Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Unraveling the link between lipid oxidation and off-flavor development in grass carp fillets (*Ctenopharyngodon idellus*) during cold storage: a non-targeted lipidomics approach

Huifang Yang[a], Ramy M. Khoder[a,b], Shanbai Xiong[a], Juan You[a], Yueqi An[c,*]

[a] Key Laboratory of Environment Correlative Dietology, Ministry of Education, College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[b] Faculty of Agriculture, Benha University, Moshtohor 13736, Egypt
[c] College of Health Science and Engineering, Hubei University, Wuhan 430062, China

ARTICLE INFO

Article history:
Received 23 August 2024
Received in revised form 15 September 2024
Accepted 17 October 2024

Keywords:
Grass carp
Solvent-assisted flavor evaporation (SAFE)-GC-MS
Volatile compounds
Lipidomics
Lipid oxidation
Cold storage

ABSTRACT

Grass carp fillets were susceptible to unpleasant fishiness during cold storage. To clarify the mechanism of fishy odor formation and achieve targeted regulation, volatile compounds, and lipid oxidation were investigated in grass carp fillets during cold storage (0–7 days). The lipid oxidation degree increased continually during cold storage, as evidenced by the change of acid value, peroxide value, thiobarbituric acid-reactive substances, and hydro-peroxide. The activities of related enzymes (lipoxygenases, lipases, phospholipases A_2, and hydroperoxide lyases) maintained a high level, reaching their maximums after 3–5 days. Free fatty acids exhibited a great downtrend, especially linoleic acid (18:2) and arachidonic acid (20:4). A total of 45 volatile compounds were identified by solvent-assisted flavor evaporation-gas chromatography-mass spectrometer, and the total contents of volatile compounds increased from 706.55 μg/kg (0 day) to 1 370.65 μg/kg (7 days). (*Z*)-4-Heptenal, (*E*,*E*)-2,4-hexadienal, 1-penten-3-ol, nonanal, and (*E*)-2-heptenal were the key volatile compounds. The Pearson correlation showed a strong correlation between 47 differentially abundant lipids (variables important in projection > 1, $P < 0.05$, and fold change > 2) and volatile compounds. During cold storage, lyso-phosphatidylcholine (20:4) and lyso-phosphatidylcholine (18:2) were selected as the main precursors of volatile compounds' formation in grass carp fillets.

1. Introduction

In recent years, pre-processed dishes have become popular with people due to the COVID-19 eruption and an increasing consumer preference for ready-to-eat options[1]. Pre-processed fish products can save the trouble of complicated processing steps. Grass carp (*Ctenopharyngodon idellus*) is the world's most widely cultivated and productive fishy commodity. Grass carp fillets containing extensive essential amino acids and polyunsaturated fatty acids (PUFA) are important ingredients for pre-processed aquatic products[2]. Refrigeration is a common method for pre-processed fillets[3]. By integrating cold chain logistics and a suitable package, it is possible to achieve short-term storage (5–10 days) of pre-processed fillets. However, it has been reported that the unpleasant fishiness increased rapidly in pre-processed fillets during cold storage[4-5]. Clarifying the mechanism of fishy odor formation in grass carp fillets is significant for expanding the market for pre-processed fillets.

The protein content of aquatic products is high, making them susceptible to decomposition by microorganisms that generate a

* Corresponding author at: College of Health Science and Engineering, Hubei University, Wuhan 430062, China.
E-mail address: anyueqi@mail.hzau.edu.cn (Y.Q. An)
Peer review under responsibility of Beijing Academy of Food Sciences.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250449

range of unpleasant odors, particularly fishy smells. Gram-negative bacteria are the primary pathogens responsible for fish deterioration, and the ideal temperature for their growth is 37 °C[6]. On the other hand, enzymatic and non-enzymatic oxidation of lipids also play an important role in forming fishy off-odors during storage[7]. Lipases (LPS) hydrolyze lipids to produce free fatty acids (FFAs). Tong et al.[8] verified that phospholipases A_1 and phospholipases A_2 (PLA_2) could break down the S_{n-1} and S_{n-2} phospholipid bonds of phospholipids (GPs) to generate fatty acids. Enzymatic oxidation of fatty acids catalyzed by lipoxygenases (LOX) and hydroperoxide lyases (HPL) can generate unsaturated ketones and alcohols, such as 5-, 6-, 8-, and 9-carbon compounds[9]. For example, 1-octen-3-ol, octanal, hexanal, and (*E*,*E*)-2,4-decadienal yielded by the oxidation of linoleic acid and arachidonic acid were described as having an unpleasant odor in grass carp. Wang et al.[10] reported that the LOX-dominated oxidation decomposition of unsaturated fatty acids (UFA) mainly produced octanal. Autoxidation of UFA produced some carbonyl compounds responsible for fishy odors, such as 6-, 7-, 8-, and 10-carbon carbonyls[11]. For example, heptanal produced by *ω*-3 fatty acids was associated with a fishy odor[12]. It has been reported that the unpleasant fishiness increased rapidly due to the oxidation of linoleic acid (18:2) in aquatic products during cold storage[13]. However, it is unclear about the state of linoleic acid (18:2) (free or bound state, GPs or triglycerides) of flavor formation in grass carp fillets. It is urgent to figure out the precise precursors of flavor formation and achieve targeted inhibition of fishy odor.

Lipidomics is a usable tool to track lipid dynamics[14]. In recent years, lipidomics has been used to determine lipid oxidation in air-fried surimi[15] as well as the mechanism of phospholipid hydrolysis in oysters during storage[16]. Several reports have been conducted on lipid profile changes in shrimp (*Penaeus vannamei*) during accelerated storage[17]. In addition, lipidomics and correlation analysis can identify metabolites at the stage of primary or secondary oxidation and examine the correlation between lipid metabolism and flavor development. Fernández-del-Río et al.[18] revealed that CoQ9 and peroxide value (POV) exhibited a positive correlation, which indicated that CoQ9 mainly produced at primary oxidation in salted egg yolk. Che et al.[19] reported that cinnamaldehyde undergone degradation into alcohols at the initial oxidation phase. Although these studies have contributed to understanding the development of lipid oxidation in aquatic products, little study has reported on the dynamic change of lipid composition in grass carp during cold storage. Therefore, it is viable to explore the possible relationship between the formation of volatile compounds and lipid oxidation and figure out the possible lipid precursors of the characteristic volatile compounds by lipidomics in grass carp fillets during cold storage.

In the present study, the grass carp fillets were prepared during cold storage at 4 °C for 7 days. The objectives of the present study are 1) to investigate the changes in volatile compounds; 2) to evaluate the degree of lipid oxidation and find the differentially abundant lipids (DALs) in grass carp during cold storage; 3) to figure out the main lipid precursors of volatile compounds. In general, this investigation provides information for understanding the path of aroma formation and lays the foundations for achieving targeted regulation of unpleasant fishiness in grass carp fillets during cold storage.

2. Materials and methods

2.1 Materials

Dithiothreitol (AR), Tris(hydroxymethyl) aminomethane (Tris, AR), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC, AR) were bought from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). High-performance liquid chromatography (HPLC)-grade dichloromethane was bought from Burdick & Jackson (Muskegon, MI, USA). [$2H_3$]-1-octen-3-ol (> 98%) was obtained from ZZBIO Biochemical Technology Co., Ltd. (Shanghai, China). Other chemical reagents (AR) were bought from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

2.2 Sample preparation

Grass carps weighing approximately 1.5 kg each were obtained from the local market in Wuhan (Hubei Province, China). Live grass carps were stunned by striking the heads, then the unnecessary parts (heads, scale, skin, viscera, and bones) were removed with the guidance of the National Institutes of Health guide for the care and use of laboratory animals, and the back meat which removed the red meat and fishbone was sliced into fillets with a thickness of 1 cm. Grass carp fillets were soaked in a 0.1% sodium azide solution to exclude microorganism effects and stored at 4 °C for 7 days.

2.3 Lipid oxidation analysis

Lipid oxidation was evaluated by the degree of acid value (AV), POV, thiobarbituric acid-reactive substances (TBARS), and hydro-peroxide (HP), and the grass carp fillets stored at 0, 1, 2, 3, 5, and 7 days were the samples.

The AV was quantified according to Zhang et al.[20]. Briefly, 5.0 g of fish sample was dissolved in 15 mL of an ether-ethanol mixture (2:1, *V*:*V*). The resulting supernatant was collected after being centrifuged at 4 000 r/min for 3 min, then the solution was titrated with 0.1 mol/L KOH, and the volume of KOH consumed was recorded. The result was presented as mg KOH/g fillets.

The POV was measured by referring to the procedures reported by Huang et al.[21]. Briefly, 0.5 g of fish sample was dissolved in 10 mL of a chloroform-methanol mixture (1:1, *V*/*V*) , then 3 mL of a 0.5% NaCl solution was added. The mixtures were centrifuged at 5 000 r/min for 5 min at 4 °C. Subsequently, 2.5 mL of the lower phase were collected, and 25 μL of a 3.94 mol/L ammonium thiocyanate and 25 μL of a 20 mmol/L iron(II) chloride containing 3.5% HCl were added. The result was expressed as mmol POV/kg fillets.

The TBARS was determined by the procedure of Shi et al.[22]. Briefly, 0.2 g of the sample was accurately weighted and dissolved in 25 mL of 7.5% trichloroacetic acid (TCA), then the mixtures were centrifuged at 4 000 r/min for 5 min at 4 °C. 4 mL of the lower phase were collected, and 4 mL of 0.02 mol/L 2-thiobarbituric acid (TBA) were added. The result was expressed as mg malonaldehyde (MDA)/kg fillets.

The HP was quantified according to Ohkawa et al.[23], and the HP content was represented as mmol HP/kg fillets.

2.4 Determination of endogenous enzyme activity

The grass carp fillets stored at 0, 1, 2, 3, 5, and 7 days were used to measure the activities of endogenous enzymes.

The activity of LOX was assessed by referring to the reported[24], and the enzyme activity was described as the increment of absorbance at 234 nm of fillet (1 g) per minute. The crude LPS was extracted according to Smichi et al.[25], and specific activities were presented as U/g of fillet sample. The activity analysis of PLA_2 was carried out following Minchiotti et al.[26]. The PLA_2 activity (U/g) was described as the increment of 1 μmol of absorbance at 340 nm of fillet (1 g) per minute. The enzyme-linked immunosorbent assay (ELISA) kit was used to determine the activity of HPL.

2.5 Analysis of FFAs composition

Grass carp fillets stored at 0, 1, 2, 3, 5, and 7 days were the samples to analyze the composition of FFA. The lipid extraction and the methylation of FFAs were performed, referring to prior work with some modifications[27]. Grass carp fillets (3 g) were reacted with 2 mL ethanol, 4 mL water solution, internal standard (undecanoyl carbonate triglyceride), and 0.1 g of pyrogallic gallic acid. The sample was added to 10 mL of a 95% ethanol solution, and then the fat was extracted by ethyl ether and petroleum ether (1:1, *V*/*V*). The fat extracted was saponified, methyl-esterified, and then fixed with *n*-heptane. Then, the fatty acid methyl esters (FAMEs) were analyzed using an Agilent 8890 gas chromatography with a column (100 m × 0.25 μm, 0.2 μm, HP-88, Agilent Technologies, USA). The machine conditions were as follows: the injection temperature was 250 °C, the initial temperature was 120 °C for 13 min, ramped to 180 °C at 10 °C/min for 6 min, increased to 200 °C at 1 °C/min and kept for 20 min, raised to 230 °C at 4 °C/min and held for 10.5 min.

The content of FFAs was quantified with the response factors of FAMEs, peak area, internal standard (the undecanoic acid methyl ester), and translation coefficient.

2.6 Sensory evaluation

Fillets stored at 0, 1, 2, 3, 5, and 7 days were used as samples for sensory evaluation according to our previous methods[28]. The sensory evaluation invited 10 experimenters (6 females and 4 males, aged 22−35) from the Freshwater Products Processing Theory and Technology Innovation Team. The members were trained according to the following steps: firstly, the members discussed the descriptors that were consistent with the aroma characteristics of grass carp fillets. “Fresh” “fishy” “grassy” “nuttyx” and “fatty” was selected as the descriptors[13]. Secondly, the members were trained by different standard compound concentrations (heptanal for the unpleasant fishy odor, hexanal for the fishy and grassy odor). Then, the members recorded the standard solutions’ strengths (0−3). The sensory evaluation was performed in triplicates over 3 days. After training, the members assessed the fillets at room temperature ((25 ± 1) °C) as the following scales: 0, no odor; 1, weak; 2, moderate; and 3, strong. Then, the strengths of each odor were recorded.

The sensory evaluation was reviewed and approved by the Huazhong Agricultural University IRB, and informed consent was obtained from each member before participating in the research.

2.7 Aroma extraction, isolation, concentration and fractionation

The aroma extraction and isolation were carried out by the solvent-assisted flavor evaporation (SAFE) device (Glasblaserei Bahr, Manching, Germany). Briefly, 100.0 g of fillets stored at 0, 3, and 7 days were blended with liquid nitrogen, and 150 mL of dichloromethane was introduced. The volatile compounds were extracted by collecting the dichloromethane layer after shaking for 2 h 3 times; [$2H_3$]-1-octen-3-ol was used as the internal standard (IS). Subsequently, the dichloromethane layer was distilled by the SAFE device at 46 °C under a 5.3×10^{-3} Pa vacuum and condensed to 500 μL.

Hydrocarbons often exhibit a high threshold, which has minimal impact on the overall smell of the sample. They constitute the majority of the total content of volatile chemicals in the sample, potentially overshadowing the signal peaks of low-threshold substances like aldehydes, ketones, and alcohols[29]. Therefore, this work utilized a fractionation process to remove hydrocarbons. The distillate was fractionated by a Solid-phase Extraction Silica-gel Column (500 mg/3 mL, Simon Aldrich). In brief, the solvent in the extract was replaced with pentane from dichloromethane in the following procedure. Pentane (1.5 mL) was added into the 100 μL of distillate, and then the complexes were concentrated to 100 μL 3 times. Then, 6 mL pentane and 6 mL dichloromethane were successively added into a solid-phase extraction silica-gel column activated by ether in a solid-phase extraction instrument, respectively. The eluate was collected and condensed to 200 L.

2.8 Identification and relative quantification of volatile compounds by gas chromatography-mass spectrometer (GC-MS)

The volatile compounds were separated and identified by an Agilent 8890 GC combined with an Agilent 7000D MS system (MSD, Agilent Technologies, Inc., Santa Clara, USA). The sample was added into the GC instrument and then separated by a column (30 m × 0.25 mm, 0.25 μm, HP-5-MS, Agilent Technologies, Inc.). The machine conditions were set as follows: the temperature of the injector was 250 °C. The initial oven temperature was 40 °C, and holding for 4 min, raised to 230 °C at 4 °C/min, and kept at 230 °C for 10 min. The ion source temperature was 230°C, and the electron impact (EI) energy was 70 eV. All samples were measured in triplicate. Volatile compounds were identified referring to mass spectra according to the standard’s NIST20 library and relative retention indices (RIs). A semi-quantitative analysis was calculated according to the amount of IS added and the mass ion response intensity according to Eq. (1).

$$C_i = \frac{I_i \times C_{IS}}{I_{IS}} \quad (1)$$

where C is concentration, I is mass ion response intensity, i is detected volatile compounds, IS represents internal standard, and the results were expressed as μg/kg samples.

2.9 Non-targeted lipidomics analysis

Grass carp fillets stored at 0, 3, and 7 days were the samples to determine the lipid profiles. Non-targeted lipidomics analysis was conducted using an ultra-high performance liquid chromatography system (Shimadzu, Japan) equipped with a Q-Exactive Plus mass spectrometer (Thermo Fisher Scientific, USA). A CSH C_{18} column separated the liquid (2.1 mm × 100 mm, 1.7 μm, Water, USA). In the

positive ion modes, the mobile phase of solvent A was composted of water/acetonitrile (4:6, *V*/*V*) with 0.1 mmol/L ammonium formate in 1% formic acid solution, and solvent B was isopropanol/acetonitrile (9:1, *V*/*V*) including 0.1 mmol/L ammonium formate in 1% formic acid solution in positive ion mode. In the negative ion mode, 0.1% formic acid was removed from solvent solvents A and B. A total of 6 replicates were determined for each sample.

Identification was carried out using electrospray ionization (ESI) in positive and negative ion modes, respectively. The ESI source conditions were as follows: heater temperature 300 °C, sheath gas flow rate 45 arb, aux gas flow rate 15 arb, sweep gas flow rate 1 arb, spray voltage 3.0kV, capillary temperature 350 °C, S-Lens RF level 50%, MS1 scan ranges: 200−1 800. Mass-to-charge ratios of lipid molecules and lipid fragments were collected: 10 fragmentation profiles were collected 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 00 at *m/z* 200.

2.10 Statistical analysis

All tests were repeated 3 times unless otherwise marked. Significant analysis ($P < 0.05$) was carried out *via* the Duncan multiple range test in the SPSS 26.0 (IBM, USA). Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLA-DA) were carried out in SIMCA 14.1 (Umetrics, Sweden). The correlation analysis was performed by Origin 2022 (OriginLab CORP, USA).

3. Results and discussion

3.1 Changes in lipid oxidation of grass carp fillets during cold storage

3.1.1 Changes in lipid oxidation degree

AV is usually described as an indicator of lipid rancidity. POV and HP values reflect the degree of lipid oxidation at the primary level. The TBARS value indicates the degree of lipid secondary oxidation. The changes of AV, POV, HP, and TBARS in fillets during storage are shown in Fig. 1A. During cold storage, the AV gradually increased from 1.85 to 3.03 mg/g. The POV values increased first and then decreased, reaching the maximum at 0.80 mmol/kg. The content of HP also presented an upward trend from 1.78 to 16.93 mmol/kg, followed by a drop to 8.78 mmol/kg throughout cold storage. The decrease in POV and HP during storage (3−7 days) might be due to the fact that the hydroperoxides were decomposed into secondary oxidation products such as aldehydes, ketones, and alcohols[30]. Generally, higher TBARs values indicate the accumulation of MDA. The initial TBARS was 0.18 mg MDA/kg and showed a slow increase to 0.25 mg MDA/kg and then a rapid increase to 0.47 mg MDA/kg. Cao et al.[31] also reported the increase in TBARS of grass carp during low temperature storage. The increase of MDA might be attributed to the rapid accumulation of the secondary oxidation products. In summary, this result indicated that the lipid oxidation degrees increased continually with the refrigeration extension period.

3.1.2 Changes in the activities of related enzymes

The LOX-catalyzed enzymatic oxidation of unsaturated primarily generated volatile flavor compounds. This occurred due to the hydrolysis of lipids by LPS, ultimately resulting in the formation of peroxides, and then the decomposition of peroxides catalyzed by HPL could produce aliphatic aldehydes[32]. The activities of LOX, LPS, PLA_2, and HPL in fillets upon cold storage are presented in Fig. 1B. The activity of LOX was increased to 101.33 U/g and then dropped to 32.2 U/g. This result could explain the change in POV and HP (Fig. 1A). Xu et al.[33] also observed the similar tendency of the LOX activity in grass carp fillets. LPS could hydrolyze ester bonds and convert triglycerides into glycerol and fatty acids. The LPS activity of fillets increased from 10.93 to 14.58 U/g and then exhibited a rapid downtrend at 3−7 days, but it still maintained a high level, which indicated that the lipid was continuously degraded. This was consistent with the changes in AV mentioned above (Fig. 1A). PLA_2 is an important component of LPS, which can promote phosphatide hydrolysis. The activity of PLA_2 presented an upward trend from 2.45 to 7.44 U/g followed by a downtrend to 2.47 U/g

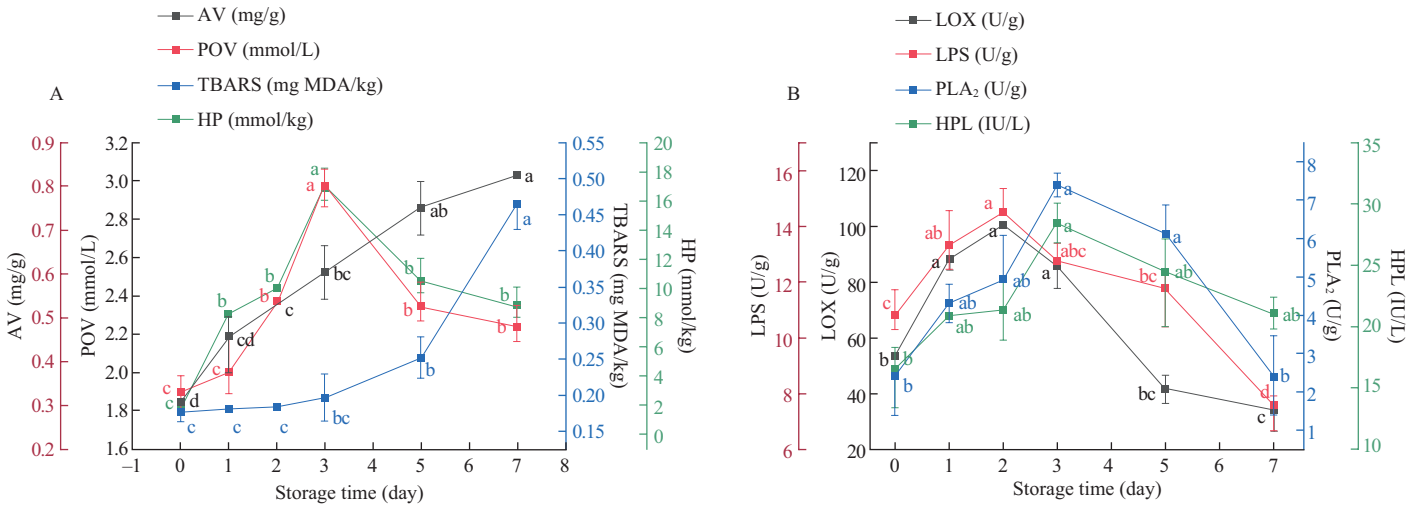

Fig. 1 Changes in AV, POV, TBARS, and HP (A), and the activities of LOX, LPS, PLA_2, and HPL (B) of fillets during cold storage. Different lowercase letters (a, b, c) represent significant differences ($P < 0.5$).

during cold storage. Meanwhile, the changes in the activity of HPL were similar to PLA_2, including the uptrend at 0−3 days and the downtrend at 4−7 days. The changes of related enzyme activities were closely related to the contents of substrates. The contents of triglycerides, GPs and FFAs were high in the early stage of cold storage, so the activities of LPS, LOX and PLA_2 remained at a high level. With the progress of oxidation, lipid primary oxidation produced a huge amount of hydroperoxide, which could be used as the substrate of HPL. That resulted to the activity increase of HPL, reaching the maximum in 3 days. In the late stage of cold storage, the levels of substrates such as triglycerides, GPs and FFAs reduced, which led to the drop of the corresponding endogenous enzyme activity. In addition, the LPS, LOX, HPL and PLA_2 activities might be triggered by other enzymes presented in the system[34]. Generally, this study showed that the activities of related enzymes increased first and then decreased but still maintained a high level.

3.2 Changes in FFA compositions in grass carp fillets during cold storage

FFAs greatly impact the flavor of grass carp. UFA, especially linoleic acid (18:2), had been reported as essential aroma precursor in the process of lipid oxidation and degradation[35]. Changes in FFA composition and content are shown in Table 1. A total of 17 FFAs were identified in the samples. It was observed that palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) were the most abundant FFAs. In general, the content of UFA consistently kept higher than saturated fatty acids (SFAs) during cold storage, and all fatty acids showed a significant downtrend. Cheng et al.[36] also revealed that the most abundant fatty acids of tilapia (*Oreochromis mossambicus*) showed a decreasing trend after cold storage.

Palmitic acid ($C_{16:0}$) was the predominant SFA in grass carp; the initial content of palmitic acid ($C_{16:0}$) was 137.50 mg/100 g, and then decreased to 100.80 mg/100 g. Among monounsaturated fatty acid (MUFA), the content of oleic acid (18:1) had shown a sharp decrease, from 189.00 to 131.52 mg/100 g. It was reported that oleic acid (18:1) could be converted into secondary oxidation products such as octanal and nonanal[37]. After cold storage, the total content of PUFA decreased by 65.72 mg/100 g, among which linoleic acid (18:2) and arachidonic acid (20:4) decreased the most, by 43.82 and 15.90 mg/100 g, respectively. Karrer et al.[38] reported that the PUFA produced unsaturated carbonyls and alcohols under LOX and hydroperoxide lyases. Furthermore, α-linolenic acid (18:3) could be decomposed and then formed unsaturated aldehydes, including (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*,*Z*)-2,6-nonadienal, and (*E*,*E*)-2,4-heptadienal, etc.[39].

3.3 Changes in aroma profiles of grass carp fillets during cold storage

As shown in Fig. 2A, the fillets stored at 0, 1, 2, 3, 5, and 7 days exhibited significant differences in aroma profiles. Fresh fillets (0 day) presented the strongest fresh odor and the weakest fishy, grassy, fatty, and nutty notes. The fishy, grassy, and nutty smell increased greatly on the 3rd day of storage. Additionally, fatty, fishy, and grassy odors masked the original fresh odor at the end of the storage period (7 days). Based on the sensory evaluation, the fillets stored at 0, 3, and 7 days were considered the typical samples to determine the composition of volatile compounds and lipidomics analysis.

Table 1
Fatty acids contents (mg/100 g) in grass carp with different storage time.

No.	Fatty acids	Storage time (days)					
		0	1	2	3	5	7
1	$C_{14:0}$	8.12 ± 0.51^{a}	5.02 ± 0.06^{b}	4.31 ± 0.23^{cd}	4.82 ± 0.03^{bc}	4.19 ± 0.07^{d}	3.54 ± 0.11^{e}
2	$C_{15:0}$	1.38 ± 0.04^{a}	0.95 ± 0.07^{b}	0.95 ± 0.07^{b}	1.00 ± 0.00^{b}	0.80 ± 0.05^{c}	0.75 ± 0.07^{c}
3	$C_{16:0}$	137.50 ± 1.41^{a}	135.00 ± 0.00^{a}	115.50 ± 7.78^{b}	128.50 ± 0.71^{a}	95.20 ± 0.42^{c}	100.80 ± 3.11^{c}
4	$C_{17:0}$	1.57 ± 0.01^{a}	1.22 ± 0.21^{b}	1.14 ± 0.06^{c}	1.08 ± 0.03^{c}	1.09 ± 0.02^{c}	0.80 ± 0.01^{d}
5	$C_{18:0}$	42.15 ± 0.64^{a}	31.60 ± 0.14^{b}	23.10 ± 0.99^{d}	29.75 ± 0.64^{c}	24.05 ± 0.49^{d}	20.75 ± 0.64^{e}
	∑SFA	190.72	173.79	145.00	165.15	125.33	126.64
6	$C_{16:1}$	50.30 ± 2.40^{a}	38.05 ± 0.07^{b}	34.70 ± 1.56^{c}	38.10 ± 0.14^{b}	24.60 ± 0.28^{e}	28.51 ± 1.27^{d}
7	$C_{18:1}$	189.00 ± 11.31^{a}	188.52 ± 2.12^{a}	168.50 ± 10.61^{b}	176.11 ± 1.41^{ab}	136.51 ± 2.12^{c}	131.52 ± 4.95^{c}
8	$C_{20:1}$	9.35 ± 0.49^{a}	ND	6.75 ± 0.03^{c}	8.18 ± 0.99^{b}	6.52 ± 0.12^{c}	4.82 ± 0.25^{d}
9	$C_{22:1n9}$	2.62 ± 0.11^{a}	2.12 ± 0.08^{b}	1.39 ± 0.08^{c}	2.66 ± 0.16^{a}	1.92 ± 0.06^{b}	1.14 ± 0.07^{d}
	∑MUFA	249.27	228.69	211.34	225.05	169.55	165.99
10	$C_{18:2}$	128.52 ± 9.19^{a}	116.53 ± 0.71^{ab}	93.70 ± 4.38^{b}	104.00 ± 1.41^{ab}	83.71 ± 0.85^{b}	84.70 ± 3.54^{b}
11	$C_{18:3}$	2.25 ± 0.71^{a}	1.61 ± 0.00^{ab}	0.71 ± 0.83^{b}	1.42 ± 0.03^{ab}	0.76 ± 0.01^{b}	1.22 ± 0.07^{b}
12	$C_{18:3}$	13.12 ± 1.13^{b}	13.95 ± 0.21^{a}	10.71 ± 0.28^{d}	11.95 ± 0.07^{c}	9.03 ± 0.23^{e}	10.62 ± 0.42^{d}
13	$C_{20:2}$	7.91 ± 0.37^{a}	6.52 ± 0.11^{b}	5.02 ± 0.26^{c}	6.11 ± 0.08^{b}	4.69 ± 0.11^{c}	4.12 ± 0.18^{d}
14	$C_{20:3}$	16.15 ± 0.42^{a}	14.15 ± 0.35^{b}	12.55 ± 0.49^{c}	14.25 ± 0.21^{b}	10.90 ± 0.14^{d}	11.35 ± 0.29^{d}
15	$C_{20:4}$	75.35 ± 2.33^{a}	66.85 ± 2.33^{b}	63.53 ± 2.12^{bc}	68.05 ± 2.33^{b}	43.65 ± 0.92^{d}	59.45 ± 2.62^{c}
16	EPA	7.06 ± 0.12^{a}	5.59 ± 0.13^{bc}	5.39 ± 0.21^{c}	5.81 ± 0.07^{b}	2.86 ± 0.06^{e}	4.58 ± 0.11^{d}
17	DHA	42.75 ± 0.78^{a}	37.51 ± 1.69^{bc}	34.95 ± 0.92^{c}	38.75 ± 0.49^{b}	22.25 ± 0.35^{e}	31.35 ± 1.63^{d}
	∑PUFA	273.11	262.71	226.56	250.34	177.85	207.39
	Total	713.1	665.3	582.90	640.54	472.73	500.02

Note: All results are presented as mean ± standard deviation ($n = 3$). ND means not detected. Different lowercase letters in each row indicate significant differences ($P < 0.05$).

3.4 Changes in volatile compounds of grass carp fillets during cold storage

A total of 45 volatile compounds were identified by SAFE-GC-MS, including 10 alcohols, 19 aldehydes, 6 ketones, 4 esters, 2 phenol, 3 acids, and 3,4-dimethylthiophenol. The contents of identified volatile compounds calculated according to the IS were shown in Table 2, and a cluster heat map was generated to visualize the changes in volatile compounds content (Fig. 2B). The results showed that the species of volatile compounds increased from 38 to 45 with the storage time, and the total contents of volatile compounds increased from 706.55 to 1 370.65 μg/kg. Overall, the volatile compounds in grass carp showed a significant change with cold storage time (Figs. 2C-E).

Table2.
Contents of odorants (μg/kg) identified by SAFE-GC-MS in grass carp during cold storage.

No.	Odorants	Content (μg/kg)			Threshold (μg/kg)	odor activity value (OAV)			FC	VIP
		0 day	3 days	7 days		0 day	3 days	7 days		
	Alcohol									
1	1-Hexanol	12.70 ± 1.21^{b}	16.99 ± 1.96^{b}	24.62 ± 3.12^{a}	250	< 1	< 1	< 1	1.94	0.20
2	1-Octene-3-ol	3.73 ± 0.65^{b}	5.79 ± 1.53^{b}	6.93 ± 1.28^{a}	1.2	3	4	6	1.86	0.21
3	(*E*)-2-Octen-1-ol	20.19 ± 1.98^{c}	26.76 ± 1.83^{b}	32.11 ± 1.51^{a}	40	1	1	1	1.62	0.67
4	3-Methyl-1-butanol	ND	26.17 ± 4.72^{b}	38.30 ± 3.34^{a}	4	< 1	7	10	> 2	1.54
5	1-Penten-3-ol	8.09 ± 2.25^{c}	15.71 ± 1.09^{b}	28.04 ± 3.10^{a}	358.1	< 1	0	0	3.46	0.76
6	Cyclopentanol	52.43 ± 14.45^{a}	ND	ND	125.1	< 1	< 1	< 1	0.00	2.44
7	4-Methyl-5-thiazoleethanol	24.14 ± 3.02^{b}	ND	61.32 ± 6.36^{a}		/	/	/	2.54	2.73
8	Phenylethyl alcohol	1.92 ± 0.16^{c}	3.49 ± 0.24^{b}	5.85 ± 1.41^{a}	564.23	< 1	< 1	< 1	3.06	0.32
9	Citronellol	ND	ND	17.57 ± 5.57^{a}		/	/	/	> 2	1.27
10	2-Ethyl-1-hexanol	ND	3.43 ± 1.74^{a}	1.19 ± 0.31^{b}	246	< 1	< 1	< 1	> 2	0.73
	Aldehyde									
11	3-Methyl-butanal	7.12 ± 0.69^{c}	9.90 ± 1.07^{b}	13.95 ± 1.43^{a}	0.15	47	66	93	1.96	0.25
12	(*E*)-2-Pentenal	4.79 ± 0.67^{c}	7.86 ± 0.92^{b}	12.66 ± 1.91^{a}	1.5	3	5	8	2.64	0.41
13	Hexanal	25.49 ± 2.74^{c}	37.98 ± 2.25^{b}	44.54 ± 1.17^{a}	2.4	11	16	19	1.75	1.10
14	(*Z*)-4-Heptenal	3.12 ± 0.98^{c}	8.18 ± 1.47^{b}	13.97 ± 3.53^{a}	0.008 7	359	940	1 605	4.47	0.62
15	Heptanal	10.10 ± 1.23^{c}	16.15 ± 2.26^{b}	29.15 ± 1.56^{a}	2.8	4	6	10	2.89	0.72
16	Benzaldehyde	62.85 ± 3.75^{a}	51.73 ± 6.53^{b}	39.13 ± 4.99^{c}	24	3	2	2	0.62	1.92
17	(*E,E*)-2,4-Hexadienal	2.74 ± 0.13^{c}	5.25 ± 0.21^{b}	9.75 ± 1.14^{a}	60	< 1	< 1	< 1	3.56	0.47
18	(*E*)-2-Heptenal	4.09 ± 1.29^{c}	8.02 ± 1.42^{b}	13.15 ± 2.15^{a}	13	< 1	1	1	3.21	0.50
19	Octanal	13.26 ± 4.49^{c}	26.37 ± 2.14^{b}	35.23 ± 3.71^{a}	3.4	4	8	10	2.66	1.93
20	(*E,E*)-2,4-Heptadienal	18.00 ± 1.08^{b}	23.52 ± 4.38^{b}	37.66 ± 3.24^{a}	15.4	1	2	2	2.09	0.43
21	(*E*)-2-Octenal	18.66 ± 3.21^{c}	35.68 ± 2.11^{b}	46.72 ± 3.98^{a}	3	6	12	16	2.50	1.09
22	Nonanal	4.68 ± 0.02^{c}	7.84 ± 0.30^{b}	15.14 ± 0.42^{a}	0.1	47	78	151	3.23	0.58
23	(*E,E*)-2,4-Octadienal	4.27 ± 0.07^{c}	6.81 ± 0.38^{b}	8.70 ± 0.27^{a}	2.6	2	3	3	2.03	0.41
24	(*E,Z*)-2,6-Nonadienal	8.99 ± 2.16^{c}	14.08 ± 0.11^{b}	17.68 ± 1.55^{a}	0.045	200	313	393	1.97	0.57
25	(*E*)-2-Nonenal	33.52 ± 2.56^{c}	50.58 ± 3.07^{b}	59.70 ± 4.47^{a}	0.19	176	266	314	1.78	1.27
26	Decanal	6.46 ± 0.23 ^{c}	9.82 ± 1.26^{b}	16.64 ± 1.71^{a}	0.1	65	98	166	2.57	0.46
27	(*E,E*)-2,4-Decadienal	5.25 ± 1.05^{c}	7.92 ± 0.07^{b}	12.82 ± 1.49^{a}	0.027	195	293	475	2.44	0.35
28	(*E*)-2-Decenal	23.61 ± 3.22^{c}	36.54 ± 2.07^{b}	52.99 ± 4.12^{a}	1	24	37	53	2.24	0.72
29	Tetradecanal	ND	ND	18.39 ± 2.44^{a}	0.64	< 1	< 1	29	> 2	1.30
	Ketone									
30	2-Hexanone	16.98 ± 0.28^{c}	24.12 ± 3.05^{b}	38.46 ± 2.60^{a}	166	< 1	< 1	< 1	2.26	0.51
31	3-Hydroxy-2-butanone	56.86 ± 5.85^{c}	63.47 ± 5.42^{b}	75.05 ± 6.35^{a}	55	1	1	1	1.32	1.24
32	1-Penten-3-one	5.69 ± 0.51^{b}	7.47 ± 0.16^{a}	8.55 ± 1.47^{a}	1	6	7	9	1.50	0.45
33	Cyclopentanone	37.55 ± 1.26^{c}	59.46 ± 2.89^{b}	78.37 ± 5.23^{a}	1 700	< 1	< 1	< 1	2.09	1.17
34	2,3-Pentanedione	ND	3.38 ± 1.19^{b}	6.62 ± 1.33^{a}	30	< 1	< 1	< 1	> 2	0.56
35	3,5-Octadien-2-one	22.57 ± 4.07^{c}	42.37 ± 5.74^{b}	57.18 ± 4.02^{a}	150	< 1	< 1	< 1	2.53	1.11
	Ester									
36	Methyl butyrate	ND	3.10 ± 0.68^{b}	4.38 ± 1.22^{a}	1	< 1	3	4	> 2	0.53
37	Ethyl propionate	ND	ND	9.93 ± 1.21^{a}		/	/	/	> 2	0.95
38	*γ*-Nonlactone	3.45 ± 0.78^{c}	5.36 ± 1.14^{b}	8.65 ± 1.76^{a}	9.7	< 1	1	1	2.50	0.31
39	Methyl palmitate	2.15 ± 1.98^{b}	6.86 ± 1.93^{b}	19.91 ± 4.55^{a}	20	< 1	< 1	1	9.27	0.90
	Phenols									
40	4-Methyl-phenol	18.53 ± 5.86^{c}	25.17 ± 1.55^{b}	33.32 ± 5.37^{a}	140	< 1	< 1	< 1	1.80	0.52
41	**Eugenol**	106.15 ± 18.81^{b}	116.42 ± 9.89^{b}	144.60 ± 23.98^{a}		/	/	/	1.36	1.52
	S-Containing compounds									
42	Dimethyl sulfide	ND	5.96 ± 0.53^{b}	13.90 ± 2.65^{a}	14.5	< 1	< 1	1	> 2	1.34
	Acid									
43	2-Methyl-propanoic acid	54.82 ± 11.35^{c}	75.66 ± 9.71^{b}	92.30 ± 12.58^{a}	6 550.5	< 1	< 1	< 1	1.68	0.28
44	Dodecanoic acid	4.07 ± 0.52^{c}	5.91 ± 1.37^{b}	7.78 ± 2.57^{a}	2.46	2	2	3	1.91	0.23
45	Octanoic acid	2.64 ± 1.55^{c}	3.93 ± 0.67^{b}	6.18 ± 1.76^{a}	3.98	1	1	2	2.34	0.75

Note: All results are presented as mean ± standard deviation (n = 3). ND means not detected. Different lowercase letters in each row indicate significant differences (P < 0.05). OAV represents odor activity value. FC means fold change. VIP means variable importance in projection.

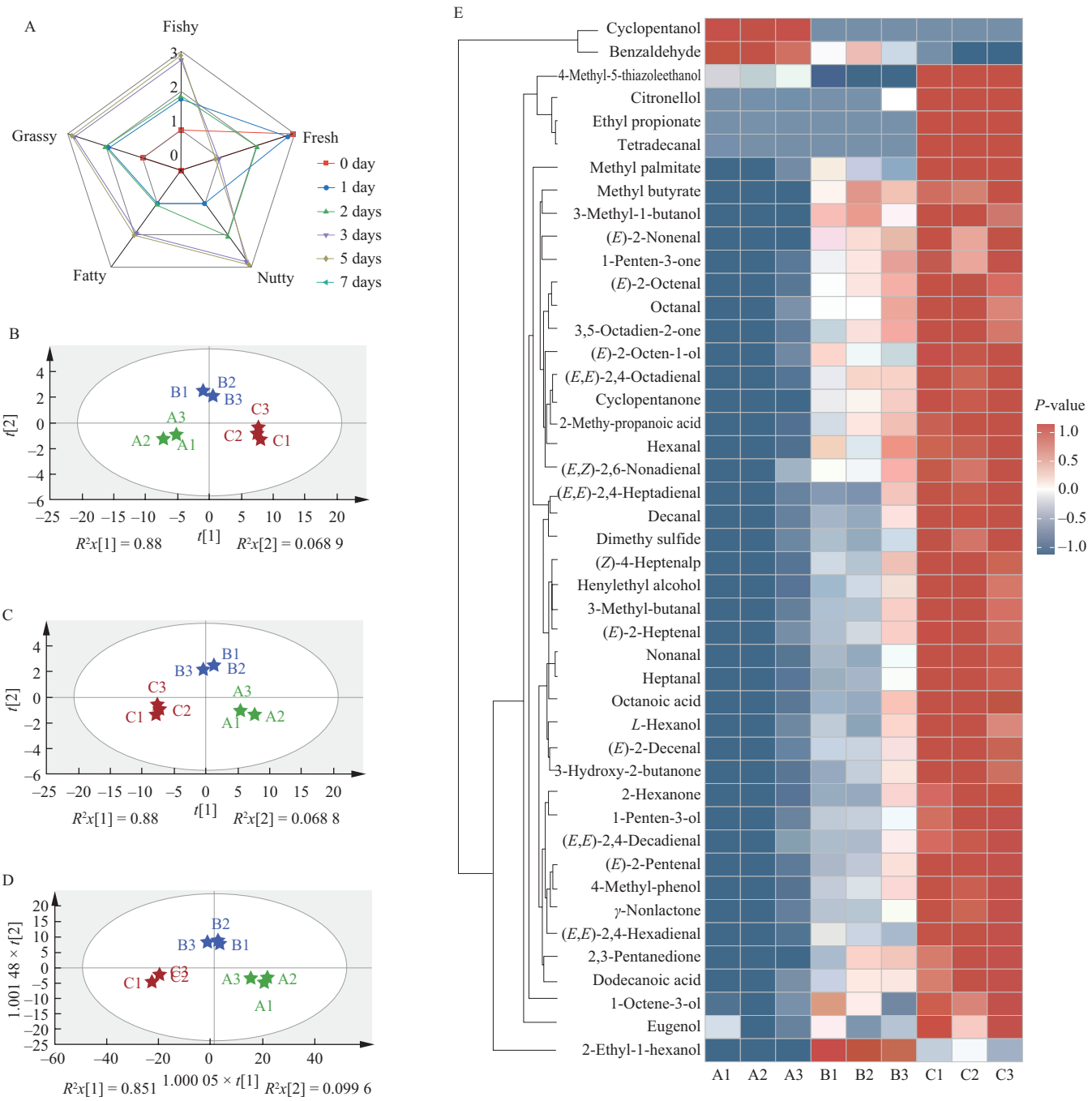

Fig. 2 Characteristics of volatile compounds of grass carp fillets during cold storage. (A) Sensory analysis, (B) PCA scores plot of volatile compounds, (C) PLS-DA scores plot of volatile compounds, (D) OPLS-DA scores plot of volatile compounds, and (E) cluster heatmap of volatile compounds. Capital letters (A, B, C) represent storage-time (0, 3, 7 days), respectively.

Among the detected odorants, hexanal, octanal, (*E*)-2-octenal, (*E*)-2-nonenal, and (*E*)-2-decenal were abundant in the samples. In sensory evaluation, the odor of grassy and fishy mostly originated from hexanal, octanal, and other compounds generated during cold storage. Hexanal and (*E*)-2-nonenal, mainly generated from linoleic acid oxidation[40], were increased by 19.05 and 26.18 μg/kg during storage, respectively. Alcohols showed the secondary peak areas in grass carp during storage. Of the 10 alcohols identified, their content gradually increased from 132.2 to 215.93 μg/kg with storage time. Among the detected alcohols, 1-octen-3-ol, mainly produced by the oxidation of arachidonic, was slightly increased to 6.93 μg/kg during cold storage. Because of the lower odor threshold of 1-octen-3-ol (mushroom, oil), it is assumed that 1-octen-3-ol was an important factor in the fishy odor[41]. 3-Hydroxy-2-butanone was the most abundant and gradually increased with cold storage. The content of 2-ethyl-1-hexanol and benzaldehyde decreased gradually at stored 3 days, combined with sensory results, it was speculated that 2-ethyl-1-hexanol and benzaldehyde had contributions to fresh odor of grass carp fillets.

The content of esters (*γ*-nonolactone, methyl palmitate) showed a steadily increasing trend during cold storage of grass carp fillets. Dimethyl sulfide compounds, described as a sign of fish deterioration,

were mainly generated from dimethyl-sulfonyl propionate and methionine. Dimethyl sulfide was detected on the 3rd day of refrigeration, and its level increased from 5.96 to 13.90 μg/kg. This result indicated that the deterioration of the quality of grass carp fillets started on the 3rd day. The fishy, nutty, grassy, and fatty odors increased on the 3rd day. In addition, a significant amount of eugenol was identified in samples, which accounted for 14.91% of the total odorant content (0 day). It was attributed to using clove oil as an anesthetic for the fish during transportation.

The effects of volatile compounds on the overall odor of grass carp fillets are not only related to the concentration but also closely related to the odor threshold. Therefore, the relative OAV was calculated based on the odor threshold value, and compounds with an OAV > 1 were considered characteristic substances constituting the overall odor of the samples. The odor threshold value thresholds mainly obtained from Gu et al.[42] and Giri et al.[43]. As shown in Table 2, 25 odorants with an OAV > 1 were screened. (*E*)-2-pentenal, hexanal, (*Z*)-4-heptenal, heptanal, (*E*,*E*)-2,4-hexadienal, (*E*)-2-heptenal, octanal, (*E*,*E*)-2,4-heptadienal, (*E*)-2-octenal, nonanal, (*E*,*E*)-2,4-octadienal, (*E*,*Z*)-2,6-nonadienal, (*E*)-2-nonenal, decanal, (*E*,*E*)-2,4-decadienal, 3-hydroxy-2-butanone, 1-octen-3-ol and 1-penten-3-ol, et al. were identified throughout the whole storage, suggesting that they had a greater influence on the overall flavor of grass carp. Among them, (*Z*)-4-heptenal (FC = 4.47), (*E*,*E*)-2,4-hexadienal (FC = 3.56), 1-penten-3-ol (FC = 3.46), nonanal (FC = 3.23), and (*E*)-2-heptenal (FC = 3.21) were selected as the primary reasons for odor deterioration in grass carp during cold storage based on the higher fold change.

3.5 *Changes in lipids of grass carp fillets during cold storage*

As shown in Fig. 3A, a total of 1 971 lipids were identified in fillet samples by ultra performance liquid chromatography-mass spectrometry (UPLC-MS) and divided into 37 subclasses. Among them, GPs accounted for more than 50% and were the most abundant. PC and PE, which played important roles in regulating lipid metabolism and lipoprotein secretion[44], were the most abundant classes of GPs. Comparison results of content in different samples were demonstrated in Fig. 3B. The content of PE decreased from 16.62% to 13.68%, increasing TBARS and POV. As a precursor for the biosynthesis of GLs and GPs, DG showed a downtrend from 3.44% to 3.21%. SPs (CerP and Cer), which could promote biological functions by regulating signal transduction[45], increased by 0.16% in grass carp fillets. In general, the results indicated that cold storage significantly affected the species and content of lipids in grass carp fillets.

As shown in Figs. 3C-E, the 3 groups of samples were separated. The PCA, PLS-DA and OPLS-DA analysis indicated the variability of lipids in grass carp fillets with cold storage time. This study used VIP > 1, FC > 2, and $P < 0.05$ to screen DALs. As

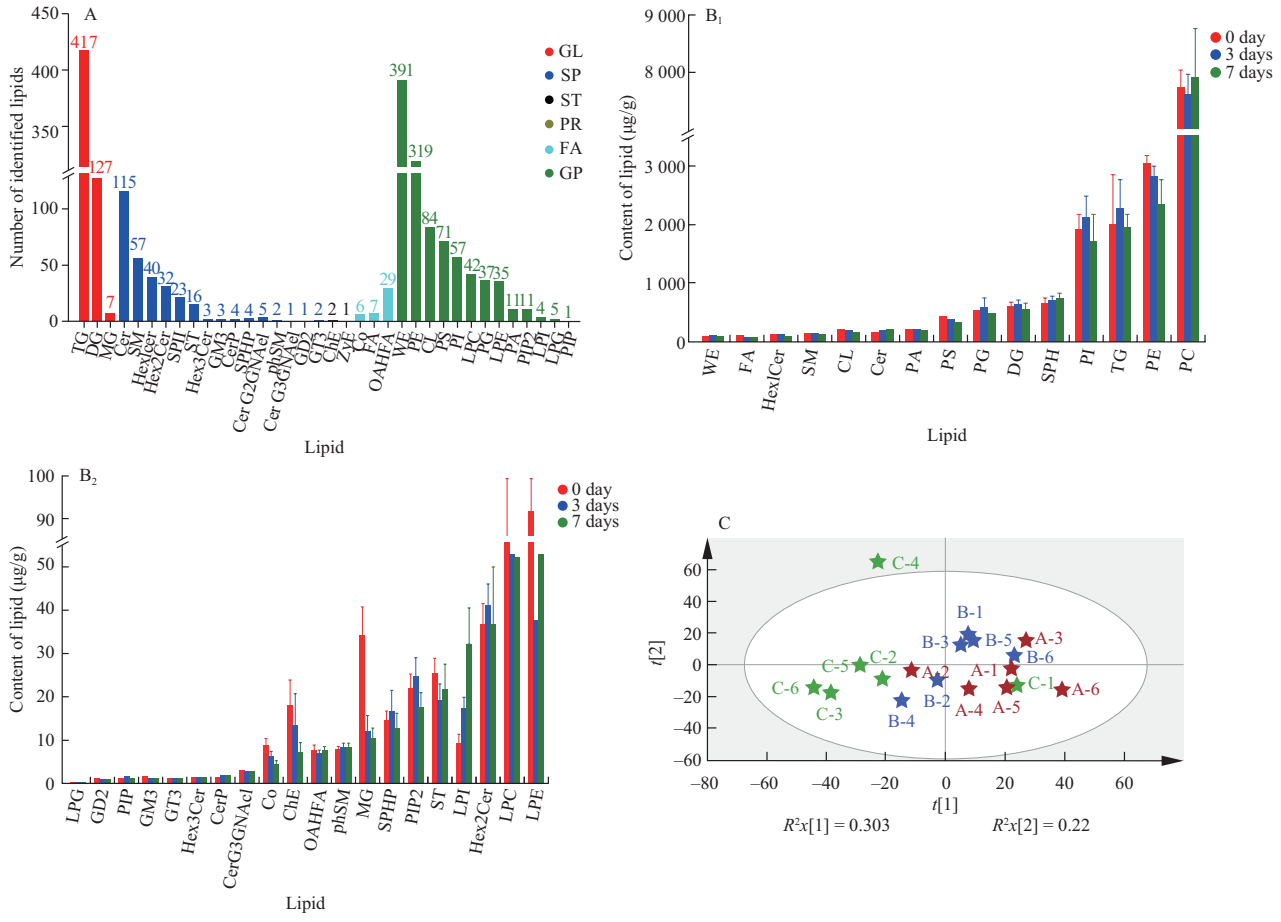

Fig. 3 Lipidomics analysis of grass carp fillets during cold storage. (A) Number of all lipid species identified, (B) the content comparison in different sample, (C) PCA scores plot of lipids, (D) PLS-DA scores plot of lipids, (E) OPLS-DA scores plot of lipids. Capital letters (A, B, C) represent storage-time (0, 3, 7 days), respectively. GL, glycerolipids; SP, sphingolipids; ST, sterol lipids; PR, prenol lipids; FA, fatty acyls; GP, glycerophospholipids.

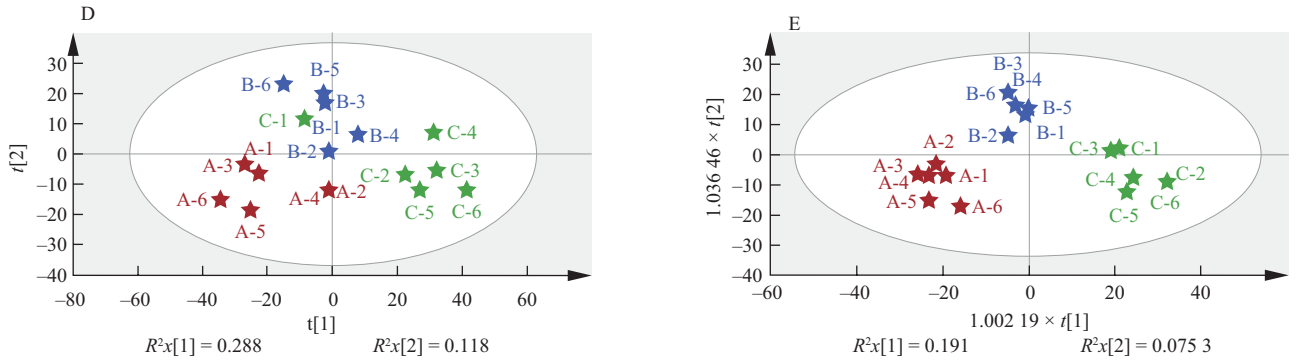

Fig. 3 (*Continued*)

shown in Fig. 4A, 47 lipid species were selected. It was found that 40 GPs (e.g., LPC, LPE, PE, and PC) had the highest percentage of the DALs. Previous studies reported that the changes in GPs may be attributed to the rearrangement and cleavage of C–C bonds in fatty acid chains[46]. To more visually represent the changes in the expression of DALs, a volcano plot was drawn (Fig. 4B). According to the volcano plot result, a total of 17 differential lipids were significantly up-regulated, including 1 LPC (16:0), 1 PI (18:0/20:5), 1 ChE (2:0), 1 Hex2Cer (42:2), 3 LPIs (LPI (16:0), LPI (18:0) and LPI (18:1), 3 PCs (PC (16:0/18:2), PC (30:1/20:4), and PC (30:1/22:6)), 3 TGs (TG (16:4/10:4/20:5), TG (22:6/12:3/12:3), and TG (48:13)), and 4 PGs (PG (38:3), PG (38:4), PG (40:5) and PG (28:0/16:0)). In addition, 30 differential lipids were significantly down-regulated, including 7 LPCs (e.g., LPC (16:1), LPC (18:1), and LPC (18:2)), 5 LPEs (e.g., LPE (16:1), LPE (18:1), and LPE (18:2)), 10 PEs (e.g., PE (8:0/11:2), PE (16:1/22:6), and PE (18:2)), as well as other differential lipids. Among the significantly down-regulated lipids, PLs were the predominant lipids species. PLs are the primary constituents of the cell membrane, which may undergo rupture during cold storage, as evidenced by scanning electron microscopy[47], leading to the release of PLs and subsequently increasing their concentration. LPCs, derived from oxidative products of PC (16:0/20:4)[48], were the main component of PLs. The most abundant FA in PLs were $C_{18:2}$, followed by $C_{18:1}$, and $C_{20:4}$, which was consistent with our findings regarding the FFAs composition. Significant differences in the expression of these lipids confirmed that oxidative changes probably occurred in grass carp fillets during cold storage.

3.6 *Correlation analysis of DALs and volatile compounds*

To verify the potential connection between odorants and DLAs, the heat map of correlation analysis of 47 DALs with 45 odorants was given in Fig. 4C. According to the Pearson correlation analysis, the 45 volatile compounds were negatively correlated with 30 lipids, including LPC (16:1), LPC (20:4), LPC (18:2), LPE (16:1), LPE (20:4), LPE (18:2), PE (8.0/11:2), PE (16:1/22:6), ChE (10:0), and PC (8:1e/10:0). Among them, 1-penten-3-ol ($|r| > 0.80$, $P < 0.05$), (*Z*)-4-heptanal ($|r| > 0.80$, $P < 0.05$), (*E*)-2-heptanal ($|r| > 0.80$, $P < 0.05$), (*E*,*E*)-2,4-hexadienal ($|r| > 0.80$, $P < 0.05$), and nonanal ($|r| > 0.90$, $P < 0.05$) were strongly negatively correlated with LPC (20:4) and LPC (18:2). Generally, we could conclude that 1-penten-3-ol, (*Z*)-4-heptenal, (*E*)-2-heptanal, and (*E*,*E*)-2,4-hexadienal and nonanal were mainly generated by the oxidative degradation of

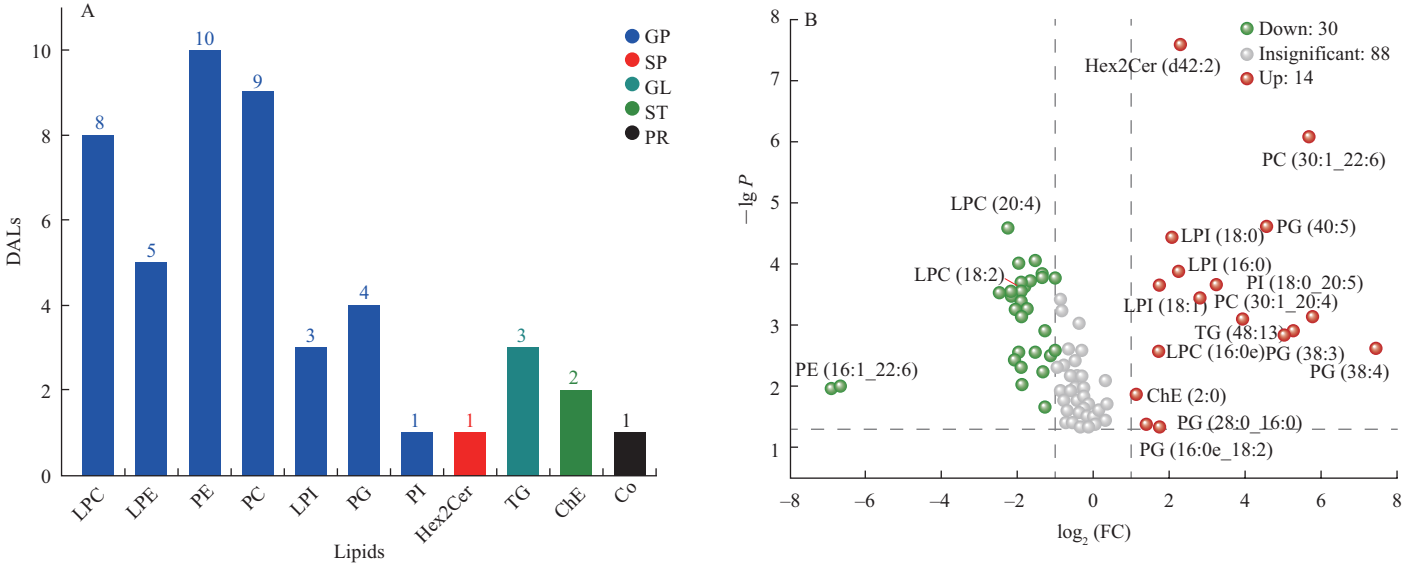

Fig. 4 (A) Species of DALs, (B) volcano plot, and (C) Pearson correlation analysis between 45 odorants and 47 DALs in grass carp fillets during cold storage. Capital letters (A, B, C) represent storage-time (0, 3, 7 days), respectively.

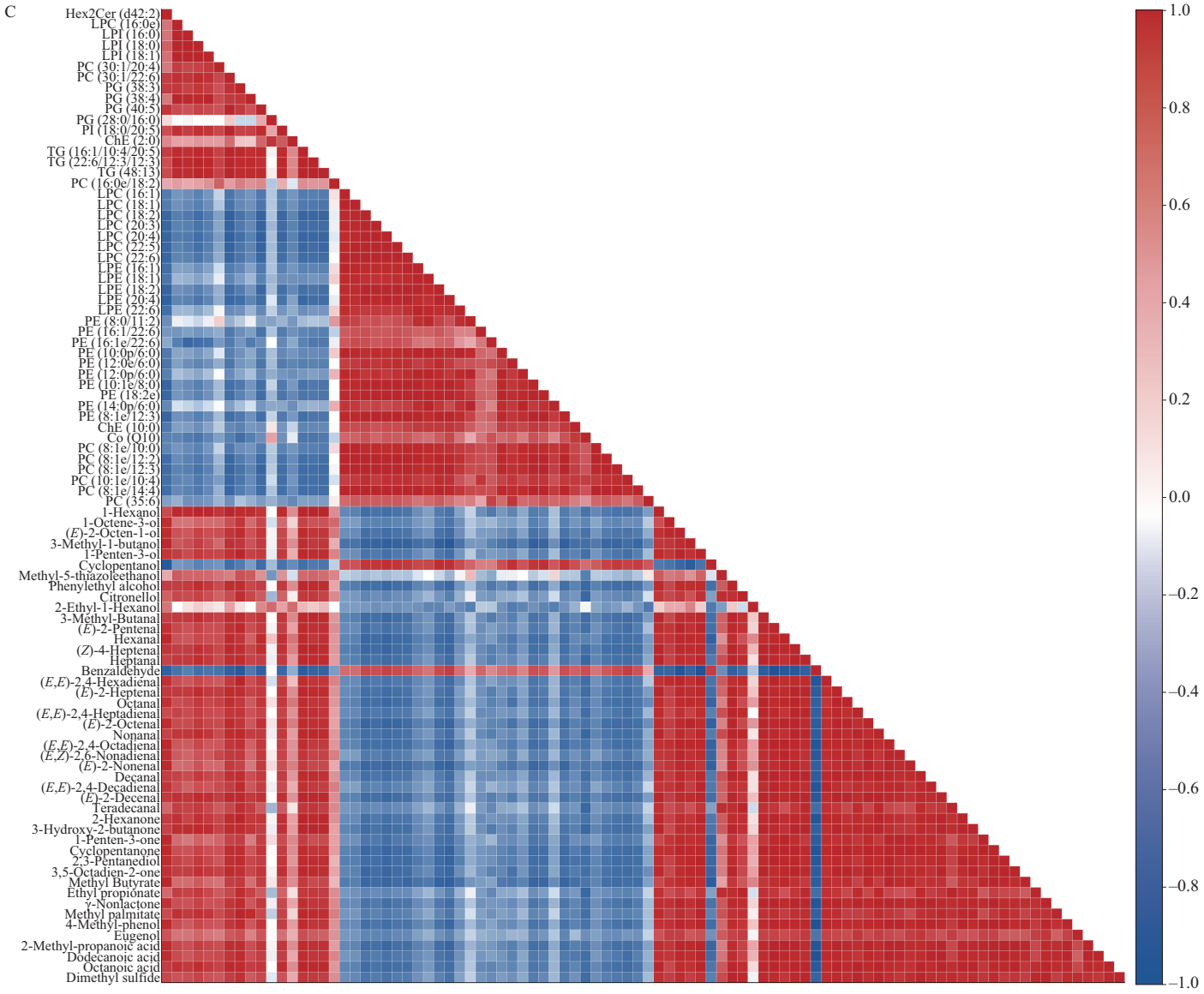

Fig. 4 (*Continued*)

LPC (20:4) and LPC (18:2). Likewise, most volatile compounds demonstrated strong negative correlations with LPC (20:4) and LPC (18:2).

4. Conclusions

During cold storage, lipid oxidation occurred, as indicated by the changes of AV, POV, TBARS, HP, and the activities of related enzymes (LOX, LPS, PLA_2, and HPL). The content of FFAs decreased greatly, especially linoleic acid (18:2) and arachidonic acid (20:4). The odor profile changed significantly in grass carp fillets. A total of 45 key volatile compounds were detected, and 47 DALs were screened, including 30 down-regulated lipids and 17 up-regulated lipids. By Pearson correlation analysis, LPC (20:4) and LPC (18:2) were verified as the main precursors of odorants' formation in grass carp fillets during cold storage. This study figured out the state of lipid oxidation substrates of flavor formation and established a foundation for our further studies to achieve targeted inhibition of unpleasant fishiness in grass carp fillets during cold storage.

Conflict of 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.

Acknowledgments

The authors gratefully acknowledge the Natural Science Foundation of Hubei Province (2023AFB311) and National Natural Science Foundation of China (32072250) for the financial supports.

Reference

[1] H.W. Zhang, S.B. Xiong, X.Y. Yu, et al., Fishy odorants in pre-processed fish fillet and surimi products made from freshwater fish: formation mechanism and control methods, Trends Food Sci. Technol. 142 (2023) 104212. https://doi.org/10.1016/j.tifs.2023.104212.

[2] N. Xiao, H. Xu, X. Jiang, et al., Evaluation of aroma characteristics in grass carp mince as affected by different washing processes using an E-nose, HS-

SPME-GC-MS, HS-GC-IMS, and sensory analysis, Food Res. Int. 158 (2022) 111584. https://doi.org/10.1016/j.foodres.2022.111584.

[3] Y.W. Diao, X.Y. Cheng, L.S. Wang, et al., Effects of immersion freezing methods on water holding capacity, ice crystals and water migration in grass carp during frozen storage, Int. J. Refrig. 131 (2021) 581-591. https://doi.org/10.1016/j.ijrefrig.2021.07.037.

[4] Y.M. Chu, J. Mei, J. Xie, Exploring the effects of lipid oxidation and free fatty acids on the development of volatile compounds in grouper during cold storage based on multivariate analysis, Food Chem. X 20 (2023) 100968. https://doi.org/10.1016/j.fochx.2023.100968.

[5] P.X. Yuan, X.N. Chen, S. Benjakul, et al., Label-free based proteomics revealed the specific changes of muscle proteins in pike eel (*Muraenesox cinereus*) under cold stress, Food Chem. X 14 (2022) 100275. https://doi.org/10.1016/j.fochx.2022.100275.

[6] E. Ringø, H.V. Doan, S. Lee, et al., Lactic acid bacteria in shellfish: possibilities and challenges, Rev. Fish. Sci. Aquacult. 28 (2020) 139-169. https://doi.org/10.1080/23308249.2019.1683151.

[7] W. Karbsri, A. Hamzeh, J. Yongsawatdigul, Changes in volatile compounds and lipid oxidation in various tissues of Nile tilapia (*Oreochromis niloticus*) during ice storage, J. Food Sci. 89 (2024) 2261-2276. https://doi.org/10.1111/1750-3841.17013.

[8] Y. Tong, N. Li, H. Liu, et al., Mechanistic studies by sum-frequency generation spectroscopy: hydrolysis of a supported phospholipid bilayer by phospholipase A_2, Angew. Chem. Int. Ed. 49 (2010) 2319-2323. https://doi.org/10.1002/anie.200904950.

[9] J.L. Li, Z.C. Tu, L. Zhang, et al., Characterization of volatile compounds in grass carp (*Ctenopharyngodon idellus*) soup cooked using a traditional Chinese method by GC-MS, J. Food Process. Preserv. 41 (2016) e13088. https://doi.org/10.1111/jfpp.12995.

[10] Y. Wang, Y. Wu, Y. Shen, et al., Metabolic footprint analysis of volatile organic compounds by gas chromatography-ion mobility spectrometry to discriminate mandarin fish (*Siniperca chuatsi*) at different fermentation stages, Front. Bioeng. Biotechnol. 9 (2021) 805364. https://doi.org/10.3389/fbioe.2021.805364.

[11] Y.Y. Zhang, E. Elam, Z.J. Ni, et al., LC-MS/MS targeting analysis of terpenoid metabolism in *Carya cathayensis* at different developmental stages, Food Chem. 366 (2022) 130583. https://doi.org/10.1016/j.foodchem.2021.130583.

[12] H.X. Wang, L. Qin, Y. Wang, et al., Effects of heating conditions on fatty acids and volatile compounds in foot muscle of abalone *Haliotis discus* hannai Ino, Fish. Sci. 80 (2014) 1097-1107. https://doi.org/10.1007/s12562-014-0779-x.

[13] X.F. Hu, J.L. Li, L. Zhang, et al., Effect of frying on the lipid oxidation and volatile substances in grass carp (*Ctenopharyngodon idellus*) fillet, J. Food Process. Preserv. 46 (2022) e16342. http://doi.org/10.1111/jfpp.16342.

[14] J.W. Deng, Y.Y. Yang, L.J. Luo, et al., Lipid analysis and lipidomics investigation by ambient mass spectrometry, TrAC-Trends Anal. Chem. 128 (2020) 115924. https://doi.org/10.1016/j.trac.2020.115924.

[15] X.N. Yu, L.Q. Li, J. Xue, et al., Effect of air-frying conditions on the quality attributes and lipidomic characteristics of surimi during processing, Innov. Food Sci. Emerg. Technol. 60 (2020) 102305. https://doi.org/10.1016/j.ifset.2020.102305

[16] Q. Chen, X. Wang, P. Cong, et al., Erratum to: mechanism of phospholipid hydrolysis for oyster *Crassostrea plicatula* phospholipids during storage using shotgun lipidomics, Lipids 52 (2017) 1059-1060. https://doi.org/10.1007/s11745-017-4308-4.

[17] L. Wang, M.W. Zang, X.Y. Cheng, et al., Evaluation of changes in the lipid profiles of dried shrimps (*Penaeus vannamei*) during accelerated storage based on chemical and lipidomics analysis, LWT-Food Sci. Technol. 191 (2024) 115564. https://doi.org/10.1016/j.lwt.2023.115564.

[18] L. Fernández-del-río, S. Rodríguez-lópez, E. Gutiérrez-casado, et al., Regulation of hepatic coenzyme Q biosynthesis by dietary omega-3 polyunsaturated fatty acids, Redox Biol. 46 (2021) 102061. https://doi.org/10.1016/j.redox.2021.102061.

[19] H.X. Che, J. Yu, J.Y. Sun, et al., Bacterial composition changes and volatile compounds during the fermentation of shrimp paste: dynamic changes of microbial communities and flavor composition, Food Biosci. 43 (2021) 101169. https://doi.org/10.1016/j.fbio.2021.101169.

[20] Q. Zhang, X.C. Chen, Y.T. Ding, et al., Diversity and succession of the microbial community and its correlation with lipid oxidation in dry-cured black carp (*Mylopharyngodon piceus*) during storage, Food Microbiol. 98 (2021) 103686. https://doi.org/10.1016/j.fm.2020.103686.

[21] Z.H. Huang, W.L. Guan, X.M. Lyu, et al., Impacts of acute ammonia-N exposure on the muscle quality of whiteleg shrimp (*Penaeus vannamei*): novel insights into lipid and protein oxidation, Food Chem. 437 (2024) 137781. https://doi.org/10.1016/j.foodchem.2023.137781.

[22] Y. Shi, P. Wei, Q. Shi, et al., Quality changes and deterioration mechanisms in three parts (belly, dorsal and tail muscle) of tilapia fillets during partial freezing storage, Food Chem. 385 (2022) 132503. https://doi.org/10.1016/j.foodchem.2022.132503.

[23] H. Ohkawa, N. Ohishi, K. Yagi, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, Anal. Biochem. 95 (1979) 351-358. https://doi.org/10.1016/0003-2697(79)90738-3.

[24] Y.Q. An, S.B. Xiong, Y.P. Qian, et al., *In vivo* and *in vitro* aroma release in surimi gel with different cross-linking degrees by proton transfer reaction-mass spectrometry, Food Chem. 373 (2022) 131502. https://doi.org/10.1016/j.foodchem.2021.131502.

[25] N. Smichi, Y. Gargouri, N. Miled, et al., A grey mullet enzyme displaying both lipase and phospholipase activities: purification and characterization, Int. J. Biol. Macromol. 58 (2013) 87-94. https://doi.org/10.1016/j.ijbiomac.2013.03.056.

[26] M. Minchiotti, M.B. Scalambro, L. Vargas, et al., Isolation of phospholipase A_2 from soybean (*Glycine max*) seeds: the study of its enzymatic properties, Enzyme Microb. Technol. 42 (2008) 389-394. https://doi.org/10.1016/j.enzmictec.2007.11.015.

[27] Y.Q. Wen, C.H. Xue, H.W. Zhang, et al., Concomitant oxidation of fatty acids other than DHA and EPA plays a role in the characteristic off-odor of fish oil, Food Chem. 404 (2023) 134724. https://doi.org/10.1016/j.foodchem.2022.134724

[28] Y.Q. An, L. Wen, W.R. Li, et al., Characterization of warmed-over flavor compounds in surimi gel made from silver carp (*Hypophthalmichthys molitrix*) by gas chromatography–ion mobility spectrometry, aroma extract dilution analysis, aroma recombination, and omission studies, J. Agric. Food Chem. 70 (2022) 9451-9462. https://doi.org/10.1021/acs.jafc.2c02119.

[29] J. Luo, M.M. Nasiru, H. Zhuang, et al., Effects of partial NaCl substitution with high-temperature ripening on proteolysis and volatile compounds during process of Chinese dry-cured lamb ham, Food Res. Int. 140 (2021) 110001. https://doi.org/10.1016/j.foodres.2020.110001.

[30] K.H. Tan, H. Awala, R.R. Mukti, et al., Inhibition of palm oil oxidation by zeolite nanocrystals, J. Agric. Food Chem. 63 (2015) 4655-4663. https://doi.org/10.1021/acs.jafc.5b00380.

[31] Q.J. Cao, H.Y. Du, Y. Huang, et al., The inhibitory effect of chlorogenic acid on lipid oxidation of grass carp (*Ctenopharyngodon idellus*) during chilled storage, Food Bioprocess Technol. 12 (2019) 2050-2061. https://doi.org/10.1007/s11947-019-02365-0.

[32] L. Feng, N. Tang, R. Liu, et al., The relationship between flavor formation, lipid metabolism, and microorganisms in fermented fish products, Food Funct. 12 (2021) 5685-5702. https://doi.org/10.1039/d1fo00692d.

[33] Z.R. Xu, Z. Gong, S. Murium, et al., Targeted screening of baicalein inhibitor with controlling lipid and protein oxidation for quality preservation of grass carp fillets, LWT-Food Sci. Technol. 191 (2023) 115526. https://doi.org/10.1016/j.lwt.2023.115526.

[34] S. Javed, F. Azeem, S. Hussain, et al., Bacterial lipases: a review on purification and characterization, Prog. Biophys. Mol. Biol. 132 (2018) 23-34. https://doi.org/10.1016/j.pbiomolbio.2017.07.014.

[35] L.Y. Zhao, S. Erasmus, P. Yang, et al., Establishing the relations of characteristic aroma precursors and volatile compounds for authenticating Tibetan pork, Food Chem. 427 (2023) 136717. https://doi.org/10.1016/j.foodchem.2023.136717.

[36] H. Cheng, J. Mei, J. Xie, Revealing the evolution of lipid extracts from tilapia (*Oreochromis mossambicus*) during cold storage: based on lipid metabolism analysis *via* GC-Q-MS and characterization of lipid oxidation, LWT-Food Sci. Technol. 201 (2024) 116268. https://doi.org/10.1016/j.lwt.2024.116268.

[37] P. Wan, J. Liu, D.W. Chen, Analysis of aroma-active compounds in bighead carp head soup and their influence on umami of a model soup, Microchem. J. 168 (2021) 106436. https://doi.org/10.1016/j.microc.2021.106436.

[38] D. Karrer, M. Gand, M. Rühl, Engineering a lipoxygenase from cyclocybe aegerita towards long chain polyunsaturated fatty acids, AMB Express 11 (2021) 37. https://doi.org/10.1186/s13568-021-01195-8.

[39] I. Peinado, W. Miles, G. Koutsidis, Odour characteristics of seafood flavour formulations produced with fish by-products incorporating EPA, DHA and fish oil, Food Chem. 212 (2016) 612-619. https://doi.org/10.1016/j.foodchem.2016.06.023.

[40] Y. Xu, Y. Liu, C. Jiang, et al., Determination of volatile compounds in turbot (*Psetta maxima*) during refrigerated storage by headspace solid-phase microextraction and gas chromatography-mass spectrometry, J. Sci. Food Agric. 94 (2014) 2464-2471. https://doi.org/10.1002/jsfa.6581.

[41] P.Y. Li, Z.J. Chen, M.T. Tan, et al., Evaluation of weakly acidic electrolyzed water and modified atmosphere packaging on the shelf life and quality of farmed puffer fish (*Takifugu obscurus*) during cold storage, J. Food Saf. 40 (2020) e12773. https://doi.org/10.1111/jfs.12773.

[42] S.Q. Gu, X.C. Wang, N.P. Tao, et al., Characterization of volatile compounds in different edible parts of steamed Chinese mitten crab (*Eriocheir sinensis*), Food Res. Int. 54 (2013) 81-92. https://doi.org/10.1016/j.foodres.2013.05.018.

[43] A. Giri, K. Osako, T.J.F.C. Ohshima, Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing, Food Chem. 120 (2010) 621-631. https://doi.org/10.1016/j.foodchem.2009.10.036.

[44] C. He, Z. Sun, X. Qu, et al., A comprehensive study of lipid profiles of round scad (*Decapterus maruadsi*) based on lipidomic with UPLC-Q-Exactive Orbitrap-MS, Food Res. Int. 133 (2020) 109138. https://doi.org/10.1016/j.foodres.2020.109138.

[45] S. Rodriguez-cuenca, V. Pellegrinelli, M. Campbell, et al., Sphingolipids and glycerophospholipids - the "ying and yang" of lipotoxicity in metabolic diseases, Prog. Lipid Res. 66 (2017) 14-29. https://doi.org/10.1016/j.plipres.2017.01.002.

[46] Y. Liu, P. Cong, B. Li, et al., Effect of thermal processing towards lipid oxidation and non-enzymatic browning reactions of Antarctic krill (*Euphausia superba*) meal, J. Sci. Food Agric. 98 (2018) 5257-5268. https://doi.org/10.1002/jsfa.9064.

[47] M. Wroniak, A. Rękas, A. Siger, et al., Microwave pretreatment effects on the changes in seeds microstructure, chemical composition and oxidative stability of rapeseed oil, LWT-Food Sci. Technol. 68 (2016) 634-641. https://doi.org/10.1016/j.lwt.2016.01.013.

[48] J.H. Lu, B.X. Chen, T.T. Chen, et al., Comprehensive metabolomics identified lipid peroxidation as a prominent feature in human plasma of patients with coronary heart diseases, Redox Biol. 12 (2017) 899-907. https://doi.org/10.1016/j.redox.2017.04.032.