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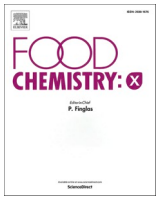

Contribution of phospholipase B to the formation of characteristic flavor in steamed sturgeon meat

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ABSTRACT

Sensory analysis and untargeted lipidomics were employed to study the impact of phospholipase B (PLB) on lipid oxidation and flavor in steamed sturgeon meat, revealing the inherent relationship between lipid oxidation and flavor regulation. The research verified that PLB effectively suppresses fat oxidation and improves the overall taste of steamed sturgeon meat. Furthermore, the PLB group identified 52 compounds, and the content of odor substances such as isoamyl alcohol and hexanal was reduced compared with other groups. Finally, lipid substances containing eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) were screened out from 32 kinds of differential phospholipids. Through Pearson correlation analysis, it was observed that certain differential phospholipids such as PC (22:6) and PC (22:5) exhibited varying correlations with odor substances like hexanal and isovaleraldehyde. These findings suggest that PLB specifically affects certain phospholipids, leading to the production of distinct volatile substances through oxidative degradation.

1. Introduction

Sturgeon, one of the largest and long-lived freshwater fish species in the world, possesses high nutritional value. Sturgeon meat is rich in fats, especially DHA and EPA (12.5%) (Chen et al., 2022), which are known to provide various health benefits such as preventing cardiovascular diseases and improving cognitive function (Tulowiecka, Kotlega, Prowans, & Szczuko, 2020). Sturgeon meat contains approximately 18% protein, along with essential amino acids. It is rich in minerals, including phosphorus, calcium, sodium, potassium, magnesium, and chlorine, making up 2% of its content. Known for its delicious flavor, sturgeon meat has an edible portion exceeding 95%, making it highly nutritious and in demand in the market.

The traditional processing methods of fish primarily include steaming, boiling, frying, and charcoal roasting. Steaming is particularly favored as it effectively preserves the original flavor of food, making it a popular choice for processing fish and other aquatic products (Wang et al., 2022). However, it is important to note that steaming can sometimes result in a decline in food quality, which may reduce consumer interest in purchasing such food. Research has shown that high-temperature processing methods, like steaming, can accelerate the oxidation of lipids in food, ultimately affecting its flavor (Huang et al., 2019). Sturgeon meat contains approximately 21% fat, primarily composed of unsaturated fatty acids, many of which are long-chain polyunsaturated fatty acids. This predisposes the fat to oxidation, resulting in the formation of unpleasant volatile compounds such as alcohols, aldehydes, acids, and ketones (Zhang et al., 2020). Therefore, steamed-sturgeon meat may lose its original nutrition and good flavor due to severe oxidation of fat.

Given that the use of antioxidants is convenient, quick, and technically mature, and can achieve effective antioxidant effects, the use of antioxidants is currently the most common method in food preservation. Natural antioxidants, in particular, are substances extracted from nature with antioxidant properties, offering a wide range of sources and safe, reliable properties. Most natural antioxidants exhibit significant antioxidant activity. Previous research has shown that tea polyphenols (Tp) have the ability to inhibit lipid oxidation effectively (Bai et al., 2018). Acidic polysaccharides extracted from *Laminaria japonica* have been found to have a significantly oxygen radical absorbance capacity (Cui et al., 2016). Chondroitin sulfate (CS) was isolated from the skull and

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backbone of large hybrid sturgeon in preliminary laboratory research, confirming its polysaccharide structure (Wang et al., 2021). Previous studies have demonstrated that CS exhibits antioxidant properties and can decrease the generation of free radicals (Campo, Avenoso, Campo, Ferlazzo, & Calatroni, 2007). Furthermore, research has shown that Phospholipase A_2 inhibits hemoglobin-mediated lipid oxidation in fish meat, with its antioxidant activity linked to the elimination of lipid-soluble preformed LOOHs in phospholipids (Tatiyaborworntham & Richards, 2015).

Numerous studies have focused on controlling lipid peroxidation in fish meat from farm to table process. However, there is limited research on regulating sturgeon meat during cooking, especially on the potential relationship between PLB and lipid oxidation, protein structure and flavor compounds. This study focuses on steamed sturgeon meat and investigates the inhibitory effect of PLB on lipid oxidation in sturgeon meat. It assesses the impact of PLB on the aroma of sturgeon meat through sensory evaluation and GC-IMS technology. The fatty acid content of phospholipids extracted from steamed sturgeon meat is analyzed using GC–MS. Non-targeted lipidomics analysis is employed to examine the lipid components in steamed sturgeon meat. Additionally, GC-IMS is utilized to identify flavor compounds in sturgeon meat. Finally, a correlation analysis between phospholipids and flavor is conducted to investigate how PLB influences lipid oxidation and regulates the formation of flavor compounds in steamed sturgeon meat.

2. Materials and methods

2.1. Experimental samples and treatment

Hybrid sturgeon (*Acipenser gueldenstaedti Brandt*♀ × *Acipenser schrenckii Brandt*♂) was purchased from the aquatic products market in Chengyang District, Qingdao. Fish Meat Processing: the fresh sturgeon was transported to the laboratory, where it was promptly stunned, slaughtered, and then had its head, tail, skin, and internal organs removed. The fish was then cut into pieces approximately 9 cm × 6 cm × 1.5 cm in size. Throughout the slaughtering process, the fish pieces were kept on crushed ice to maintain a low temperature. Following slaughter, the fish pieces were rinsed three times with clean water, drained, and stored in a − 65 °C freezer for future use.

PLB was purchased from Xia Sheng Biological Technology Co., Ltd. Marinating process: PLB group: 0.0625 g PLB; Cp group: 3.0 g cartilage polysaccharide; Tp group: 7.5 g Tp; laminarin group: 7.5 g laminarin (L). Each of the above groups was accurately weighed and dissolved in 100 mL of water. Twenty grams of fish meat was marinated in each group. The marinated fish meat was steamed with the lid on for 12 min. After steaming, the fish meat was immediately removed, placed in crushed ice to cool and stop the reaction. Once cooled to room temperature, it was homogenized for 1 min using a homogenizer and then prepared for experimental measurements.

2.2. Selection of flavor regulators for steamed sturgeon meat

Regulators that could influence the flavor of steamed sturgeon meat were selected based on their antioxidant capacity, overall flavor, and the impact on volatile flavor compounds in the meat.

Antioxidant capacity measurement: fat oxidation indicators, including acid value (AV), peroxide value (PV), thiobarbituric acid value (TBA), were determined according to AOCS (2011) official methods Cd 3d-63, Cd 8b-90, and Cd 18–90, respectively. All samples were tested in triplicate.

Sensory analysis was approved by Ocean University of China Institutional Review Board Committee. Sensory evaluation was performed by fifteen well-trained panelists from the College of Food Science and Engineering Department in Ocean University of China (eight males and seven females, aged from 24 to 40 years) according to Sensory Analysis-Guidelines for the use of quantitative response scales, (GB/T 39501–2020/ISO 4121:2003). Before the experiment, each panelist had to complete a 60-h of sensory evaluation training with steamed sturgeon meat in 20 days. All participants received written information about the study, and they signed informed consent to participate. Sensory quantitative description analysis (QDA) was performed according previous study. In earlier stages of the study, characteristic odor attributes of sturgeon meat were explored, and ultimately, ten characteristic odor attributes were selected, including meat, rancid/cheesy, visceral/bloody, oily/fatty, umami, fishy, salty, faint, grassy, and earthy (Li et al., 2022). The scoring scale consisted of six levels, as shown in Table S1.

Overall rating of Flavor was assigned to the steamed sturgeon meat, with a total score of 10 points. The scoring criteria are provided in Table S2. Undesirable flavors such as rancid, sourness, visceral, and bloody were considered negative attributes and resulted in a deduction of 1 point each if detected. Pleasant flavors like freshness, saltiness, fatty, and fragrant were considered positive attributes and added 1 point each to the score.

The volatile flavor compounds were determined using GC-IMS (Gas Chromatography-Ion Mobility Spectrometry), with three replicates for each meat sample. The experimental method was adapted from Arroyo-Manzanares et al. (2018) with certain modifications. A metal capillary column (MXT-WAX, 15 m × 0.53 mm × 1.0 μm) was used, and the headspace incubation temperature was set at 60 °C with shaking at 500 r/min for 20 min. Subsequently, 500 μL of gas was injected into the injection port through the headspace of the injection needle (PAL3-SYH-207853). The volatile flavor components of steamed sturgeon meat were analyzed using Laboratory Analytical Viewer, Reporter, and Gallery Plot plug-ins, along with the GC-IMS Library Search NIST 14 database. The retention index of volatile flavor compounds was determined using N-ketone (C4 ~ C9) as an external standard.

2.3. Determination of optimal treatment conditions for flavor regulators

PLB and Tp were chosen as the subjects of study to examine their antioxidant properties, with an untreated blank group serving as a control. The optimal treatment time and concentration of the antioxidants were determined using GC-IMS to measure volatile flavor compounds, along with sensory analysis results. The processing method of steamed sturgeon meat, intensity rating of aroma, odor composite score, and identification of volatile flavor compounds were consistent with the details in section 2.2.

For determining antioxidant treatment time: PLB and Tp (prepared at specified concentrations) were applied at different times (h): 0.5, 1.0, 1.5, 2.0, and 2.5. To establish the treatment concentration of antioxidants, the treatment time was set to optimal time. The treatment concentrations of PLB and Tp can be found in Table S3.

2.4. Effect of PLB on protein structure and function

In this experiment, the blank control group and the group treated with the maximum concentration of PLB were selected and compared with the group treated with the optimum concentration to analyze the effect of PLB on protein structure and function. Extraction of myofibrillar proteins (MP) from sturgeon meat was carried out by referring to the method of Takahashi, Takahashi, and Konno (2005) with slight modifications. 120 g of fish meat was weighed, PLB-treated, and steamed for 12 min. The fish meat was stirred, and 5 times the volume of pre-cooled Tris buffer A (pH 7.5, containing 0.1 mol KCl) was added to the fish meat, and centrifuged (8000 r/min, 4 °C, 20 min) after shaking for 5 min, the supernatant was removed and the lower precipitate was retained, and the process was repeated three times. Then add pre-cooled Tris buffer B (0.6 mol/L NaCl, pH 7.5) to the precipitate, shake for 3 min, put it into the fresh layer of the refrigerator at 4 °C for 30 min, then centrifuged (8000 r/min, 4 °C, 20 min), and the supernatant was taken as MP in sturgeon meat.

To determine the structure of MP, a biochemical kit (purchased from

Nanjing Jiancheng Bioengineering Institute) was used to determine the changes in the carbonyl and sulfhydryl content of MP; a certain mass of MP sample was weighed, and 2 mL of phosphate buffer was added to make a protein solution with a concentration of 1 mg/mL. 80 μL of the protein solution and 20 μL of 5 × sampling buffer were taken in 1 mL centrifugal tubes, and then the sample was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to observe the changes of MP subunits in steamed sturgeon meat after PLB treatment.

Determination of MP solubility was based on the method of Agyare, Xiong, and Addo (2007) with slight modifications. Accurately weighed 1 g of MP was dissolved in 20 mL of pre-cooled phosphate buffer (pH 6.25, 50 mmol/L, 0.6 mol/L NaCl), shaken well for 3 min, and the protein concentration was determined by the Caulmers Brilliant Blue method, and was recorded as C_1. Protein solutions were allowed to stand at 4 °C for 1 h and then centrifuged (6000 r/min, 4 °C, 15 min), and the supernatant was taken to determine the protein concentration, noted as C_2. The formula for MP solubility was as follows:

Solubility (%) $= C_1/C_2 \times 100$.

To determine the emulsification properties of MP, MP was dissolved in 5 mL of phosphate buffer (0.1 mol/L, pH 6.5) to produce a protein solution; 20.0 ml of protein solution was added to 5 mL of soybean oil, homogenized for 1 min, and 50 μL of the homogenate was added to 5 mL of 0.1% SDS solution, shaken and mixed, and then left to stand for 10 min, and measure the absorbance value at 500 nm as A_0; after the homogenate was left for 10 min, measure the absorbance value according to the same method as A_{10}, and use the 0.1% SDS as blank control. The emulsification activity (m^2/g) and emulsification stability (%) of MP were calculated as follows:

$$\text{Emulsification activity } \left(m^2/g\right) = 2 \times 2.303 \times A_0 \times DF/\left(C(1-\Phi) \times 10,000\right)$$

$$\text{Emulsification stability } (\%) = A_{10}/A_0 \times 100$$

DF-Dilution factor (101);

C-protein mass concentration (g/mL);

Φ-volume fraction occupied by the oil phase in the emulsion, 0.25;

The surface hydrophobicity of MP was determined by referring to the method of Chin, Go, and Xiong (2009) with slight modifications. MP was dissolved in phosphate buffer (0.02 mol/L, pH 6.0) to make a 5 mg/mL MP solution; 1 mL of protein solution (the blank was replaced by phosphate buffer) was added with bromophenol blue (200 μL, 1 mg/mL) in a centrifuge tube, and then centrifuged after shaking for 10 min (8000 r/min, 4 °C, 10 min). The supernatant was diluted 10-fold and the absorbance value at 595 nm was determined A. Surface hydrophobicity was expressed by the amount of bromophenol blue bound, which was calculated by the following formula:

$$\text{Bromophenol Blue Binding Amount } (\mu g) = 40 \times (A - A_0/A_0.$$

2.5. Effect of PLB on antioxidant effect of lipids

In this experiment, the blank control group and the group treated with the maximum concentration of PLB were also selected for comparison with the group treated with the optimal concentration, and the effect of PLB on the antioxidant effect of lipids was explored by determining TBA、AV、PV. The determination method was the same as 2.2.

2.6. Modelling of phospholipid thermal oxidation in steamed sturgeon meat

Phospholipid extraction in steamed sturgeon meat was carried out by referring to the method of Bligh and Dyer (1959) for extraction of fish phospholipids with slight modifications. Weigh 0.4 g of fish meat sample, add 12 mL of chloroform-methanol solution (2:1, *v*/v), add 6 mL of ultrapure water, centrifuge (8000 r/min, 10 °C, 10 min), collect the organic phase (lower layer), the remaining add 6 mL of extracting solution to repeat the extraction twice, combine the organic phases and blow-dry under nitrogen. After drying, 1 mL of phospholipid dissolution solution was added to redissolve.

To model the thermal oxidation of phospholipids, 200 μL of phospholipid sample was taken in a 20 mL headspace bottle, and the organic solvent was removed by nitrogen blowing with a nitrogen purging apparatus, sealed and then steamed in a steamer for 12 min. After the end of the steaming process, the sample was removed and cooled to room temperature. The odor components of the phospholipid oxidation model were subsequently analyzed by GC-IMS.

2.7. Determination of fatty acids by GC–MS

Take the phospholipid sample, add 15 mL of chloroform-methanol solution (2:1, *v*/v), centrifuge after shaking (7000 r/min, 4 °C, 15 min), collect the lower layer of solution, add 10 mL of extraction solution to repeat the extraction of lipids in the filtrate, and repeat the extraction twice according to the above conditions, combine the lower layer of solution (chloroform-lipid solution), and nitrogen blowing to obtain the crude oil and grease. Add KOH-methanol solution (5 mL, 0.5 mol/L) to the extracted lipid, water bath at 65 °C for 20 min, shaking for 3 min. Add boron trifluoride-methanol solution (2 mL, 14%), water bath at 65 °C again for 5 min, followed by adding 2 mL of n-Hexane, 1 mL of ultrapure water, shaking for 3 min, and then left to stand, retaining the upper layer. The extraction was repeated 3 times, nitrogen-blown to constant weight, and fixed to 10 mL with hexane.

GC–MS (QP2010-SE; Shimadzu, Kyoto, Japan) was used with an HP-5 ms capillary column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, Santa Clara, CA, USA). The conditions were an injection port temperature of 250 °C and a column temperature program as follows: holding at 60 °C for 1 min, increasing to 160 °C at 10 °C/min, holding for 5 min; increasing to 200 °C at 3 °C/min, holding for 10 min, increasing to 280 °C at 6 °C/min and holding for 5 min. The carrier gas was helium with a flow rate of 1.5 mL/min. The split ratio was 10:1, and the injection volume was 1 μL. MS was performed with an ion source temperature of 250 °C, an electron energy of 70 eV, and a mass scan range of 35–500 *m/z* (Ma et al., 2020).

2.8. Identification of lipid components

Weigh 1 g of fish, add 12 mL of chloroform-methanol solution (2:1, *v*/v), shake and mix for 3 min, then add 6 mL of ultrapure water and shake for 1 min, then ultrasonic extraction at 20 °C for 20 min, centrifugation at 4 °C and 5000 r/min for 10 min, collect the lower phase, and repeat the extraction twice by adding 12 mL of the extraction solution to the aqueous phase. The aqueous phase was extracted with 12 mL of extraction solution for two times. The three extracts were combined, nitrogen-blown to a constant weight, and dissolved in 1 mL of isopropanol-acetonitrile (2:1, v/v).

Chromatographic conditions: Hypersil Gold C18 reversed-phase column (100 mm × 2.1 mm × 1.9 μm); mobile phase A: 0.1% formic acid+10 mmol/L ammonium formate+acetonitrile/water (60:40, v/v); mobile phase B: 0.1% formic acid+10 mmol/L ammonium formate+isopropanol/acetonitrile (90:10, v/v); The injection volume was 2 μL, the injection rate was 300 μL/min, and the column temperature was 55 °C.

2.9. Statistical analysis

All data were processed by one-way analysis of variance (ANOVA) in SPSS 25.0 to determine the differences between these groups. Data were expressed as mean and standard error of the mean (SEM). In this study, $P < 0.01$ was considered highly significant and $P < 0.05$ was considered significantly different. Radar chart and bar graphs (mean ± standard error) were plotted using Origin 2017 and ChiPlot (https://www.chiplot.online/). The potential metabolic pathway in Fig. 6(b) by Figdraw

(https://www.figdraw.com/).

3. Results and discussion

3.1. Screening of flavor modifiers for steamed sturgeon meat

The degradation of fats is a crucial pathway for changes in food flavor, and excessive lipid oxidation is a major cause of food spoilage and the development of unpleasant odors (Li et al., 2022). As shown in Fig. 1 (a, b, c), there were significant differences in plasma malondialdehyde (MDA) and PV between the control group and the groups treated with PLB/Tp ($P < 0.05$). TBA is one of the recognized indicators for assessing the degree of lipid peroxidation, and it is typically represented by MDA. PV is an indicator used to detect the products of early-stage lipid oxidation, and Fig. 1(b) shows that there was a significant difference between the PLB group (0.01 g/100 g) and the control group (0.06 g/100 g) ($P < 0.05$). These results are consistent with a study by Tatiyaborworntham and Richards (2015), which showed that adding phospholipase to codfish and pork reduced TBA values and PV. On the 12th day, the addition of PLB reduced the TBA value and PV of salted pork by 24% and 67%, respectively ($P < 0.05$). The results showed that compared with other antioxidants, PLB showed good antioxidant properties and was able to inhibit fat oxidation of steamed sturgeon meat.

Trained sensory evaluators assessed the samples of steamed sturgeon meat treated with different methods. As shown in Fig. 1(e), Tp group had the lowest scores for "oily/fatty" and "visceral/bloody" compared to the other groups. PLB group reduced "grassy" "earthy" "visceral/bloody"

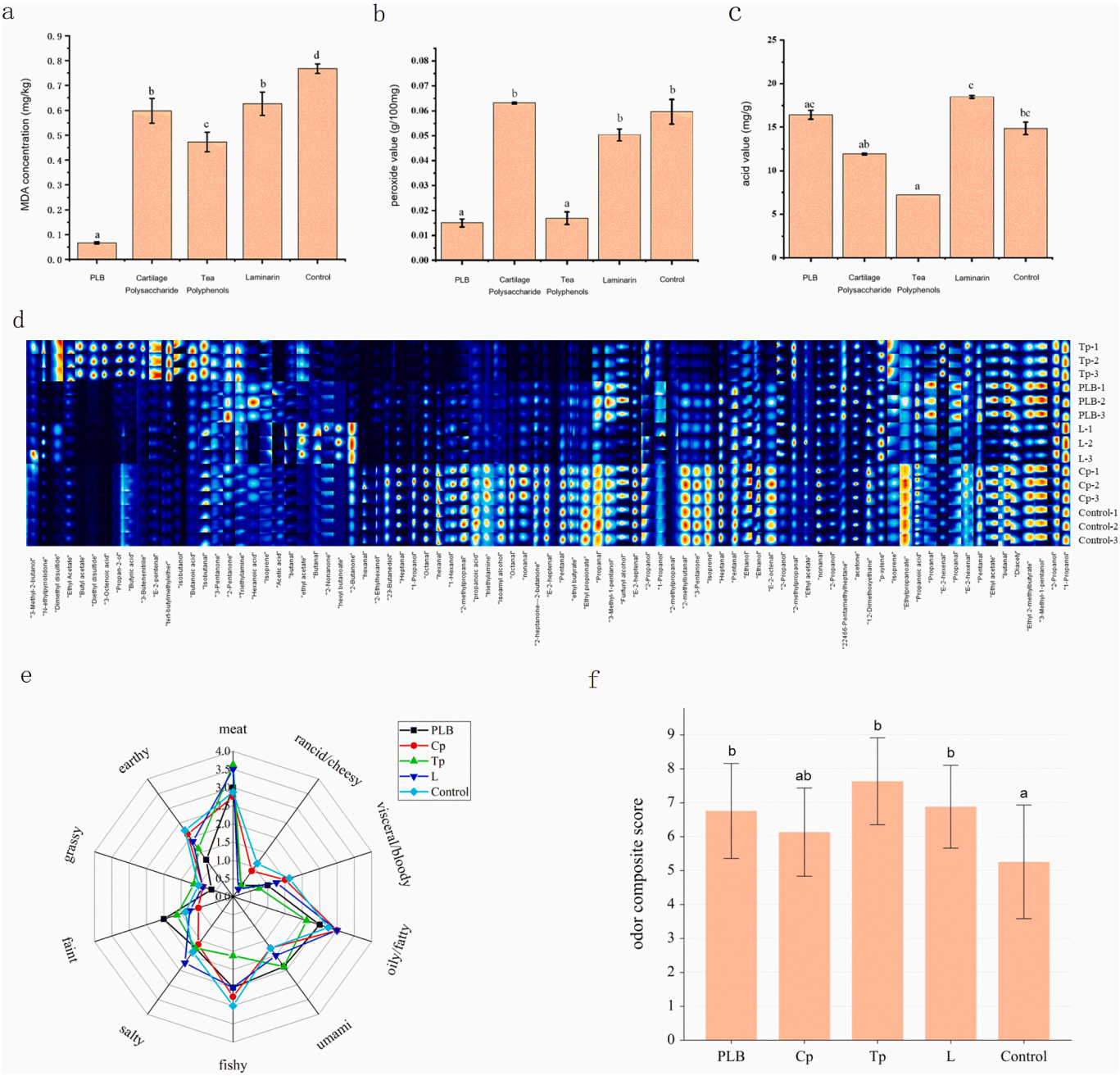

Fig. 1. Sensory evaluation of sturgeon meat treated with different antioxidants. Changes of TAB (a); Changes of peroxide value (b); Changes of acid value (c); Fingerprints of volatile compounds based on odor information from IMS signal intensity (d); Intensity values of ten odor attributes (e) and Overall odor score (f). PLB: phospholipase B; Cp: Cartilage polysaccharide; Tp: Tea polyphenols; L: Laminarin. The data are expressed as the means ± standard deviations ($n = 3$).

and “rancid/cheesy” while increasing “faint” and “umami”. The overall sensory evaluation score (Fig. 1f) also indicated that Tp, L and PLB group were the three highest-scoring groups. This suggests that PLB had a positive effect on enhancing freshness and reducing undesirable odors. Combining the results of the antioxidant experiments, MDA is the final product of lipid peroxidation, and MDA can further react with amino acids in a non-enzymatic browning reaction, leading to an increase in the intensity of undesirable odors such as “fishy” (Nilsuwan, Chantakun, Chotphruethipong, & Benjakul, 2021). This indicates that PLB can influence food flavor by inhibiting lipid oxidation.

GC-IMS was used to analyze the volatile flavor compounds in steamed sturgeon meat samples treated differently. The fingerprint spectra of volatile organic compounds (VOC_S) detected by GC-IMS are shown in Fig. 1(d). A total of 52 compounds were identified, including ester (6), aldehydes (12), alcohols (11), ketones (9), acid (5), ethers (4) and others (5). The volatile compounds are shown in Table S4. In this experiment, aldehydes (12) and alcohols (11) were detected, which are the major flavor substances in steamed sturgeon meat. Through fingerprint spectrum analysis, it was observed that in the Tp and the PLB group, the content of alcohols and aldehydes significantly decreased compared to the control group, and the concentration of isoamyl alcohol and 2-ethylhexanol was reduced compared with the control group, and both isoamyl alcohol and 2-ethylhexanol had unpleasant taste at high concentration (Miao et al., 2023). Additionally, aldehydes like heptanal, octanal, and (E)-2-octenal decreased in content. It has been found in previous research that the presence of C7-C11 unsaturated aldehydes is a major factor contributing to the development of unpleasant odors in meat (Zhang, Zhang, Liu, Zhao and Luo, 2020). This is consistent with the results of the sensory evaluation mentioned earlier.

3.2. Establishment of optimal treatment conditions for flavor modifiers

In this experiment, PLB and Tp were selected to establish the optimal treatment conditions for flavor modifiers. As shown in Fig. 2(a, b, c), sensory evaluation of odor intensity combined with comprehensive score results indicated that prolonged treatment with PLB and Tp could lead to a deterioration in the odor of steamed sturgeon meat. PLB and Tp groups exhibited the strongest “meat” and “faint” at 1 h, with weaker undesirable odors such as “fishy” and “earthy” resulting in the highest comprehensive scores. This suggests that the odor of steamed sturgeon meat is improved after treatment with PLB and Tp for 1 h.

The fingerprint spectra of VOC_S detected by GC-IMS are shown in Fig. 2(d), identifying a total of 63 compounds, including esters (14), aldehydes (12), alcohols (13), ketones (6), acids (5), ethers (4), and other compounds (9). The volatile compounds are shown in Table S5. Among these, esters, aldehydes, and alcohols were the major flavor substances. In the steamed sturgeon meat treated with PLB for 1 h, compounds like ethyl pentanoate and ethyl propanoate were detected at the highest levels. These esters, containing short-chain fatty acids, are known to contribute to a “fruity” (Jeleń & Gracka, 2016). Additionally, in the steamed sturgeon meat treated with Tp for 1 h, compounds like 3-methyl-2-butanol and 3-methyl-1-butanol were detected at the lowest levels. These unsaturated aldehydes can produce unpleasant odors such as spiciness and have low odor thresholds, which can have a significant impact on food flavor (Akakabe, Matsui, & Kajiwara, 2005). Therefore, it is inferred that treating samples with PLB and Tp for 1 h has the best flavor control effect for steamed sturgeon meat.

Similarly, as shown in Fig. 2(e, f, g), sensory evaluation of odor intensity combined with comprehensive score results indicated that treatment with 0.625 mg/mL PLB significantly improved the production of unpleasant odors such as “fishy” “rancid/cheesy” and “oily/fatty” in steamed sturgeon meat while enhancing “umami” and “meat”. In contrast, Tp at a concentration of 25 mg/mL effectively reduced “grassy” and “fishy” and enhanced “umami” and “faint”. The fingerprint spectra of VOC_S detected by GC-IMS are shown in Fig. 2(h), identifying a total of 57 compounds, including ester (10), aldehydes (11), alcohols (10), ketones (9), acid (6), ethers (4) and others (7). The volatile compounds are shown in Table S6. In the steamed sturgeon meat treated with 0.625 mg/mL PLB, esters, aldehydes, ketones, and pyridines were abundant and had high concentrations. Although the 3.750 mg/mL PLB group had a higher concentration of volatile compounds, it also contained high levels of unpleasant odors such as hexanoic acid (“oily/fatty”) and 2-ethylhexanol (“earthy”), which adversely affected the flavor of sturgeon meat. Different concentrations of Tp did not have a significant effect on the types of volatile flavor compounds, but treatment with 25 mg/mL Tp significantly reduced the content of undesirable flavor compounds such as diethyl disulfide and diallyl sulfide. Therefore, it is inferred that 0.625 mg/mL PLB and 25 mg/mL Tp have the best flavor control effect on steamed sturgeon meat.

3.3. Effects of PLB on protein structure and function

The carbonyl and thiol content can be used to characterize the degree of protein oxidation. As shown in Fig. 3(a), the lowest carbonyl content was observed in steamed sturgeon meat treated with 0.625 mg/mL PLB, indicating that this treatment group experienced the least oxidation attack on sturgeon meat. Research has shown that the carbonyl content of proteins in meat tends to increase after heat treatment, and this increase becomes more significant with longer heating times (Traore et al., 2012). This suggests that 0.625 mg/mL PLB can better inhibit protein oxidation during the high-temperature steaming process of sturgeon meat. Comparing with other groups, the 0.625 mg/mL PLB treatment group exhibited the highest thiol content (Fig. 3b). Protein oxidation can lead to the degradation of thiols in meat, forming disulfide bonds, and antioxidants can play a protective role by inhibiting thiol degradation during meat processing (Hernández-López, Rodríguez-Carpena, Lemus-Flores, Galindo-García, & Estévez, 2016). To further observe the impact of PLB on the subunits of MP, SDS-PAGE analysis was conducted. As shown in Fig. 3(d), different treatment groups exhibited significant differences in the molecular weights of Myosin heavy chain (MHC), Actin, and Tropomyosin (Tm). These large protein molecules tend to degrade into smaller proteins during processing, which is reflected as lighter protein bands in electrophoresis. Both the 3.750 mg/mL PLB group and the control group experienced varying degrees of degradation in large protein molecules. Considering the results of carbonyl and thiol content, it can be inferred that 0.625 mg/mL PLB has a better antioxidative effect, contributing to the stability of protein structures.

The binding amount of Bromophenol blue to MP to indicate the hydrophobicity of proteins, with higher binding indicating stronger hydrophobicity (Noh, Kang, Hong, & Yun, 2005). Hydrophobicity is an important indicator of protein denaturation. As shown in Fig. 3(c), PLB reduced the hydrophobicity of proteins, especially at a concentration of 0.625 mg/mL, where protein hydrophobicity was the weakest. Protein denaturation occurs due to external factors such as pressure, high temperature, etc., leading to changes in protein conformation and the exposure of hydrophobic amino acid residues on the protein surface, resulting in increased surface hydrophobicity (Liu, Ma, Liu, & Zeng, 2023). Protein solubility is a fundamental property of proteins. As seen in Fig. 3(e), PLB improved protein solubility, particularly at a concentration of 0.625 mg/mL, where protein solubility was the highest. Research has shown that protein denaturation, which occurs during heating, can cause proteins to aggregate, making them less soluble. The application of antioxidants can help retain protein solubility (Santos et al., 2019). The effect of PLB on the protein emulsifying properties in steamed sturgeon meat is shown in Fig. 3(f, g). It can be observed that the 0.625 mg/mL PLB treatment group had the highest emulsifying activity and emulsifying stability index. Previous research has shown that protein oxidation can disrupt the original spatial structure of proteins in fish meat, leading to reduced emulsifying properties (Padial-Domínguez, Espejo-Carpio, Pérez-Gálvez, Guadix, & Guadix, 2020). In summary, PLB can effectively inhibit protein oxidation and degradation, and a PLB concentration of 0.625 mg/mL results in the most stable

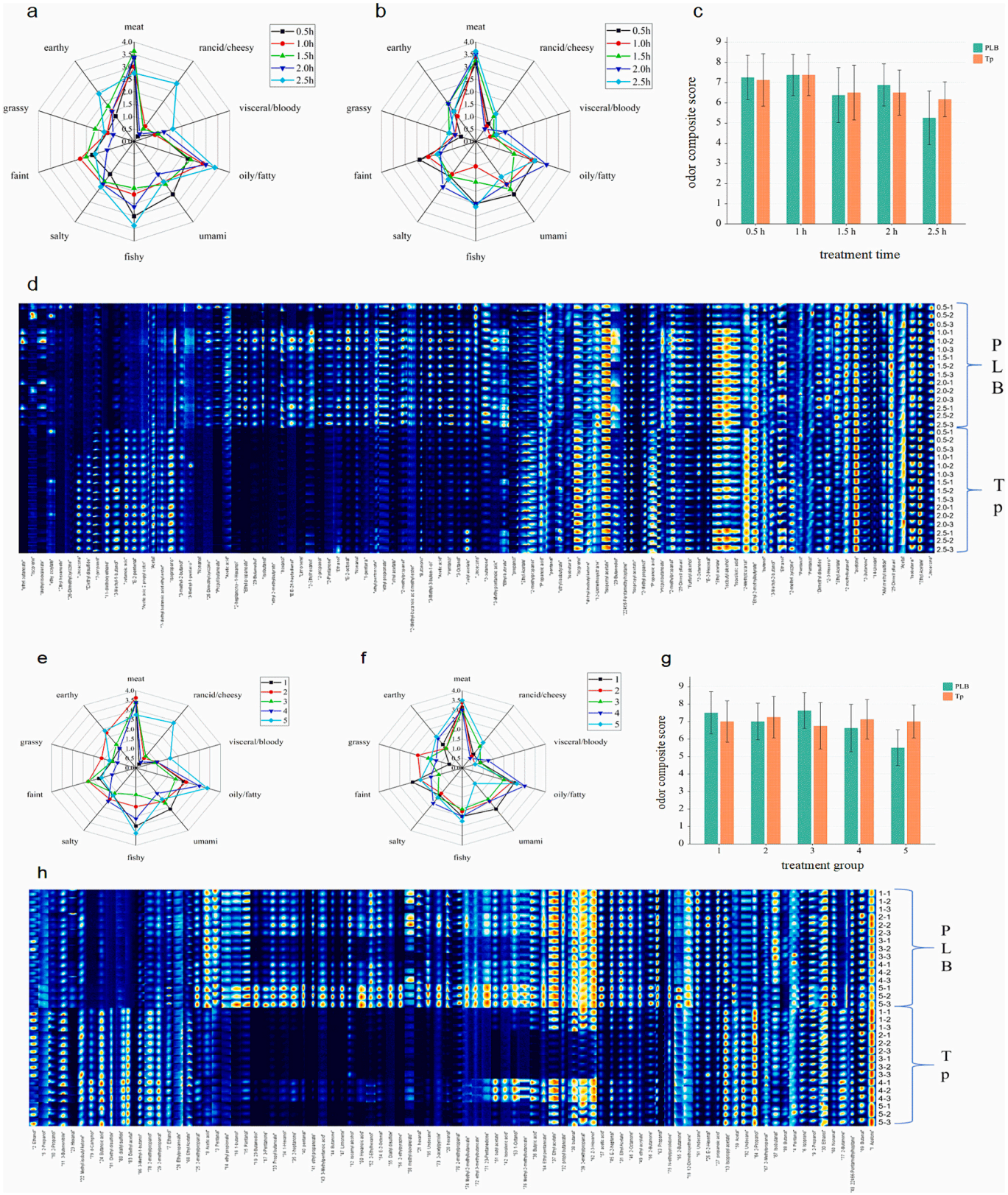

Fig. 2. Sensory evaluation and flavor changes under different treatment time. Intensity values of ten odor attributes: PLB (a) and Tp (b); Overall odor score (c); Fingerprints of volatile compounds based on odor information from IMS signal intensity (d). Sensory evaluation and flavor changes under different treatment content. Intensity values of ten odor attributes: PLB (e) and Tp (f); Overall odor score (g); Fingerprints of volatile compounds based on odor information from IMS signal intensity (h). PLB: phospholipase B; Tp: Tea polyphenols.

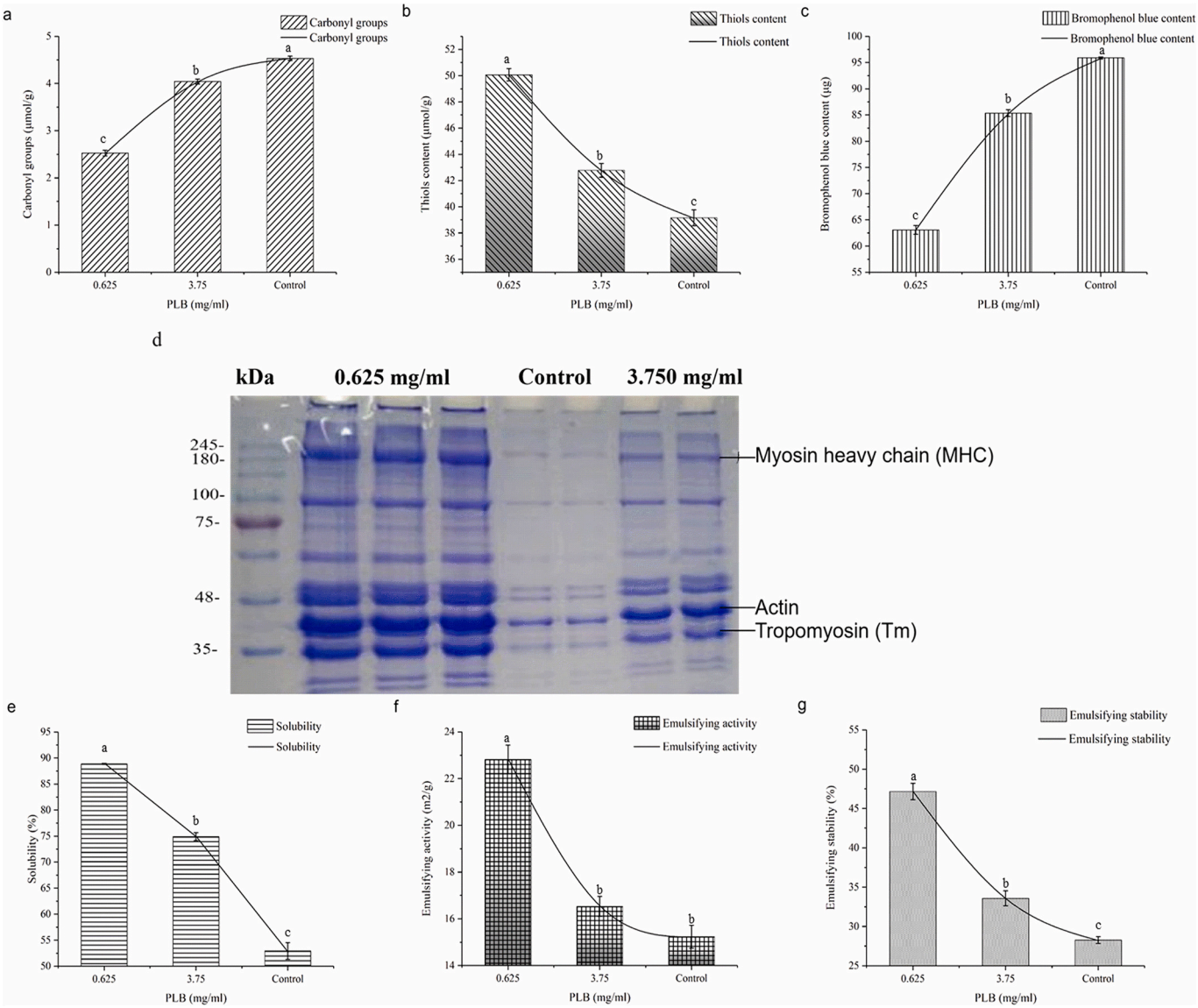

Fig. 3. Effect of PLB on Protein Structure and Properties. Carbonyl content (a); Thiols content (b); Hydrophobicity (c); SDS-PAGE (d); Solubility (e); Emulsifying activity (f); Emulsifying stability (g).

protein properties in steamed sturgeon meat.

3.4. Effects of PLB on fat oxidation inhibition

As shown in Fig. 4(a, b, c), the changes in MDA, AV and PV of steamed sturgeon meat after treatment with different concentrations of PLB are represented to characterize the degree of fat oxidation. As shown in the figure, the 0.625 mg/mL concentration of PLB treatment group exhibited lower MDA, AV and PV. Based on the analysis of the experiment in Section 3.1, it can be concluded that a concentration of 0.625 mg/mL PLB has better antioxidative properties, effectively inhibiting fat oxidation and maintaining the freshness of steamed sturgeon meat. Fig. 4(d) shows the brief process of fat oxidation and the principle of using MDA, AV and PV to characterize the degree of fat oxidation. PLB can inhibited fat degradation pathway, However, the experimental results indicate that when the PLB concentration is too high, the PLB treatment may not effectively inhibit fat oxidation. Although the scientific literature does not provide information on the specific concentration of PLB that may impact fat oxidation, it is noteworthy that the degree of fat oxidation in samples treated with high-concentration PLB was significantly higher than that in the group treated with 0.625 mg/mL.

3.5. Identification of volatile compounds in the phospholipid thermal oxidation model by GC-IMS

Phospholipids are the primary lipid compounds that undergo oxidation during the steaming process of sturgeon meat. GC-IMS was used to analyze the volatile flavor compounds in different treatments of the phospholipid model. The GC-IMS analysis revealed distinctive fingerprint spectra of VOC_S. Notably, the flavor compounds detected after the addition of PLB showed significant differences compared to those detected in the control group. Furthermore, the concentrations of VOCs in the two different PLB groups also exhibited significant changes (Fig. 5a). A total of 35 compounds were identified, including esters (5), aldehydes (11), alcohols (6), ketones (2), acids (3), ethers (2) and others (5). Among them, aldehydes (11) were detected, which are the major flavor compounds in the phospholipid model. The volatile compounds are shown in Table S7. Through fingerprint analysis, the content of aldehydes, such as hexanal, butanal, (E)-2-hexenal, and 2-methylbutanal, decreased after PLB treatment. Aldehydes are the main products of lipid oxidation, and the decrease in their content indicates a reduction in the

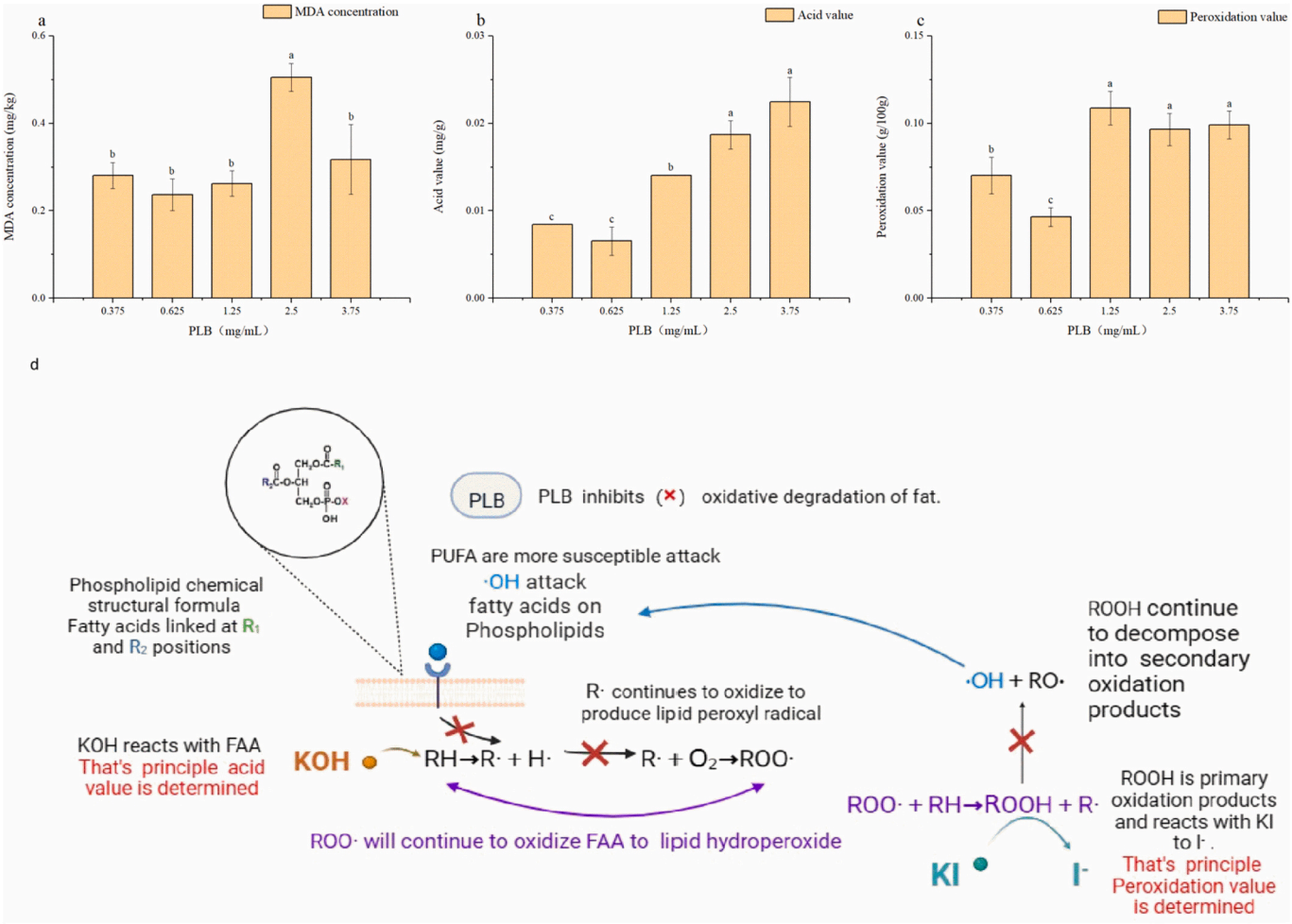

Fig. 4. Inhibitory effect of PLB on lipid oxidation. MDA concentration (a); Acid value (b); Peroxidation value (c); Principle of detection of fat oxidation indicators (d).

degree of lipid oxidation. The accumulation of n-alkane aldehydes, such as hexanal and butanal, during lipid oxidation, may result in undesirable flavors like rancidity and pungency (Han et al., 2023). However, after PLB treatment, the contents of alcohols, esters and acids were relatively high, especially ethyl isovalerate, ethyl acetate, butyl acetate, isopropanol, 2-methylbutanoic acid and other VOCs, which were only detected in 0.625 mg/mL PLB group. These compounds primarily contribute to "grassy" and "fruity" (Miao et al., 2023). In conclusion, the inhibition of fat oxidation by PLB plays a crucial role in regulating the aroma formation of steamed sturgeon meat. PLB effectively hinders the ongoing production of aldehydes, while the abundant presence of aromatic compounds such as esters and alcohols is likely responsible for the delightful taste of steamed sturgeon meat.

3.6. Effects of PLB on the fatty acid profile of steamed sturgeon meat

As shown in Fig. 5(b), a total of 21 fatty acids (FAs) were identified in steamed sturgeon meat, including 6 Saturated fatty acids (SFAs), 4 Monounsaturated fatty acids (MUFAs), and 11 Polyunsaturated fatty acids (PUFAs). Docosahexaenoic acid (DHA, 22:6n-3) was the most abundant fatty acid, followed by trans-linoleic acid (C18:2n-6 t), oleic acid (C18:1n-9), linoleic acid (C18:2n-6c), and palmitic acid (C16:0). These four FAs are also the most abundant types of FAs in Siberian sturgeon and beluga sturgeon (Pyz-Aukasik & Kowalczyk-Pecka, 2017). Compared to the control group, the PLB-treated sturgeon meat exhibited an increase in FAs, indicating that PLB could inhibit the degradation of FAs. It has been reported that the composition of FAs can affect the nutritional value of fish meat (Mekonnen, Desta, Alemayehu, Kelikay, & Daba, 2020). The content of UFAs, PUFAs, increased in the PLB-treated groups. UFAs can influence the quality and flavor characteristics of food. The oxidation of oleic acid and linoleic acid can result in the production of unsaturated aldehydes, ketones, and alcohols, such as nonanal and octanal. These compounds are known to contribute to the odor of aquatic products and provide attributes of "grassy" and "fruity" aroma (Li et al., 2020). However, some studies have found no significant correlation between the content of long-chain polyunsaturated fatty acids and the abundance of flavor substances (Zhang et al., 2023). Therefore, further investigation and discussion are needed to determine the changes in non-volatile precursors of flavor compounds and their relationship to aroma formation.

3.7. Lipidomics analysis of phospholipid thermal oxidation model

Non-targeted lipidomic approaches can be used to infer the identity of volatile flavor compound precursors to reveal their relationship with metabolites. In this experiment, 0.625 mg/mL and 3.750 mg/mL PLB group as well blank control group were selected for non-targeted lipidomics determination. According to the classification of metabolites in the Human Metabolome Database, the lipids in the two groups of samples could be classified into 11 lipid classes, and a total of 191 lipid components were identified, of which Triglycerides, Glycerophosphocholine and Glycerophosphoethanolamine were the major lipid fractions in the phospholipid thermal oxidation model (Fig. 5c). Some metabolites ($P < 0.05$, VIP score > 1.0) were selected as potential

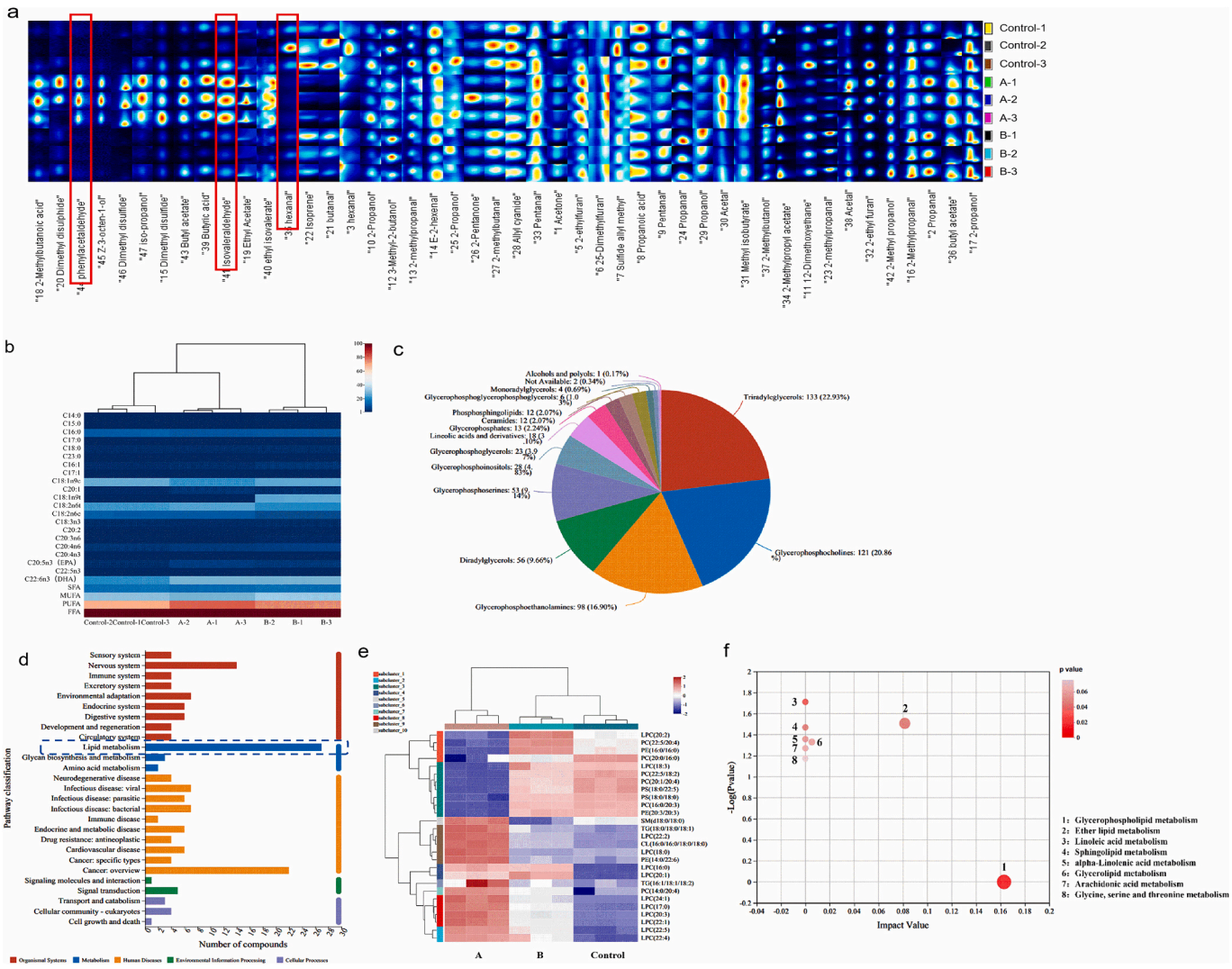

Fig. 5. Fingerprints of volatile compounds based on odor information from IMS signal intensity (a); Fatty acid profile of phospholipid thermal oxidation model (b); Distribution of phospholipid metabolites (c); Differential lipid KEGG pathway (d); Differential lipid content (e); Differential lipids KEGG enrichment analysis (f). A: 0.625 mg/mL PLB treatment groups; B: 3.750 mg/mL PLB treatment groups.

differential compounds, and a total of 32 differential metabolites were identified. KEGG functional pathway analysis was performed on the screened 32 differential lipids, as shown in Fig. 5(d). The analysis revealed that lipid metabolism was the predominant pathway, with 27 lipids involved. The specific contents of these 27 differential lipids were depicted in Fig. 5(e). These findings indicate a significant impact of PLB on the differential lipids content. Furthermore, KEGG pathway enrichment analysis revealed that the differential lipids primarily participated in Glycerolphospholipid metabolism (Fig. 5f), suggesting that PLB primarily influenced phospholipid metabolism in steamed sturgeon meat.

The oxidative degradation of UFAs in phospholipids is an important pathway for the production of aromatic compounds (Xu et al., 2022). Aldehydes are the main VOCs formed from the oxidation of UFAs in phospholipids, especially EPA and DHA (Sicuro, 2019). Therefore, we selected lipids rich in PUFAs from the 27 differential lipids and observed their changes in content, including PC (22:5/20:4), PC (20:1/20:4), PC (22:5/18:2), and LPC (18:3) content decreased, while PE (14:0/22:6) and PC (14:0/20:4) content increased. These alterations may be attributed to the varying enzymatic effects of PLB on phospholipids with distinct structures. To further explore this, Pearson correlation coefficients were calculated to determine metabolites significantly correlated with changes in aldehyde and ketone content (Fig. 6a), confirming a correlation between the oxidation of unsaturated fatty acids in phospholipids and the formation of aldehydes and ketones. The contents of hexanal, isovaleraldehyde, and phenylacetaldehyde showed a significant correlation with phospholipid oxidation. Aldehydes such as hexanal can be produced through the metabolic pathways of linoleic acid, α-linolenic acid and arachidonic acid (Huang et al., 2024). Combined with the content change information of phospholipids, FFAs and VOCs in this experiment, the potential metabolic pathway of VOCs in steamed sturgeon meat are depicted is described in Fig. 6 (b), from which the mechanism of flavor regulation is speculated (Fig. 6c).

The composition and acylation position of fatty acids in phospholipids can lead to different flavor compounds. As markers of lipid oxidation initiation, can impact the production of volatile flavor compounds (Grandois, Marchioni, Ennahar, Giuffrida, & Bindler, 2010). However, the impact of PLB on the distribution and content of fatty acids in phospholipids, particularly in different acylation positions, has not been studied. Some studies have indicated that Phospholipases have specific activity on acylation positions in different lipids. For example, Phospholipase A_1 can selectively enrich omega-3 fatty acids from Triglycerides (Moharana, Byreddy, Puri, Barrow, & Rao, 2016), and Phospholipase A_3 can reshape Triglycerides and phospholipids in animal lipids. In the absence of enzymatic oxidation, the degradation rate of certain specific acylation positions slows down, affecting the production of volatile flavor compounds (Mitsche, Hobbs, & Cohen, 2018).

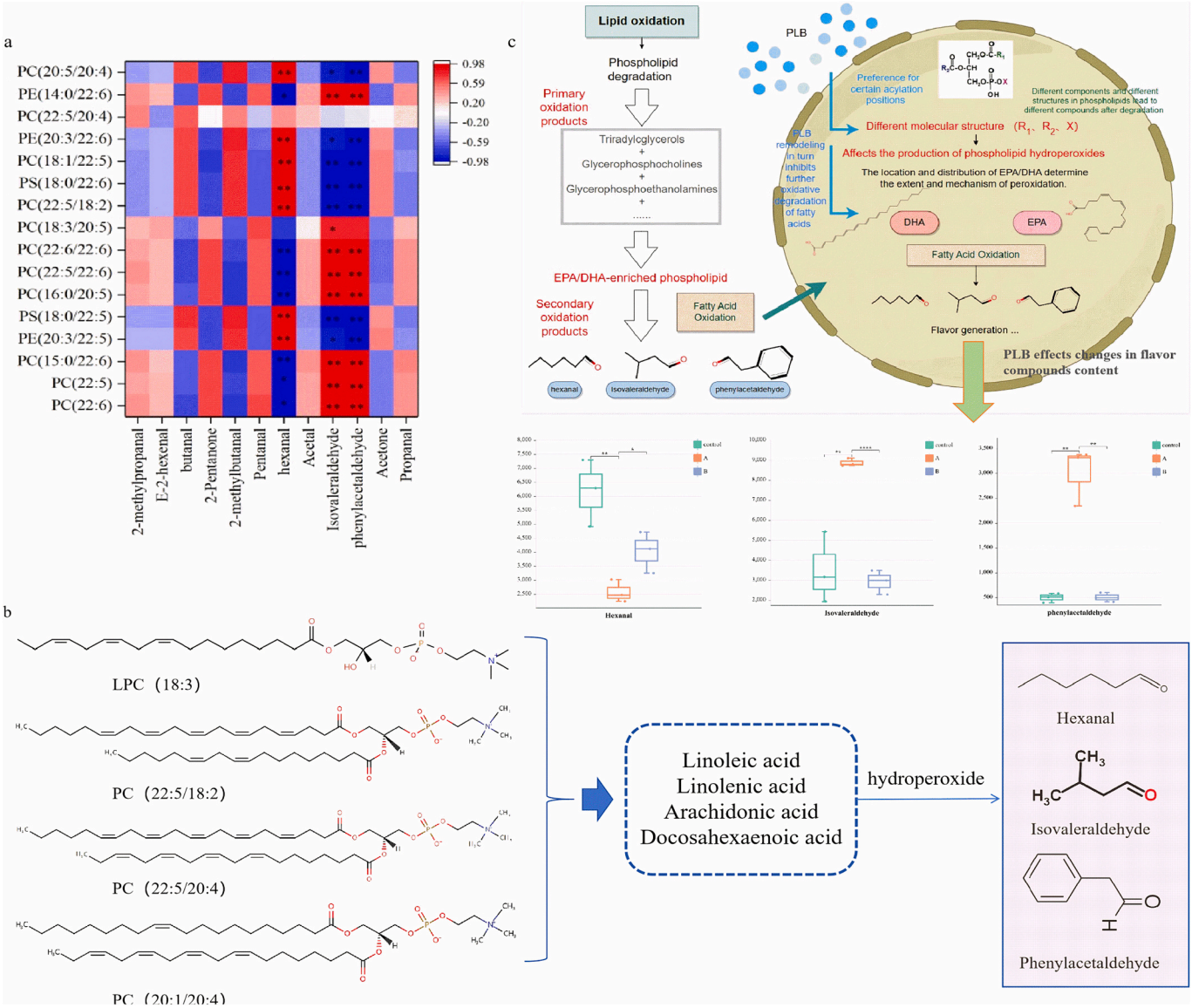

Fig. 6. Heat map representation of the results of Pearson correlation analysis between phospholipids with difference in abundance and aldehydes and ketones (a); A possible pathway for PLB to regulate phospholipid oxidation to form flavor compounds was proposed and regulation of PLB on the content of major volatile flavor compounds (b); The potential metabolic pathway of PLB regulating the flavor change of steamed sturgeon meat (c). The correlation coefficient ($-1 \leq r \leq 1$) is shown in different colors. Significance levels are as follows: * indicates $0.01 < P \leq 0.05$; ** indicates $0.001 < P \leq 0.01$; A: 0.625 mg/mL PLB treatment groups; B: 3.750 mg/mL PLB treatment groups.

Therefore, it is speculated that the mechanism of action of PLB as a flavor modulator involves a preference for specific structural fatty acids at certain acylation positions, resulting in a decrease in phospholipids containing these fatty acids. Additionally, PLB can regulate phospholipids remodeling. For example, Phospholipases can hydrolyze eicosanoids from PE and replenish them on PC through transacylation reactions (Astudillo, Balboa, & Balsinde, 2019). While reshaping phospholipids, it also inhibits further oxidative degradation of fatty acids, reducing the accumulation of undesirable flavors such as aldehydes, thus improving the taste of steamed sturgeon meat. The mechanism of inhibiting lipid oxidation and regulating aroma after high concentration PLB treatment is still unclear in comparison to 0.625 mg/mL PLB treatment. Further research is required to elucidate this phenomenon.

4. Conclusion

This study utilized non-targeted lipidomics to demonstrate that PLB treatment primarily affects the glycerophospholipid metabolic pathway in steamed sturgeon meat. The Pearson correlation coefficients also indicated that the peroxidation of unsaturated fatty acids, particularly EPA and DHA, in phospholipids leads to the formation of aldehydes and ketones. Furthermore, GC-IMS identified aldehydes and ketones as the major volatile flavor compounds generated from phospholipid oxidation during the steaming process of sturgeon meat. It is speculated that PLB may affect the degradation and remodeling of phospholipids due to its specific activity at the phospholipid acylation position, and at the same time inhibit the excessive oxidative degradation of fatty acids. Overall, PLB effectively inhibits oxidation in steamed sturgeon meat and regulates phospholipid oxidation, reducing the generation of undesirable odors and improving the overall flavor of steamed sturgeon meat. However, further research is needed on the specific activity of PLB at the phospholipids acylation position.

CRediT authorship contribution statement

Zhuyu Yang: Writing – review & editing, Writing – original draft,

Visualization, Validation, Software, Methodology, Investigation, Data curation. **Yahui Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Data curation. **Fan Bai:** Formal analysis. **Jinlin Wang:** Formal analysis. **Ruichang Gao:** Writing – review & editing, Conceptualization. **Yuanhui Zhao:** Supervision, Resources, Project administration, Funding acquisition. **Xinxing Xu:** Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101391.

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