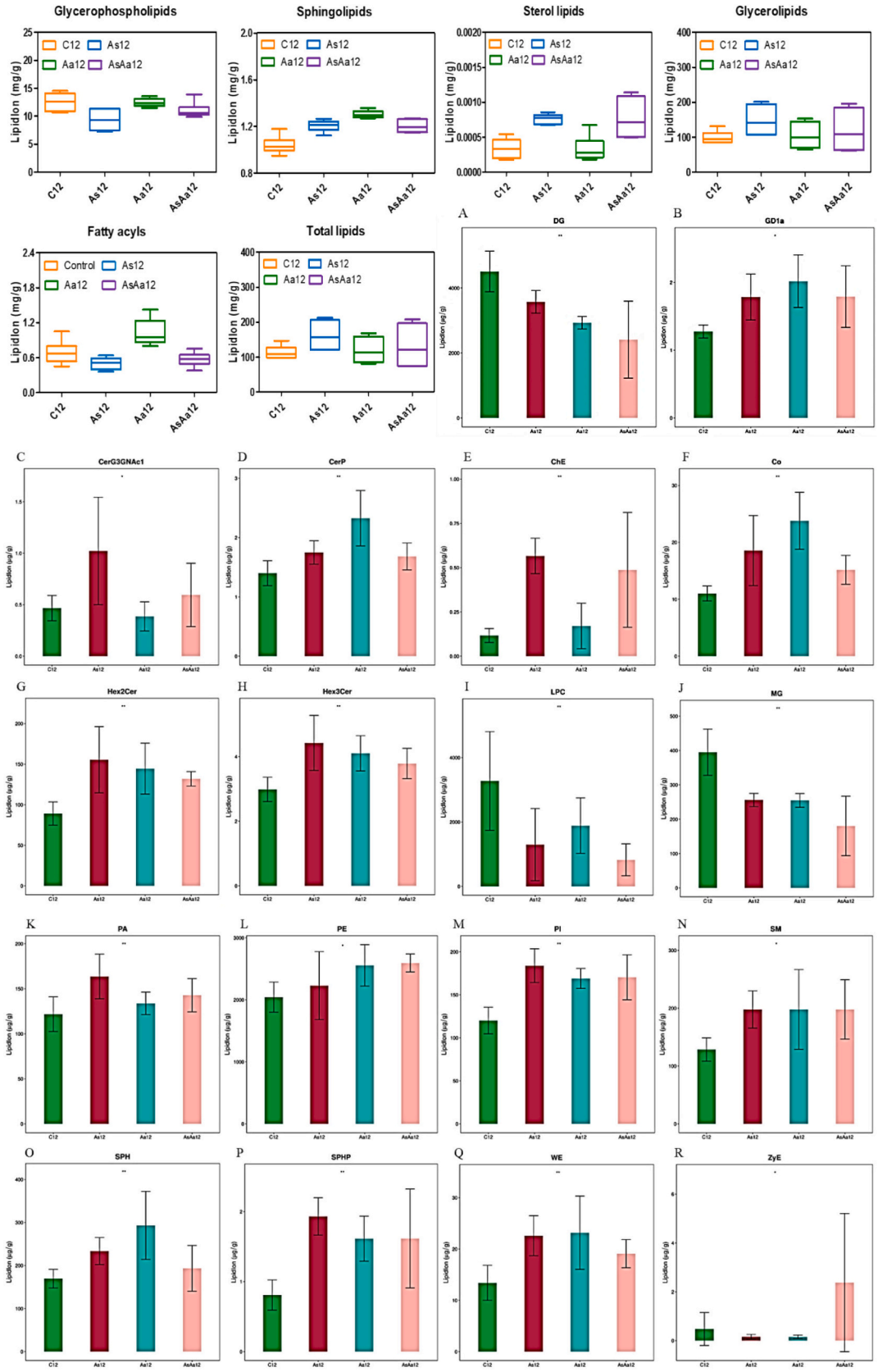


Fig. 4. Changes in lipid subclasses (A) and Class levels of significantly different categories (B) in sturgeon fillets from different inoculated groups during refrigeration.

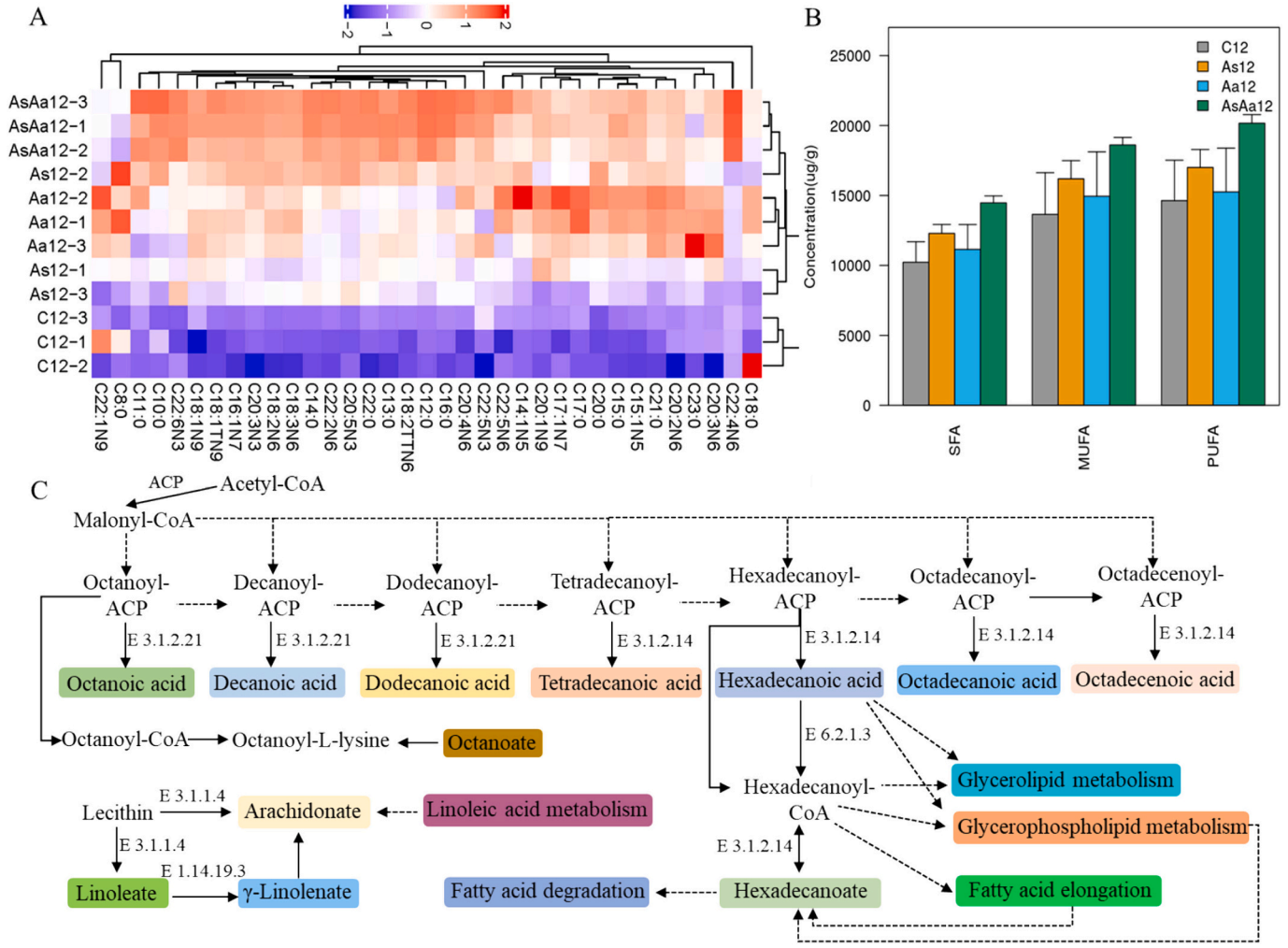


Fig. 5. Clustering heat map of differential fatty acids (A), changes in different fatty acid contents (B), and KEGG metabolic pathways involved in differential fatty acids (C) of sturgeon fillets from different inoculated groups during refrigeration at 4 °C.

in a cluster, which suggests that the fatty acid metabolic processes between samples from the same inoculation group are relatively close, while large differences were observed between the different inoculation groups, especially between the control group and the co-cultured group. The spoilage bacteria significantly changed the composition of medium- and long-chain fatty acids in sturgeon fillets, and *A. albensis* had a greater effect on fatty acids than *A. sobria*. In addition, saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in the fish samples increased after refrigerated storage (Fig. 5B). For samples from the control group, *A. sobria* inoculated group, *A. albensis* inoculated group, and co-inoculated group, SFA increased from 7.07 mg/g sample on day 0 to 10.23, 12.29, 11.15, and 14.47 mg/g on day 12, respectively. The results are consistent with the studies of Al-Dalali et al. (2022) and Nazemroaya et al. (2009), who found that the SFA, MUFA, and PUFA content in sturgeon fillets increased with storage time. Unsaturated fatty acids are easily oxidized during storage, which may be one of the reasons for the significant increase in TBARS and POV values in the inoculated groups compared to the control group. Taken together, spoilage microorganisms significantly promoted fatty acid production compared to the control group, especially when *A. albensis* was co-presented with *A. sobria*.

Based on the relationship between the significantly different fatty acid molecules detected in the samples and the KEGG pathway, the fatty acid metabolism network was schematically mapped (Fig. 5C). Fatty acid metabolism by spoilage microorganisms involves the synthesis, degradation, and chain elongation of fatty acids. The lipids in fish fillet are hydrolyzed into free fatty acids by endogenous enzymes and microbial enzymes, which are further metabolized and oxidized to generate acetyl-CoA and involved in the production of a series of fatty acids under the action of related synthases. The intermediate products of fatty acid metabolism, palmitic acid and palmitoyl-CoA are related to glyceride metabolism, glycerophospholipid metabolism and chain elongation of fatty acids, which in turn produced methyl palmitate and palmitoyl coenzyme A (Fig. 5C). The metabolism of unsaturated fatty acids in fish fillets is also affected by spoilage microorganisms, such as linoleic acid metabolism and lecithin metabolism to produce arachidonic acid.

3.5.5. *Lipid metabolic pathways of sturgeon fillets*

Lipid molecules with significant differences were selected for KEGG pathway analysis and potential pathways for lipid transformation were schematically mapped in combination with the identified intermediates (Fig. 6). The related metabolic pathways are mainly involved in glycerophospholipid metabolism, glyceride metabolism, linoleic acid and other fatty acid metabolism, and sterol lipid biosynthesis. It is reported that cholesterol biosynthesis is influenced by cytochrome P450 aromatase, cholesterol sidechain lyase and 17α-hydroxylase/lyase (Tokarz et al., 2015). The formation of cholesterol ester in this study may be caused by the cholesterol O-acyltransferase secreted by spoilage

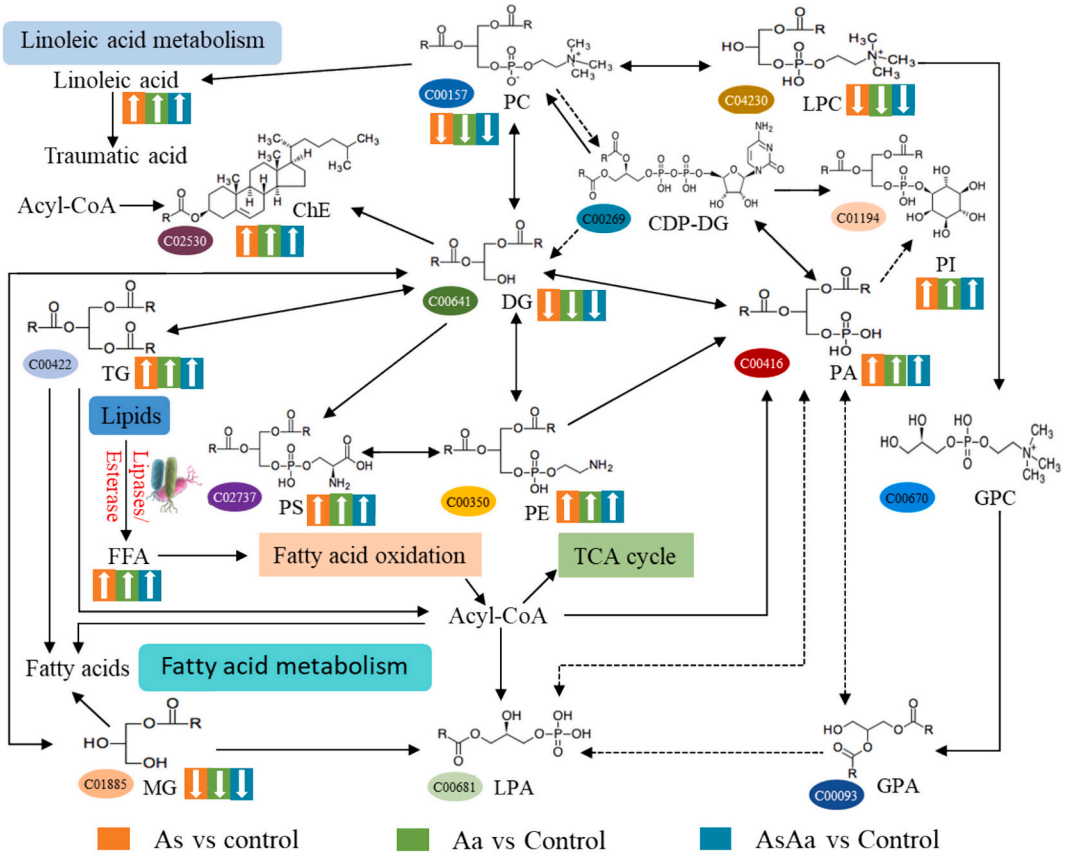


Fig. 6. Potential pathways involved in the lipid transformation in sturgeon fillets induced by spoilage bacteria during refrigeration. Numbers represent the ID of each lipid subclass.

microorganisms that transfers long-chain fatty acyl from DG to cholesterol, and at the same time release 1-acylglycerol (Wang et al., 2022). Glycerophospholipids play a key role in cell composition and metabolic regulation (Hugues Chap, 2016). The results suggested that PE, PA, PC, LPC, and PI were the major lipids associated with glycerophospholipid metabolic pathways in sturgeon fillets. PC and LPC are transformed into each other through the action of enzymes (Fig. 5), the phospholipase A2 breaks down PC molecules to produce LPC (207), and LPC and acetyl-CoA can form PC by the action of lysophospholipid acyltransferase (Wang et al., 2022). CDP-DG, the main precursor of phospholipid synthesis, also plays a critical role in the lipid transformation process of fish fillets, the CDP-DG can convert PA into PC, PI, PG, and cardiolipin (CL) through CDP-DG synthase (Xu et al., 2019). PA is a key intermediate metabolite in the cell membrane glycerophospholipid synthesis pathway and is involved in a variety of cellular processes (Zhang et al., 2023), and it can be generated and metabolized by various types of lipolytic enzymes (e.g., phospholipases, lipid kinases, and phosphatases) (Tei & Baskin, 2020). The increase in PA content during the refrigeration process is mainly caused by microorganisms using other substances to synthesize their own cell membranes for reproduction.

TG and DG are the main lipids identified in the refrigerated sturgeon fillets that are related to the glyceride metabolism pathway, where DG can be synthesized by phospholipase C-catalyzed hydrolysis of PE and PC, and converted by PA, TG, MG, and CDP-DG (Chiang et al., 2021). DG can also act as a lipid anchor for PE and PC synthesis in the presence of the corresponding phosphotransferases (Geiger et al., 2013). The results of this study showed that the DG content in fish fillets decreased while the TG content increased under the action of microbial enzymes, and the conversion of DG took place in the direction of TG production. TG is formed mainly through the covalent binding of DG to fatty acyl-CoA, which is catalyzed by diacylglycerol acyltransferase (DGAT) (Lu et al., 2015). Among them, DGAT1 preferentially esterifies preformed FA and is in charge of the assembly and secretion of TG-rich very low-density lipoprotein particles, whereas DGAT2 esterifies FA to synthesize TG (Bhatt-Wessel et al., 2018; Hu et al., 2020). In addition, the lipids in fish fillets are decomposed by microbial extracellular enzymes to generate free fatty acids, which further undergo fatty acid metabolism and oxidation during refrigerated storage. This is due to the action of lipolytic enzymes and lipoxygenases (Feng et al., 2021). When the concentration of oxidation products of lipids in fish meat reaches a certain level, they begin to decompose, forming alkoxyl groups, which then pass through different pathways to form hydrocarbons, alcohols, aldehydes, acids and other compounds (Feng et al., 2021). In summary, significant lipid changes took place in sturgeon fillets during refrigeration, and such changes are associated with several metabolic pathways and various enzymes. However, the role of specific related enzymes in refrigerated fish fillets needs to be further investigated.

3.6. Analysis of VOCs

Lipolysis and lipid oxidation are the main sources of flavor compounds in fish products. Fish meat oxidation and microbial action will produce a large number of VOCs, so this study used GC-IMS to detect VOCs in sturgeon fillets of different inoculation groups on days 0, 6, and 12. A total of 82 VOCs were identified in the fillets, mainly including alcohols, aldehydes, ketones, esters, and sulfur-containing compounds, as well as 20 unclassified compounds (Table S2). Many of these VOCs existed in different degrees of polymerization, including monomers, dimers, and trimers, depending on their concentration. These aggregates showed similar retention times but different drift times.

The contour plot of volatiles was shown in Fig. 7A, where the spot intensity of the VOCs distribution in the samples reflects the changes in

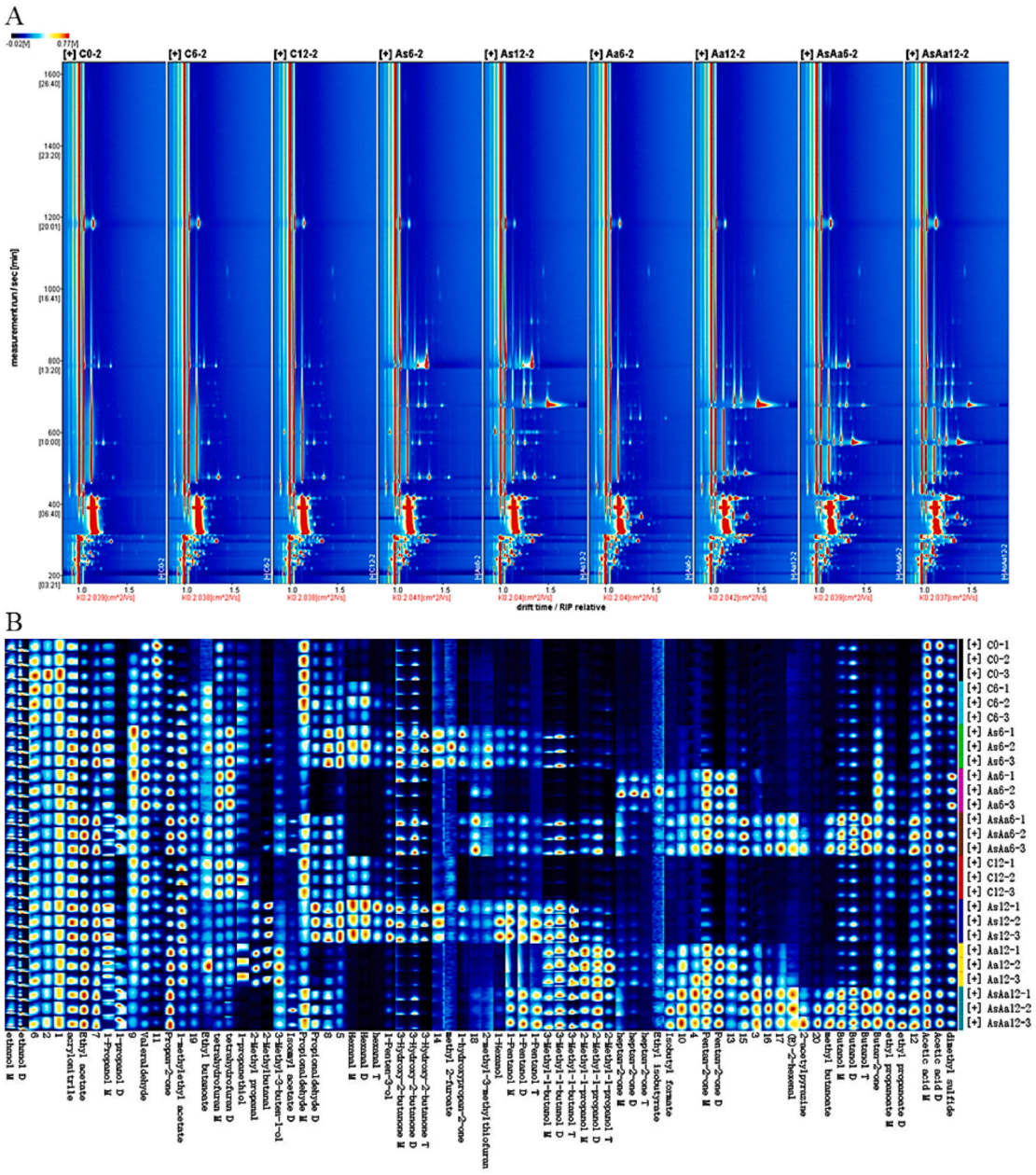


Fig. 7. The VOCs identified in different inoculated fillets on days 0, 6 and 12 during storage at 4 °C. (A) Contour plots corresponding to the signals detected in the sample, (B) Comparison of the fingerprints of VOCs in the sample determined by GC-IMS.

compounds during storage. The volatiles in the fillets increased as the storage time increased. The production of VOCs in fillets during refrigeration is mainly due to the degradation of its own substances and the metabolism of microorganisms (Parlapani et al., 2017). The comparative fingerprint analysis of VOCs was shown in Fig. 7B, inoculation of specific spoilage bacterium *A. sobria* and dominant bacterium *A. albensis* both increased a large amount of VOCs. On the 6th day of refrigeration, VOCs such as 1-hexanol, 1-pentanol, 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-3-methylthiofuran, 1-hydroxypropan-2-one, 2-methylbutanal, and 3-hydroxy-2-butanone were substantially increased in the *A. sobria* inoculated group and the co-cultured group; VOCs such as ethyl isobutyrate, isobutyl formate, ethyl propanoate, heptan-2-one, pentan-2-one, propan-2-one, 3-methyl-3-buten-1-ol, and dimethyl sulfide increased significantly in the *A. albensis* inoculated group and the co-cultured group. However, VOCs such as ethyl acetate, isoamyl acetate, methyl butanoate, 1-propanol, 2-methyl-1-propanol, butanol, 2-acetylpyrazine, and (E)-2-hexenal increased significantly only in the co-cultured group. These results suggested that the co-cultured group had a greater effect on volatiles than the single inoculated group, and the VOCs such as ethyl isobutyrate, ethyl propanoate, isobutyl formate, pentan-2-one, propan-2-one, 2-butanone, 3-methyl-3-buten-1-ol, and dimethyl sulfide were mainly associated with *A. albensis*, while 1-hexanol, 1-pentanol, 1-penten-3-ol, 3-methyl-1-butanol, 2-methyl-3-methylthiofuran, 1-hydroxypropan-2-one, 2-methylbutanal, 3-hydroxy-2-butanone, and propionaldehyde were mainly related to *A. sobria*. Microbial interactions significantly affected the composition and content of volatiles such as ethyl acetate, ethyl propionate, isoamyl acetate, methyl butyrate, 1-propanol, 2-methyl-1-propanol, butanol, 2-acetylpyrazine, (E)-2-hexenal.

In this study, pentan-2-one and heptan-2-one may be formed by oxidation of fillets, as they are often produced in high-oxygen packaged meat (Domínguez et al., 2019). Ketones such as heptan-2-one, aldehydes such as 2-methylbutanal, and sulfur-containing compounds are related to the production of off-odors (Parlapani et al., 2017), which might also

be responsible for the development of irritating odors in the late stage of refrigeration, and their production is related to both *A. sobria* and *A. albensis*. The oxidative decomposition of lipids, the thiamine degradation, and the interaction of metabolites produced by these reactions form most of the flavor components in meat and meat products (Ramalingam et al., 2019). For example, some aldehydes and alcohols are common in meat products because they have a low threshold and most of them come from enzymatic oxidation or auto-oxidation of unsaturated fatty acids (Yin et al., 2021). Aldehydes can be partially reduced to the corresponding alcohols by aldehyde reductase (Wu et al., 2020). Linoleic acid, linolenic acid, and eicosapentaenoic acid in fish meat generate various hydroperoxides after homolysis, which further produce VOCs such as alcohols, esters, and aldehydes (Benet et al., 2015). Among them, hexanal is generally considered to be an excellent predictor of the level of fat oxidation (Yin et al., 2021). 1-Hexanol is produced by the reduction of hexanal or by oxidation of linoleic acid (Merlo et al., 2021). Furans are usually produced during carbohydrate catabolism due to lipid oxidation and amino acid catabolism, and they might be produced by thermal decomposition (Yin et al., 2021).

4. Conclusion

In present study, the biochemical changes and lipid metabolism profiles of sturgeon fillets interplayed between spoilage and dominant bacteria during the refrigeration were investigated. *A. sobria* reduced fatty acyls and glycerophospholipids while increasing glycerolipids, sphingolipids, and sterol lipids via extracellular lipases and esterases. *A. albensis* mainly elevated sphingolipids and fatty acyls. Spoilage bacteria in sturgeon fillets mainly utilize DG, LPC and MG lipids and convert them into PE, PS, TG, PA and other categories. Remarkably, *A. albensis* exhibited stronger extracellular lipase and esterase production than *A. sobria*, promoting lipid metabolism and oxidation through interaction. The lipid metabolic pathways were mainly involved in glycerophospholipid metabolism, glyceride metabolism, linoleic acid and other fatty acid metabolism, and accompanied by the production of VOCs. Microbial interactions significantly affected the composition and content of VOCs, resulting in the production of more ketones, aldehydes, and sulfur containing compounds that cause off flavors in refrigerated sturgeon fillets. However, further studies are needed to investigate the precise roles of relevant microbial enzymes on the lipid profiles of sturgeon fillets during storage.

CRediT authorship contribution statement

Chunming Tan: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Shiqi Zhang:** Resources, Data curation. **Fanglei Zou:** Software, Investigation. **Boya Gao:** Investigation. **Yujin Li:** Writing – review & editing. **Pinglan Li:** Writing – review & editing, Project administration, Funding acquisition. **Nan Shang:** 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.2024.101714.

References

Al-Dalali, S., Li, C., & Xu, B. (2022). Effect of frozen storage on the lipid oxidation, protein oxidation, and flavor profile of marinated raw beef meat. *Food Chemistry, 376*, Article 131881. https://doi.org/10.1016/j.foodchem.2021.131881

AOCS.. (2003). *Official method Cd 8-53. Champaign-Urbana.* Illinois, USA: American Oil Chemists' Society.

Balakrishna, M., Ma, J., Liu, T., Geng, Z., Li, P., Wang, D., Zhang, M., & Xu, W. (2020). Hydrolysis of oxidized phosphatidylcholines by crude enzymes from chicken, pork and beef muscles. *Food Chemistry, 313*, Article 125956. https://doi.org/10.1016/j.foodchem.2019.125956

Benet, I., Guàrdia, M. D., Ibañez, C., Solà, J., Arnau, J., & Roura, E. (2015). Analysis of SPME or SBSE extracted volatile compounds from cooked cured pork ham differing in intramuscular fat profiles. *LWT- Food Science and Technology, 60*(1), 393–399. https://doi.org/10.1016/j.lwt.2014.08.016

Bhatt-Wessel, B., Jordan, T. W., Miller, J. H., & Peng, L. (2018). Role of DGAT enzymes in triacylglycerol metabolism. *Archives of Biochemistry and Biophysics, 655*, 1–11. https://doi.org/10.1016/j.abb.2018.08.001

Bora, L., Gohain, D., & Das, R. (2013). Recent advances in production and biotechnological applications of thermostable and alkaline bacterial lipases. *Journal of Chemical Technology and Biotechnology, 88*(11), 1959–1970. https://doi.org/10.1002/jctb.4170

Chap, H. (2016). Forty five years with membrane phospholipids, phospholipases and lipid mediators: A historical perspective. *Biochimie, 125*, 234–249. https://doi.org/10.1016/j.biochi.2016.04. 002

Chen, Y., Cai, W., Shi, Y., Dong, X., Bai, F., Shen, S., Jiao, R., Zhang, X., & Zhu, X. (2020). Effects of different salt concentrations and vacuum packaging on the shelf-stability of Russian sturgeon (*Acipenser gueldenstaedti*) stored at 4 °C. *Food Control, 109*, Article 106865. https://doi.org/10.1016/j.foodcont.2019.106865

Chiang, Y.-P., Li, Z., Chen, Y., Cao, Y., & Jiang, X.-C. (2021). Sphingomyelin synthases 1 and 2 exhibit phosphatidylcholine phospholipase C activity. *Journal of Biological Chemistry, 297*(6), Article 101398. https://doi.org/10.1016/j.jbc.2021.101398

Domínguez, R., Pateiro, M., Gagaoua, M., Barba, F. J., Zhang, W., & Lorenzo, J. M. (2019). A comprehensive review on lipid oxidation in meat and meat products. *Antioxidants, 8*, 429. https://doi.org/10.3390/antiox8100429

Feng, L., Tang, N., Liu, R., Gong, M., Wang, Z., Guo, Y., Wang, Y., Zhang, Y., & Chang, M. (2021). The relationship between flavor formation, lipid metabolism, and microorganisms in fermented fish products. *Food & Function, 13*, 5685–5702. https://doi.org/10.1039/d1fo00692d

Geiger, O., López-Lara, I. M., & Sohlenkamp, C. (2013). Phosphatidylcholine biosynthesis and function in bacteria. *Biochimica et Biophysica Acta (BBA) - Molecular and cell Biology of Lipids, 1831*(3), 503–513. https://doi.org/10.1016/j.bbalip.2012.08.009

Hu, S., Gao, S., Zhu, J., Gan, X., Chen, X., He, H., & Wang, J. (2020). Differential actions of diacylglycerol acyltransferase (DGAT) 1 and 2 in regulating lipid metabolism and progesterone secretion of goose granulosa cells. *The Journal of Steroid Biochemistry and Molecular Biology, 202*, Article 105721. https://doi.org/10.1016/j.jsbmb.2020.105721

Jia, S., Li, Y., Zhuang, S., Sun, X., Zhang, L., Shi, J., Hong, H., & Luo, Y. (2019). Biochemical changes induced by dominant bacteria in chill-stored silver carp (*Hypophthalmichthys molitrix*) and GC-IMS identification of volatile organic compounds. *Food Microbiology, 84*, Article 103248. https://doi.org/10.1016/j.fm.2019.103248

Jia, W., Li, R., Wu, X., Liu, L., Liu, S., & Shi, L. (2021). Molecular mechanism of lipid transformation in cold chain storage of Tan sheep. *Food Chemistry, 347*, Article 129007. https://doi.org/10.1016/j.foodchem.2021.129007

Li, X.-L., Zhang, W.-H., Wang, Y.-D., Dai, Y.-J., Zhang, H.-T., Wang, Y., … Lu, F.-P. (2014). A high-detergent-performance, cold-adapted lipase from *Pseudomonas stutzeri* PS59 suitable for detergent formulation. *Journal of Molecular Catalysis B: Enzymatic, 102*, 16–24. https://doi.org/10.1016/j.molcatb.2014.01.006

Li, Y., Jia, S., Hong, H., Zhang, L., Zhuang, S., Sun, X., Liu, X., & Luo, Y. (2020). Assessment of bacterial contributions to the biochemical changes of chill-stored blunt snout bream (*Megalobrama amblycephala*) fillets: Protein degradation and volatile organic compounds accumulation. *Food Microbiology, 91*, Article 103495. https://doi.org/10.1016/j.fm.2020.103495

Lou, X., Zhai, D., & Yang, H. (2021). Changes of metabolite profiles of fish models inoculated with *Shewanella baltica* during spoilage. *Food Control, 123*, Article 107697. https://doi.org/10.1016/j. foodcont.2020.107697

Lu, J., Boeren, S., van Hooijdonk, T., Vervoort, J., & Hettinga, K. (2015). Effect of the DGAT1 K232A genotype of dairy cows on the milk metabolome and proteome. *Journal of Dairy Science, 98*(5), 3460–3469. https://doi.org/10.3168/jds.2014-8872

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International, 94*, 90–100. https://doi.org/10.1016/j.foodres. 2017.02.003

Merlo, T. C., Lorenzo, J. M., Saldaña, E., Patinho, I., Oliveira, A. C., Menegali, B. S., … Contreras-Castillo, C. J. (2021). Relationship between volatile organic compounds,

free amino acids, and sensory profile of smoked bacon. *Meat Science, 181*(8), Article 108596. https://doi.org/10.1016/j.meatsci.2021.108596

Mi, H., Guo, X., & Li, J. (2016). Effect of 6-gingerol as natural antioxidant on the lipid oxidation in red drum fillets during refrigerated storage. *LWT - Food Science and Technology, 74*, 70–76. https://doi.org/10.1016/j.lwt.2016.07.029

Nazemroaya, S., Sahari, M. A., & Rezaei, M. (2009). Effect of frozen storage on fatty acid composition and changes in lipid content of *Scomberomorus commersoni* and *Carcharhinus dussumieri*. *Journal of Applied Ichthyology, 25*, 91–95. https://doi.org/10.1111/j.1439-0426.2008.01176.x

Okpala, C. O. R., Bono, G., Cannizzaro, L., & Jereb, P. (2016). Changes in lipid oxidation and related flesh qualities of white shrimp (*Litopenaeus vannamei*) during iced storage: Effects on the use of increasing ozone exposures. *European Journal of Lipid Science and Technology, 118*(12), 1942–1951. https://doi.org/10.1002/ejlt.201500347

Parlapani, F. F., Mallouchos, A., Haroutounian, S. A., & Boziaris, I. S. (2017). Volatile organic compounds of microbial and non-microbial origin produced on model fish substrate un-inoculated and inoculated with gilt-head sea bream spoilage bacteria. *LWT- Food Science and Technology, 78*(1–2), 54–62. https://doi.org/10.1016/j.lwt.2016.12.020

Pinheiro, A. C. D. A. S., Silvia, T., Patrignani, F., Lanciotti, R., Romani, S., & Rocculi, P. (2020). Effects of novel modified atmosphere packaging on lipid quality and stability of sardine (*Sardina pilchardus*) fillets. *International Journal of Food Science & Technology, 56*(2), 945–953. https://doi.org/10.1111/ijfs.14747

Ramalingam, V., Song, Z., & Hwang, I. (2019). The potential role of secondary metabolites in modulating the flavor and taste of the meat. *Food Research International, 122*, 174–182. https://doi.org/10.1016/j.foodres.2019.04.007

Sarkar, P., Yamasaki, S., Basak, S., Bera, A., & Bag, P. K. (2012). Purification and characterization of a new alkali-thermostable lipase from *Staphylococcus aureus* isolated from *Arachis hypogaea* rhizosphere. *Process Biochemistry, 47*(5), 858–866. https://doi.org/10.1016/j. procbio.2012.02.023

Tan, C., Hu, J., Gao, B., Zhang, B., Li, P., & Shang, N. (2023). Effects of the interaction between *Aeromonas sobria* and *Macrococcus caseolyticus* on protein degradation of refrigerated sturgeon fillets: Novel perspective on fish spoilage. *LWT - Food Science and Technology, 183*(4), Article 114908. https://doi.org/10.1016/j.lwt.2023.114908

Tan, C., Li, P., & Shang, N. (2023). Novel perspective on the spoilage metabolism of refrigerated sturgeon fillets: Nonspecific spoilage dominant organisms play an important role. *LWT - Food Science and Technology, 173*, Article 114292. https://doi.org/10.1016/j.lwt.2022.114292

Tan, C., Xiao, M., Wu, R., Li, P., & Shang, N. (2022). Unraveling the effects of biochemical drivers on the bacterial communities and volatile profiles in refrigerated sturgeon fillets at 4 °C. *Frontiers in Microbiology, 13*, Article 849236. https://doi.org/10.3389/fmicb.2022.849236

Tei, R., & Baskin, J. M. (2020). Spatiotemporal control of phosphatidic acid signaling with optogenetic, engineered phospholipase Ds. *The Journal of Cell Biology, 219*(3), Article e201907013. https://doi.org/10.1083/jcb.201907013

Tokarz, J., Möller, G., Hrabě de Angelis, M., & Adamski, J. (2015). Steroids in teleost fishes: A functional point of view. *Steroids, 103*, 123–144. https://doi.org/10.1016/j.steroids.2015.06.011

Wang, H., Wu, Y., Xiang, H., Sun-Waterhouse, D., Zhao, Y., Chen, S., Li, L., & Wang, Y. (2022). UHPLC-Q-Exactive Orbitrap MS/MS-based untargeted lipidomics reveals molecular mechanisms and metabolic pathways of lipid changes during golden pomfret (*Trachinotus ovatus*) fermentation. *Food Chemistry, 396*, Article 133676. https://doi.org/10.1016/j.foodchem.2022. 133676

Wang, M., Liu, Y., Zhao, T., Xiao, F., Yang, X., & Lu, B. (2021). Back cover: Dietary sterols and sterol oxidation products on atherosclerosis: An insight provided by liver proteomic and lipidomic. *Molecular NutritionFood Research, 65*(20), Article 2170055. https://doi.org/10.1002/mnfr. 202170055

Wang, X., Xiang, X., Wei, S., & Li, S. (2023). Multi-omics revealed the formation mechanism of flavor in salted egg yolk induced by the stages of lipid oxidation during salting. *Food Chemistry, 398*, Article 133794. https://doi.org/10.1016/j.foodchem.2022.133794

Wang, X., & Xie, J. (2020). Assessment of metabolic changes in *Acinetobacter johnsonii* and *Pseudomonas fluorescens* co-culture from bigeye tuna (*Thunnus obesus*) spoilage by ultrahigh-performance liquid chromatography-tandem mass spectrometry. *LWT - Food Science and Technology, 123*, Article 109073. https://doi.org/10.1016/j.lwt.2020.109073

Wang, Y., Jiang, Y. T., Cao, J. X., Chen, Y. J., Sun, Y. Y., Zeng, X. Q., … Gan, N. (2016). Study on lipolysis-oxidation and volatile flavour compounds of dry-cured goose with different curing salt content during production. *Food Chemistry, 190*, 33–40. https://doi.org/10.1016/j.foodchem.2015.05.048

Wu, S., Yang, J., Dong, H., Liu, Q., Li, X., Zeng, X., & Bai, W. (2020). Key aroma compounds of Chinese dry-cured Spanish mackerel (*Scomberomorus niphonius*) and their potential metabolic mechanisms. *Food Chemistry, 342*(3), Article 128381. https://doi.org/10.1016/j.foodchem.2020. 128381

Xu, Y., Mak, H. Y., Lukmantara, I., Li, Y. E., Hoehn, K. L., Huang, X., … Yang, H. (2019). CDP-DAG synthase 1 and 2 regulate lipid droplet growth through distinct mechanisms. *Journal of Biological Chemistry, 294*, 16740–16755. https://doi.org/10.1074/jbc.ra119.009992

Yin, X., Wen, R., Sun, F., Wang, Y., Kong, B., & Chen, Q. (2021). Collaborative analysis on differences in volatile compounds of Harbin red sausages smoked with different types of woodchips based on gas chromatography–mass spectrometry combined with electronic nose. *LWT- Food Science and Technology, 143*, Article 111144. https://doi.org/10.1016/j.lwt.2021.111144

Yu, M. M., Gang, K. Q., Li, C., Wang, J. H., Liu, Y. X., Zhou, D. Y., & Zhu, B. W. (2020). Change of lipids in whelks (*Neptunea arthritica cumingi* Crosse and *Neverita didyma*) during cold storage. *Food Research International, 136*, Article 109330. https://doi.org/10.1016/j.foodres.2020.10 9330

Zhang, M., Xie, D., Wang, D., Xu, W., Zhang, C., Li, P., & Sun, C. (2023). Lipidomic profile changes of yellow-feathered chicken meat during thermal processing based on UPLC-ESI-MS approach. *Food Chemistry, 399*, Article 133977. https://doi.org/10.1016/j.foodchem.2022.133977