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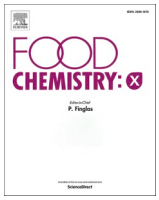

Multi-omics combined approach to analyze the mechanism of flavor evolution in sturgeon caviar (*Acipenser gueldenstaedtii*) during refrigeration storage

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ABSTRACT

Multi-omics techniques were combined with microstructure, molecular sensory science and non-volatile matrices for the first time to investigate variations in organic macromolecules and flavor in caviar during preservation. After 4–6 weeks of storage, the peroxide value was 35.38 mg/g and the accumulation of thiobarbiturates was significant with caviar membranes exhibiting a decrease in elasticity and an increase in viscosity. Sixteen key volatile compounds were detected by GC–MS, while the volatile compounds that contributed to the differences in caviar flavor at different storage times were mainly tetradecane, (*E*)-2-hexenal, and heptanal. The pathways associated with flavor release during storage were mainly abundant in the linolenic acid metabolism, alanine metabolism, and glycerophospholipid metabolism pathways. The correlation of 11 differential proteins and 24 differential lipids with odorants was further explored, such as arginine, proline, alanine, PE (20:4/22:6), PE (16:1/18:2), and PE (20:5/18:2). Overall, Aspartate, glutamate, oleic acid, linoleic acid, and phospholipids enriched in C22:6 and C18:2 chains are potential metabolic markers. This study provides a basis from a multi-omics perspective for the investigation of the relationship between quality deterioration and precursor metabolism in caviar storage process.

1. Introduction

Caviar is a microbially-fermented food obtained by slightly salting sturgeon eggs, which is known as one of the world's top three delicacies along with foie gras and black truffle (Haraharap et al., 2023). China produces 70% of the world's caviar and is the largest producer and exporter in the world. Caviar possesses proteins (24.75%), an abundance of amino acids (such as arginine, histidine, lysine and methionine), and lipids (16.41%, such as cholesterol and phospholipids with rich ω-3 fatty acids), which have high food value and potential healthy function (Brambilla et al., 2020; Lee et al., 2020). However, the production of wild sturgeon has been drastically reduced due to overfishing, ecological destruction and other factors. The long growth cycle of farmed sturgeon (7–10 years), the high cost of farming, the late identification period of male and female, the strict control of the processing technology of caviar, and the high technical level of the preservation process led to the expensive price of caviar (Palamarchuk, Nikolayenko, Ivanyuta, Zabolotnaya, & Bal, 2023). Therefore, the high standard of caviar quality and the maintenance of storage have become the new requirements of global consumers.

Although caviar is manufactured in a highly clean environment, specifically, caviar has a high protein and lipid content, which decomposes and oxidizes under microbial activity affecting the quality of caviar (Alak, Kaynar, & Atamanalp, 2021a) The main microorganisms in

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caviar include bacteria such as cocci, yeasts, molds, and *Escherichia coli* (Altug & Bayrak, 2003). Several spoilage microorganisms, such as *Listeria monocytogenes* and *Shewanella* spp., can also negatively affect the quality of caviar (Shi et al., 2019). Furthermore, small molecules of protein peptides increased with muscle degradation. It was reported that caviar stored at −4 degrees Celsius for 14 days showed a significant a decrease in pH and increase in oxidation, thiobarbituric acid (TBA), and psychrotrophic bacterial populations with the flavor deterioration. Further, oxidation of lipids produces aldehydes with rancid and fishy odors, exacerbating the decline in caviar texture, flavor, and nutritional value (Kido, Chosa, & Tanaka, 2023). Therefore, the metabolites produced by the above decomposition and oxidation deeply affect the quality of caviar, and problems associated with metabolic processes have seriously hampered the development of the caviar industry.

Non-targeted metabolomics is an important subfield critical to the exploration of flavor substances and functional components in foods (Wang, Guo, Zhao, & Feng, 2024). From complex metabolomics data, non-volatile components associated with key flavor substances can be identified. Lipidomics techniques have emerged as a result of the rapid development of mass spectrometry in the field of lipid detection and analysis and have been widely applied to explore the type, distribution and function of lipids (Yu et al., 2024). Sun et al. (2022) comparatively analyzed the differences in lipids between fresh and oxidized hazelnut oils and summarized the rules of flavor deterioration and the mechanisms of oxidative failure. Proteomics has emerged as a promising approach to determine the correlation between the levels of differentially expressed proteins and flavor changes in aquatic products by identifying molecular pathways and molecular interactions (Liu et al., 2022). In practical studies, the integration of multi-omics data is essential to enable the application of foodomics in food safety, flavor and nutritional health (Huang et al., 2024). However, no study has been conducted to monitor the changes in flavor quality of caviar during storage by means of multi-omics correlation analysis. Therefore, it is necessary to jointly investigate the evolution of flavor markers in caviar during storage from the perspectives of lipidomics and proteomics. The high content of marine phospholipids in aquatic products is highly susceptible to oxidation leading to flavor deterioration, resulting in quality decline during storage. Caviar as a good source of unsaturated fatty acids has always suffered from difficult storage quality control. Recent studies have reported that lipid peroxidation and microbial metabolism occurred in white caviar at different stages of maturation, resulting in significant differences in volatile compounds (aldehydes and alcohols) (Lopez et al., 2020). Lipidomics revealed that phospholipid PEs (20:2/20:4) and (22:6/22:5) in caviar were positively correlated with the formation of volatile aldehydes (Xu et al., 2022). A previous study reported that linolenic acid and oleic acid were oxidated to (*E, E*)-2,4-heptadienal and nonanal, respectively, which were associated with lipoxygenase (Fu, Xu, & Wang, 2009). Further studies are necessary to investigate the process of lipid changes during storage and the mechanism of flavor formation. In addition, in order to provide a reference for standardization of the quality of caviar, it is necessary to dynamically monitor indices associated with changes in quality.

In this study, the dynamics of caviar with different storage times were monitored (i) to comprehensively identify variations in indices associated with the quality of caviar; (ii) qualify and quantify key volatile compounds of caviar in different storage periods, and (iii) to investigate associations between lipid molecules, especially phospholipids and volatile compounds, during storage. This study aimed to select metabolic markers that represents the quality change during storage and reveal the mechanisms of change by metabolomics and lipidomics for providing a reference for the precise control of the quality of caviar.

2. Materials and methods

2.1. Preparation of caviar and chemicals

Freshly caviar of sturgeon was supplied by sturgeon aquatic food science and technology development Co., Ltd. (Quzhou, China) and transported to the laboratory on ice. After salting with 3.8% edible salt, the caviar was packed in vacuum cans in quantities of 30 g in each can and was then delivered to the laboratory on ice. The caviar was stored in sealed flat dishes at −4 °C and sampled once a week for a total of 6 times. The samples were freeze-dried and stored in a refrigerator at −60 °C for further testing. The experiment was repeated three times. The purity of the 16 standards was above 95% (Aladdin Biochemical Technology Co., Ltd., Shanghai, China).

2.2. Determination of acid value

The caviar (1.0 g) was added to the petroleum ether mixture and shaken for 30 min. The mixture was allowed to stand for 48 h, then filtered and the upper oil sample was blown dry with nitrogen and weighed. A few drops of phenolphthalein indicator were dissolved in ethanol (95%) and the solution was brought to a light boil in a 95 °C water bath. At this point, titration was immediately performed with a standard solution of KOH (0.01 mol/L). The mixture was shaken vigorously in a water bath at 95 °C to form a suspension. The mixture was titrated with the standard titration solution of KOH (0.01 mol/L). The acid value was determined from the amount of KOH consumed and was expressed in mg/100 g.

2.3. Determination of peroxide value

The constant weight caviar oil sample was mixed with trichloromethane, glacial acetic acid (2:3, *v*/v) and saturated potassium iodide. The mixed solution was placed under light-free conditions for 15 min before an addition of 10 mL of ultrapure water. Immediately, the solution was titrated with $Na_2S_2O_3$ (0.01 mol/L) until the blue color of the solution disappeared. The peroxide value (POV) was determined from the amount of $Na_2S_2O_3$ consumed and was expressed in meq/kg.

2.4. Determination of TBA concentration

Caviar samples (1000.23 mg) were thoroughly mixed with trichloroacetic acid (TCA; 7.6%, *w/v*) for 30 min at 50 °C and filtered through a double layer of quantitative filter paper. The upper filtrate sample was mixed with TBA (0.02 mol/L, 1:1, *v*/v) and heated in a water bath at 95 °C for 30 min and then cooled to room temperature. The absorbance of the sample solution was obtained at 532 nm and substituted into the standard curve (MDA) to calculate the content (mg/kg).

2.5. Determination of free amino acids (FAA) contents

The determination of amino acids was performed according to the previous method with minor modifications (Chu, Mei, & Xie, 2023). After a 0.5 g sample was homogenized for 30 min in a blender with 5 mL of 0.1 mol/L HCl, the homogenate was centrifuged at 10,000 r/min for 10 min at 4 °C. An automated amino acid analyzer (L-8900, HITACHI Limited, Kyoto, Japan) was used for the determination of caviar FAAs. The clear solution was combined in a 10 mL volumetric flask and volume-filled with ultrapure water. The volume of the solution was mixed with trichloroacetic acid (TCA) solution (10%, 1:1, *v*/v) and centrifuged at 10,000 r/min for 10 min. The precipitate was discarded, and the pH of the supernatant was adjusted to 2.0 with NaOH (6 mol/L) and then volume-fixed to 15 mL. The final solution was passed through a 0.22 μm filter membrane to the upper stage.

2.6. Determination of fatty acids contents

Different caviar samples were mixed with chloroform/methanol (2:1, v/v) for 20 min and allowed to stand at 4 °C for 2 h (Folch, Lees, & Stanley, 1957). The sample solution was passed through medium speed filter paper to obtain a filtrate. The filtrate was mixed with NaCl solution (0.9%) and centrifuged at 4000 r/min for 10 min. Total lipids were obtained by nitrogen blowing the lower organic phase at 65 °C, followed by saponification with potassium hydroxide methanol solution (0.125 mol/L) for 30 min at 60 °C in a water bath. The saponified solution was mixed with a methanol solution containing boron trifluoride (14%) and held at 60 °C for 30 min. After the solution cooled, the extraction was repeated three times with hexane and ultrapure water. After blowing with nitrogen to a constant weight, hexane was added to a volume of 10 mL to obtain a methyl esterified sample.

The GC–MS (QP2010 SE; Shimadzu, Kyoto, Japan) with a column (HP-5MS, 30 m × 0.25 mm × 0.25 μm) was used for fatty acid analysis. The heating sequence was as follows: an initial holding time of 60 °C for 1 min, followed by an increase in temperature at 10 °C/min to 160 °C for 5 min, an increase in temperature at 3 °C/min to 200 °C for 10 min, and a final increase in temperature at 6 °C/min to 280 °C for 5 min. The MS was scanned at a mass-to-charge ratio of 30–500 *m/z* with an ion source temperature of 250 °C at an electron energy of 70 eV.

2.7. Analysis of texture profile

A texture analyzer (TMS-TOUCH, FTC, Virginia, USA) equipped with a 50 mm diameter cylindrical probe was used to determine the textural properties of caviar. A caviar sample (1.0 cm × 1.0 cm) was positioned for texture measurement and was compressed twice to 20% of its initial volume. The starting force of the probe was 0.05 N, and its speed was 1 mm/s. The values of textural parameters (adhesiveness, spring, and cohesiveness) were calculated.

2.8. Morphological analysis

The surface morphology of caviar was characterized using scanning electron microscopy (SEM; JSM-5800 LV, JEOL Corporation, Tokyo, Japan) operating at an acceleration voltage of 20.00 kV. Before imaging, the caviar was coated with a thin gold layer under vacuum.

2.9. Sensory analysis

Trained sensory panelists (15 in total, 8 females and 7 males, aged 20–45 years) participated in the sensory scoring. The caviar was scored every week for 6 weeks using a digital scoring method. Five sensory indices, namely, glossiness, fullness, odor, umami, and spring, were each rated on a 5-point scale (1 = not at all, 5 = overwhelmingly). The sensory analysis was conducted in individual booths in a sensory evaluation room to make sure that there was no interference. This sensory assessment required no ethical clearance and was taken with the consent of the subject.

2.10. GC-IMS determination of caviar at different storage periods

GC-IMS conditions for the monitor were determined as previously reported (Li et al., 2024). Freeze-dried caviar samples (1 g) were dissolved in water and added to a headspace bottle (20 mL). After pre-incubation of the sample at 60 °C for 20 min, headspace solid-phase microextraction coupled with gas chromatography-ion mobility spectrometry (GC-IMS) was used to analyze the volatile compounds in the caviar.An MXT-5 capillary column (15 m × 0.53 mm × 1 μm; RESTEK, Bellefonte, USA) was used for separation of ion streams in the mass spectrometer. The initial temperature of the gas phase was 60 °C and the carrier gas was helium with flow rates ranging from 2 mL/min to 10 mL/min and finally 150 mL/min. The total programmed setup time was 45 min. Laboratory Analytical Viewer software (G.A.S., Dortmund, Germany) was used for data processing and the samples were replicated in three experiments.

2.11. Gaschromatography-mass spectrometry (GC–MS) detection

Freeze-dried caviar powder (1.0 g) was kept in a 20 mL headspace bottle with 1 mL ultrapure water and was stored at −80 °C until analyzed. Another sample of 1.0 g was accurately placed into a 20 mL headspace bottle with 1 mL ultrapure water, and then 2-methyl-3-heptanone (5 μL, 100 μg/mL) was added to the bottle as an internal standard. The bottle was placed in a heater to equilibrate for 20 min at 60 °C, and then a divinylbenzene/carboxen/polydimethylsiloxane solid-phase microextraction fiber (2 cm, 50/30 μm; Supelco, Bellefonte, PA, USA) was exposed to the headspace above the samples for 30 min at 60 °C. GC–MS was detected using the previous method (Zhang et al., 2024). A GC–MS (QP2010 SE; Shimadzu, Kyoto, Japan) equipped with an HP-5MS column (60 m × 0.25 mm × 0.25 μm) was used for the analysis of volatile compounds in caviar. The GC conditions were initially held at 50 °C for 3 min, ramped to 100 °C at 3 °C/min and held for 2 min, then increased to 180 °C at 6 °C/min and held for 2 min, and finally raised to 250 °C at 10 °C/min held for 3 min. The MS was performed on an ion source at 230 °C with a mass scan range of 35–500 *m/z* and an electron energy of 70 eV. Identification of volatile compounds was performed using the NIST08 database (match scores >80%).

2.12. Standard quantification of volatile compounds (OAV > 1)

The odor activity value (OAV, μg/L) of volatile compounds was the relative amount of the compound divided by the threshold of the compounds. Sixteen volatile compounds (with OAVs of >1) were chosen for standard quantification and detected by previous study (Liu et al., 2023), namely 3-methylbutanal, hexanal, heptanal, (*E*, *E*)-2,4-heptadienal, (*E*)-2-octenal, nonanal, phenylacetaldehyde, (*E*, *Z*)-2,6-nonadienal, 2-dodecenal, 1-penten-3-ol, 1-octen-3-ol, 2-heptanone, 2,3-butanedione, 3-hydroxy-2-butanone, caryophyllene, and ocimene. Substances with an OAV value >1 were screened out for precise quantification using the selective ion monitoring (SEM) mode. Adsorption was performed in triplicate under the same conditions as the SPME described above. The response rates of standard compounds and internal standards were plotted against their respective concentrations for standard quantification.

2.13. Untargeted metabolomics analysis

Non-targeted metabolomics of caviar from different storage periods was performed as previously reported (Schlotterbeck, Cebo, Kolb, & Lammerhofer, 2019). Grinding beads were added to a mixture of caviar samples (0.2 g) and 80% methanol with L-2-chlorophenylalanine (0.02 mg/mL). The mixture was ground in a cryogenic tissue mill at −10 °C for 6 min and then sonicated at 4 °C. After placed at −20 °C for 30 min, the sample was centrifuged at 13,000 r/min for 15 min. Furthermore, the 20 μL supernatant was collected from each sample and mixed to obtain a quality control sample. The supernatant was injected into a UPLC-QExactive Plus mass spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with a HSS T3 column (100 mm × 2.1 mm × 1.8 μm) for analysis. Mobile phase A was 5% acetonitrile containing 1% formic acid and mobile phase B was a mixture of acetonitrile, isopropanol and ultrapure water (47.5:47.5:5, *v*/v/v) with 1% formic acid. The flow rate was 0.4 mL/min and the temperature of the column was 40 °C. The separation was carried out at a flow rate of 0.4 mL/min.

2.14. Proteomics analysis by LC-MS/MS

After mixing the caviar samples with protein lysate to inhibit protease activity, the samples were ground three times using a high-

throughput tissue mill. The mixture was sonicated and left to stand for 30 min at 4 °C. Subsequently, it was centrifuged at 12,000 *g* for 30 min at 4 °C and the concentration of the protein supernatant was determined using the BCA Protein Assay Kit (Pierce, Thermo, USA).

Protein samples were reacted in triethylammonium bicarbonate buffer (TEAB, 100 mM) and tris(2-carboxyethyl) phosphine (TCEP, 10 mM) for 60 min at 37 °C. The reaction solution was mixed with iodoacetamide (40 mM) and reacted for 40 min at room temperature, protected from light. Pre-cooled acetone was added in a volume of 6 times that of the samples, and then precipitated for 4 h at −20 °C. The protein supernatants were extracted with trypsin at 37 °C and the samples were incubated with tryptic acid. Finally, the mixture was digested with trypsin (1:50) at 37 °C overnight. Digested peptides were analyzed by LC-MS on a reversed-phase C18 column (1.7 μm, 2.1 mm × 150 mm, Waters, USA) as reported (Abril et al., 2024). The second dimension was performed using an Evosep One coupled to an Obitrap Exploris 480 mass spectrometer. The sample was injected and separated on the column for 44 min at a flow rate of 300 nL/min. Scanning was performed between 350 and 1500 (*m/z*) with a primary resolution of 60,000 and a secondary resolution of 15,000, with a dynamic exclusion time of 30 s.

2.15. Untargeted lipidomics analysis by UPLC-Q Exactive Orbitrap MS

Mix caviar of different storage periods with ml of chloroform/methanol (2:1, *v*/v) and homogenise. The mixture was allowed to stand for 1 h at 4 °C and centrifuged at 5000 r/min for 10 min at 4 °C. The upper layer was extracted twice with chloroform/methanol (2:1, v/v). The bottom layer was combined and blown under nitrogen to a constant weight. Caviar oil samples were prepared with isopropanol/acetonitrile (2:1, v/v) for uptake. Quality control samples were made by mixing equal amounts of all samples.

A UPLC-Q Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific, USA) equipped with a ZORBAX Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm) was used for non-targeted lipidomics. Mobile phase A was a mixture of acetonitrile and water (4:6, v/v) containing 0.77 g ammonium acetate. Mobile phase B was acetonitrile and isopropanol (1:9, v/v). The flow rate was 0.30 mL/min and the column temperature was 45 °C. Three replicates were performed for each set of samples.

2.16. Statistical analysis

All values were expressed using analysis of variance (ANOVA) and Duncan's multiple range test (SPSS 22.0 software, SPSS Inc). Data analyses were obtained by calculations on the majorbio choud platform (cloud.majorbio.com). Metabolic pathways were designed according KEGG pathway database (https://www.kegg.jp/kegg).GO (gene ontology, http://geneontology.org/) was chosen to analyze all differential proteins in terms of biological processes.

3. Results and discussion

3.1. Variation of caviar morphology and degree of oxidation

Authentic samples of caviar were analyzed by scanning electron microscopy (SEM) to observe the changes in microscopic morphology caused by different storage times (Fig. 1A). The surface of caviar assumed a complete and smooth form within 2 weeks. After 2–3 weeks of storage, the overall form of caviar became flat, the film was broken and flaky. At the later stages of storage (4–6 weeks), the caviar membrane gradually fragmented and became more layered. The membrane of caviar comprised a collagen layer consisting of a large quantity of insoluble (cross-linked) collagen (Vilgis, 2020). This collagen endowed caviar with spring and an intact morphology. Endogenous proteases induced the proteolysis of myofibrillar proteins and collagen (Singh & Benjakul, 2018). This result was supported by a study by Alak, Kaynar, and Atamanalp (2021b), which reported that in the later stages of caviar degradation, the surface loses the properties and turns into a sticky substance. With longer storage time, the protein contracted, resulting in muscle cross-linking and dehydration. Intercellular connections were thus weakened. During storage, cohesiveness and spring declined constantly (Fig. 1E and F). The spring values of caviar in this study ranged from 17.61 to 5.97 mm due to the loss of water from the stored caviar. This observation was caused by protein degradation or weakening of protein cross-linking. Adhesion was the ability to adhere or bond to a substrate. Caviar had an initial adhesion value of approximately 2.24, while the adhesiveness increased continually (Fig. 1D). It was highest after the 6 weeks of storage (7.73). According to reports, in the presence of Na and Ca ions in cured caviar, the inhibitory effect of salt ions on proteolysis increased with storage time and affected the composition and molecular weight of proteins (Gagliano, Sudmalis, Temmink, & Plugge, 2020). Degradation of myofibrillar proteins during storage leaded to a decrease in the affinity of the surface for water molecules (Wang, Guo, et al., 2024). Hence, the originally tight structure of myofibrillar proteins and collagen became loose and softened with continuous degradation.

The changes in the acid value of caviar during storage at −4 °C for 6 weeks were shown in Fig. 1B. The acid value increased overall during storage, which increased significantly after 5–6 weeks and reached 3.752 (KOH)/(mg/g). This phenomenon was reported and studies reported that hydrolysis and oxidation of lipids intensify during the later stages of fish storage because of the action of lipoxygenases, leading to a rapid increase in the concentration of free fatty acids (Yang et al., 2017). Furthermore, the peroxide value (POV) could also reflect the accumulation of intermediates during lipid oxidation. During storage for 0–5 weeks, the POV of caviar exhibited a slow increasing trend (Fig. 1C). In comparison with the value after storage for 5 weeks, the POV of caviar increased significantly after 6 weeks from 28.80 mg/100 g to 35.38 mg/100 g. A study reported that the continued accumulation of primary oxidation products during the early stages. The lipids were oxidized and hydroperoxides were produced with the extension of storage time, causing the increase of POV (Custodio-Mendoza, Ares-Fuentes, & Carro, 2023). As illustrated in Fig. 1D, the concentration of Thiobarbiturates (TBA) increased slowly during the 5 weeks and the value reached 13.36 mg/kg at 6 weeks, which was 1.74 times the initial value. This result was consistent with the findings of Wang et al. (2021). The accumulation of TBA was associated with the instability of hydroperoxides and their decomposition to from malondialdehyde. The height of the TBA value reflected the rate of lipid oxidation. Overall, significant variations in the surface characteristics of caviar after 5 weeks of storage accompanied by an increase in the degree of lipid oxidation, indicating the occurrence of caviar quality deterioration.

3.2. Dynamic changes in concentrations of free amino acids and fatty acids

Free amino acids were produced by protein degradation as important flavor substances in aquatic products (Li et al., 2022). Most of the amino acids showed an increasing trend during storage and increased significantly after 3 weeks of storage (Fig. 1I and Table A1). TAV of Glu increased to 1.165, and the TAV of other free amino acids (e.g., Asp, Ser, Met, and Val) also reached the highest level at 3 weeks of storage (Table A2). Moreover, the amino acid content of stored caviar exhibited a decreasing trend after the 3 weeks of storage. A study reported that enzymatic reactions and oxidation during storage resulted in protein degradation. The decrease in amino acid concentration was attributed to the proteins being solubilised from the muscle at a slower rate than the decarboxylation to by-products (i.e. biogenic amines) (Cerbu et al., 2023). Interestingly, the Asp and Glu content of caviar decreased significantly after 6 weeks of storage, with Lys, His, Met, Arg, Leu, Ala, and Tyr as the main components instead. Met and Leu caused the formation of methyl mercaptan and dimethyl sulfide molecules, which

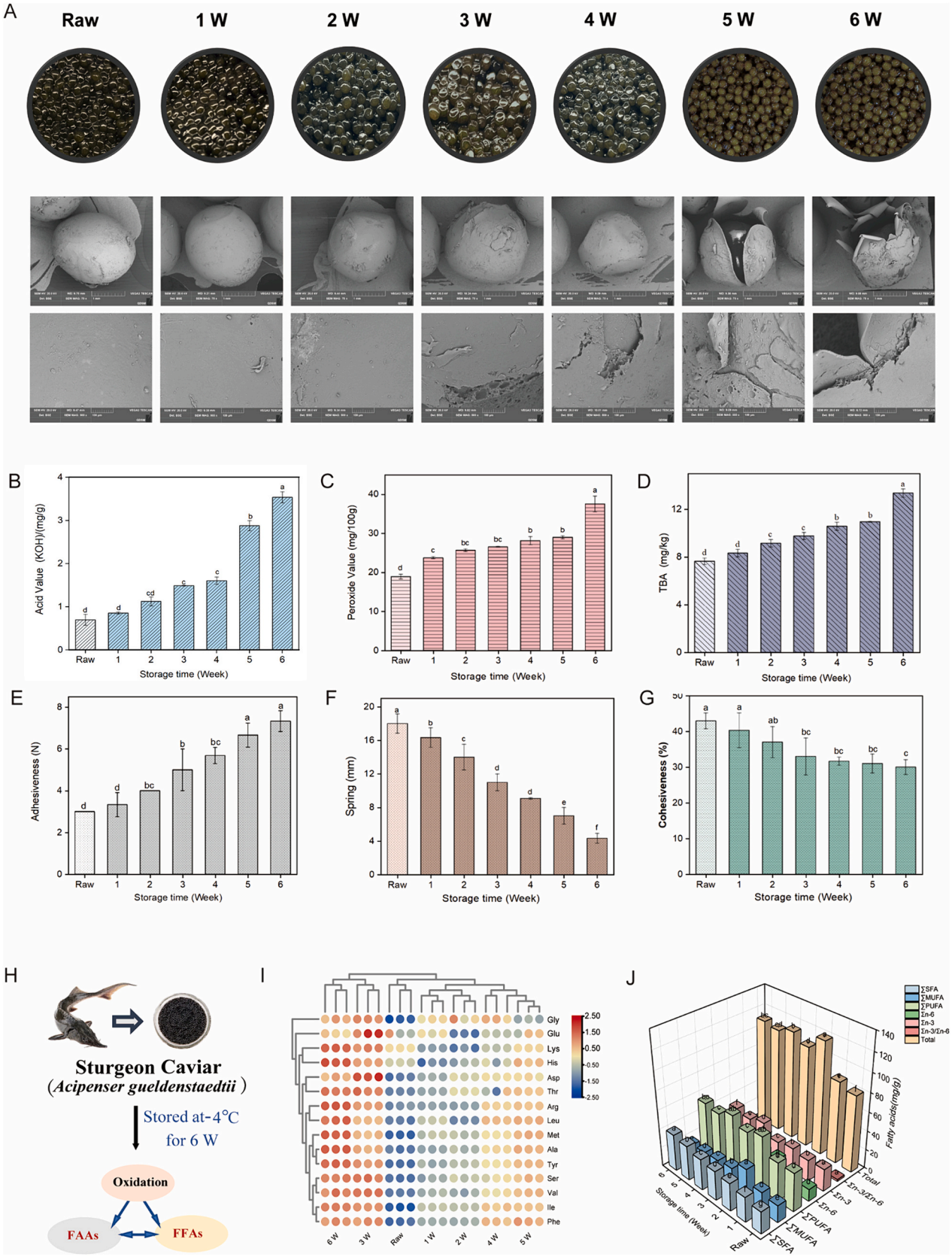

Fig. 1. Sample object and scanning electron microscope for caviar with different storage periods (A). Changes in acid value (B), peroxide value (C), thiobarbituric acid concentration (D), adhesiveness (E), spring (F), and cohesiveness (G). Non-volatile correlation, free amino acid, and free fatty acid classification in caviar stored at −4 °C for 6 weeks. A difference between the letters indicates a significant difference ($p < 0.05$).

were closely associated with the development of off-flavors (Marcinkowska & Jeleń, 2022). This phenomenon was mainly caused by the catabolic action of bacterial growth (Xiao, et al., 2020). There were two distinct phases of microbial growth that alter the quality of the food; one was a lag phase, when the concentration of some amino acids undergoes fluctuations. In another stage, microbial growth expands exponentially, accompanied by the production of off-flavors. The results indicated that caviar had the riches taste after 3 weeks and displayed a significant deterioration in quality after 6 weeks of storage. Furthermore, Glu and Asp were considered to be metabolic markers for determining the storage period of caviar.

In terms of different types of fatty acids, the content of saturated fatty acids (SFAs) continued to increase to 38.476 ± 0.278 mg/g. The content of monounsaturated fatty acid (MUFAs) reached the highest level after 2 weeks (33.169 ± 0.154 mg/g) and decreased from 3 to 6 weeks. The content of polyunsaturated fatty acid (PUFAs) was highest after 4 weeks (59.731 ± 0.602 mg/g) (Fig. 1J). PUFAs and monounsaturated fatty acids (MUFAs) underwent oxidation and were transformed into SFAs (Chaijan, Benjakul, Visessanguan, & Faustman, 2006). The changes in the composition and contents of fatty acids in caviar during storage are shown in Table S4. Palmitic acid (C16:0) was the most abundant fatty acid, followed by DHA, oleic acid (C18:1n9t), linoleic acid (C18:2n6t), and eicosapentaenoic acid (EPA). During storage, the content of palmitic acid increased gradually while that of hexadecenoic acid (C16:1, cis-9) decreased slowly. The descending trend was due to the high sensitivity of oleic acid and linoleic acid to autoxidation (Ajmal, Nadeem, Imran, & Junaid, 2018). The content of DHA underwent a sharp rise to 28.529 ± 0.303 mg/g after 4 weeks of storages. The content of EPA reached 9.958 ± 0.224 mg/g after 2 weeks but decreased significantly to 4.046 ± 0.213 mg/g after 6 weeks. Obviously, the contents of MUFAs, PUFAs, EPA, oleic acid, and linoleic acid exhibited a tendency to increase and then decrease. Zhang et al., (2021) reported that linoleic acid and arachidonic acid were both ω-6 polyunsaturated fatty acid acyl chains and excellent sources of aldehydes obtained by cleavage of carbon-carbon bonds. The lipolytic action of lipase and the hydrogenation of lipids caused these contents to increase (Wang et al., 2014). Oxidation resulted in the reaction and autoxidative degradation of the fatty acids in caviar with free radicals, producing secondary volatile products such as aldehydes and ketones (Lopez et al., 2020). Therefore, variation in flavor profile of caviar during storage and correlation with non-volatile metabolites remained to be explored.

3.3. Flavor properties of caviar in storage

The sensory profiles of caviar during storage were shown Fig. 2A-2C. The total score for five sensory attributes roughly decreased with an increase in the storage period (Fig. 2C). Based on the organoleptic characteristics of caviar, caviar with different storage times were classified into six groups. The caviar after 3 and 4 weeks of storage were grouped together, which illustrated the organoleptic similarity as well as quality degradation due to prolonged storage time. (Fig. 2B). The fullness, odor, umami, and spring of caviar increased from 0 to 2 or 3 weeks, and decreased subsequently, while the glossiness gradually decreased. The umami score of caviar reached its highest level after 3 weeks (4.231 ± 0.127) and then decreased to 2.385 ± 0.165 after 6 weeks (Fig. 2A). The umami of aquatic products mainly come from Glu and Asp, which was consistent with the trend of changes in free amino acids. The flavor of aquatic products in storage as changed from freshness to bitterness. These results were in consonance with the research of Mabuchi, Ishimaru, Tanaka, Kawaguchi, and Tanimoto (2019). The variation in taste values was mainly attributed to the loss of electrolytes and changes in the content of taste compounds in caviar resulting from quality deterioration during storage (Li et al., 2023).

The volatile compounds responsible for caviar flavor differences between storage times were mainly tetradecane, (*E*)-2-hexenal, and heptanal, followed by hexanal, benzaldehyde, 1-octen-3-ol, 1-penten-3-ol, (*E*, *E*)-2,4-heptadienal, and dodecane (Fig. 2E). As shown in Fig. 2D and F, in fresh caviar, the flavor profile consists mainly of aldehydes (hexanal, heptanal, and nonanal), olefins (undecane), and acids (octadecanoic acid and tetradecanoic acid), causing the oily, green, fat, fresh, fatty, and fishy odors. Caviar after 3 weeks of storage exhibited an increase in C7-C11 unsaturated aldehydes (e.g., (*E*)-2-heptenal and (*E*, *E*)-2,4-decadienal) and the presence of C8 ketones (e.g., 3,5-octen-2-one) primarily resulting in undesirable attributes, such as rancid, oxidization, earthy, and musty. Moreover, 3-methyl-butyraldehyde (rancid and fishy odor), 3-methyl-bmoutyric acid, 1-octen-3-ol (mushroom odor), and 2,3-butanedione (oxidation and earthy odors) increased significantly after 6 weeks of storage. 3-methyl-1-butanol and 3-methylbutyraldehyde were contributed to rancid odor and were markers of fish freshness evaluation. The 2,3-butanedione, which played an important role among the ketones, was derived from the amino transferasemia of α-amino acids buttery and creamy odor (Liu, Holland, & Crow, 2004). 3-Methylbutyraldehyde was formed via Strecker degradation of dicarbonyl compounds while 1-octen-3-ol was degraded from lipids (Tian, Xiong, Yu, Chen, & Lou, 2023). Thus, aroma compounds could be produced from different α-amino acids and reducing sugars via the Strecker pathway. Furthermore, oxidation of free fatty acids was also important for flavor variation during storage and could contribute as important aroma precursors for ketones, alcohols, and esters (Liu et al., 2022).

Volatile compounds with an OAV of >1 was regarded to be in the human detectable range and could be perceived. Sixteen volatile compounds (with OAVs of >1) were chosen for standard quantification and are listed in Table S3. 3-Methylbutanal ("fatty, green, nutty") had the highest OAV from 0 to 3 weeks, exhibited grassy, nutty, oily, and fruity odors. (*E*, *Z*)-2,6-Nonadienal ("green, fatty") made the greatest contribution to the odor of caviar at weeks 3 and weeks 4. 1-Octen-3-ol and 2-heptanone was produced after 3 weeks, casuing the mushroom and cheesy odors. After 5–6 weeks, ketones began to be generated and endowed caviar with cheesy, buttery, and dairy odors. Xu et al. (2022) demonstrated that nonanal and hexanal possessed strong fatty odors and were aromatic active compounds in sturgeon caviar. Previous studies indicated that the levels of ethyl acetate, 2-methylbutyraldehyde and 3-methylbutyraldehyde increased with storage time during caviar storage, which were derived from free amino acids through Strecker degradation reactions (e.g., 2-methylbutyraldehyde was derived from isoleucine) (Jiang, et al., 2022).

A total of 41 volatile compounds were identified by GC-IMS, (Fig. 2G and H). Many volatile compounds exhibited an upward and then a downward trend (such as butanal, 2-pentanone, and 2,3-pentanedione). At 2–4 weeks, 2-pentanone ("sweet, fruity, and banana"), 2,3-pentanedione ("buttery, nutty, and toasted"), butanoic acid ("dairy, cheesy, buttery, and fruity"), (*E*)-2-hexenal ("fresh, green, leafy"), (*E*)-2-heptenal ("green, fatty, oily"), and (*E*)-2-octenal ("fresh, cucumber, and fatty") had high contents, which was consistent with GC–MS results. From 5 to 6 weeks, the contents of the abovementioned volatile substances decreased markedly. However, the contents of propyl acetate ("fruity, banana"), 3-methyl-1-butanol ("fermented"), ethyl 2-methylpropanoate ("sweet"), pentanoic acid ("sour"), and 2-butanone ("fruity") increased. This indicated that the caviar exhibited significant off-flavors at the later stages of storage. Similar results were found in the report of Frank et al. (2020). Ethyl caprylate, 2-pentylfuran, valeric acid and hexanoic acid were identified as potential shelf-life markers. In frozen beef, 3-methyl-1-butanol, 2,3-butanediol and 2-ethyl-1-hexanol, benzaldehyde and 2-butanone accumulated with increased storage period. Therefore, more research was required to confirm the relationship between key volatile compounds and non-volatile changes to caviar quality.

3.4. Association analysis of molecular sensory, volatile compounds and their precursors

Differences in volatile compounds in caviar with different storage

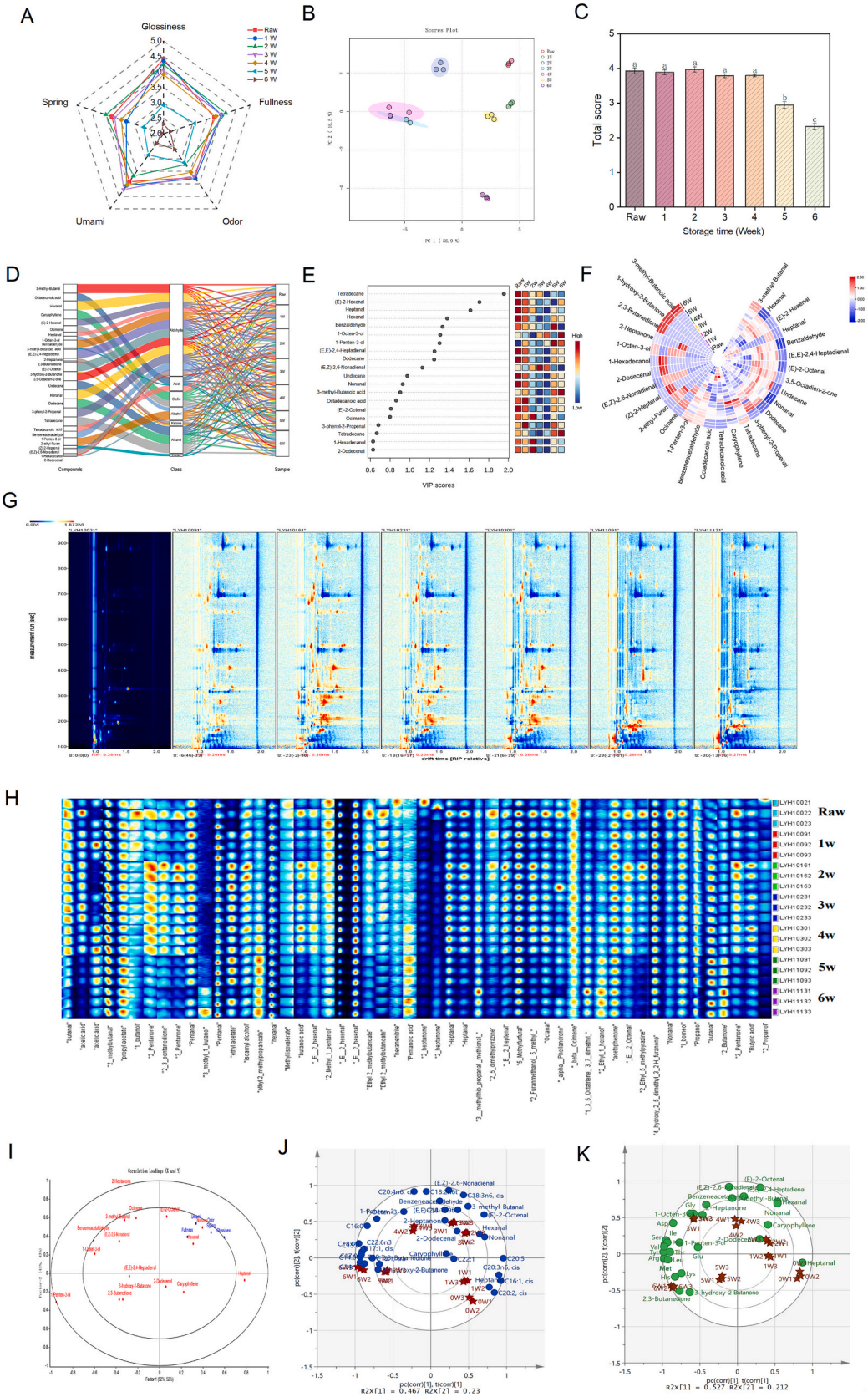

Fig. 2. Sensory score radar chart (A), PCA analysis (B), and overall score (C) for caviar with different storage periods. Mulberry plot (D), VIP score plot (E), and clustered heat map (F) of volatile compounds in caviar stored at −4 °C for 6 weeks detected by GC–MS. Comparison (G) and gallery plot (H) of ion flow detected by GC-IMS. PLSR analysis of key volatile compounds and sensory attributes for OAV > 1 (I), correlation double-loading plots of fatty acids (J), amino acids (K), and key volatile compounds, respectively.

times were further explored. The relationships between sensory descriptors, key volatile compounds (rOAV>1) and their precursors were analyzed by PLS-DA plots (Fig. 2I-K). The grassy, fatty, and fishy odors of fresh caviar mainly > ω-3 of C20 were closely related to the flavor composition of caviar stored for 2–4 weeks mainly with unsaturated fatty acids (C18:3, C18:2, and C20:4), resulting in the enrichment of aldehydes and ketones (Fig. 2J). Some compounds responsible for caviar off-flavors such as 2,3-butanedione, 3-hydroxy-2-butaone, and 1-penten-3-ol were highly associated with the later stages of storage (5–6 weeks) mainly (Fig. 2K). Similar results were reported in the study of Kritikos et al. (2020). The relative concentrations of 2,3-butanedione, 3-hydroxy-2-butanone (Ket-3 group) and acetic acid started to increase significantly after 5 days of storage, which can be used as markers of spoilage in stored sea bass. At the same time, the metabolism and succession of amino acids are inextricably linked to the production of these compounds, including (Met, His, and Lys, etc.). The composition and content of amino acids during storage can be attributed to changes in protein structure and transformations during metabolism (Zhao et al., 2022). Previous studies demonstrated an increase in the carbonyl content of red trout during cold storage (Babic Milijasevic et al., 2023). Strecker aldehyde and 3-methylbutyraldehyde were originated as a result of interactions between amino acids and sugars, as well as possibly as a result of oxidized lipids and protein interactions (Cheng, Wang, & Xie, 2023). Therefore, the oxidation processes of proteins and lipids during metabolism and their correlation with caviar quality deterioration required further investigation.

3.5. Metabolite profiles and bioinformatics analysis of differential abundance proteins

Changes in the contents of small and medium molecules in caviar during storage were analyzed by untargeted metabolomics (Fig. 3). The respective proportions of the superclasses of organic acids and their derivatives, lipids and lipid-like molecules, and organoheterocyclic compounds were 49.55%, 22.32%, and 8.93% in all samples based on HMDB (Fig. 3A). A total of 226 potential differential metabolites were selected from different caviar samples. In the KEGG pathways, 38 differential metabolites were annotated to beta-alanine metabolism, alpha-linolenic acid metabolism, sphingolipid metabolism, biosynthesis of unsaturated fatty acids, and alanine, aspartate and glutamate metabolism. The key metabolites comprised organic acids (14), lipids (7), amino acids (7), nucleotides (6), and other compounds (4). The variations during storage are shown in Fig. 3B.

Based on the identification of all proteins, the three groups of samples were quantitatively differentiated using Principal Component Analysis (PCA) (Fig. 3 C). The PCA score plot indicated that samples with different storage times were far enough apart to be distinguished from each other, suggesting that there were significant differences in their proteomes in agreement with the results of the heat map analysis. Proteins in the range of FC > 1.20 or < 0.83 with $p < 0.05$ were considered differential proteins compared to controls. Screening based on the above criteria yielded 436 differential proteins.

According to KEGG classification, the differentially expressed proteins were involved in metabolic processes mainly for fatty acids and amino acids, followed by vitamins, monosaccharides, and nucleotides (Fig. 3D). The key metabolic pathways of the differentially expressed proteins were further resolved by pathway enrichment analysis. The highest pathways included Citrate cycle (TCA cycle), Glucagon signaling pathway, Glutathione metabolism, and beta-Alanine metabolism (Fig. 3E). Protein and KEGG pathway enrichment correlations were analyzed in depth by the creation of chordal plots. The metabolic pathways with higher *Z*-values were ranked as xenobiotic metabolism of cytochrome P450, the citric acid cycle (TCA cycle), and pyruvate metabolism, suggesting that these pathways are activated and expressed during caviar storage (Fig. 3F). This result was consistent with the study of Li et al., (2017). Four metabolic pathways were detected to be significantly upregulated in oysters with different glycogen content, including alanine, aspartate and glutamate metabolism (map00250), D-glutamine and D-glutamate metabolism (map00471), arginine and proline metabolism' (map00330), and citric acid cycle (TCA cycle). Based on the metabolic pathways of the KEGG pathway pooled by iPath, 11 major differential proteins were selected to further explore the evolution in caviar at different storage times (Fig. 3G). The enzymes related to fatty acid metabolic pathways (e.g. A0A444V2R9 and A0A444UQ88) were key enzymes involved in fatty acid β-oxidation. Compounds produced by lipid degradation were the main volatile components of caviar at different storage times. In particular, the action of lipoxygenases on polyunsaturated fatty acids produces characteristic volatile compounds such as 1-octanol, 1-heptanol, and other alcohols (Jin, Gouda, Jin, and Ma, 2019). 1-Octen-3-ol, a signature volatile compound in aquatic products, originates from the reaction of alkoxyl groups with lipid molecules in lipid oxidation (Huang et al., 2021). In the metabolic network of stored caviar, enzymes involved in arachidonic acid metabolism include A0A444UPP0 and A0A444V5E0. Whereas straight chain aldehydes (octanal) were mainly produced by the oxidation of linoleic acid and arachidonic acid, of which arachidonic acid can be obtained by linoleic acid metabolism (Liu et al., 2020).

3.6. Phospholipids in caviar and their associations with volatile metabolites

The relationships between phospholipids and flavor generation in caviar were analyzed. According to the HMDB, the metabolites included glycerophospholipids (49.56%), glycerolipids (41.90%), sphingolipids (4.75%), and other compounds (Fig. 4A). Metabolites ($p < 0.05$, VIP > 1.0) were selected as differential substances of caviar samples in different storage periods. A total of 394 metabolites were selected, which consisted of 292 metabolites in positive detection mode and 102 metabolites in negative detection mode. However, only 146 metabolites were annotated to the HMDB. The metabolites were classified into lipids according to their biological functions by KEGG. In the class of lipids, PC (15:0/18:2), PE (16:0/16:0), PE (18:0/18:0), PE (16:1/20:4), PE (20:3/20:5), PE (14:0/22:6), LPC (17:0), LPC (22:4), and cardiolipin (CL) (16:0) were significantly differentially abundant metabolites (Fig. 4B). After KEGG pathway annotation was performed, they all belonged to lipid metabolism (Fig. 4C). Similar results were observed in Chen et al., (2023). Glycerophospholipid metabolism was reported to dominate lipid metabolism in fermenting mandarin fish. PC and LPE were produced by PE. PC was degraded to LPC and sn-glycerol-3-phosphate choline, which was further degraded to choline and sn-glycerol 3-phosphate.

A total of 61 metabolites were annotated to lipids in KEGG (Fig. 4D). Different phospholipids (24) containing EPA or DHA of which the relative contents changed significantly during storage in Fig. 4E and F. Except for PC (22:4/22:6), lysophosphatidylethanolamine (LPE) (22:6), and LPE (20:5), the contents of the other 21 phospholipids that were rich in DHA or EPA exhibited a trend of first decreasing and then increasing. Most of the precursors of flavor compounds are derived from the food itself, mainly via the oxidative degradation of lipids and non-enzymatic browning reactions. Of these, oxidative degradation of lipids can give specific aromas to foods (Li et al., 2022). In aquatic products, phospholipids were important precursors for the oxidation of lipids to generate volatile compounds, with the main pathway being the oxidation of unsaturated acyl chains (Chen, Balagiannis, & Parker, 2019). Aldehydes were important oxidation products of phospholipid degradation that had a strong and specific smell, a low threshold, and a fatty fragrance (Calkins & Hodgen, 2007). Reis and Spickett (2012) reported that hydroperoxides are likely to be generated at the C9 position in phospholipids. Triglycerides and their methyl ester derivatives in the C9 and C13 positions produced hydrogen peroxide in the presence of enzymes and oxygen. C9 hydrogen peroxide further generated 2,4-decadienal, and hexanal was oxidized from C13 hydrogen peroxide as a

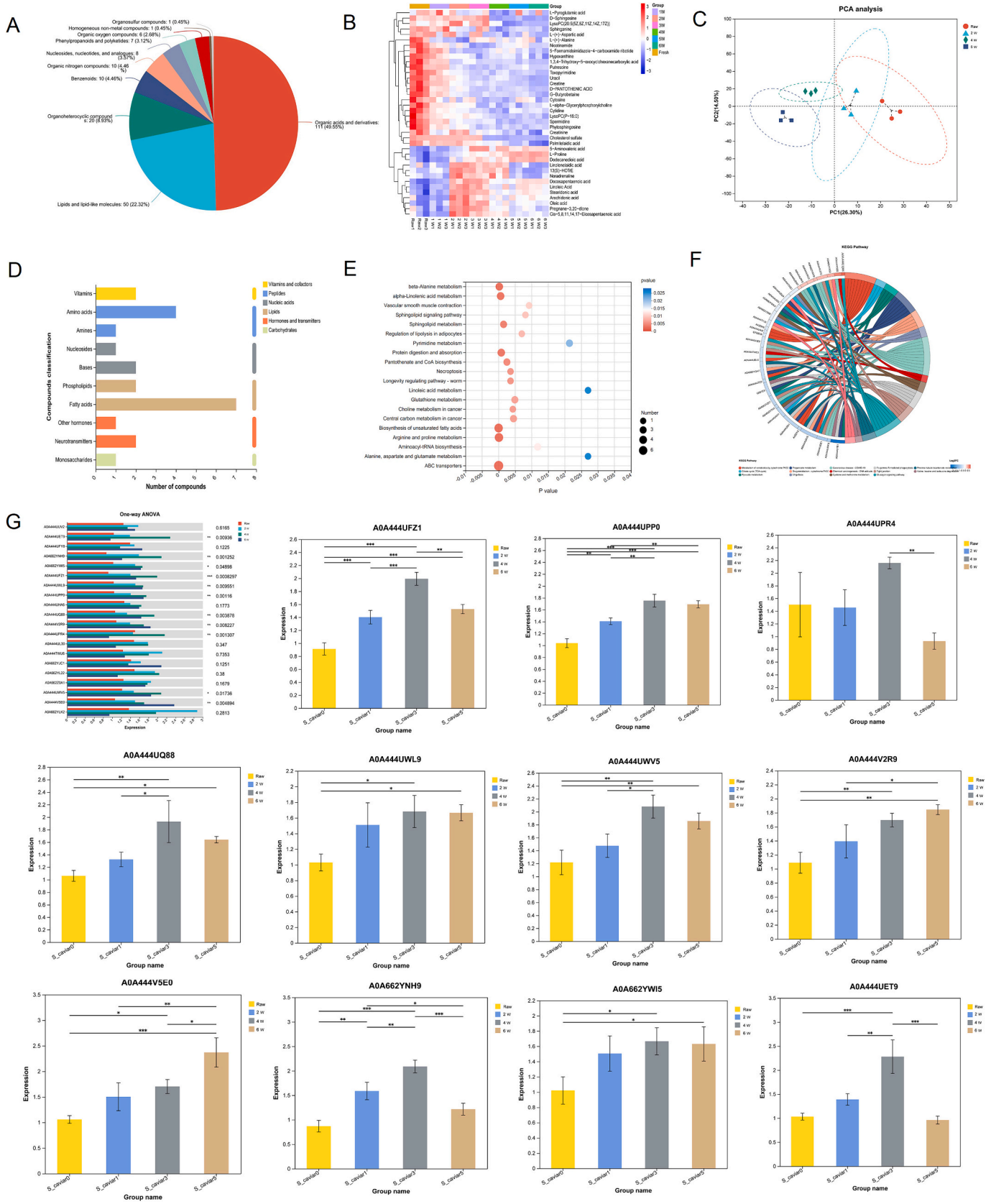

Fig. 3. Proportions of metabolites in caviar based on Human Metabolome Database (HMDB) superclasses (A), Heat map of variations in key metabolites in caviar annotated according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) (B), and PCA analysis of key metabolites (C). KEGG pathway-based differential metabolite classification (D), pathway annotation (E), and pathway chordography (F). Differential proteins (G) and their changes in re-stored caviar based on one-way ANOVA test. Levels of significance are shown as follows: *$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; ***$p \leq 0.001$.

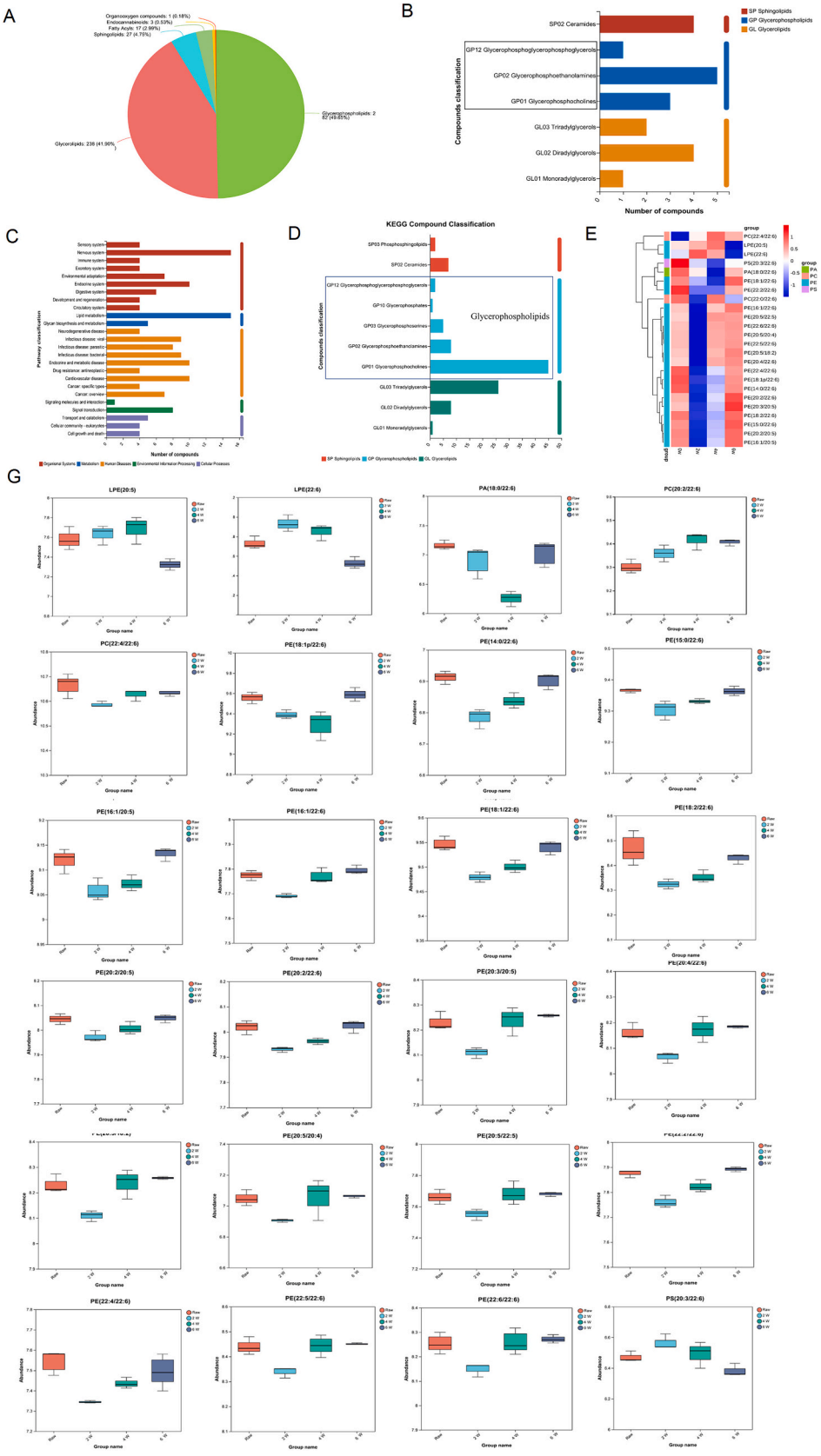

Fig. 4. Proportions of lipid metabolites in caviar based on HMDB classes (A). KEGG compound classification of lipids (B). KEGG pathway classification of differential lipid metabolites (C). KEGG-based annotation of lipid metabolic pathways (D). Metabolites annotated by KEGG belonging to the classification of lipids (E). Variation of 24 differential lipids in stored caviar (F).

precursor (Chen et al., 2019). This indicated that the site of hydrogenation could lead to the production of different volatile compounds. Liu, Ye, Liu, Wang, and Chen (2021) observed that phospholipids in pork suppressed the formation of 1-octen-3-ol while increased that of (*E, E*)-2,4-decadienal slightly. (*E, E*)-2,4-Decadienal ("fatty, fried") was formed by the autoxidation of ω-6 fatty acids, such as linoleic acid and arachidonic acid (Chen et al., 2019). PE (18:0/18:2), LPE (18:1), and CL (16:0) exhibited significant negative correlations with the formation of ketones. PE (20:4/22:6), PE (16:1/18:2), PE (20:5/18:2), PE (16:1/22:6), and PE (22:5/22:6) exhibited significant negative correlations with the generation of aldehydes, which could also be metabolic markers because the main volatile compounds in caviar were aldehydes.

3.7. Differential metabolite and non-volatile and volatile metabolite correlations

By calculating the Pearson's coefficients associated with the different free compounds, metabolites that were significantly associated with changes in these non-volatiles could be identified. As shown in Fig. 5A, the free amino acids in caviar were positively correlated with organic acids and derivatives such as dodecanedioc acid, oleic acid, arachidonic acid, docosapentaenoic acid, linoleic acid, and stearidonic acid. However, in Fig. 5B, most fatty acids were positively correlated with amino acids, organic acids, and their derivatives but negatively correlated with lipids and their derivatives.

During storage, decomposed proteins can be degraded into polypeptides and further into amino acids. Some amino acids are associated with the flavor and taste of foods, such as Asp and Glu (Li et al., 2022). Among amino acids and derivatives, during storage the contents of Ala and Asp declined gradually while that of Pro increased. Among lipids and derivatives, lysophosphatidylcholines (LPCs) were intermediate products in the metabolism of phosphatidylcholines (PCs). The relative content of LPC (20:5) increased dramatically from 0 to 3 weeks and then declined rapidly from 4 to 6 weeks, whereas that of LPC (18:0) decreased gradually. Among organic acids and derivatives, the contents of linolenelaidic acid, hydroxyoctadecatrienoic acid, docosapentaenoic acid, linoleic acid, stearidonic acid, arachidonic acid, oleic acid, EPA, and creatinine exhibited first a rising trend then a decreasing trend. The contents of palmitelaidic acid, creatine, and D-pantothenic acid exhibited decreasing trends with the prolongation of the storage time. In combination with the abovementioned research results, the evidence that Asp, oleic acid, and linoleic acid was metabolic markers was sufficient. Glycerides, phospholipids, and free fatty acids were the three major lipid components in foods. Among metabolites, organic acids, lipids, and their derivatives accounted for the largest proportion. Phospholipids in aquatic products contained a large amount of ω-3 long-chain PUFAs, such as arachidonic acid, EPA, and DHA, which were easily oxidized (Lu et al., 2017). The double bonds in phospholipid unsaturated fatty acids reacted with hydroxyl radicals to form hydroperoxides in three ways: autoxidation, photooxidation, or enzymatic oxidation. Hydroperoxides generate multimers, ketones, and secondary oxidation products by polymerization, dehydration, and oxidation, respectively. The secondary oxidation products could further degrade to produce aldehydes, ketones, alcohols, carboxylic acids, aliphatic hydrocarbons, furans and other micromolecular compounds and can further form compounds with various aroma components (Al-Dalali, Li, & Xu, 2022).

Correlation network analysis could be used to assess the association between identified key volatiles and metabolites. As shown in Fig. 5C, A0A444UQ88 and PE (22:5/22:6) were positively correlated with all key volatile compounds, whereas AOA444V5E0 was negatively correlated with most of the compounds, such as (*E*)-2-octenal, Heptanal, and Hexanal. In addition, microorganisms such as *Staphylococcus* spp. and *Erythrobacter erythropolis* during caviar storage provide enzymes for the lipid metabolism to provide lipolytic enzymes and amino acid metabolism to provide amino acid catabolic/synthetic enzymes (Xu et al., 2022). Glycerophospholipid metabolism was the main pathway for aldehyde formation. PC was degraded to LPC and sn-glycerol-3-phosphate choline (and then to choline and sn-glycerol-3-phosphate). This result was in accordance with previous results found in fermented Mandarin fish (Chen et al., 2023). sn-Glycerol-3-phosphate choline and hypoxanthine were positively correlated with all the key volatile compounds, whereas creatine and TMAO were negatively

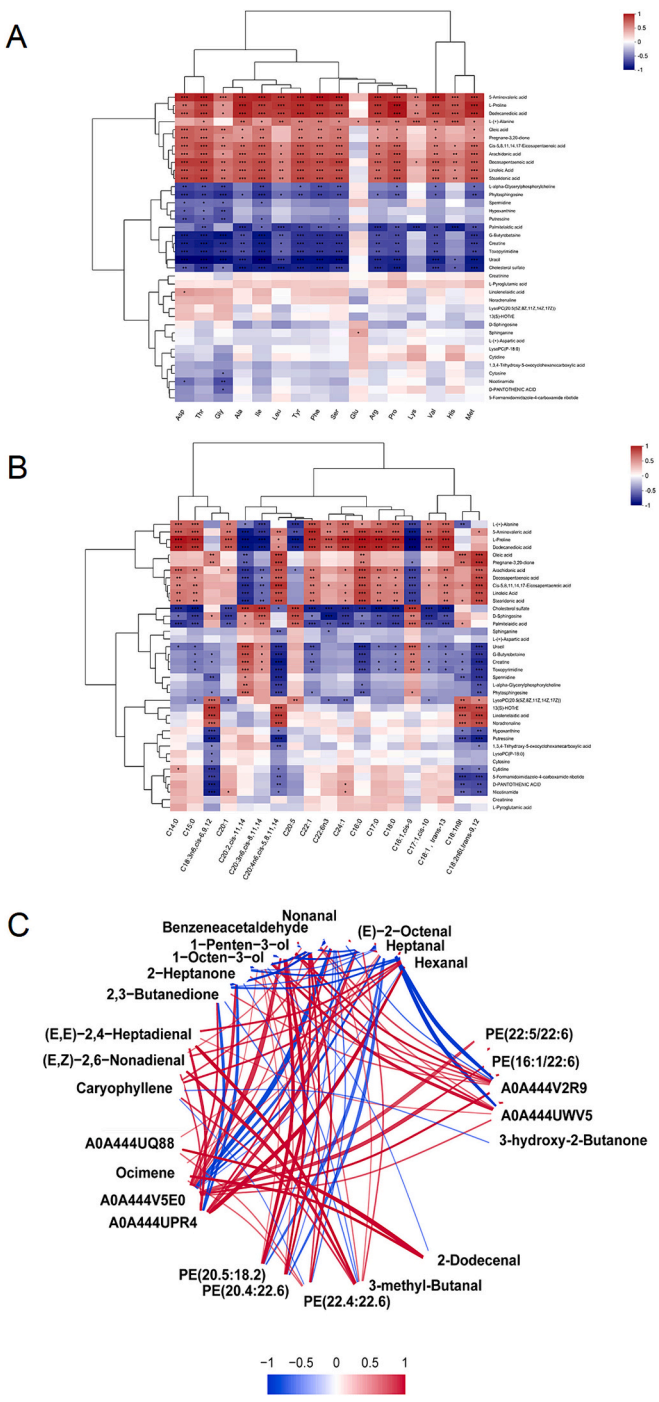

Fig. 5. Heat map of Pearson correlation analysis between amino acids and top 30 differential metabolites in terms of significance (A). Heat map of Pearson correlation analysis between amino acids and top 30 differential metabolites in terms of significance (B). Pearson correlation of differential proteins, differential lipids and key volatile compounds (C). The correlation coefficient ($-1 \leq r \leq 1$) is shown in different colors. Levels of significance are shown as follows: *$0.01 < p \leq 0.05$; **$0.001 < p \leq 0.01$; ***$p \leq 0.001$.

correlated with them. These key metabolites can directly or indirectly affect aroma production. Re-thermal oxidation of PE results in the formation of a large number of aldehydes such as nonanal, octanal, (*E*,*E*)-2,4-decadienal, and (E)-2-octenal, depending on the structural differences of PE. Summarily, these key metabolites can directly or indirectly affect aroma production.

3.8. Proposed formation pathways of volatiles derived from key metabolites

To better illustrate the changes in proteins, lipids, and flavor compounds in caviar during storage, we investigated the main processes and metabolites involved in metabolism (Fig. 6). The main pathway involved six metabolic pathways, including unsaturated fatty acid biosynthesis, glycerophospholipid metabolism, linoleic acid metabolism, α-linolenic acid metabolism, β-alanine metabolism, arginine and proline metabolism. During different storage periods, the proteins in caviar generated a large number of free amino acids and derivatives through degradation, mainly involving arginine, proline, alanine, β-alanine, aspartate and glutamate metabolism, which were used as precursors to react with fat oxidative products again to obtain volatile compounds. Furthermore, creatine was converted to phosphocreatine and creatinine, which were involved in metabolic activities mainly through ATP supplementation.

Fatty acid degradation, glycerophospholipid metabolism and α-linolenic acid metabolism are the main processes involved in lipid oxidation. The evolution of key volatile compounds in caviar is closely related to the oxidation of phospholipids. Secondary hydroperoxides were produced by the oxidation of phospholipids, whereas alkoxy radicals were cleaved to produce aldehydes. 2-Alkenals were present in a large number of seafood products. From 2-hexenal to 2-undecenal, 2-alkenals were produced by the oxidation of oleic acid in phospholipids (Varlet, Prost, & Serot, 2007). Heptanal was produced by the oxidation of an 11-hydroperoxide, which was generated from oleic acid by lipoxygenase (Al-Dalali et al., 2022). Xu et al. (2022) reported that DHA/EPA-enriched phospholipids were also precursors of 2-alkenals. 2-Heptanone, 2,3-butanedione, and 3-hydroxy-2-butanone had a buttery and fatty odor. Wu and Wang (2019) observed that the presence of

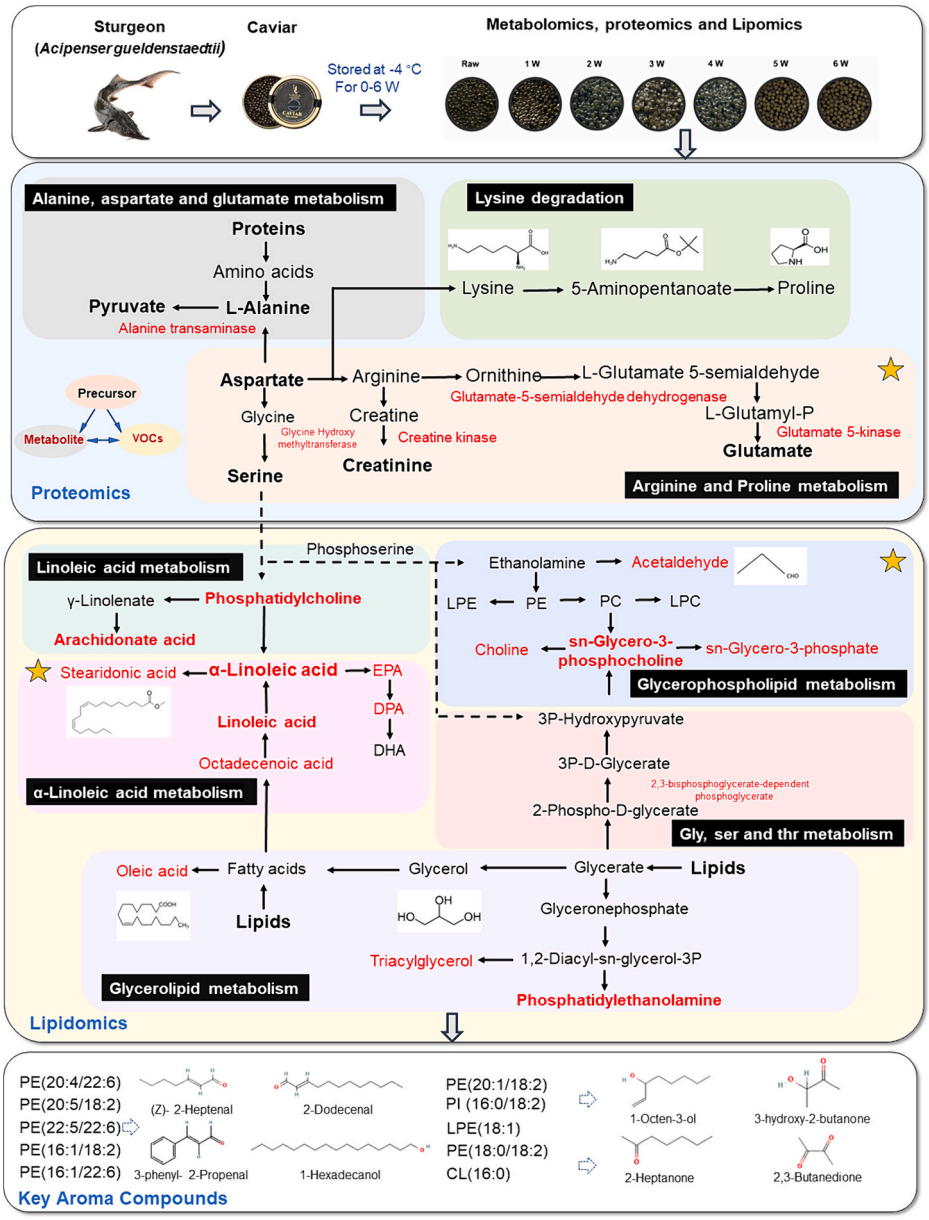

Fig. 6. Proposed pathways involved in deterioration of the quality of caviar during storage based on multi-omics.

phospholipids with C18:2 or C18:1 chain would increase the contents of ketones in *Eriocheir sinensis,* which was consistent with the results of this study. The precursors for the synthesis of 1-octen-3-ol were ω-6 fatty acids such as linoleic acid and arachidic acid. The oxidation of methyl linoleic acid produces hydroperoxides was at the C13 and C9 positions in the fatty acid chain and also at the C10 and C12 positions. Methyl linoleic acid was a precursor of hexanal, 1-octen-3-ol, and other volatile compounds (Al-Dalali et al., 2022). Although most hydroperoxides were generated at the C13 and C9 positions in methyl linoleate, the quantity of hydroperoxides generated at the C10 and C12 positions could reach about half that of the C13 and C9 hydroperoxides. Moreover, it has been shown that the hydroperoxides produced at the C10 position are also among the sources of 1-octen-3-ol (Ladikos & Lougovois, 1990). In conclusion, the metabolic processes involved in the spoilage of caviar and this formation of flavor compounds have been revealed. On the basis of the changes in the abovementioned indices, Glu, Asp, oleic acid, linoleic acid, PE (20:4/22:6), PE (16:1/18:2), PE (20:5/18:2), PE (16:1/22:6), and PE (22:5/22:6) can be regarded as metabolic markers for determining the storage period of caviar.

4. Conclusions

The overall quality of caviar showed an increasing and then decreasing trend during 6 weeks of storage. The storage period of 3–4 weeks was considered optimal for maintaining the quality of caviar. Correlation analyses of lipids and proteins with flavor compounds showed that flavor release from caviar during storage was closely related to fatty acid degradation and amino acid metabolism. The changes in lipids and proteins during storage varied with the deterioration of flavor. This study has given a comprehensive description of changes in the quality of caviar for the first time, which has provided a reference for optimizing caviar storage methods, predicting the storage period, and controlling the quality of caviar. However, more detailed investigations, such as validation and application of metabolic markers, need to be carried out in further studies. These results were analyzed by multivariate statistical analysis to obtain markers of flavor deterioration during caviar storage. These results were combined with the analysis of biological information, allowing us to further understand the interactions of volatile components and related precursors in caviar during routine cold storage. Overall, this study provides new insights into the control of off-flavors in caviar.

CRediT authorship contribution statement

Li Liu: Writing – review & editing, Writing – original draft, Methodology, Conceptualization. **Yihuan Liu:** Writing – original draft, Software, Methodology, Data curation. **Fan Bai:** Resources, Formal analysis, Conceptualization. **Jinlin Wang:** Validation, Resources. **He Xu:** Validation, Methodology. **Xiaoming Jiang:** Validation, Software. **Shixue Lu:** Software, Methodology, Formal analysis, Data curation. **Jihong Wu:** Validation, Methodology. **Yuanhui Zhao:** Writing – review & editing, Funding acquisition. **Xinxing Xu:** Writing – review & editing, Funding acquisition, 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

No data was used for the research described in the article.

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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.2024.101688.

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