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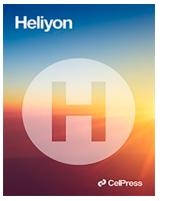

Research article

Lipidomics analysis reveals new insights into crisp grass carp associated with meat texture

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ABSTRACT

Feeding faba beans to grass carp could crisp its muscle texture to avoid softening, the relationship between texture formation throughout the crisping process and the critical lipids regulating the fish quality has not yet been clarified. Herein, an 60-day nutritional trial and untargeted lipidomic analysis was used to study the changes of lipids in crisp grass carp dorsal muscle. A total of 1036 lipids were remarkably different between ordinary and crisp grass carp. The concentrations of the LPC, LPE, PG, Cer, Hex2Cer, SM, MG and MGMG were positively correlated with hardness and springiness, and the CL, TG, PMe, WE, dMePE and AcCa were negative correlation. High content of lipids involved in storage in ordinary grass carp, such as glycerophospholipids, polyunsaturated and saturated fatty acid content. In contrast, high content of membrane components in crisp grass carp, such as monounsaturated fatty acid, sphingolipid and glycerolipids content, and the distribution of PUFA in lipid molecules was related to lipid biosynthesis. This study might provide some insights into improved knowledge of the association between meat texture and lipid molecules in fish fed with faba bean.

1. Introduction

The crisp grass carp, known for its crunchy texture, were exclusively nourished with whole faba beans (*Vicia faba, L.*), an unexpected byproduct from aquaculture farmers in Guangdong province of China. Faba beans, boasting an impressive seed protein content ranging from 26 % to 33 %, were the sole dietary source [1]. The consumption of faba beans can induce alterations in the muscle fiber properties of grass carp. The ability of grass carp muscles to retain water is diminished, and transcriptome analysis indicated that the genes that were up-regulated primarily involved the stimulation of myofibroblast growth [2]. The microstructure examination additionally revealed that there was an augmentation in muscle fiber density and a reduction in muscle fiber diameter when comparing the crisp grass carp with the common grass carp [3]. Exclusively providing grass carp with intact faba beans resulted in the occurrence of muscle fiber hyperplasia, along with a decline in the prevalence of fatty acid degradation and calcium signaling pathways [4]. The firmness of grass carp primarily hinges on the process of myogenesis, extracellular matrix (ECM) composition, and myocyte function [5].

The inclusion of faba beans in the diet has a significant impact on the fat distribution and fatty acid composition in grass carp. Grass carp that consume faba beans have lower levels of crude fat and free fatty acid in their muscle tissues, resulting in a distinctive and appealing flavor profile [6]. In contrast to the commercial diet, fish fed with faba beans exhibited a higher accumulation of fat in the

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liver and mesenteric adipose tissue. Additionally, the decrease in muscle fiber size could potentially result in reduced swimming ability [7]. Including more faba beans in the diet may lead to a reduction in levels of saturated and monounsaturated fatty acids, while simultaneously increasing polyunsaturated fatty acids. Additionally, an increase in muscle firmness was observed over time [8]. There is a general consensus among researchers that lipids can be classified into eight distinct classes, namely fatty acyls, glycerolipids, glycerolipids, saccharolipids, sphingolipids, sterol lipids, polyketides, and prenol lipids. It is widely acknowledged within academic circles that these categories encompass the wide range of lipid compounds found in biological systems [9].

Lipidomics proves to be a powerful and delicate approach in deciphering biological reactions through the examination of distinct lipid species. The scrutiny of lipidomics fluctuations can offer precious insights into the exploration of lipid metabolism [10]. Analyzing lipid profiles of biological samples in depth is an essential component of lipid research. It plays a crucial role in unraveling the mysteries surrounding the functions of individual lipid molecules, as well as the metabolic processes occurring within organisms [10]. Nevertheless, the utilization of lipidomics to examine the traits of lipid metabolism in the muscular tissue of crisp grass carp has not been explored in any research.

Interestingly, faba beans have a lower lipid content than conventional diets but faba bean feeding can induce excess lipid accumulation in the viscera of grass carp. We assume that faba bean supplementation obviously influenced muscle fatty acid composition in the grass carp, then may be an effective measure for improving fish muscle quality. However, the lipid regulatory mechanism of muscle hardness increase in crisp grass carp is still unclear. Therefore, crisp grass carp were exclusively fed with intact faba beans, and the lipid profile of grass carp that consumed a diet rich in faba beans was compared to those that followed a regular diet. This investigation offers valuable insights for the cultivation of crisp grass carp and enhancing the overall quality of fish meat.

2. Materials and methods

2.1. Chemicals, reagents and instruments

LC-MS grade isopropyl alcohol (IPA) was purchased from Fisher Scientific (Loughborough, UK). LC-MS grade methanol (MeOH) was purchased from Dikma Technologies (51 Massier Lane, USA). Chloroform was obtained from Chron Chemicals (Sichuan, China). LC-MS grade acetonitrile (ACN) was purchased from Dikma Technologies (51 Massier Lane, USA). Formic acid was obtained from TCI (Shanghai, China). Ammonium formate was obtained from Sigma-Aldrich (Shanghai, China) Ultrapure water was purchased from watsons (Guangdong, China). High speed freezing centrifuge was obtained from Hunan Xiangyi Experiment Equipment Co., Ltd. (Hunan, China). Vortex mixer was obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Tissue grinder was obtained from Zhejiang Meibi Experiment Equipment Co., Ltd. (Zhejiang, China). Centrifugal vacuum evaporator was from Beijing JM Technology Co., Ltd (Beijing, China). Microporous membrane filters (0.22 μm) was purchased from Tianjin Jinteng Experiment Equipment Co., Ltd. (Tianjin, China). The LC analysis was performed on a ACQUITY UPLC System (Waters, Milford, MA, USA). Mass spectrometric detection of metabolites was performed on Q Exactive (Thermo Fisher Scientific, USA).

2.2. Experimental diets, feeding trial and experimental conditions

Healthy grass carp were purchased from an aquaculture farm in Zunyi, Guizhou Province, China. The fish were initially placed in a temporary cement pond (5 m × 5 m × 1.5 m) for a duration of one week, during which they were provided with a daily feed ration equivalent to 2–3% of their body weight. A total of 180 fish, initially weighing 768 ± 75 g, were randomly allocated into two groups: Group A, consisting of crisp grass carp, and Group B, consisting of ordinary grass carp. Each group consisted of three replicates. 30 fish were raised in each of the six cement ponds, measuring 2 m in length, 2 m in width, and 1.5 m in depth. The cultured crisp grass carp were exclusively nourished with whole faba beans. To prepare the feed, the beans were first soaked in a solution of salt water, with a concentration of approximately 0.15 %, for a duration of 24 h. Subsequently, the beans were soaked in plain water for an additional 12 h, until the faba beans had sprouted. For the ordinary grass carp, a commercially prepared diet was provided (containing 329.9 g kg^{-1} of crude protein and 43.8 g kg^{-1} of crude lipid, from Tongwei Company in China). The fish were given two meals daily, at 8:00 and 17:00. The water temperature was kept at 25–30 °C, pH was 6.5–7.5, and dissolved oxygen was above 5.0 mg/L. The final weights of crisp grass carp and ordinary grass carp were 1230 ± 78 g and 1436 ± 92 g after 60 days, respectively.

2.3. Sample collection

Upon completion of the feeding trial, the researchers proceeded to dissect the muscle tissue of six fish from each dietary group. Subsequently, approximately 300 mg of muscle tissue from each fish was collected and combined, and the muscle filet at the junction of the fourth dorsal fin and lateral line scales was sampled with the aid of a scalpel blade, resulting in a total of three samples per dietary treatment. These samples were then placed in 2 mL cryogenic vials (CryoKing, Biologix Group, China), which were specially designed to be free of DNase and RNase. To preserve the samples, they were promptly frozen using liquid nitrogen and stored at −80 °C.

Once the fish were slaughtered, the laboratory proceeded to directly assess the meat color, pH, cooking loss, and water loss in the fish muscle. Meat color parameters including lightness (L*), redness (a*) and yellowness (b*) were measured at 45 min and 24 h postmortem at 4 °C with a CR-400 chroma meter (Konica Minolta Inc., Tokyo, Japan). After the carcass was cooled, the chroma meter probe was positioned perpendicularly on the incised fish muscle. It was crucial to minimize the exposure of the fish muscle's cut surface to ambient air, ensuring it did not exceed a duration of 10 min. Each sample was subjected to three separate measurements. The

pH was measured at 24 h postmortem with a TESTO 205 pH meter (TESTO AG Inc., Lenzkirch, Germany). The analysis of the dorsal muscle texture, which encompassed characteristics such as firmness, elasticity, toughness, and binding ability, was conducted utilizing the texture profiles analysis (TPA) method, using a Universal TA device from Tengba Company. Test conditions were as follows: a 25 mm × 25 mm flat-bottomed cylindrical probe, a compression ratio of 30 %, a test speed of 1 mm/s and a post-test speed of 1 mm/s with the staying time of 2 s. Test conditions of shear force were as follows: Compression distance of 20 mm, a test speed of 1 mm/s and a post-test speed of 1 mm/s with a staying time of 2 s.

2.4. Untargeted lipidomic analysis

The untargeted lipidomic analysis was performed by Suzhou Panomix Biomedical Tech Co. Ltd. (Suzhou, China), with two steps approach that included lipid extraction, as well as liquid chromatograph-mass spectrometry (LC-MS) analysis.

2.4.1. Lipid extraction

A centrifuge tube was utilized to hold a 100 mg sample of fish muscle. Two glass beads were inserted into the tube, followed by the precise addition of 750 μL of a mixed solvent (chloroform: methanol, 2:1, v/v) at a temperature of −20 °C. The contents were then thoroughly mixed for a duration of 30 s using a vortex. The sample was swiftly frozen in liquid nitrogen for a duration of 5 min, followed by a subsequent process of freezing and thawing at ambient temperature, and vigorously agitated for 2 min at a frequency of 50 Hz. Allow the tube to solidify for a period of 40 min, introduce 190 μL of H_2O, agitate for 30 s, and continue to incubate at freezing temperature for 10 min. Utilize centrifugal force at 12000 rpm for 5 min at ambient temperature, and subsequently transfer 300 μL of the organic layer into a newly acquired centrifuge tube. Add 500 μL of mixed solvent (chloroform: methanol, 2:1, v/v), vortex for 30 s. Centrifuge at 12000 rpm for 5 min at room temperature and transfer 400 μL organic layer into the same centrifuge tube. The samples underwent vacuum evaporation to achieve dryness. The dissolved samples were mixed with 200 μL of isopropanol, and the resulting liquid was passed through a 0.22 μm membrane filter to obtain the prepared samples for LC-MS analysis [11].

2.4.2. Liquid chromatography-mass spectrometry (LC-MS) analysis

Chromatographic separation was used with an ACQUITY UPLC® BEH C18 (2.1 × 100 mm, 1.7 μm, Waters) column maintained at 50 °C. The temperature of the autosampler was 8 °C. Gradient elution of analytes was carried out with acetonitrile: water = 60: 40 (0.1 % formic acid+10 mM ammonium formate) (A2) and isopropanol: acetonitrile = 90: 10 (0.1 % formic acid +10 mM ammonium formate) (B2) at a flow rate of 0.25 mL/min. Injection of 2 μL of each sample was done after equilibration. Separation was conducted under the following gradient: 0–5 min, 70∼57 % A2; 5∼5.1 min, 57 %∼50 % A2; 5.1–14 min, 50 %∼30 % A2; 14∼14.1 min, 30 % A2; 14.1–21 min, 30 %∼1 % A2; 21–24 min, 1 % A2; 24∼24.1 min, 1 %∼70 % A2; 24.1–28 min, 70 % A2.

For the ESI-MSn experiments, the spray voltage was adjusted to 3.5 kV and 2.5 kV in positive and negative modes, respectively, to ensure optimal results. The sheath gas and auxiliary gas were carefully calibrated to 30 and 10 arbitrary units, respectively, to enhance the overall performance. To maintain a stable environment, the capillary temperature was precisely set at 325 °C. The orbitrap analyzer meticulously scanned a wide mass range between *m/z* 150 and 2,000, providing a comprehensive full scan with an impressive mass resolution of 35,000. Data dependent acquisition (DDA) MS/MS experiments were performed with HCD scan. The collision energy was adjusted to 30 eV, resulting in normalized collision energy. To declutter the MS/MS spectra, a dynamic exclusion method was used to eliminate extraneous details [12,13].

2.5. Correlation analysis

The type and amount of lipids play a significant role in determining the quality of meat found in fish dorsal muscle. To investigate the relationship between lipids and meat quality, Pearson correlation analysis was conducted using SPSS 27.0. Statistical significance was considered at $P < 0.05$, and a correlation coefficient exceeding 0.8 or falling below −0.8 indicated a strong correlation.

2.6. LION-PCA heatmap and LION enrichment analysis

LION-PCA was implemented as a module of the web application LION/web (https://heatmap.lipidontology.com/) and visualizes the most characteristic LION-signatures of a given lipidomic dataset in a heatmap. Enrichment analysis was performed using the ranking mode as described in Molenaar et al. (2019) [14], on normalized lipid log-ratio concentrations to compare the lipidomic fingerprint of ordinary and crisp grass carp and report any potential lipid LION term enrichments in the condition of interest.

2.7. Statistical analysis

The values of a*45min, b*45min, L*45min, a*24h, b*24h, L*24h, pH24h, cooking loss and water holding were analyzed by independent-sample T-test in SPSS 27.0 (SPSS Inc, Chicago, IL, USA) and were represented as mean ± standard deviation (SD) of the mean.

After formatting conversion, the raw data underwent a series of data preprocessing steps, including data collection and alignment, before being imported into LipidSearch (version 4.2.28). The LipidSearch database was then utilized to tentatively identify annotated lipids by comparing the precursor ion *m/z* values and the product ion pattern of the data. The elimination of batch effects was achieved through normalization based on the total peak area. Moreover, log transformation and Pareto scaling were implemented. To determine

the false discovery rate (FDR), a *t*-test was conducted using the Benjamini-Hochberg procedure.Statistical significance was defined as P values < 0.05 and FDR values < 0.25. Two distinct models for multivariate statistical analysis, namely unsupervised and supervised, were employed to differentiate the groups (PCA and OPLS-DA). Ultimately, crucial features for distinguishing the groups were identified based on the criteria of Lipids P-value <0.05 and VIP value > 1. LipidomeR was used to analyze the structural characteristics of the identified differential lipids [15]. Briefly, the lipid quantitative value findings were analyzed using a regression model, which determined the carbon atom count and level of saturation (number of double bonds) in the lipid. A comparison was made between the treatment group and lipid to examine the relationship, while the multivariate model characteristics of the lipid structure were further investigated.

3. Results

3.1. Muscle physical parameters and texture characteristics

In this study, the muscle physical parameters and texture characteristics of ordinary and crisp grass carp was presented in Table 1. Crisp grass carp demonstrated significantly lower cooking loss and L*45min values compared to ordinary grass carp ($P < 0.05$). The crisp grass carp exhibited markedly higher levels of hardness, springiness, gumminess, and a*45min compared to the ordinary grass carp ($P < 0.05$). In contrast, no significant differences were observed in muscle pH, chewiness, and cohesiveness between the two groups ($P > 0.05$).

3.2. Global composition of lipid metabolites in muscle

According to the LC-MS analysis results, a total of 1623 lipids were detected, clearly identified, and quantified in ordinary and crisp grass carp (Supplementary Table S1). A visual representation of the distribution of lipid types was shown in Fig. 1. The dominant lipids were phosphatidylcholine (PC), accounting for 29.31 % and 29.42 % respectively, followed by triglyceride (TG) with 18.14 % and 18.08 %, and phosphatidylethanolamine (PE) with 14.77 % and 14.78 %. In addition, a comprehensive analysis revealed the presence of thirty-nine distinct lipid classes (Supplementary Table S2 and Table S3).

Fig. 2A and B displays the score plots of PCA and OPLS-DA for lipids in ordinary and crisp grass carp, respectively. The PCA analysis demonstrated a clear separation of lipid distribution between ordinary and crisp grass carp, as evidenced by the score scatterplot. The total variance explained by PCA was 81.6 %, with component 1 accounting for 73.6 % and component 2 contributing 8 %. The cumulative R2X value was 0.816. On the other hand, the score scatterplot of OPLS-DA showed a total variance of 79.8 %, with component 1 explaining 73.6 % and component 2 explaining 6.2 %. The permutation test results indicated an R2X value of 0.798 and a Q2 value of 0.991 (Fig. 2C).

3.3. Differential of lipid metabolites in muscle

A significant difference was observed between the ordinary and crisp grass carp groups, with a total of 1036 lipids (VIP of >1 and $P < 0.05$) found to be remarkably distinct (Supplementary Table S4 and Fig. 3). Among these lipids, 539 were upregulated and 497 were downregulated in the crisp grass carp. The result of differential lipid analysis was shown in Fig. 4A, B and 4C, further highlighting the pronounced disparity between the two groups. The dominant lipid category was PC (257 lipids), closely followed by phosphatidylethanolamine (PE, 140 lipids), TG (136 species), Methyl phosphatidylcholine (MePC, 91 species), Cardiolipin (CL, 45 lipid species), Sphingomyelin (SM, 42 lipid species), and Diglyceride (DG, 40 lipid species). Most of the other lipid classes identified contained a range of 2–38 individual lipids, while only Glycerophosphoethanolamine-N-(biotinyl) (BiotinylPE), Coenzyme (Co), Gangliosides

Table 1
Muscle physical and texture parameters of grass carp after 60-day feeding experiment.

Parameters	Crisp grass carp	Ordinary grass carp
a*45min	−2.11 ± 0.79	−3.89 ± 0.93
b*45min	3.37 ± 0.62	3.59 ± 0.06
L*45min	45.91 ± 1.01	54.49 ± 1.42*
a*24h	−3.48 ± 0.20	−3.80 ± 0.44
b*24h	7.18 ± 1.83	5.35 ± 0.60
L*24h	50.62 ± 1.05	53.80 ± 2.43
pH	6.68 ± 0.02	6.56 ± 0.03
Water holding capacity (%)	11.19 ± 2.14	9.16 ± 1.14
Cooking loss (%)	21.36 ± 2.48	29.28 ± 0.53*
Hardness (Kg)	0.99 ± 0.14*	0.65 ± 0.10
Springiness (mm)	4.32 ± 0.35*	2.99 ± 0.44
Gumminess (Kg)	2.91 ± 0.50*	1.98 ± 0.22
Chewiness (mJ)	32.85 ± 3.74	28.90 ± 3.40
Cohesiveness (mm)	0.57 ± 0.11	0.53 ± 0.04

Results are presented as the mean ± SEM (n = 3). Values with * indicate significant differences ($P < 0.05$); L*, a* and b* represent lightness, redness and yellowness, respectively.

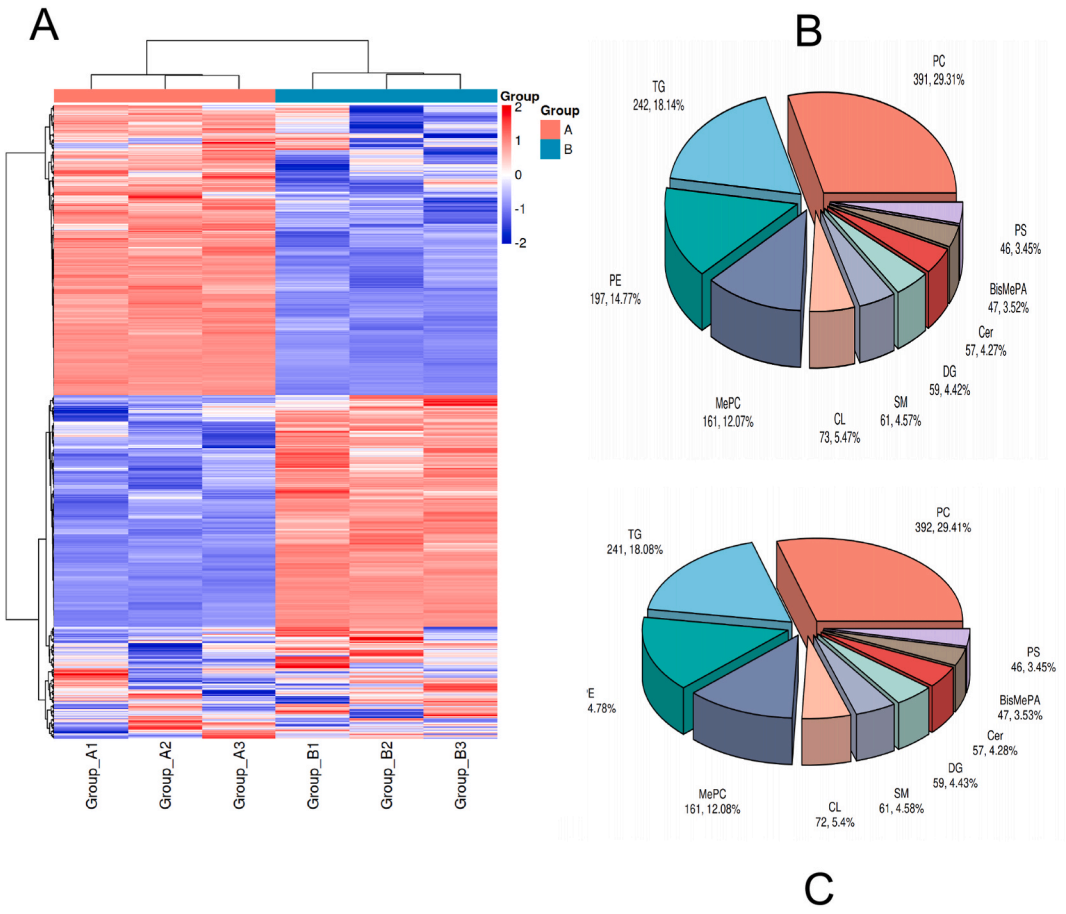

Fig. 1. The overall lipid composition and distribution in muscle of grass carp. (A) Lipid clustering heat map. The size of quantitative values is shown by the difference of colors. The more red the color, the higher the expression level, and the more blue the expression level, the lower. (B) Lipid subclasses and numbers of lipid molecules identified in muscle of crisp grass carp. (C) Lipid subclasses and numbers of lipid molecules identified in muscle of ordinary grass carp. Group A, crisp grass carp group; Group B, ordinary grass carp group. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

(GM3), Bis (monooleoylglycero) phosphate (LBPA), and Phosphatidylmethanol (PMe) were represented by a single lipid species.

Table 2 displays the prominent 30 lipids that exhibit noticeable regulation between the two groups. The lipidomics findings reveal that Phospholipids, Neutral lipids, Glycoglycerolipids, Sphingolipids, Sphingolipids, and Derivatized lipids are the lipid classes most profoundly impacted in the muscle. Remarkably, the crisp grass carp exhibited notably elevated levels of certain lipids compared to the ordinary grass carp. Specifically, the content of PE (20:1e_20:4; 18:2e_18:1 and 18:2p_22:5), PC (18:2e_22:6; 35:4e; 16:1e_18:1; 25:3; 16:0_22:1; 11:0_24:0 and 16:1e_18:2), Triglyceride (TG, 18:0_20:4_24:1 and 25:0_18:1_18:1), Diglyceride (DG, 16:0_22:1; 18:1_22:1 and 20:1_20:2), Monogalactosyldiacylglycerol (MGDG, 40:8e and 38:5e), Sphingomyelin (SM, d43:2), Phosphatidylethanol (PEt, 40:5e and 42:7e), and Bis-methyl phosphatidic acid (BisMePA, 18:2_22:6) were significantly higher in the crisp grass carp. Conversely, the ordinary grass carp experienced increased levels of PC (22:5_22:5; 14:0_18:2; 42:7; 19:0 and 18:1), PE(16:1_20:4; 18:1e_22:5 and 16:1_18:1), and SM (t40:7).

3.4. The correlation of lipids with meat texture

Fig. 5 displays the correlations between important lipids and meat texture. The concentration of Cardiolipin (CL), Simple Glc series (Hex1Cer), TG, Phosphatidylmethanol (PMe), Wax esters (WE), Glycerophosphoethanolamine-N-(biotinyl) (BiotinylPE) Lysodimethylphosphatidylethanolamine (LdMePE), Dimethylphosphatidylethanolamined (MePE) and Acyl Carnitine (AcCa) exhibited a positive correlation with cooking loss. On the other hand, Lysophosphatidylcholine (LPC), Lysophosphatidylethanolamine (LPE), Phosphatidylglycerol (PG), Ceramides (Cer), Gangliosides (GM3), Simple Glc series (Hex2Cer), Sulfatide (ST), Sphingomyelin (SM), Cholesterol Ester (ChE), Monoglyceride (MG), OAcyl-(gamma-hydroxy) FA (OAHFA), Phosphatidylethanol (PEt), Monogalactosylmonoacylglycerol (MGMG) and Sulfoquinovosyldiacylglycerol (SQDG) displayed a negative correlation with cooking loss. The concentration of LPC, LPE, PG, Cer, Hex2Cer, ST, SM, DG, MG, Coenzyme (Co), OAHFA, PE, MGMG, and LdMePE demonstrated a significant positive correlation with hardness. Conversely, CL, PC, Hex1Cer, PMe, WE, BiotinylPE, Dimethylphosphatidylethanolamine

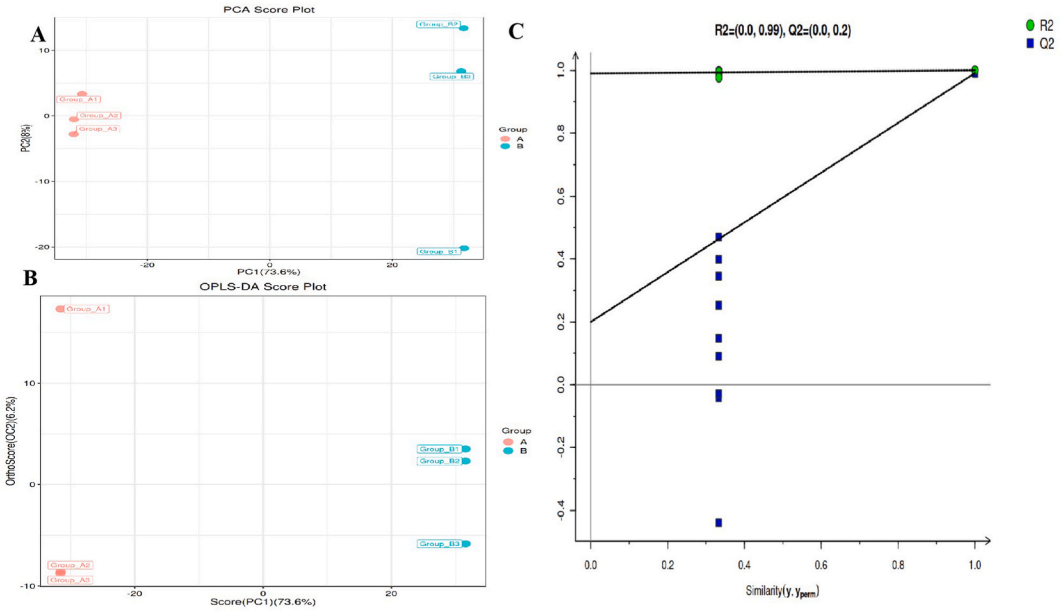

Fig. 2. The multivariate statistical analysis. (A) The PCA analysis of lipids in muscle of grass carp group. (B) The OPLS-DA analysis of lipids in muscle of grass carp group. (C) The OPLS-DA analysis of lipids in muscle of grass carp group.

(dMeP), Bis (monooleoylglycero) phosphate (LBPA), and AcCa exhibited a notable negative correlation with hardness. The concentration of LPC, LPE, PG, Hex2Cer, Cer, GM3, SM, Sphingosine (SPH), DG, ChE, MG, Co, PEt, OAHFA, MGMG, SQDG, LdMePE, BisMePA and Methyl phosphatidylcholine (MePC) showed a positive correlation with springiness, while CL, PC, Hex1Cer, TG, Phosphatidylmethanol (PMe), WE, BiotinylPE, dMePE, LBPA, and AcCa exhibited a negative correlation. The association between springiness and the concentration of these compounds was apparent. Gumminess was positively correlated with the concentration of LPE, GM3, SPH, DG, MG, PEt, BisMePA and MePC; which was negatively correlated with Co, TG, WE, dMePE and AcCa.

3.5. Analysis of LION-PCA heatmap and LION enrichment

We first performed a heat map to visualize the lipidomic fingerprint of each grass carp using the LION-PCA heat map module of LION/web, which is a modified version from a gene ontology-PCA specifically developed for global and in-depth lipidomic data mining (Supplementary Table S5, Fig. 6A and B). LION-PCA heatmap was able to reduce the lipidomes containing 1623 lipids into 101 LION signatures. LION/web was set to group LION signatures with similar dynamics into twelve clusters. The lipidomic fingerprints of grass carp appeared to cluster into two distinct groups (Fig. 6B). The first group is composed with enriched lipid content compared with the second group composed with depleted lipid content. We further performed lipid ontology (LION) enrichment analysis to compare the lipidomic fingerprint of both ordinary and crisp grass carp. Out of the 1036 differential molecular lipid species, 886 were matched to the LION/web database (i.e., 85.52 %), meaning that 150 lipid species were not considered in the LION/web analysis. This includes AcCa (n = 1; lipid class: Fatty acyl and other lipids), BiotinylPE (n = 1; Derivatized lipids), BisMePA (n = 22; Derivatized lipids), CerG2GNAc1 (n = 3; Sphingolipids), Co (n = 1; Fatty acyl and other lipids), DGDG (n = 1, Glycoglycerolipids), Hex1Cer (n = 6; Sphingolipids), MGDG (n = 11; Glycoglycerolipids), MGMG (n = 2; Glycoglycerolipids), MePC (n = 80; Derivatized lipids), OAHFA (n = 3; Fatty acyl and other lipids), PEt (n = 4; Fatty acyl and other lipids), PMe (n = 1; Fatty acyl and other lipids), SQDG (n = 8; Glycoglycerolipids), dMePE (n = 4; Derivatized lipids) and phSM (n = 2; Sphingolipids). Our results indicated significant enrichments in crisp grass carp (compared with ordinary grass carp) of the following LION terms: "diradylglycerols" (associated with n = 40 lipid species), "diacylglycerols" (n = 38), "C18:1" (n = 128), "fatty acid with 18 carbons" (n = 268), "monounsaturated fatty acid" (n = 280), "sphingolipids" (n = 212) and "glycerolipids" (n = 178), (Fig. 6C). By contrast, our results indicated significant depletions in crisp grass carp (compared with ordinary grass carp) of the following LION terms: "diacylglycerophosphocholines" (n = 182), "diacylglycerophosphoethanolamines" (n = 79), "fatty acid with 16 carbons" (n = 162), "fatty acid with 3–5 double bonds" (n = 166), "glycerophospholipids" (n = 542), "polyunsaturated fatty acid" (n = 410) and "saturated fatty acid" (n = 242) (Fig. 6C, SupplementaryTable S6 and S7).

3.6. Analysis of lipid structure characteristics

Fig. 7 presents the statistical findings regarding variations in lipid structure characteristics between the ordinary and crisp grass carp. Notably, a marked disparity was observed in the distribution of lipid molecules in DG and PC. Specifically, the crisp grass carp exhibited a considerably higher presence of lipid molecules with greater carbon atom amounts and reduced unsaturation, as compared

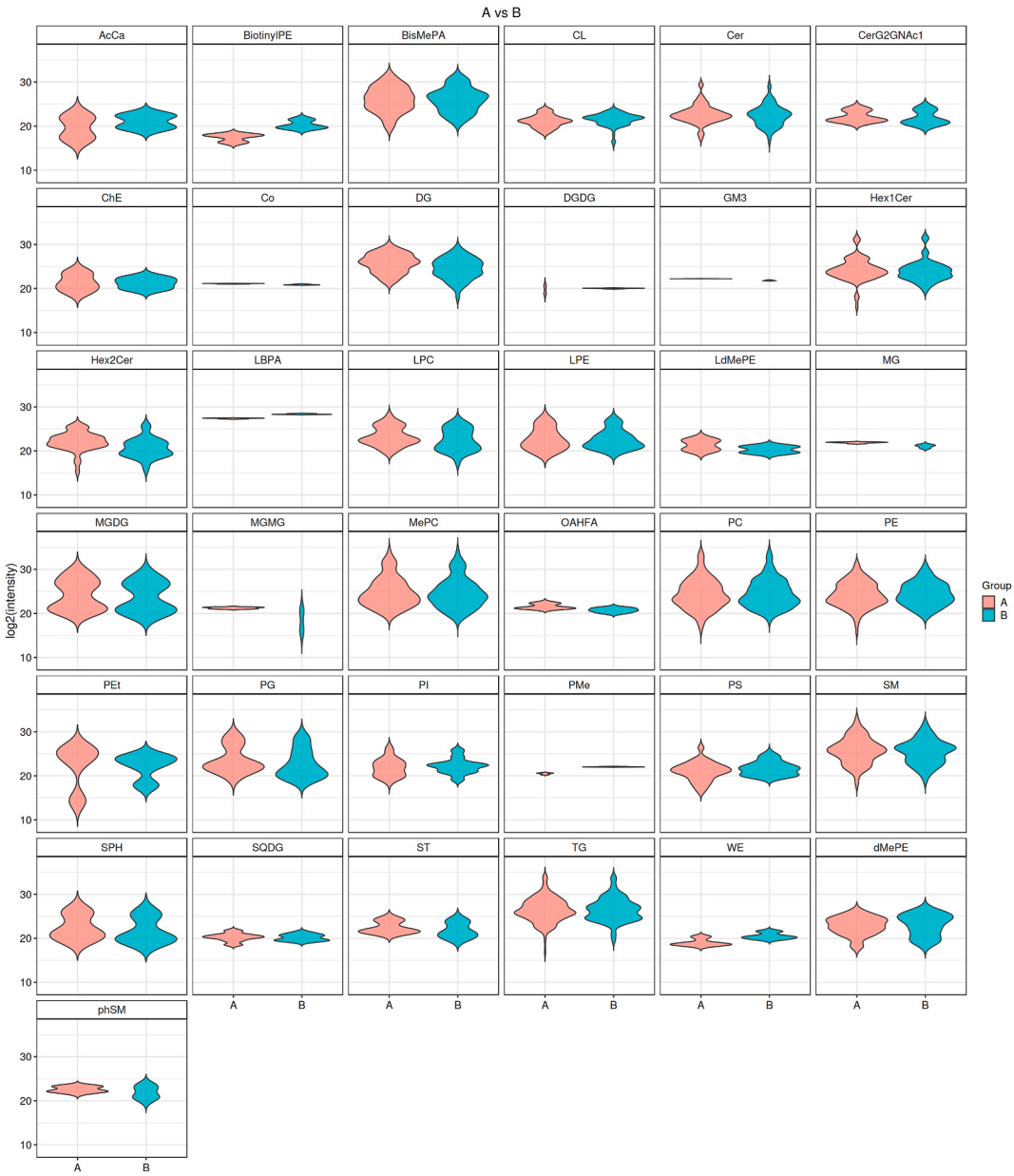

Fig. 3. The dfferential lipid classification violin diagram. Each subgraph shows the differential lipid outcomes under different lipid classifications. The horizontal coordinate is different group, and the vertical coordinate is the range of lipid signal values.

to the ordinary grass carp. A similar pattern was also evident in PC. Additionally, the distribution of lipid molecules with lower carbon atom amounts and reduced unsaturation in PG of the crisp grass carp was notably higher than that observed in the ordinary grass carp. Compared to the ordinary grass carp the crisp grass carp exhibited a notable decrease in the distribution of lipid molecules with lower carbon atom numbers and unsaturation in the PS. Conversely, there was a significant increase in the distribution of lipid molecules with higher carbon atom numbers or unsaturation. In the TG, the crisp grass carp displayed significantly lower levels of lipid molecules with lower carbon atom numbers and unsaturation compared to the ordinary grass carp, while higher carbon atom numbers or lower unsaturation levels were remarkably higher.

4. Discussion

The evaluation of muscle quality heavily relies on the lipids found in meat, and the amount of lipids in muscle can impact various aspects of meat quality [16]. Nutrient control has been proven to be advantageous in enhancing the quality of fish flesh, according to Dong et al. (2022) [17]. The introduction of faba bean (*Vicia faba* L.) into the diet has been associated with improved muscle quality in

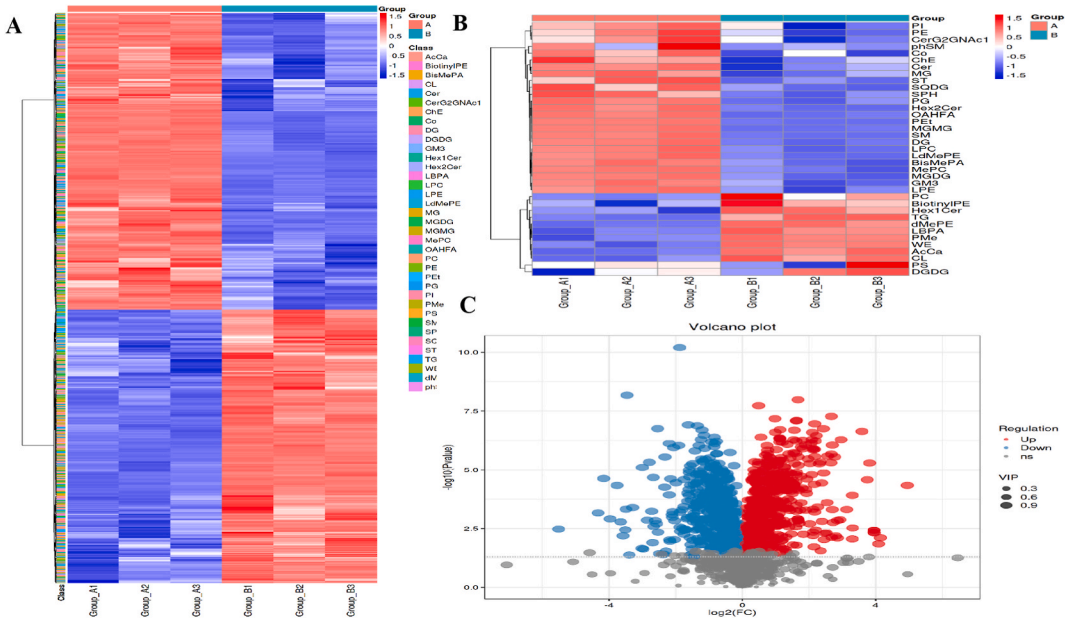

Fig. 4. The differential lipid analysis. (A) The differential lipid Class clustering heat map. The size of quantitative values in the figure is shown by the difference of colors. (B) The differential lipid class classification heat map. (C) The lipid volcano map. Each point in the figure represents a lipid, and the horizontal coordinate represents the Log2 value of the multiple of quantitative difference between the two groups of lipids; The ordinate represents the -log10 value of the *p*-value. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 2
The top 30 markedly regulated lipids between the ordinary and crisp grass carp groups.

Accession	Fold	*p*-value	VIP	Class	Regulation
PC(22:5_22:5)	0.27	6.17175E-11	1.16542244	PC	Down
PC(14:0_18:2)	0.09	6.62031E-09	1.165349061	PC	Down
TG(18:0_20:4_24:1)	3.19	1.02974E-08	1.165335842	TG	Up
PE(20:1e_20:4)	1.41	1.84872E-08	1.165303322	PE	Up
PC(18:2e_22:6)	6.37	5.3587E-08	1.165208912	PC	Up
PC(35:4e)	1.98	6.71071E-08	1.165185411	PC	Up
PC(16:1e_18:1)	3.09	7.97062E-08	1.165167155	PC	Up
MGDG(40:8e)	3.07	8.2409E-08	1.165155834	MGDG	Up
SM(d43:2)	4.5	1.10498E-07	1.165110127	SM	Up
SM(t40:7)	0.33	1.22269E-07	1.165093056	SM	Down
PC(42:7)	0.39	1.33444E-07	1.165073621	PC	Down
PC(19:0)	0.17	1.75983E-07	1.165030793	PC	Down
DG(16:0_22:1)	5.4	1.7777E-07	1.165039175	DG	Up
PEt(40:5e)	2.19	1.95554E-07	1.165012741	PEt	Up
PE(16:1_20:4)	0.43	2.01865E-07	1.164994864	PE	Down
TG(25:0_18:1_18:1)	2.95	2.05457E-07	1.165003121	TG	Up
DG(18:1_22:1)	12.02	2.30722E-07	1.164978787	DG	Up
PC(25:3)	1.98	2.52882E-07	1.164949971	PC	Up
PEt(42:7e)	4.3	2.55339E-07	1.164950022	PEt	Up
PC(18:1)	0.45	3.36608E-07	1.164874546	PC	Down
BisMePA(18:2_22:6)	4.64	3.48791E-07	1.16486155	BisMePA	Up
PC(16:0_22:1)	1.47	4.09689E-07	1.164820551	PC	Up
PE(18:1e_22:5)	0.56	4.11022E-07	1.164808276	PE	Down
PC(11:0_24:0)	2.17	4.34822E-07	1.164799891	PC	Up
PE(18:2e_18:1)	2.19	4.62362E-07	1.164778698	PE	Up
PE(18:2p_22:5)	2.17	4.71926E-07	1.164785955	PE	Up
PC(16:1e_18:2)	4.26	5.13547E-07	1.164749969	PC	Up
PE(16:1_18:1)	0.42	5.17862E-07	1.164748052	PE	Down
DG(20:1_20:2)	7.78	5.23675E-07	1.164729948	DG	Up
MGDG(38:5e)	2.91	5.23955E-07	1.16474259	MGDG	Up

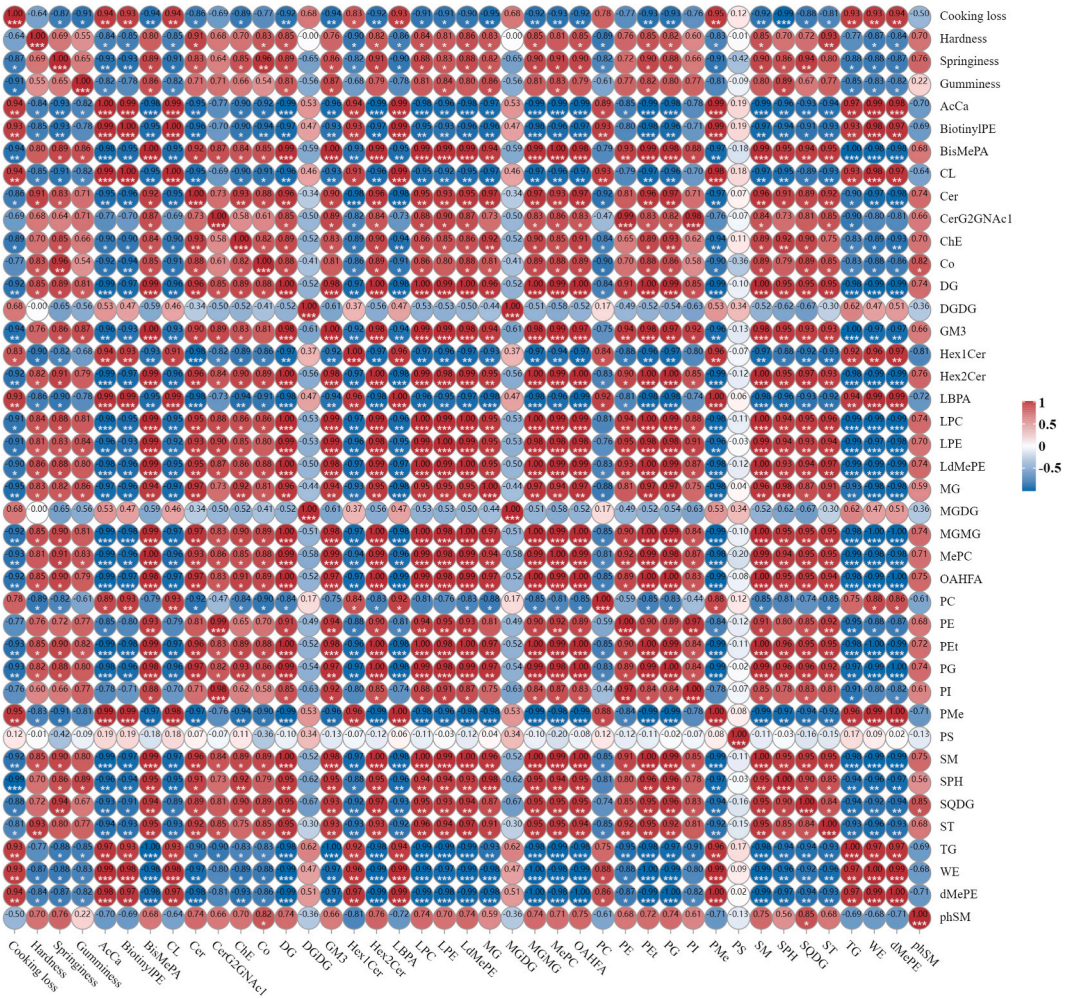

Fig. 5. The correlations of crucial lipids with meat quality. Red and blue represent positive and negative correlations, respectively. The deeper the color, the higher the correlation. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

fish, characterized by increased muscle hardness and crispiness. This dietary supplementation with faba bean has also been found to significantly alter the fatty acid composition of grass carp muscle, as reported by several studies [7,18–21]. Our present study revealed noticeable distinctions in texture parameters between crisp and ordinary grass carp. The crisp grass carp exhibited significantly higher levels of hardness, springiness, and gumminess ($P < 0.05$) compared to the ordinary grass carp. This was mainly associated with increased collagen content and decreased muscle fiber diameter in crisp grass carp, revealing that the main effect of feeding faba beans on the muscle texture was hardness and chewiness. Additionally, a decrease in lower cooking loss and L*45min was observed in the muscle of the crisp grass carp. These findings suggest that the inclusion of faba bean in the diet is an effective approach to enhance the muscle texture of grass carp, which is consistent with previous research [2,4,22].

Lipidomics, which has been derived from metabolomics, proves to be a valuable tool in deciphering the lipid composition by capitalizing on the distinctive properties of lipids. In this study, an innovative approach grounded on untargeted lipidomics was employed to ascertain and assess the alterations in the overall lipid composition and distribution in the muscle of grass carp under the influence of diverse dietary regimes. A total of 1623 lipids were successfully identified, with the prominent ones being PC, TG, and PE. Furthermore, an additional 39 lipid classes were successfully discerned. Other fish species displayed a comparable result, although the occurrence rate of these findings varied. In Nile tilapia (*Oreochromis niloticus*) and orange-spotted grouper (*Epinephelus coioides*) muscular tissues, TG emerged as the dominant lipid class, with PC and PE following closely behind [23,24]. Given that TG primarily functions as a source of energy [25], PC and PE play crucial roles in the proper integration of membrane proteins and membrane fusion and fission processes [24,26]. Furthermore, the fatty acyl chain composition in PC has been found to influence the secretion of very low-density lipoproteins (VLDL) [27].

In this study, the PCA showed that the ordinary and crisp grass carp clustered quite separately. The results obtained from the Principal Component Analysis (PCA) and Orthogonal Partial Least Squares-Discriminant Analysis (OPLS-DA) indicated significant

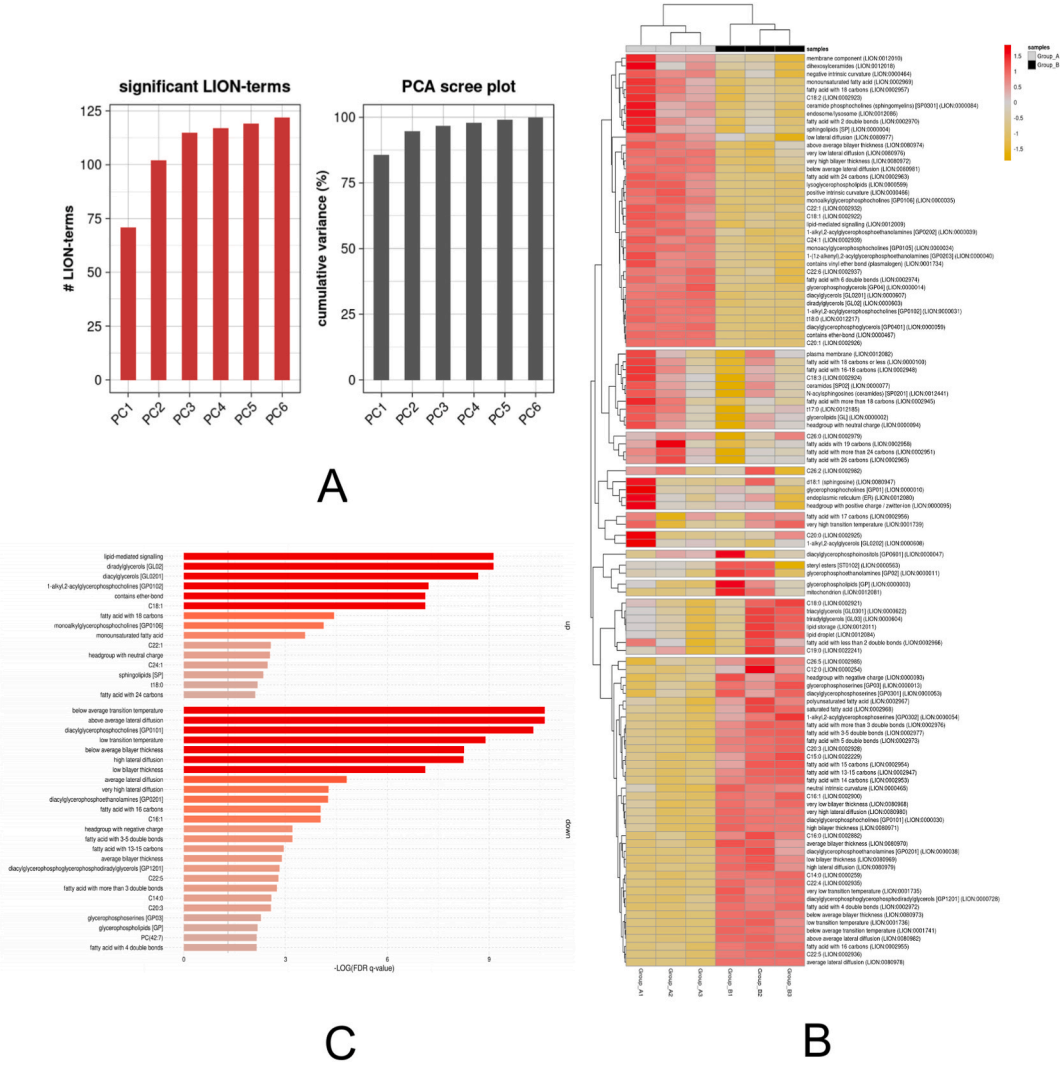

Fig. 6. The LION-PCA heatmap and LION enrichment analysis. A and B, LION-PCA heatmap analysis of lipidomics data. (A) Bar graphs showing the number of significant LION-terms (left) or the cumulative variance explained (right), per set number of principal components. (B) Heatmap generated by the LION-PCA heatmap module in LION/web with the number of principal components set to 6. Heatmap colors (from yellow to red) indicate the mean z-score for a given LION-signature per sample. (C) LION enrichment analysis. The gray vertical line indicates the cutoff value of significant enrichments. The size and color (from gray to red) of the horizontal bars (x-axis) are scaled with the enrichment. Ordinary grass carps were considered as the control condition and crisp grass carps as the condition of interest. "up" refers to an enrichment, and "down" refers to a depletion. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

variations in lipid profiles between the ordinary and crisp grass carp. Moreover, the diverse diets administered to the grass carp led to a significant disruption in lipid composition within their muscle tissue. Notably, the predictive capability of the model was excellent, suggesting its dependable utility in identifying distinctive metabolites. The OPLS-DA model demonstrates remarkable accuracy and strong predictive capability, as evidenced by these exceptional values. In particular, a striking total of 1036 lipids were identified to exhibit significant disparities between the ordinary and crisp grass carp. Among these, 539 lipids were found to be notably upregulated, while 497 lipids were observed to be downregulated. The top 30 markedly regulated lipids to be concerned, thereinto, 21 lipids content in the crisp grass carp was significantly upregulated (see Table 2), and PC (22:5_22:5; 14:0_18:2; 42:7; 19:0 and 18:1), PE (16:1_20:4; 18:1e_22:5 and 16:1_18:1) and SM (t40:7) levels was significantly downregulated. The above results demonstrated that the lipid composition is highly responsive and significantly directs the lipid compositions towards an atypical condition for crisp grass carp. It is suggested that they play a pivotal role in upholding the fundamental structure of cell membranes, facilitating both the smooth movement of molecules in and out of cells, and controlling the transmission of signals between the interior and exterior of cells [28]. PC and PE are classified as a prevalent type of polar lipids, primarily serving as constituents of cell membranes. TG are reported to play a central role in numerous physiological processes, including energy supply, formation of cellular membranes, key components of lipid-transport and storage vesicles, and as signaling molecules [29,30].

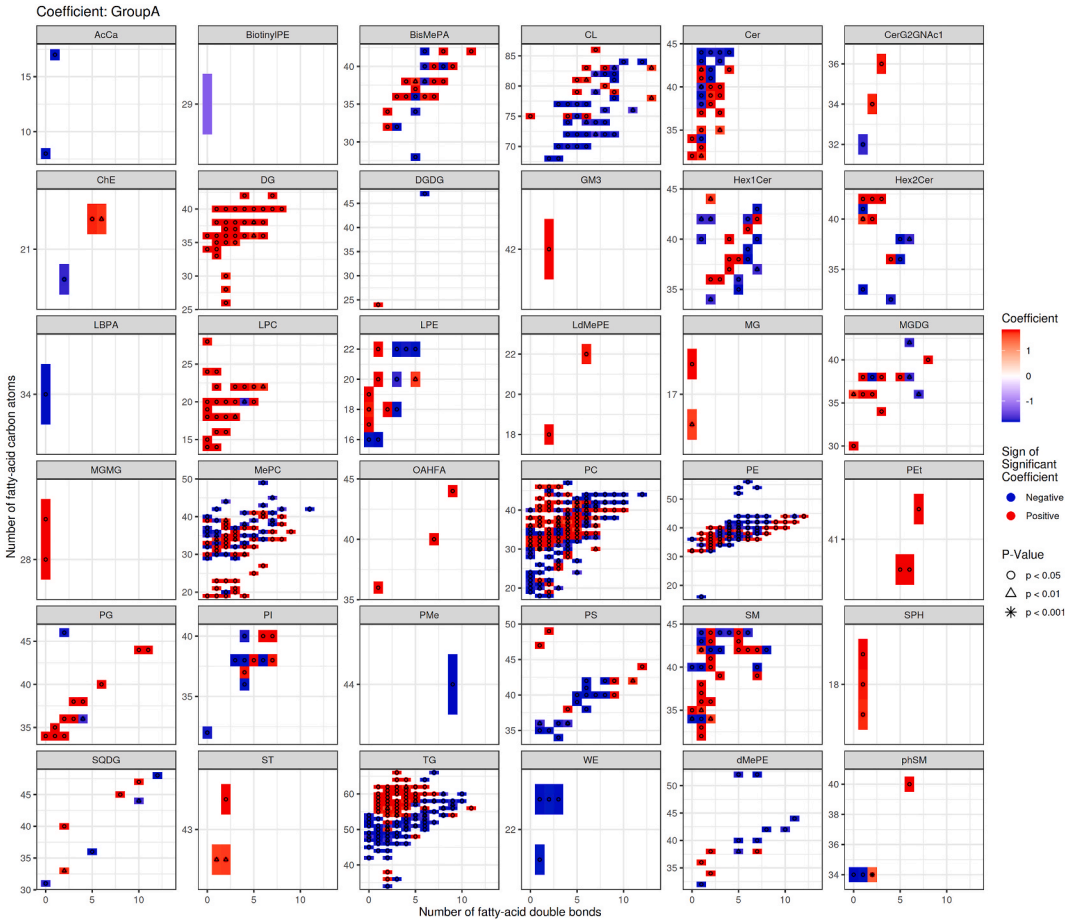

Fig. 7. The statistical heat map of differences in lipid structural characteristics. Each subgraph represents differences in lipid structural characteristics within a single group of a lipid classification. The X-axis represents the level of carbon saturation (number of double bonds) and the Y-axis represents the number of lipid carbon atoms. The rectangle represents different lipids of the same classification, the color represents the significance of the difference expression, the red represents the significant up-regulation, the blue represents the significant down-regulation, and the lipids with significant statistical differences between the comparison group are highlighted with different symbols. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

The impact of diets on the quality of fish flesh is widely acknowledged [31]. Crisp grass carp exhibited noteworthy changes in muscle fiber characteristics, with a notable rise in density and a reduction in diameter [4,8]. Muscle cellularity, including muscle fiber diameter and density, plays a crucial role in shaping the textural properties of muscle [4,32]. Additionally, the density of muscle fibers exhibited a positive association with muscle firmness, stickiness, elasticity, resilience, and tenderness [33]. In this study, we observed a notable association between the levels of LPC, LPE, PG, Cer, Hex2Cer, SM, MG and MGMG and the attributes of hardness and springiness. Conversely, these attributes displayed an inverse correlation with cooking loss. It is worth mentioning that among these compounds, LPC and LPE are categorized as Lysophospholipids (LPL) and are produced through the Phospholipase A (PLA) reaction [34]. Specifically, LPE serves as a minor component of cell membranes, playing a crucial role in facilitating cell-to-cell communication and activating various enzymes [35]. On the other hand, PG are responsible for executing vital functions within cells [35]. Cer and Hex2Cer are biologically active lipids that participate in disrupting mitochondrial function, promoting cell growth, and inducing oxidative stress. Our results suggested that this was primarily attributed to the fact that in addition to anti-nutritional factors such as tannins, faba beans also contained undesirable factors such as trypsin inhibitor, which was able to cause an increase in free radicals, leading to the production of oxidative stress. These factors contribute to the development of an inflammatory environment, which is closely associated with NAFLD and the onset of hepatic insulin resistance [36]. Until recently, SM was primarily regarded as a structural lipid, but it serves a multitude of other functions within specific organelles. These functions encompass promoting molecular order in membranes, serving as a vital source of ceramide for cell signaling and apoptosis, as well as forming clusters/nanodomains in conjunction with cholesterol and ceramide [37]. MGs are compounds resulting from the combination of fatty acid and glycerol molecules, and they have the ability to disrupt phospholipid membranes, thereby exerting a wide array of biological effects [38]. In this study, the concentrations of CL, TG, PMe, WE, dMePE and AcCa exhibited a negative correlation with the characteristics of hardness, springiness, and gumminess, while displaying a positive correlation with cooking loss. CL holds a significant role as a

phospholipid in mitochondria, playing a crucial part in generating cellular energy in the form of ATP [39]. AcCa, which are produced during the transportation of lengthy fatty acids into the mitochondria [40], play a vital role in energy metabolism and β-oxidation [41]. Our results indicate that feeding faba beans also damaged the mitochondrial respiratory chain of crisp grass carp, which accelerated the release of proapoptotic factors and activated the caspase cascade reaction to hasten the apoptosis process, thereby leading to the myofibrils gap substitutionally filled by the collagen, and influencing the texture quality of crisp grass carp. The surge in acylcarnitine levels is likely attributed to the promotion of lipolysis [42], Our results indicate that the lipid accumulation caused by faba bean consumption is a combined outcome of boosted lipogenesis and reduced lipid oxidation. This phenomenon can be attributed to a rise in the synthesis of fats and a decrease in their breakdown within the body, leading to the gradual accumulation of lipids [43].

The lipidomic fingerprint differs between ordinary and crisp grass carp. LION/web is an ontology database containing information related to lipid metabolism, which associates >50,000 lipid molecular species to biophysical, chemical, and cell biological features (referred as "LION terms"). In the present study, the difference in the lipidomic fingerprint composition ordinary and crisp grass carp is consistent with their contrasted feed-nutrition strategy. Specifically, we found high content of lipids involved in storage in ordinary grass carp, such as glycerophospholipids, polyunsaturated and saturated fatty acid content. Our results suggested that the hardness and chewiness of crisp grass carp muscle were significantly increased, probably due to the changes in the polyunsaturated fatty acid composition of fish muscle after feeding faba beans. In contrast, crisp grass carp showed high content of membrane components, such as monounsaturated fatty acid, sphingolipid and glycerolipids content. Additionally, the examination of lipid structure characteristics in ordinary and crisp grass carp groups has provided further evidence indicating that the presence of PUFA in lipid molecules (particularly TG, PC, and DG) is associated with lipid biosynthesis. However, the precise biochemical and physiological mechanisms responsible for the varying distributions of lipid molecules remain unclear. More extensive research is necessary to validate any potential direct correlation.

5. Conclusion

Our study first revealed that the incorporation of faba bean increased the crispness of muscle in grass carp, accompanied by changes in the lipidome. This suggests that some important molecules, such as monounsaturated fatty acid, sphingolipid and glycerolipids, are involved in increased dorsal muscle hardness, springiness and gumminess. Overall, our study provided new insights into the formation of crisp grass carp and shed new light on innovative farming and quality improvement of aquatic products.

Data availability statement

The raw data are available online on the Mendeley database are available at the following link: https://data.mendeley.com/datasets/xyzbwbwpc7/1.

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Ethics statement

All experimental procedures in this study strictly adhered to the regulations outlined by Chinese law regarding animal research. Furthermore, the comprehensive protocol received official approval from the Ethics Scientific Committee for Experiments on Animals at Zunyi Normal College (Protocol number: ZUNSHIFA[2018]08).

CRediT authorship contribution statement

Meilin Hao: Writing – review & editing, Writing – original draft, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis. **Lanlan Yi:** Writing – original draft, Validation, Software. **Wenjie Cheng:** Formal analysis. **Junhong Zhu:** Validation, Resources. **Sumei Zhao:** Writing – review & editing, Resources, Project administration, Investigation, Data curation.

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.

Abbreviations

AcCa Acyl Carnitine
BiotinylPE Glycerophosphoethanolamine-N-(biotinyl)
BisMePA Bis-methyl phosphatidic acid

Cer	Ceramides
ChE	Cholesterol Ester
CL	Cardiolipin
Co	Coenzyme
DG	Diglyceride
dMeP	Dimethylphosphatidylethanolamine
GM3	Gangliosides
Hex1Cer	Simple Glc series
LBPA	Bis(monooleoylglycero)phosphate
LdMePE	Lysodimethylphosphatidylethanolamine
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolamine
MePC	Methyl phosphatidylcholine
MePE	Dimethylphosphatidylethanolamined
MG	Monoglyceride
MGDG	Monogalactosyldiacylglycerol
MGMG	Monogalactosylmonoacylglycerol
OAHFA	OAcyl-(gamma-hydroxy)FA
PC	Phosphatidylcholine
PEt	Phosphatidylethanol
PG	Phosphatidylglycerol
PMe	Phosphatidylmethano
SM	Sphingomyelin
SPH	Sphingosine
SQDG	Sulfoquinovosyldiacylglycerol
ST	Sulfatide
TG	Triglyceride
WE	Wax esters

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e32179.

References

[1] I.C.M. Labba, H. Frøkiær, A.S. Sandberg, Nutritional and antinutritional composition of fava bean (Vicia faba L., var. minor) cultivars, Food Res. Int. 140 (2021) 110038, https://doi.org/10.1016/j.foodres.2020.110038.
[2] W. Xu, H. Guo, S. Chen, Y. Wang, Z. Lin, X. Huang, H. Tang, Y. He, J. Sun, L. Gan, Transcriptome analysis revealed changes of multiple genes involved in muscle hardness in grass carp (Ctenopharyngodon idellus) fed with faba bean meal, Food Chem. 314 (2020) 126205, https://doi.org/10.1016/j.foodchem.2020.126205.
[3] B. Fu, G. Kaneko, G. Wang, H. Yang, J. Tian, Y. Xia, Z. Li, W. Gong, K. Zhang, E. Yu, MicroRNA-dependent regulation of targeted mRNAs for improved muscle texture in crisp grass carp fed with broad bean, Food Res. Int. 155 (2022) 111071, https://doi.org/10.1016/j.foodres.2022.111071.
[4] E. Yu, H. Zhang, Z. Li, G. Wang, H. Wu, J. Xie, D.G. Yu, Y. Xia, K. Zhang, W. Gong, Proteomic signature of muscle fibre hyperplasia in response to faba bean intake in grass carp, Sci. Rep. 7 (2017) 45950, https://doi.org/10.1038/srep45950.
[5] J. Tian, M. Ji, J. Liu, Y. Xia, K. Zhang, H. Li, W.B. Gong, Z.F. Li, W. Xie, G. Wang, J. Xie, E. Yu, N-glycosylomic analysis provides new insight into the molecular mechanism of firmness of fish fillet, Food Chem. 424 (2023) 136417, https://doi.org/10.1016/j.foodchem.2023.136417.
[6] J. Zhang, G. Kaneko, J. Sun, G. Wang, J. Xie, J. Tian, Z. Li, W. Gong, K. Zhang, Y. Xia, E. Yu, Key factors affecting the flesh flavor quality and the nutritional value of grass carp in four culture modes, Foods 10 (2021) 2075, https://doi.org/10.3390/foods10092075.
[7] J. Tian, B. Fu, E. Yu, Y. Li, Y. Xia, Z. Li, K. Zhang, W. Gong, D. Yu, G. Wang, J. Xie, Feeding faba beans (Vicia faba L.) reduces myocyte metabolic activity in grass carp (Ctenopharyngodon idellus), Front. Physiol. 11 (2020) 391, https://doi.org/10.3389/fphys.2020.00391.
[8] Q. Li, Y. Huang, X. Zhang, C. Zou, L. Lin, Improvement of muscle quality in tilapia (Oreochromis niloticus) with dietary faba bean (Vicia faba L.), Front. Nutr. 10 (2023) 1153323, https://doi.org/10.3389/fnut.2023.1153323.
[9] E. Fahy, S. Subramaniam, H.A. Brown, C.K. Glass, A.H. Merrill Jr., R.C. Murphy, C.R. Raetz H, D.W. Russell, Y. Seyama, W. Shaw, T. Shimizu, F. Spener, G. V. Meer, M.S. VanNieuwenhze, S.H. White, J.L. Witztum, E.A. Dennis, A comprehensive classification system for lipids, J. Lipid Res. 46 (2005) 839–861, https://doi.org/10.1194/jlr.E400004-JLR200.
[10] Y. Yuan, F. Xu, M. Jin, X. Wang, X. Hu, M. Zhao, X. Cheng, J. Luo, L. Jiao, M.B. Betancor, D.R. Tocher, Q. Zhou, Untargeted lipidomics reveals metabolic responses to different dietary n-3 PUFA in juvenile swimming crab (Portunus trituberculatus), Food Chem. 354 (2021) 129570, https://doi.org/10.1016/j.foodchem.2021.129570.
[11] E.R. Werner, M.A. Keller, S. Sailer, D. Seppi, G. Golderer, G. Werner-Felmayer, R.A. Zoeller, K. Watschinger, A novel assay for the introduction of the vinyl ether double bond into plasmalogens using pyrene-labeled substrates, J. Lipid Res. 59 (2018) 901–909, https://doi.org/10.1194/jlr.D080283.
[12] J. Dalli, R.A. Colas, M.E. Walker, C.N. Serhan, Lipid mediator metabolomics via LC-MS/MS profiling and analysis, Clinical Metabolomics: Methods and Protocols (2018) 59–72, https://doi.org/10.1007/978-1-4939-7592-1_4.
[13] G. Dasilva, S. Muñoz, S. Lois, I. Medina, Non-targeted LC-MS/MS assay for screening over 100 lipid mediators from ARA, EPA, and DHA in biological samples based on mass spectral fragmentations, Molecules 24 (2019) 2276, https://doi.org/10.3390/molecules24122276.
[14] M.R. Molenaar, A. Jeucken, T.A. Wassenaar, C.H. Van De Lest, J.F. Brouwers, J.B. Helms, LION/web: a web-based ontology enrichment tool for lipidomic data analysis, GigaScience 8 (6) (2019) giz061, https://doi.org/10.1093/gigascience/giz061.

[15] V.S. Olund, R. Benfeitas, A.D. Knudsen, M. Gelpi, J. Høgh, M. Thomsen, Integrative lipidomics and metabolomics for system-level understanding of the metabolic syndrome in long-term treated HIV-infected individuals, Front. Immunol. 12 (2022) 742736, https://doi.org/10.3389/fimmu.2021.742736.
[16] M. Gagaoua, M. Bonnet, B. Picard, Protein array-based approach to evaluate biomarkers of beef tenderness and marbling in cows: understanding of the underlying mechanisms and prediction, Foods 9 (2020) 1180, https://doi.org/10.3390/foods9091180.
[17] M. Dong, L. Zhang, P. Wu, L. Feng, W.D. Jiang, Y. Liu, S. Kuang, S. Li, H. Mi, L. Tang, X. Zhou, Dietary protein levels changed the hardness of muscle by acting on muscle fiber growth and the metabolism of collagen in sub-adult grass carp (Ctenopharyngodon idella), J. Anim. Sci. Biotechnol. 13 (2022) 109, https://doi.org/10.1186/s40104-022-00747-7.
[18] M.S. Azaza, K. Wassim, F. Mensi, A. Abdelmouleh, B. Brini, M.M. Kraïem, Evaluation of faba beans (Vicia faba L. var. minuta) as a replacement for soybean meal in practical diets of juvenile Nile tilapia Oreochromis niloticus, Aquaculture 287 (2009) 174–179, https://doi.org/10.1016/j.aquaculture.2008.10.007.
[19] F. Lun, X. Leng, X. Meng, X. Liu, Effect of feeding broad bean on muscle quality of tilapia, J. Shanghai Fish. Univ. 16 (2007) 83–86, https://doi.org/10.3390/ani13233705.
[20] J. Feng, W. Lin, L. Li, X. Yang, J. Wang, H. Huang, X. Hu, S. Hao, Y. Wu, Advances in effects of broad bean on crispness in fish, Sci Techno Food Ind 37 (2016) 395–399, https://doi.org/10.13386/j.issn1002-0306.2016.14.070.
[21] X. Li, S. Chen, J. Sun, X. Huang, H. Tang, Y. He, Q. Pan, L. Gan, Partial substitution of soybean meal with faba bean meal in grass carp (Ctenopharyngodon idella) diets, and the effects on muscle fatty acid composition, flesh quality, and expression of myogenic regulatory factors, J. World Aquacult. Soc. 51 (2020) 1145–1160, https://doi.org/10.1111/jwas.12671.
[22] Y. Wang, H. Ji, H. Chen, E. Yu, Effects of feeding broad bean on muscle texture characteristics, lipid accumulation and tissue fatty acid composition of grass carp (Ctenopharynodon idellus), Journal of Southern Agriculture 46 (2015) 2040–2045, https://doi.org/10.3969/j:issn.2095-1191.2015.11.2040.
[23] F. Bai, X. Wang, X. Niu, G. Shen, J. Ye, Lipidomic profiling reveals the reducing lipid accumulation effect of dietary taurine in groupers (Epinephelus coioides), Front. Mol. Biosci. 8 (2021) 814318, https://doi.org/10.3389/fmolb.2021.814318.
[24] Y. Liu, J. Jiao, S. Gao, L. Ning, S. Limbu, F. Qiao, L. Chen, M. Zhang, Z. Du, Dietary oils modify lipid molecules and nutritional value of fillet in Nile tilapia: a deep lipidomics analysis, Food Chem. 277 (2019) 515–523, https://doi.org/10.1016/j.foodchem.2018.11.020.
[25] C. Zou, N. Su, J. Wu, M. Xu, Z. Sun, Q. Liu, L. Chen, Y. Zhou, A. Wang, C. Ye, Dietary Radix Bupleuri extracts improves hepatic lipid accumulation and immune response of hybrid grouper (Epinephelus lanceolatus♂× Epinephelus fuscoguttatus♀), Fish Shellfish Immunol. 88 (2019) 496–507, https://doi.org/10.1016/j.fsi.2019.02.052.
[26] G. Meer, A.I.P.M. Kroon, Lipid map of the mammalian cell, J. Cell Sci. 124 (2011) 5–8, https://doi.org/10.1242/jcs.071233.
[27] J.N. Van der Veen, J.P. Kennelly, S. Wan, J.E. Vance, D.E. Vance, R.L. Jacobs, The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease, Biochim. Biophys. Acta Biomembr. 1859 (2017) 1558–1572, https://doi.org/10.1016/j.bbamem.2017.04.006.
[28] N. Wellner, T.A. Diep, C. Janfelt, H.S. Hansen, N-acylation of phosphatidylethanolamine and its biological functions in mammals, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1831 (2013) 652–662, https://doi.org/10.1016/j.bbalip.2012.08.019.
[29] J.P. Koelmel, C.Z. Ulmer, C.M. Jones, A.R. Yost, J.A. Bowden, Common cases of improper lipid annotation using high-resolution tandem mass spectrometry data and corresponding limitations in biological interpretation, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862 (2017) 766–770, https://doi.org/10.1016/j.bbalip.2017.02.016.
[30] S.M. Lam, H. Tian, G. Shui, Lipidomics, en route to accurate quantitation, Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1862 (2017) 752–761, https://doi.org/10.1016/j.bbalip.2017.02.008.
[31] J. García-Romero, R. Ginés, M.S. Izquierdo, R. Haroun, R. Badilla, L. Robaina, Effect of dietary substitution of fish meal for marine crab and echinoderm meals on growth performance, ammonia excretion, skin colour, and flesh quality and oxidation of red porgy (Pagrus pagrus), Aquaculture 422 (2014) 239–248, https://doi.org/10.1016/j.aquaculture.2013.11.024.
[32] M.J. Periago, M.D. Ayala, O. López-Albors, I. Abdel, C. Martínez, A. García-Alcázar, G. Ros, F. Gil, Muscle cellularity and flesh quality of wild and farmed sea bass, Dicentrarchus labrax L, Aquaculture 249 (2005) 175–188, https://doi.org/10.1016/j.aquaculture.2005.02.047.
[33] I.A. Johnston, R. Alderson, C. Sandham, A. Dingwall, D. Mitchell, C. Selkirk, D. Nickell, R. Baker, B. Robertson, D. Whyte, J. Springate, Muscle fibre density in relation to the colour and texture of smoked Atlantic salmon (Salmo salar L, Aquaculture 189 (2000) 335–349, https://doi.org/10.1016/S0044-8486(00)00373-2.
[34] K. Makide, H. Kitamura, Y. Sato M. Okutani, J. Aoki, Emerging lysophospholipid mediators, lysophosphatidylserine, lysophosphatidylthreonine, lysophosphatidylethanolamine and lysophosphatidylglycerol, Prostag. Other Lipid Mediat. 89 (2009) 135–139, https://doi.org/10.1016/j.prostaglandins.2009.04.009.
[35] Z.J. Struzik, A.N. Weerts, J. Storch, D.H. Thompson, Stereospecific synthesis of phosphatidylglycerol using a cyanoethyl phosphoramidite precursor, Chem. Phys. Lipids 231 (2020) 104933, https://doi.org/10.1016/j.chemphyslip.2020.104933.
[36] M.C. Petersen, G.I. Shulman, Roles of diacylglycerols and ceramides in hepatic insulin resistance, Trends Pharmacol. Sci. 38 (2017) 649–665, https://doi.org/10.1016/j.tips.2017.04.004.
[37] F.M. Goñi, Sphingomyelin: what is it good for? Biochem. Biophys. Res. Commun. 633 (2022) 23–25, https://doi.org/10.1016/j.bbrc.2022.08.074.
[38] B.K. Yoon, J.A. Jackman, S. Park, N. Mokrzecka, N.J. Cho, Characterizing the membrane-disruptive behavior of dodecylglycerol using supported lipid bilayers, Langmuir 35 (2019) 3568–3575, https://doi.org/10.1021/acs.langmuir.9b00244.
[39] E.M. Mejia, H. Nguyen, G.M. Hatch, Mammalian cardiolipin biosynthesis, Chem. Phys. Lipids 179 (2014) 11–16, https://doi.org/10.1016/j.chemphyslip.2013.10.001.
[40] C. Indiveri, V. Iacobazzi, A. Tonazzi, N. Giangregorio, V. Infantino, P. Convertini, L. Console, F. Palmieri, The mitochondrial carnitine/acylcarnitine carrier: function, structure and physiopathology, Mol. Aspect. Med. 32 (2011) 223–233, https://doi.org/10.1016/j.mam.2011.10.008.
[41] Z.R. Jarrell, M.R. Smith, X. Hu, M. Orr, K.H. Liu, A.A. Quyyumi, D.P. Jones, Y.M. Go, Plasma acylcarnitine levels increase with healthy aging, Aging (Albany NY) 12 (2020) 13555, https://doi.org/10.18632/aging.103462.
[42] K. Fujisawa, T. Takami, H. Shintani, N. Sasai, T. Matsumoto, N. Yamamoto, I. Sakaida, Seasonal variations in photoperiod affect hepatic metabolism of medaka (Oryzias latipes), FEBS Open bio 11 (2021) 1029–1040, https://doi.org/10.1002/2211-5463.13095.
[43] J. Tian, H. Ji, Y. Wang, J. Xie, G.J. Wang, Z. Li, E. Yu, D. Yu, K. Zhang, W. Gong, Lipid accumulation in grass carp (Ctenopharyngodon idellus) fed faba beans (Vicia faba L.), Fish Physiol. Biochem. 45 (2019) 631–642, https://doi.org/10.1007/s10695-018-0589-7.