Detection and characterization of lipids in eleven species of fish by non-targeted liquid chromatography/mass spectrometry

Siddabasave Gowda B. Gowda[a,b#], Yusuke Minami[c#], Divyavani Gowda[a], Hitoshi Chiba[d], and Shu-Ping Hui[a*]

a. Faculty of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
b. Graduate School of Global Food Resources, Hokkaido University, Kita-9, Nishi-9, Kita-Ku, Sapporo 060-0809, Japan
c. Graduate School of Health Sciences, Hokkaido University, Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan
d. Department of Nutrition, Sapporo University of Health Sciences, Nakanuma, Nishi-4-3-1-15, Higashi-ku, Sapporo 007-0894, Japan.

# Equally contributing authors

Email addresses of all authors:

1. SGBG: siddabasavegowda.bommegowda@hs.hokudai.ac.jp
2. YM: minami.yusuke.t2@elms.hokudai.ac.jp
3. DG: divyavani@hs.hokudai.ac.jp
4. HC: chiba-h@sapporo-hokeniryou-u.ac.jp

* Correspondence: Shu-Ping Hui.

Faculty of Health Sciences, Hokkaido University,

Kita-12, Nishi-5, Kita-ku, Sapporo 060-0812, Japan.

E-mail: keino@hs.hokudai.ac.jp

Tel: +81-11-706-3693.

1. Abstract

Fish is an important nutrition source because its lipids, which are rich in ω-3 fatty acids, are beneficial for human health. However, studies focusing on their detection, composition, and nutritional value are limited. In this study, we applied a non-targeted lipidomic approach based on ultra-high performance liquid chromatography coupled with linear-ion trap-Orbitrap mass spectrometry (UHPLC/LTQ-Orbitrap-MS) to comprehensively profile, compare, and detect unknown lipids in eleven types of dietary fish. A total of 287 molecular species from five major lipid classes were characterized by MS/MS analysis. Multivariate principal component analysis revealed the distinct lipid composition in shishamo smelt and Japanese sardine compared to other fish types. The assessment of nutritional indices based on the levels of free fatty acid suggested that among the eleven fish types, shishamo smelt is highly beneficial for health. Further, lipids such as N-acyl lysophosphatidylethanolamine were detected and characterized for the first time in fish fillets. Hierarchical cluster correlations indicated the predominance of glycerophospholipids (GPs) and sphingolipids in sardine, whereas fatty acyls and triacylglycerols (TAGs) were predominant in shishamo smelt. The high levels of polyunsaturated fatty acid-enriched GPs and TAGs in dietary fish endow it with great potential as a health-promoting food for human consumption. This study offers a comprehensive analysis of lipids and their compositions in fish fillets, demonstrating their potential use in the nutritional assessment of functional foods.

Keywords: Fish fillet, lipidomics, nutritional indices, N-acyl lyso-phosphatidylethanolamine, liquid chromatography, mass spectrometry, correlation analysis

1. Introduction

Fish are an important component of human nutrition and contain many valuable nutrients, such as bioactive lipids, essential amino acids, minerals, vitamins, and proteins(Neff et al., 2014). The benefit of this meat matrix is highly related to the quality of their lipid content. Lipids such as long-chain fatty acids account for one of the key constituents of fish fillets with potential health benefits (Xu et al., 2020). Fatty acids are classified into saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA), depending on the number of double bonds. Fish and its products are a known source of long-chain ω-3 PUFAs such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Xu et al., 2020). Extensive studies have demonstrated that the intake of fish products is associated with the reduction of major chronic diseases, inflammation, and cardiovascular events (Virtanen et al., 2008). The fatty acid content and types mainly vary according to species, seasons, and geographic conditions (Zhang et al., 2020).

Diets rich in SFAs increase the blood cholesterol levels and cardiovascular disease risks (Garonzi et al., 2021). Conversely, ω-3 PUFAs offers protection against cardiovascular disease (Yagi et al., 2017), diabetes (Farrell et al., 2021), and neurodegeneration to reduce inflammation (Borsini et al., 2021). However, men with high blood levels of ω-3 PUFAs are reported to have an increased risk of prostate cancer (Brasky et al., 2013). Hence, a balanced intake of SFA and PUFA in the diet is preferred to reduce the disease risk (Kang et al., 2005). In addition to the PUFA:SFA ratio, several nutritional indices have been developed to provide further knowledge on fish lipid quality and its association with diseases (Fernandes et al., 2014). Moreover, several functional lipids other than fatty acids, such as phospholipids (PLs), glycerolipids (GLs), and sphingolipids, are yet to be characterized well in dietary fishes. Notably, the abundant nutritional and healthy characteristics of fish are primarily attributed to the abundance of PLs and GLs rich in high-level of ω-3 PUFAs (Schuchardt & Hahn, 2013).

Although several analytical methods focus on the targeted determination of lipids in various fish types by gas or liquid chromatography/mass spectrometry(LC/MS), they are limited to the analysis of a specific class of lipids such as fatty acids (de Souza et al., 2020; Devadason et al., 2016). Recently, untargeted mass spectrometric approaches have been widely used in comprehensive food and fish lipidome profiling (Siddabasave et al., 2021; Zang et al., 2018; Zhou et al., 2018). However, studies focusing on the comprehensive lipid fingerprinting of dietary fish are limited. In our previous study, we profiled fatty acids and annotated furan-fatty acids in seafood by nontargeted analysis (Siddabasave et al., 2021). In this study, we present a comprehensive lipidome profiling of dietary fish using a highly sensitive ultra-high performance liquid chromatography coupled with linear-ion trap-Orbitrap mass spectrometry (UHPLC/LTQ-Orbitrap-MS) technique. Comparative correlations of multiple lipids among the characterized eleven common dietary fish were studied, and their nutritional significance from the dietary aspect was discussed. Further, previously unknown lipids such as N-acyl lysophosphatidylethanolamine were characterized in fish fillets using the un-targeted technique.

2. Materials and method

2.1. Materials

Solvents such as isopropanol, chloroform, and methanol of LC/MS grade were purchased from Wako Pure Chemical Industries, Ltd., (Osaka, Japan). Ammonium acetate (eluent additive for LC/MS, 1 M) was obtained from Sigma-Aldrich (St. Louis, USA). Zirconium ceramic oxide bulk beads (1.4 mm, catalog no. 15-340-159, Fisherbrand) for fillet homogenization were purchased from Thermo Fisher Scientific (Tokyo, Japan). The EquiSPLASH LIPIDOMIX quantitative standard for mass spectrometry and oleic acid-d9 internal standards were purchased from Avanti Polar Lipids, Inc (Alabaster, USA).

2.2. Fish samples

Commercially available refrigerated fish such as Pacific cod (*Gadus macrocephalus,* Madara in Japanese, Hokkaido, Japan), saffron cod (*Eleginus gracilis*, Komai, Hokkaido, Japan), Lindberg skate (*Raja pulchra,* Makasube, Hokkaido, Japan), blackin flounder (*Microstomus achne,* Nametagarei, Hokkaido, Japan), Japanese sardine (*Sardinops melanostictus,* Maiwashi, Hokkaido, Japan), Japanese pond smelt (*Hypomesus nipponensis,* Wakasai, Hokkaido, Japan), red seabream (*Pagrus major,* Madai, Ehime, Japan), flatfish (*Paralichthys olivaceus,* Hirame, Hokkaido, Japan), shishamo smelt (*Spirinchus lanceolatus,* Shisyamo, Hokkaido, Japan), skipjack tuna (*Katsuwonus pelamis,* Katuo, Shizuoka, Japan), and Okhotsk Atka mackerel (*Pleurogrammus azonus,* Mahokke, Hokkaido, Japan) were purchased from different supermarkets located in Sapporo, Hokkaido, Japan and were stored at -80 °C until the analysis. Triplicate analysis was performed for each fish species.

2.3. Lipid extraction

Total lipid extraction of the fish fillet was performed by the method described by Folch with minor modifications (Folch et al., 1957; Siddabasave et al., 2021). A freeze-dried fish fillet was weighed and homogenized in 10 volumes of methanol in a homogenizer (Bead Mill 4, Fisherbrand, Tokyo, Japan) for 30 s (× 2 cycles). Subsequently, 100 µL of the homogenate (10 mg) was transferred to a 1.5 mL Eppendorf tube, and 100 µL of the pre-mixed internal standard in methanol was added (10 ng of each of the following: phosphatidylcholine (PC) (15:0-18:1(d7)), phosphatidylethanolamine (PE) (15:0-18:1(d7)), phosphatidylglycerol (PG) (15:0-18:1(d7)), phosphatidylserine (PS) (15:0-18:1(d7)), phosphatidylinositol (PI) (15:0-18:1(d7)), lysophosphatidylethanolamine (LPE) (18:1(d7)), lysophosphatidylcholine (LPC) (18:1(d7)), sphingomyelin (SM) (d18:1/18:0(d9)), ceramide (Cer) (d18:1/15:0 (d7)), triacylglycerol (TAG) (15:0-18:1(d7)-15:0), diacylglycerol (DAG) (15:0-18:1(d7)), cholesterol ester (18:1(d7)), and monoacylglycerol (MAG) (18:1(d7)) and 100 ng of oleic acid (d9)) and vortexed at 3500 rpm for 30 s. Then, 400 µL of chloroform was added and vortexed for

5 min; subsequently, 100 µL of milli-Q was added and the solution was further vortexed for 30 s. The lipid extracts were centrifuged at 15,000 rpm for 10 min. After centrifugation, the chloroform layer was transferred to a new Eppendorf tube, and the aqueous layer was re-extracted with an additional 400 µL of chloroform. The combined chloroform layer containing lipids was collected and evaporated under a vacuum in a centrifuge evaporator, which was then dissolved in 100 µL of methanol, centrifuged, and transferred to an LC/MS vial.

2.4. LC/MS analysis

The UHPLC system (Shimadzu Corporation, Kyoto, Japan) was equipped with an Atlantis T3 C18 column (2.1 × 150 mm, 3 µm, Waters, Milford, USA). The LC/MS conditions were slightly modified compared to those reported in our previous studies (Gowda et al., 2021; Siddabasave et al., 2021). The column temperature was maintained at 40 °C; the flow rate and injection volume were 0.2 mL/min and 10 µL, respectively. Mobile phases used were 10 mM aq. CH_3COON_4 (A), isopropanol (B), and methanol (C). The gradient elution is as follows: 0–1 min, 30% B and 35% C; 1–9 min, 75% B and 15% C; 9–21 min, 82.5% B and 15% C; 21–25 min, 95% B and 5% C; 25–26 min, 30% B and 35% C. The column was pre-equilibrated for 4 min; the total run time was 30 min. Mass spectrometry analysis was performed on a LTQ Orbitrap mass spectrometer (Thermo-Fisher Scientific Inc., San Jose, USA) in the positive and negative ionization modes. The electron spray ionization (ESI) conditions were set as follows: capillary temperature 330 °C; sheath gas flow 50 units; and auxiliary gas 20 and 30 units for the positive and negative modes, respectively. For the negative mode, the source and capillary voltage was set to 3 kV and 10 V, respectively. For the positive mode, source and capillary voltage was set to 4 kV and 25 V, respectively. The MS^1 scan range was set at *m*/*z* 160–1900 and 100–1750 for negative and positive modes, respectively in a Fourier transform mode with a resolving power of 60,000 collision energy

of 35 V to obtain MS spectra for high-resolution masses. Low-resolution MS/MS spectra were obtained at a collision energy of 40 V in the ion-trap mode.

2.5. Data processing for identification of lipids

The raw data obtained from the MS was processed for alignment, peak extraction, identification, and peak area integration using MS-DIAL software (ver 4.2). The following parameters were set during data processing in MS-DIAL: peak height cut-off 1000 amplitude, mass slice width 0.1 Da, smoothing level and minimum peak width 3 and 5 scans, sigma window 0.5, and signal intensity 5 folds (> blank) (Siddabasave et al., 2021). The retention time and MS^1 tolerance were 0.5 min and 0.015 Da, respectively. The identification of lipid molecular species was confirmed by their MS/MS spectra. Extracted ion chromatograms and MS spectra illustrated herein were obtained using Xcalibur 2.2 (Thermo Fisher Scientific, Waltham, USA). The relative quantification of lipid metabolites was performed according to the guidelines of Lipidomics Standards Initiative level 2 and level 3. Relative quantification was achieved considering that the lipid molecule was quantified by the labeled internal standard of similar lipid subclasses or a typical lipid class. The relative amount was calculated by considering peak area ratios of the analyte to the internal standard and multiplying it using the added internal standard.

2.6. Calculation of lipid health quality indexes

The nutritional assessment of eleven fish types was conducted based on their values of index of atherogenicity (IA), health promotion index (HPI), hypo/hypercholesterolemic (HH) ratio, and fish lipid quality (FLQ). These parameters were calculated based on the equations suggested in a previous study (J. Chen & Liu, 2020). IA indicates the relationship between the total SFAs and the total unsaturated fatty acids (UFAs); it assesses the atherogenic potential of FA (Ulbricht & Southgate, 1991). The SFAs, which include C12:0, C14:0, and C16:0, are considered pro-atherogenic whereas UFAs are considered to be

anti-atherogenic properties. The IA value is ranges from 0.21 to 1.41 for fish (S. Chen et al., 2004) and was calculated using the following equation:

$$IA = [C12:0+(4 \times C14:0) +C16:0]/\Sigma UFA.$$

The HPI is the reciprocal of IA and is an index for evaluating the nutritional value of dietary fat (S. Chen et al., 2004). This value determines the effect of fatty acid composition on cardiovascular diseases (CVD). It is mainly used in research on dairy products and a value ranges from 0.16-0.68 (S. Chen et al., 2004). The HPI was calculated using the following equation:

$$HPI = \Sigma UFA/[C12:0+(4\times C14:0)+ C16:0].$$

Further, the HH ratio was used to assess the effect of FA composition on cholesterol and predict the cardiovascular risk. It characterizes the relationship between hypocholesterolemic fatty acid (C18: 1 and PUFA) and hypercholesterolemia FA. It is assumed that, HH ratio could accurately reflect the effect of the FA composition on CVD. The HH ratio is ranges from 1.54 to 4.83 for fish (S. Chen et al., 2004). and was calculated using the following equation:

$$HH =（C18:1+\Sigma PUFA/(C12:0+C14:0+C16:0).$$

In the HH equation, the term “cis-C18:1” was revised to “C18:1” because we did not characterize cis or trans isomers in the present method. FLQ calculates the sum of EPA and DHA as a percentage of total fatty acids. This parameter is used to assess the quality of the fish lipids and is more suitable for marine products because of their high proportions of EPA (FA 22:6) and DHA (FA 20:5). FLQ is widely used for marine products and it calculated using the ratio of sum of EPA and DHA as percentage of total FAs. The FLQ is ranges from 13.01 to 36.37 for fish (S. Chen et al., 2004) and was calculated using the following equation:

$$FLQ =100\times(C22:6 + C20:5)/\Sigma FA.$$

2.7 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), principal component analysis (PCA), and cluster correlation analysis using MetaboAnalyst (ver 5.0). The data were plotted in GraphPad Prism 8 software as the mean ± standard error (analysis of triplicate of each sample). Student's *t*-test ($p < 0.05$) or one-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant) were used to study statistically significant differences between the groups.

3. Results and Discussion

3.1. Lipid annotation and principal component analysis of eleven types of fish

The lipidomic profiles of fish fillets were analyzed by UHPLC/LTQ-Orbitrap-MS in both positive and negative modes. A total of 287 lipid molecular species were annotated based on their exact masses and MS/MS behavior by MS-DIAL software. After the identification and relative quantification using the internal standard method, the multivariant analysis such as one-way ANOVA ($p < 0.05$) with Tukey's post-hoc analysis was applied. The results are shown in **Fig 1a**; 282 lipid molecular species (red) were statistically significant, and 5 were insignificant (green). The PCA demonstrated the differences based on the liquid composition changes of the groups. The PCA analysis of the 287 annotated lipids from eleven types of fish was performed, and the results are illustrated in **Fig 1b**. A previous study demonstrated comparative lipid profiles of various commercial fishes from Japan. However, their analysis was limited to only the fatty acid class (Devadason et al., 2016). The score plots showed distinct grouping and clear separation between the Japanese sardine and the shishamo smelt, suggesting their distinct lipid composition compared to other analyzed fish types. Although these two species were previously studied for their lipid content, a comprehensive class-specific determination was not conducted (Shirai et al., 2002). The two components, PC1 and PC2, accounted for 90.3% of the total model variance, which was described by 56.9% of PC1. The loading plot signifies the distribution of the important variables, the two first principal components, and the groupings among the

samples. A loading plot of components that affects the differences is visualized in the score plot, as shown in **Fig 1b.** The larger positive or negative loading scores indicate that a variable strongly affects the components. Lipids such as triacylglycerols (TAG (16:0/18:1/18:1)), cholesterol esters (CE 20:5, CE 22:6), and sphingomyelin (SM (d18:1/24:0)) have large positive loading scores (for PC1) and contributed mainly for the group separation. These results indicate the differential lipids responsible for group separation in the two-dimensional PCA plot.

3.2 Distributions of lipid classes in eleven different types of fish

The percentage distributions based on the total amount of each lipid class for all the fish types are represented by pie charts, and the results are illustrated in **Fig 2a**. The lipid molecular species were grouped based on their main categories, such as glycerophospholipids (GP), sphingolipids (SP), glycerolipids (GL), fatty acyls (FA), and sterols (ST), according to the LIPID MAPS guidelines. Although these lipids are beneficial to human health, their percentage distribution studies in fish are limited. Profiling of phospholipids and eicosanoids in Atlantic salmon was attempted using shotgun lipidomics; however, other lipid classes were not examined (Yeo & Parrish, 2021). Recently, interest in GPs has dramatically increased owing to their biochemical features in the lipid fraction (Pongsetkul et al., 2017). In our results, the Lindberg skate (83.61%), skipjack tuna (74.80%), and Okhotsk atka mackerel (70.68%) demonstrated the highest abundance of GPs. In contrast, shishamo smelt (86.39%), Japanese sardine (64.27%), and red seabream (50.64%) had higher levels of GLs. The abundance of different molecular species of GPs has been reported in fermented fish (Zang et al., 2018). However, information on the lipid molecular species in commercially available dietary fish is still limited. Hence, in our analysis, we annotated the lipids from multiple classes at molecular species level using state-of-the-art mass spectrometry. Blackin flounder (34.40%) and flatfish (24.48%) demonstrated the highest abundance of sphingolipids, whereas saffron cod (49.23%) had the highest percentage of fatty acyls. In a previous study, the amount of fatty acids in

saffron cod was present in the milligram-scale with a higher amount of neutral lipids than polar lipids (Copeman et al., 2020). Remarkably, we also determined sterols (mainly CEs as major lipids in Pacific cod (68.89%). Although sterols, including various cholesterols esters, were reported in the red fish muscle (Voronin et al., 2021) and cod liver (Hammann et al., 2015), they were not observed in the Pacific cod fillets. Thus, the present study is the first to demonstrate the abundance of sterols in fillets.

3.3 Fish fatty acid compositions and aspects of nutritional indices

Untargeted analysis of fish samples detected 36 fatty acid molecular species from C_{12} to C_{28} carbon chain. These species were further classified as saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), and poly-unsaturated fatty acid (PUFA), based on their number of double bonds. The relative amounts of total SFAs, MUFAs, and PUFAs in different types of fish studied are illustrated in **Fig 2b**. The highest amount of SFAs (10.50±0.21 mg/100g) and MUFAs (11.59±0.32 mg) was observed in shishamo smelt followed by saffron cod (8.22±0.36 mg, 3.23±0.06 mg), whereas Japanese sardine had the lowest amount of MUFAs (0.15±0.01 mg). Furthermore, the highest amount of bioactive PUFAs was observed in shishamo smelt (12.7±0.30 mg) and the lowest in skipjack tuna (0.16±0.01 mg). A previous study had established that fish-derived ω-3 PUFAs, particularly DHA and EPA, had favorable effects on cardiovascular, neurological, inflammatory, and metabolic disorders (Parolini, 2019). Considering this aspect, shishamo smelt could be a promising source as a dietary supplement of sufficient amount of PUFAs compared to other groups. Subsequently, the evaluation of PUFA/SFA (P:S) ratio was conducted, and the results are shown in **Fig 2c**.

The P:S ratio is considered as the most common index to assess the dietary food nutritional value. It is also used to evaluate the impact of diet on the cardiovascular health; a value in the range 1–1.5 in salmon trout is favorable for reducing the risk of cardiac diseases(Kang et al., 2005). Our results indicate that shishamo smelt has the highest P:S ratio (1.21)

279 followed by saffron cod (0.79), whereas skipjack tuna had the lowest value (0.04).
280 Therefore, shishamo smelt could be considered a valuable source in diet due to its
281 appropriate levels of P:S ratio. Recently, the P:S ratio of various food products was
282 extensively reviewed, and the ranges were as follows: sea weeds: 0.42–2.12, meat: 0.11–
283 2.04, fish: 0.11–1.62, and shellfish: 0.20–2.10 (J. Chen & Liu, 2020). Our previous study
284 also demonstrated the highest P:S value of 1.52 in salmon trout (Siddabasave et al., 2021).
285 Hence, the P:S value of dietary fish evaluated in this study could be useful to assess their
286 potential nutritional value. The relative concentrations of each identified fatty acid are
287 represented by a heatmap visualization, as shown in **Fig 3a**, classifying each group and
288 clustered with each other. The intense red color indicates the high concentration of fatty
289 acids in that group. Conversely, the intense blue color implies the low concentration of fatty
290 acids. These results suggest that fatty acids, including FA 20:5 and FA 22:6, are
291 considerably high in shishamo smelt and Saffron cod.

292 Fish with different fatty acid profiles may contribute differently toward human health. These
293 benefits can be evaluated using the nutritional indices, such as the IA, HPI, HH ratio, and
294 FLQ; their respective equations are described in Section 2.6. The nutritional qualities of the
295 lipid profiles from this study and related indices are shown in **Fig 3b**. The IA value was
296 higher in skipjack tuna (4.86±0.39) followed by Lindberg skate (3.09±0.15) and lower in
297 shishamo smelt (0.30±0.01) and saffron cod (0.48±0.02). A previous study reported that
298 consuming foods with lower IA values could decrease the total cholesterol and low-density
299 lipoprotein-cholesterol in human blood plasma (Yurchenko et al., 2018). Additionally, the IA
300 value of fish was reported to range from 0.21 to 1.41 (J. Chen & Liu, 2020). Among all the
301 fish analyzed, the highest HPI value was in shishamo smelt (3.30±0.06) and saffron cod
302 (2.09± 0.11), and the lowest value was in skipjack tuna (0.21±0.02) and Lindberg skate
303 (0.32±0.02). Previously, the HPI was mainly used to evaluate the nutritional value of milk
304 and cheese; the value ranges from 0.16 to 0.68 (J. Chen & Liu, 2020).

Additionally, the HH ratio was the highest in shishamo smelt (4.18 ± 0.09) and saffron cod (2.22 ± 0.13) and the lowest in skipjack tuna (0.21 ± 0.02). Compared with the P:S ratio, the HH ratio can precisely reflect the effect of the fatty acid composition on cardiovascular diseases. The HH ratio indicates the effect of fatty acids on cholesterol metabolism, and the higher values are desirable for human health. A recent review reported that the HH levels in fish were in between 0.87 and 4.83 (J. Chen & Liu, 2020). Finally, the quality of lipids is assessed by FLQ indices. The highest value was obtained for saffron cod (23.51±0.65), Pacific cod (22.45±0.34), and shishamo smelt (21.95±0.79). Skipjack tuna (1.29 ± 0.07) demonstrated the lowest FLQ indices among all fish types. Fish lipid quality is the percentage of EPA and DHA in total fatty acids. These fatty acids reduce the risk of CVD (Breslow, 2006) and dementia (Johnson & Schaefer, 2006). Previous studies reported the quality of fish lipids by FLQ indices, with the value in the 13.01–36.37 range (J. Chen & Liu, 2020). Additionally, the FLQ value assessed the seasonal changes in the fatty acid profile of the fillet of red bream (Senso et al., 2007). Because this index was high in saffron cod, this fish has a great scope for further development as a nutritional supplement.

3.4. Identification of N-acyl-lysophosphatidylethanolamine (LNAPE) in fish fillets

Untargeted LC/MS analysis of total lipid extracts from eleven different types of fish detected the previously discovered lipids, particularly N-acyl-lysophospahtidylethanolamine (LNAPE), which were the partially hydrolyzed products of N-acyl-phospahtidylethanolamine (NAPE). Although these lipids belong to the family of phosphatidylethanolamines (PEs), their amine moieties are esterified with fatty acids. NAPEs were previously detected in the brain of bony fish; however, they were not detected in cartilaginous skeletons (Schmid et al., 1990). Nevertheless, the study lacks a complete characterization of the lipids. The occurrence of NAPEs in mammals and other organisms, such as yeast, insects, reptiles and worms, were also reported (Wellner et al., 2013). However, to the best of our knowledge, no studies have detected LNAPEs in fish fillets. NAPEs are the precursors of bioactive N-acylethanolamines (NAE), which are endogenous signaling molecules. The incorporation of NAPE-producing

bacteria into the gut retards high-fat diet-induced obesity in wild-type mice (Chen et al., 2014) and improves cardiometabolic disease indices in low-density lipoprotein null mice (May-Zhang et al., 2019). Furthermore, multiple studies demonstrated the anti-inflammatory effects of NAPEs (Dalle Carbonare et al., 2008; Solorzano et al., 2009). Despite their great benefits, the characterization of NAPEs in food is still limited.

In this study, we detected seven molecular species of LNAPE with FA 20:5 as the predominating N-acylated fatty acid. The extracted ion chromatograms of LNAPE are shown in **Fig 4a**; their relative levels and other lysophospholipids in each fish type are visualized by a heatmap, as illustrated in **Fig 4b**. The identification of these lipids was confirmed by their high-resolution masses obtained from their MS spectra and fragmentation behavior observed in low-resolution MS/MS spectra. The MS and MS/MS spectra of all the seven major LNAPE species are shown in **Fig 5**. These lipids ionized in negative mode to provide $[M-H]^-$ ion as the precursor ion. The compound elutes with a retention time of 13.92 min and as a *m/z* value of 688.4929 [$C_{37}H_{72}NO_8P$, calculated *m/z*: 688.4923, error: -0.05 ppm], and ionizes to generate *m/z* 452.3 by the neutral loss of FA 16:1 and H_2O. Additionally, peaks at *m/z* 255.3 (FA16:0) and 253.3 (FA 16:1) in the MS/MS spectra suggest that the detected compound is LNAPE-(N-(FA16:0)/16:1). The lipid eluted at 14.25 min has *m/z* 740.5242 [$C_{41}H_{76}NO_8P$, calculated *m/z*: 740.5236, error: 0.28 ppm] and ionizes to generate *m/z* 478.5 by the neutral loss of FA 18:2 and H_2O. Moreover, predominant peaks at *m/z* 279 (FA 18:2) and FA *m/z* 281 (FA 18:1) in the MS/MS spectra indicate that the detected compound is LNAPE (N-(FA18:1)/18:2). The lipid eluted at 13.91 min has *m/z* 736.4936 [$C_{41}H_{72}NO_8P$, calculated *m/z*: 736.49231, error: 1.23 ppm] and ionizes to provide *m/z* 452.3 by the neutral loss of FA 20:5 and water. Further, peaks at *m/z* 255 (FA 16:0 and *m/z* 301 (FA 20:5) in the MS/MS spectra imply that the detected compound is LNAPE-(N-(FA16:0)/20:5).

The compound eluted at 13.64 min has *m/z* 748.4928 [$C_{42}H_{72}NO_8P$, calculated *m/z*: 748.4923, error: 2.28 ppm] and demonstrates an MS behavior similar to that mentioned

earlier; it ionizes to afford *m/z* 464.4 (neutral loss of FA 20:5 and water), 267 (FA 17:1), and 301 (FA 20:5) in the MS/MS spectra, suggesting that the detected compound is LNAPE (N-(FA17:1)/20:5). Similarly, the lipid eluted at 13.96 min has *m/z* 762.5084 [$C_{43}H_{74}NO_8P$, calculated *m/z*: 762.5079, error: 1.39 ppm] and ionizes to afford *m/z* 478 by the neutral loss of FA 20:5 and water. Peaks at *m/z* 281(FA 18:1) and 301(FA 20:5) in the MS/MS spectra suggest that the detected compound is LNAPE (N-(FA18:1)/20:5). The lipid eluted at 13.27 min has *m/z* 758.4775 [$C_{43}H_{70}NO_8P$, calculated *m/z*: 758.4766, error: 1.18 ppm] and ionizes to provide *m/z* 474 (neutral loss of FA 20:5 and water); the peaks at *m/z* 277 (FA 18:3) and *m/z* 301(FA 20:5) in the MS/MS spectra suggest that it is also an isomeric mixture and mainly comprises LNAPE (N-(FA18:3)/20:5). Finally, the lipid eluted at 13.90 min has *m/z* 810.5081 [$C_{47}H_{74}NO_8P$, calculated *m/z*: 810.5078, error: 2.65 ppm], and it ionizes to give *m/z* 520 (loss of FA 20:5 and water) and *m/z* 500 (loss of FA 22:6 and water). Moreover, peaks of FA 22:6 (*m/z* 327), FA 20:5 (*m/z* 301), FA 20:4 (*m/z* 303), FA 22:6 (*m/z* 283), and FA 20:5 (*m/z* 301) in the MS/MS spectra indicate that it is an isomeric mixture of LNAPE (N-(FA 20:4)/22:6) and LNAPE (N-(FA 22:5)/20:5).

The concentrations (mean ± SE, in mg/100g) of total LNAPE were as follows: red seabream (0.25±0.01), flatfish (0.33±0.05), Okhotsk atka mackerel (0.44±0.02 mg), Japanese sardine (0.75±0.02 mg), blackin flounder (0.75±0.02 mg), Lindberg skate (0.25±0.01 mg), skipjack tuna (0.19±0.03 mg), saffron cod (0.15±0.00 mg), shishamo smelt (0.33±0.02 mg), Pacific cod (0.19±0.01 mg), and Japanese pond smelt (0.83±0.03 mg). LNAPE (N-(FA18:1)/18:2) is abundant in red seabream, LNAPE (N-(FA20:4)/22:6) and LNAPE (N-(FA16:0)/16:1) were abundant in blackin flounder. LNAPE (N-(FA16:0)/20:5), LNAPE (N-(FA18:1)/20:5), and LNAPE (N-(FA17:1)/20:5) were abundant in Japanese pond smelt, whereas LNAPE (N-(FA18:3)/20:5) and LPE (N-(FA17:1/20:5) were abundant in Japanese sardine. The abundance of PUFAs as LNAPEs acyl chains suggest that these lipids are also a potential source of dietary PUFAs. To the best of our knowledge, the present study is the first to

characterize and demonstrate the relative levels of LNAPEs in multiple fish types. Further investigations are necessary to determine their biological effects.

3.5. Hierarchical cluster analysis of complex lipids profiled in eleven types of fish

Hierarchical clustering heatmaps visualizes the concentrations of complex lipids detected in fish fillets. The visualization of lipids such as phosphatidylcholines (PC), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidylglycerols (PG), and cardiolipins (CL) in eleven types of fish is represented by a heatmap and is shown in **Fig 6a, 6b**. A tight clustering among the sample groups was observed. The concentration of PC, PS, and PI was relatively higher in Japanese pond smelt and Lindberg skate compared to other fish types. These phospholipids are highly enriched with PUFAs such as FA 22:6, FA 20:5, and FA 20:4. Hence, these membrane lipids could be the potential sources of PUFAs in the diet. Moreover, fish-derived ω-3 PUFAs were reported to significantly affect intestinal microbiota and regulate immune function (Parolini, 2019).

The energy metabolism associated lipids, such as PGs and CLs, were relatively high in shishamo smelt. Further, lipids such as Cer, SM, CE, MAG, DAG, TAG, and NAE in eleven types of fish are visualized by a heatmap, and the results are illustrated in **Fig 6c,6d**. The concentration of sphingolipids (Cer and SM) and sterols (CE) was higher in Japanese pond smelt whereas that of MAG, DAGs, and TAGs was higher in shishamo smelt. Remarkably, TAGs were also enriched with many PUFAs as acyl chains. The NAE lipids were relatively high in shishamo smelt and saffron cod. NAEs are a functionally diverse family of signaling lipids that have been implicated as metabolic signals regulating the nutritional status and lifespan (Lucanic et al., 2011). These lipids may have important implications for biomedical research on aging and obesity (De Petrocellis & Di Marzo, 2011). A previous study demonstrated the application of the untargeted technique for comprehensive lipid profiling in round scad (He et al., 2020) and sea urchin (Zhou et al., 2018); several studies focused on profiling specific lipids such as phospholipids (Yeo & Parrish, 2021; Zang et al., 2018) or

fatty acids (Devadason et al., 2016; Zhang et al., 2020) in various other fish types. However, no evident studies have demonstrated a comprehensive profile of lipids in the fish types explored in the present study.

4. Conclusion

In summary, various lipid species and their relative content in the eleven types of fish were analyzed using UHPLC/LTQ-Orbitrap MS. GP was the most abundant lipid class in many groups. The PUFAs, P:S ratio, and HPI was significantly higher in shishamo smelt, suggesting it as a potential health-beneficial dietary fish type. The previously uncharacterized and biologically beneficial lipids such as LNAPEs were detected and characterized for the first time in fish fillets. In addition, the comprehensive profile of multiple lipid classes and their distribution among each fish type was revealed by the cluster correlation analysis. Shishamo smelt was enriched with TAGs, and Japanese sardines had a relatively large amount of PC, PI, SM, and CEs. Therefore, lipids in dietary fishes can account for their nutritional and health-beneficial functions. Because exploring the sources of these lipids is of significant interest, we aim to apply the untargeted technique to determine the unidentified lipids in other food types.

CRediT authorship contribution statement

Siddabasave Gowda B. Gowda: Conceptualization, Methodology, Supervision, Writing - original draft. Yusuke Minami: Formal analysis, Visualization, Writing - original draft. Divyavani Gowda: Data curation, visualization, Writing - review & editing. Hitoshi Chiba: Supervision, Writing - review & editing. Shu-Ping Hui: Supervision, Resources, Writing - review & editing.

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.

436 **Acknowledgements**

437 This work is supported by the Japanese Society for the Promotion of Science KAKENHI
438 Grants (18K0743408, 19H0311719, 19K0786109 and 21K1481201). SGBG thanks editage,
439 Japan for English language editing of the manuscript and Graduate School of Global Food
440 Resources, Hokkaido University for the financial aid.

441 **References**

442 Borsini, A., Nicolaou, A., Camacho-Muñoz, D., Kendall, A. C., Di Benedetto, M. G.,
443 Giacobbe, J., Su, K. P., & Pariante, C. M. (2021). Omega-3 polyunsaturated fatty acids
444 protect against inflammation through production of LOX and CYP450 lipid mediators:
445 relevance for major depression and for human hippocampal neurogenesis. *Molecular*
446 *Psychiatry 2021 26:11*, *26*(11), 6773–6788. https://doi.org/10.1038/s41380-021-01160-
447 8

448 Brasky, T. M., Darke, A. K., Song, X., Tangen, C. M., Goodman, P. J., Thompson, I. M.,
449 Meyskens, F. L., Goodman, G. E., Minasian, L. M., Parnes, H. L., Klein, E. A., & Kristal,
450 A. R. (2013). Plasma phospholipid fatty acids and prostate cancer risk in the SELECT
451 trial. *Journal of the National Cancer Institute*, *105*(15), 1132–1141.
452 https://doi.org/10.1093/JNCI/DJT174

453 Breslow, J. L. (2006). n−3 Fatty acids and cardiovascular disease. *The American Journal of*
454 *Clinical Nutrition*, *83*(6), 1477S-1482S. https://doi.org/10.1093/AJCN/83.6.1477S

455 Chen, J., & Liu, H. (2020). Nutritional Indices for Assessing Fatty Acids: A Mini-Review.
456 *International Journal of Molecular Sciences*, *21*(16), 1–24.
457 https://doi.org/10.3390/IJMS21165695

458 Chen, S., Bobe, G., Zimmerman, S., Hammond, E. G., Luhman, C. M., Boylston, T. D.,
459 Freeman, A. E., & Beitz, D. C. (2004). Physical and sensory properties of dairy
460 products from cows with various milk fatty acid compositions. *Journal of Agricultural*

461 *and Food Chemistry*, *52*(11), 3422–3428. https://doi.org/10.1021/JF035193Z

462 Chen, Z., Guo, L., Zhang, Y., Walzem, R. L., Pendergast, J. S., Printz, R. L., Morris, L. C.,
463 Matafonova, E., Stien, X., Kang, L., Coulon, D., McGuinness, O. P., Niswender, K. D.,
464 & Davies, S. S. (2014). Incorporation of therapeutically modified bacteria into gut
465 microbiota inhibits obesity. *The Journal of Clinical Investigation*, *124*(8), 3391–3406.
466 https://doi.org/10.1172/JCI72517

467 Copeman, L., Spencer, M., Heintz, R., Vollenweider, J., Sremba, A., Helser, T., Logerwell,
468 L., Sousa, L., Danielson, S., Pinchuk, A. I., & Laurel, B. (2020). Ontogenetic patterns in
469 lipid and fatty acid biomarkers of juvenile polar cod (Boreogadus saida) and saffron
470 cod (Eleginus gracilis) from across the Alaska Arctic. *Polar Biology*, *43*(8), 1121–1140.
471 https://doi.org/10.1007/S00300-020-02648-9/FIGURES/8

472 Dalle Carbonare, M., Del Giudice, E., Stecca, A., Colavito, D., Fabris, M., D'Arrigo, A.,
473 Bernardini, D., Dam, M., & Leon, A. (2008). A Saturated N-Acylethanolamine Other
474 than N-Palmitoyl Ethanolamine with Anti-inflammatory Properties: a Neglected Story….
475 *Journal of Neuroendocrinology*, *20*(SUPPL. 1), 26–34. https://doi.org/10.1111/J.1365-
476 2826.2008.01689.X

477 De Petrocellis, L., & Di Marzo, V. (2011). Why fasting worms age slowly. *Nature 2011*
478 *473:7346*, *473*(7346), 161–162. https://doi.org/10.1038/473161a

479 de Souza, A. F. L., Petenuci, M. E., Camparim, R., Visentainer, J. V., & da Silva, A. J. I.
480 (2020). Effect of seasonal variations on fatty acid composition and nutritional profiles of
481 siluriformes fish species from the amazon basin. *Food Research International*, *132*,
482 109051. https://doi.org/10.1016/J.FOODRES.2020.109051

483 Devadason, C., Jayasinghe, C., Sivakanesan, R., Senarath, S., Beppu, F., & Gotoh, N.
484 (2016). Comparative Analysis of Lipid Content and Fatty Acid Composition of
485 Commercially Important Fish and Shellfish from Sri Lanka and Japan. *Journal of Oleo*

Science, *65*(7), 543–556. https://doi.org/10.5650/JOS.ESS16056

Farrell, S. W., DeFina, L. F., Tintle, N. L., Leonard, D., Cooper, K. H., Barlow, C. E., Haskell, W. L., Pavlovic, A., & Harris, W. S. (2021). Association of the Omega-3 Index with Incident Prostate Cancer with Updated Meta-Analysis: The Cooper Center Longitudinal Study. *Nutrients 2021, Vol. 13, Page 384*, *13*(2), 384. https://doi.org/10.3390/NU13020384

Fernandes, C. E., Vasconcelos, M. A. D. S., De Almeida Ribeiro, M., Sarubbo, L. A., Andrade, S. A. C., & Filho, A. B. D. M. (2014). Nutritional and lipid profiles in marine fish species from Brazil. *Food Chemistry*, *160*, 67–71. https://doi.org/10.1016/J.FOODCHEM.2014.03.055

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *The Journal of Biological Chemistry*.

Garonzi, C., Forsander, G., & Maffeis, C. (2021). Impact of Fat Intake on Blood Glucose Control and Cardiovascular Risk Factors in Children and Adolescents with Type 1 Diabetes. *Nutrients*, *13*(8), 2625. https://doi.org/10.3390/NU13082625

Gowda, S. G. B., Tsukui, T., Fuda, H., Minami, Y., Gowda, D., Chiba, H., & Hui, S. P. (2021). Docosahexaenoic Acid Esters of Hydroxy Fatty Acid Is a Novel Activator of NRF2. *International Journal of Molecular Sciences 2021, Vol. 22, Page 7598*, *22*(14), 7598. https://doi.org/10.3390/IJMS22147598

Hammann, S., Wendlinger, C., & Vetter, W. (2015). Analysis of Intact Cholesteryl Esters of Furan Fatty Acids in Cod Liver. *Lipids*, *50*(6), 611–620. https://doi.org/10.1007/S11745-015-4019-7

He, C., Sun, Z., Qu, X., Cao, J., Shen, X., & Li, C. (2020). A comprehensive study of lipid profiles of round scad (Decapterus maruadsi) based on lipidomic with UPLC-Q-Exactive Orbitrap-MS. *Food Research International (Ottawa, Ont.)*, *133*.

https://doi.org/10.1016/J.FOODRES.2020.109138

Johnson, E. J., & Schaefer, E. J. (2006). Potential role of dietary n-3 fatty acids in the prevention of dementia and macular degeneration. *The American Journal of Clinical Nutrition*, *83*(6 Suppl). https://doi.org/10.1093/AJCN/83.6.1494S

Kang, M. J., Shin, M. S., Park, J. N., & Lee, S. S. (2005). The effects of polyunsaturated:saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats. *The British Journal of Nutrition*, *94*(4), 526–532. https://doi.org/10.1079/BJN20051523

Lucanic, M., Held, J. M., Vantipalli, M. C., Klang, I. M., Graham, J. B., Gibson, B. W., Lithgow, G. J., & Gill, M. S. (2011). N-acylethanolamine signaling mediates the effect of diet on lifespan in C. elegans. *Nature*, *473*(7346), 226. https://doi.org/10.1038/NATURE10007

May-Zhang, L. S., Chen, Z., Dosoky, N. S., Yancey, P. G., Boyd, K. L., Hasty, A. H., Linton, M. R. F., & Davies, S. S. (2019). Administration of N-Acyl-Phosphatidylethanolamine Expressing Bacteria to Low Density Lipoprotein Receptor−/− Mice Improves Indices of Cardiometabolic Disease. *Scientific Reports 2019 9:1*, *9*(1), 1–13. https://doi.org/10.1038/s41598-018-37373-1

Neff, M. R., Bhavsar, S. P., Braekevelt, E., & Arts, M. T. (2014). Effects of different cooking methods on fatty acid profiles in four freshwater fishes from the Laurentian Great Lakes region. *Food Chemistry*, *164*, 544–550. https://doi.org/10.1016/J.FOODCHEM.2014.04.104

Parolini, C. (2019). Effects of Fish n-3 PUFAs on Intestinal Microbiota and Immune System. *Marine Drugs*, *17*(6). https://doi.org/10.3390/MD17060374

Pongsetkul, J., Benjakul, S., Vongkamjan, K., Sumpavapol, P., & Osako, K. (2017). Changes in lipids of shrimp (Acetes vulgaris) during salting and fermentation.

536 *European Journal of Lipid Science and Technology*, *119*(11), 1700253.
537 https://doi.org/10.1002/EJLT.201700253

538 Schmid, H. H. O., Schmid, P. C., & Natarajan, V. (1990). N-Acylated glycerophospholipids
539 and their derivatives. *Progress in Lipid Research*, *29*(1), 1–43.
540 https://doi.org/10.1016/0163-7827(90)90004-5

541 Schuchardt, J. P., & Hahn, A. (2013). Bioavailability of long-chain omega-3 fatty acids.
542 *Prostaglandins, Leukotrienes, and Essential Fatty Acids*, *89*(1), 1–8.
543 https://doi.org/10.1016/J.PLEFA.2013.03.010

544 Senso, L., Suárez, M. D., Ruiz-Cara, T., & García-Gallego, M. (2007). On the possible
545 effects of harvesting season and chilled storage on the fatty acid profile of the fillet of
546 farmed gilthead sea bream (Sparus aurata). *Food Chemistry*, *101*(1), 298–307.
547 https://doi.org/10.1016/J.FOODCHEM.2006.01.036

548 Shirai, N., Terayama, M., & Takeda, H. (2002). Effect of season on the fatty acid
549 composition and free amino acid content of the sardine Sardinops melanostictus.
550 *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*,
551 *131*(3), 387–393. https://doi.org/10.1016/S1096-4959(01)00507-3

552 Siddabasave, S. G., Minami, Y., Gowda, D., Furuko, D., Chiba, H., & Hui, S. P. (2021).
553 Lipidomic analysis of non-esterified furan fatty acids and fatty acid compositions in
554 dietary shellfish and salmon by UHPLC/LTQ-Orbitrap-MS. *Food Research*
555 *International*. https://doi.org/10.1016/j.foodres.2021.110325

556 Solorzano, C., Zhu, C., Battista, N., Astarita, G., Lodola, A., Rivara, S., Mor, M., Russo, R.,
557 Maccarrone, M., Antonietti, F., Duranti, A., Tontini, A., Cuzzocrea, S., Tarzia, G., &
558 Piomelli, D. (2009). Selective N-acylethanolamine-hydrolyzing acid amidase inhibition
559 reveals a key role for endogenous palmitoylethanolamide in inflammation. *Proceedings*
560 *of the National Academy of Sciences of the United States of America*, *106*(49), 20966–

20971. https://doi.org/10.1073/PNAS.0907417106/SUPPL_FILE/0907417106SI.PDF

Ulbricht, T. L. V., & Southgate, D. A. T. (1991). Coronary heart disease: seven dietary factors. *Lancet (London, England)*, *338*(8773), 985–992. https://doi.org/10.1016/0140-6736(91)91846-M

Virtanen, J. K., Mozaffarian, D., Chiuve, S. E., & Rimm, E. B. (2008). Fish consumption and risk of major chronic disease in men. *The American Journal of Clinical Nutrition*, *88*(6), 1618. https://doi.org/10.3945/AJCN.2007.25816

Voronin, V. P., Nemova, N. N., Ruokolainen, T. R., Artemenkov, D. V., Rolskii, A. Y., Orlov, A. M., & Murzina, S. A. (2021). Into the Deep: New Data on the Lipid and Fatty Acid Profile of Redfish Sebastes mentella Inhabiting Different Depths in the Irminger Sea. *Biomolecules 2021, Vol. 11, Page 704*, *11*(5), 704. https://doi.org/10.3390/BIOM11050704

Wellner, N., Diep, T. A., Janfelt, C., & Hansen, H. S. (2013). N-acylation of phosphatidylethanolamine and its biological functions in mammals. *Biochimica et Biophysica Acta*, *1831*(3), 652–662. https://doi.org/10.1016/J.BBALIP.2012.08.019

Xu, H., Turchini, G. M., Francis, D. S., Liang, M., Mock, T. S., Rombenso, A., & Ai, Q. (2020). Are fish what they eat? A fatty acid's perspective. *Progress in Lipid Research*, *80*. https://doi.org/10.1016/J.PLIPRES.2020.101064

Yagi, S., Fukuda, D., Aihara, K. I., Akaike, M., Shimabukuro, M., & Sata, M. (2017). n-3 Polyunsaturated Fatty Acids: Promising Nutrients for Preventing Cardiovascular Disease. *Journal of Atherosclerosis and Thrombosis*, *24*(10), 999. https://doi.org/10.5551/JAT.RV17013

Yeo, J., & Parrish, C. C. (2021). Shotgun Lipidomics for the Determination of Phospholipid and Eicosanoid Profiles in Atlantic Salmon (Salmo salar L.) Muscle Tissue Using Electrospray Ionization (ESI)-MS/MS Spectrometric Analysis. *International Journal of*

586 *Molecular Sciences*, *22*(5), 1–14. https://doi.org/10.3390/IJMS22052272

587 Yurchenko, S., Sats, A., Tatar, V., Kaart, T., Mootse, H., & Jõudu, I. (2018). Fatty acid
588 profile of milk from Saanen and Swedish Landrace goats. *Food Chemistry*, *254*, 326–
589 332. https://doi.org/10.1016/J.FOODCHEM.2018.02.041

590 Zang, J., Xu, Y., Xia, W., Jiang, Q., Yang, F., & Wang, B. (2018). Phospholipid molecular
591 species composition of Chinese traditional low-salt fermented fish inoculated with
592 different starter cultures. *Food Research International*, *111*, 87–96.
593 https://doi.org/10.1016/J.FOODRES.2018.05.016

594 Zhang, X., Ning, X., He, X., Sun, X., Yu, X., Cheng, Y., Yu, R. Q., & Wu, Y. (2020). Fatty
595 acid composition analyses of commercially important fish species from the Pearl River
596 Estuary, China. *PloS One*, *15*(1). https://doi.org/10.1371/JOURNAL.PONE.0228276

597 Zhou, X., Zhou, D. Y., Lu, T., Liu, Z. Y., Zhao, Q., Liu, Y. X., Hu, X. P., Zhang, J. H., &
598 Shahidi, F. (2018). Characterization of lipids in three species of sea urchin. *Food*
599 *Chemistry*, *241*, 97–103. https://doi.org/10.1016/J.FOODCHEM.2017.08.076

600 **Table 1:** Amount (mg/100 g of fillet) of lipid characterized in eleven types of dietary fish. (A-

601 LPE: Alkyl lysophosphatidylethanolamine, AA-PE: Alkyl acyl phosphatidylethanolamine)

Lipids	Red seabream	Flatfish	Okhotsk atka mackerel	Japanese sardine	Blackin flounder	Lindberg skate	Skipjack tuna	Saffron cod	Shishamo smelt	Pacific cod	Japanese pond smelt
LPC	4.17±0.28	0.09±0.00	0.43±0.10	2.29±0.05	2.25±0.18	0.04±0.01	1.35±0.11	0.40±0.05	0.56±0.03	3.20±0.37	1.31±0.22
LPE	0.23±0.01	0.08±0.00	0.57±0.00	0.59±0.03	1.33±0.04	0.15±0.01	0.19±0.02	0.44±0.01	0.51±0.02	0.17±0.01	0.53±0.01
A-LPE	0.03±0.00	0.04±0.00	0.02±0.00	0.01±0.00	0.39±0.01	0.04±0.00	0.09±0.01	0.29±0.02	0.28±0.01	0.06±0.00	0.22±0.01
LNAPE	0.25±0.01	0.33±0.03	0.44±0.02	0.75±0.02	0.75±0.02	0.25±0.00	0.19±0.01	0.15±0.00	0.34±0.02	0.39±0.02	0.83±0.03
LPI	-	-	0.01±0.00	0.01±0.00	0.05±0.01	0.01±0.00	0.01±0.00	0.03±0.00	0.04±0.00	0.03±0.01	0.02±0.01
PC	42.3±2.39	25.05±0.57	20.44±2.29	25.46±1.84	21.36±1.52	53.24±1.95	16.93±2.33	4.06±0.33	23.47±0.97	4.18±0.22	197.01±10.49
AA-PC	10.12±0.13	8.53±0.20	8.52±1.26	8.68±0.55	5.98±0.47	17.74±3.7	11.59±2.02	1.53±0.07	6.69±0.61	2.46±0.12	10.84±2.23
PE	9.95±0.58	6.12±0.19	12.9±2.58	17.78±0.90	8.54±0.80	20.86±2.95	1.62±0.20	0.77±0.01	4.98±0.17	1.52±0.08	11.60±0.20
AA-PE	3.98±0.21	3.00±0.14	2.28±0.47	3.01±0.16	3.80±0.42	13.16±1.99	3.05±0.36	0.49±0.01	1.94±0.06	0.69±0.04	4.22±0.03
PS	1.81±0.07	1.85±0.07	1.44±0.04	1.95±0.05	1.69±0.05	2.23±0.05	1.33±0.14	0.88±0.01	1.72±0.04	0.27±0.01	1.51±0.04
PI	3.27±0.73	3.07±0.07	1.83±0.04	5.43±0.10	3.17±0.06	3.03±0.07	2.32±0.21	2.13±0.04	2.46±0.07	0.55±0.02	11.17±0.15
PG	0.04±0.00	0.02±0.00	0.01±0.01	0.14±0.00	0.08±0.00	0.04±0.00	0.07±0.01	0.08±0.00	0.32±0.01	0.01±0.00	0.20±0.01
CL	0.62±0.03	0.11±0.00	0.06±0.00	0.33±0.01	0.11±0.01	0.34±0.02	0.21±0.02	0.08±0.00	0.17±0.01	0.11±0.01	0.35±0.01
Cer	1.16±0.50	1.03±0.09	0.75±0.11	1.39±0.06	1.94±0.04	1.27±0.16	0.29±0.04	0.30±0.01	3.02±0.15	0.61±0.05	10.21±0.94
SM	14.72±2.06	16.56±1.06	5.28±1.54	14.88±1.92	21.31±0.57	12.00±1.56	6.05±0.88	1.53±0.25	9.79±0.36	0.75±0.07	299.00±38.36
CE	1.25±0.39	3.67±0.18	1.54±0.03	2.52±0.46	31.56±2.23	10.09±1.16	1.99±0.11	6.54±0.61	37.43±7.8	64.27±4.60	210.94±31.76
MAG	0.11±0.00	0.08±0.01	0.14±0.01	0.16±0.01	0.02±0.00	0.03±0.00	0.12±0.03	0.15±0.01	1.29±0.04	0.06±0.00	0.32±0.01
NAE	0.17±0.01	0.52±0.03	1.28±0.06	0.45±0.02	1.46±0.02	0.20±0.01	0.16±0.01	1.92±0.10	2.16±0.06	1.78±0.07	1.14±0.00
TAG	98.16±9.61	0.16±0.00	2.79±0.07	182.33±11.09	0.15±0.01	0.16±0.01	0.11±0.03	0.34±0.06	845.60±0.06	0.47±0.04	329.68±13.55
Total lipid (mg/100g)	192.34±17.03	70.33±2.63	60.82±8.65	268.16±17.28	105.96±6.45	134.89±13.64	47.68±6.55	22.11±1.58	942.77±133.26	81.56±5.74	1124.43±98.67

Figure legends:

Fig 1 Multivariant analysis of lipid metabolites annotated in fish fillet samples. **a**. One-way ANOVA analysis of 287 identified lipid molecular species (Tukey's HSD with $p<0.05$). **b**. Principal component analysis score plot and loadings plot of lipid metabolites from eleven fish types.

Fig 2 Distribution of lipid classes and fatty acid compositions. **a.** Percentage distribution of five major lipid classes in eleven fish types (GP: Glycerophospholipids, SP: Sphingolipids, GL: Glycerolipids, FA: Fatty acyls, St: Sterols). **b**. Fatty acid composition a based-on unsaturation in eleven fish types ($p<0.05$, are considered as statistically significant. The groups which are significant are shown here: **SFA:** a_1-[c_1,e_1,h_1,i_1,j_1,k_1] i.e. group a1 is significantly different with the groups shown in square bracket, b_1-[c_1,e_1,h_1,i_1,j_1,k_1], c_1-[d_1,f_1,g_1,h_1,i_1], d_1-[e_1,h_1,i_1,j_1,k_1], e_1[f_1,g_1,h_1,i_1], f_1-[h_1,i_1,j_1,k_1], g_1-[h_1,i_1,j_1,k_1], h_1-[i_1,j_1,k_1], i-[j,k]. **MUFA:** a_2-[e_2,h_2,i_2,j_2,k_2], b_2-[e_2,h_2,i_2,j_2,k_2], c_2-[d_2,f_2,g_2,h_2,I_2,j_2,k_2], d_2-[e_2,h_2,i_2,j_2,k_2], e_2[f_2,g_2,h_2,I_2,j_2], f_2-[h_2,i_2,j_2,k_2], g_2-[h_2,i_2,j_2,k_2], h_2-[i_2,j_2,k_2], i_2-[j_2,k_2], j_2-k_2. **PUFA:** a_3-[c_3,e_3,h_3,i_3,j_3,k_3], b_3-[c_3,e_3,h_3,i_3,j_3,k_3], c_3-[d_3,e_3,f_3,g_3,h_3,I_3,j_3], d_3-[e_3,h_3,i_3,j_3,k_3], e_3[f_3,g_3,h_3,I_3,k_3], f_3-[h_3,i_3,j_3,k_3], g_3-[h_3,i_3,j_3,k_3], h_3-[i_3,j_3,k_3], i_3-[j_3,k_3], j_3-k_3]. **c.** PUFA to MUFA ratio (P:S) in dietary fish [a_0-[c_0,e_0,g_0,h_0,i_0,j_0,k_0], b_0-[c_0,e_0,f_0,g_0,h_0,i_0,j_0], c_0-[d_0,e_0,f_0,g_0,h_0,I_0,j_0,k_0], d_0-[e_0,h_0,i_0,j_0,k_0], e_0-[f_0,g_0,h_0,I_0,k_0], f_0-[h_0,i_0,j_0,k_0], g_0-[h_0,i_0,j_0,k_0], h_0-[i_0,j_0,k_0], i_0-[j_0,k_0], j_0-k_0]. (Blackin founder

Fig 3 Heatmap visualization of free fatty acids and evaluation of nutritional indices of fish. **a.** Hierarchical cluster correlation analysis of free fatty acids, **b.** Index of atherogenicity [IA], **c.** Health promotion index [HPI], **d.** Hypo/Hyper-cholesterolemic ratio [HH], and e. Fish lipid quality [FLQ]. (Pacific cod (PC), saffron cod (SC), Lindberg skate (LS), blackin flounder (BF), Japanese sardine (JS), Japanese pond smelt (JP), red seabream (RB), flatfish (FF), shishamo smelt (SS), skipjack tuna (ST), and Okhotsk Atka mackerel (OM).

Fig 4 a. Extracted ion chromatograms of LNAPEs **b**. Hierarchical cluster correlation analysis of lyso-phospholipids detected in fish fillet samples.

628 **Fig 5** The MS and MS/MS spectra of LNAPEs detected in various fish types [*isomeric
629 mixture].

630 **Fig 6** Hierarchical cluster correlation analysis of glycerophospholipids. **a.** PC and PS. **b.** PI,
631 PG, and CL **c.** sphingolipids, N-acyl ethanol amines, cholesterol esters, mono-and di-
632 acylglycerols. **d.** Triacylglycerols characterized in eleven fish types. (clustering method: ward,
633 distance measure: Euclidean)

Figures

Fig 1

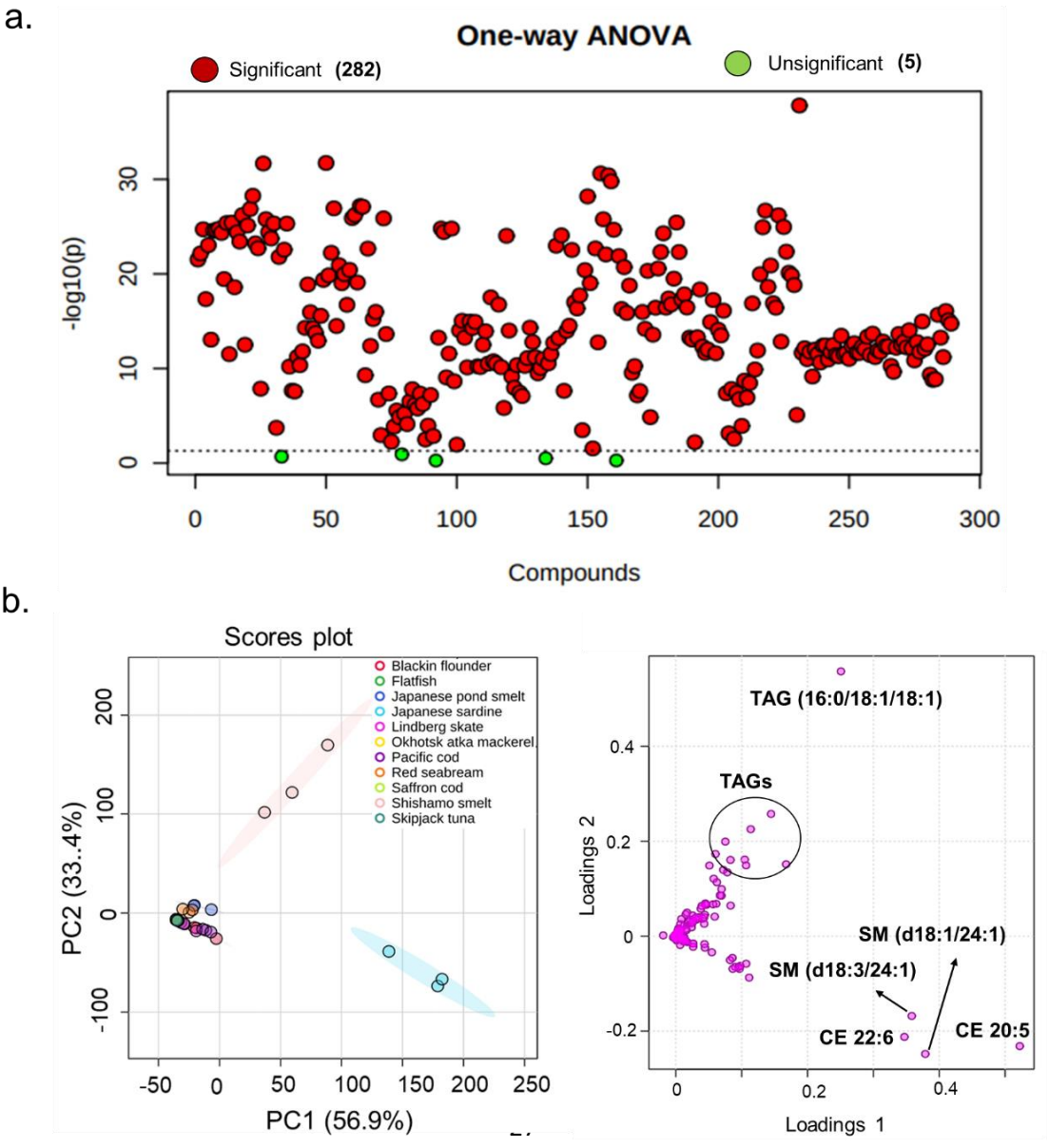


Fig 2

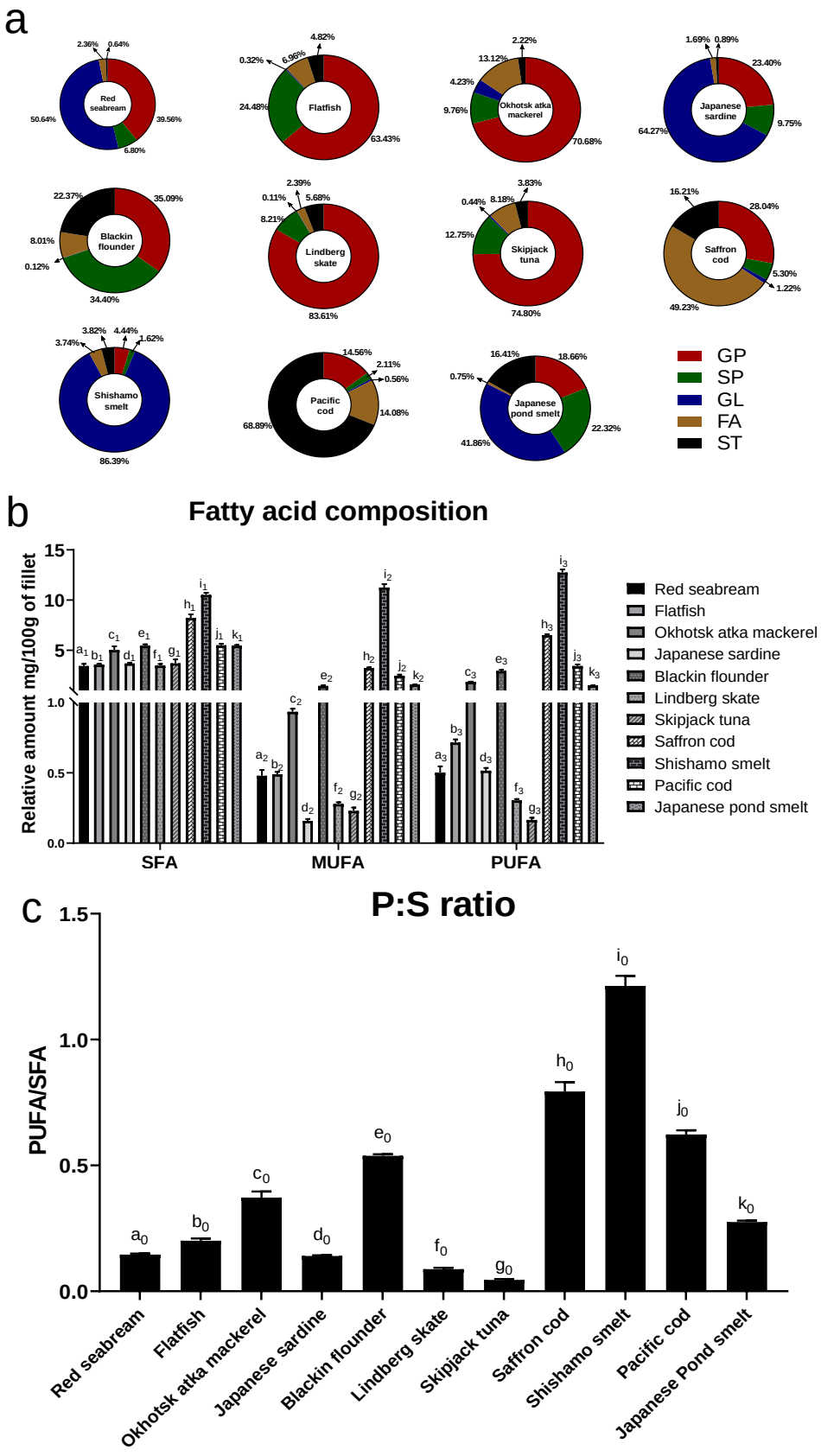

a
Red seabream
2.36%
0.64%
50.64%
39.56%
6.80%
Flatfish
4.82%
0.32%
6.96%
24.48%
63.43%
Okhotsk atka mackerel
2.22%
13.12%
4.23%
9.76%
70.68%
Japanese sardine
1.69%
0.89%
23.40%
64.27%
9.75%
Blackin flounder
22.37%
35.09%
8.01%
0.12%
34.40%
Lindberg skate
2.39%
0.11%
5.68%
8.21%
83.61%
Skipjack tuna
3.83%
0.44%
8.18%
12.75%
74.80%
Saffron cod
16.21%
28.04%
5.30%
1.22%
49.23%
Shishamo smelt
3.82%
4.44%
3.74%
1.62%
86.39%
Pacific cod
14.56%
2.11%
0.56%
14.08%
68.89%
Japanese pond smelt
16.41%
18.66%
0.75%
22.32%
41.86%
GP
SP
GL
FA
ST
b
Fatty acid composition
Relative amount mg/100g of fillet
15
10
5
1.0
0.5
0.0
SFA
MUFA
PUFA
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japanese pond smelt
c
P:S ratio
PUFA/SFA
1.5
1.0
0.5
0.0
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japanese Pond smelt

Fig 3

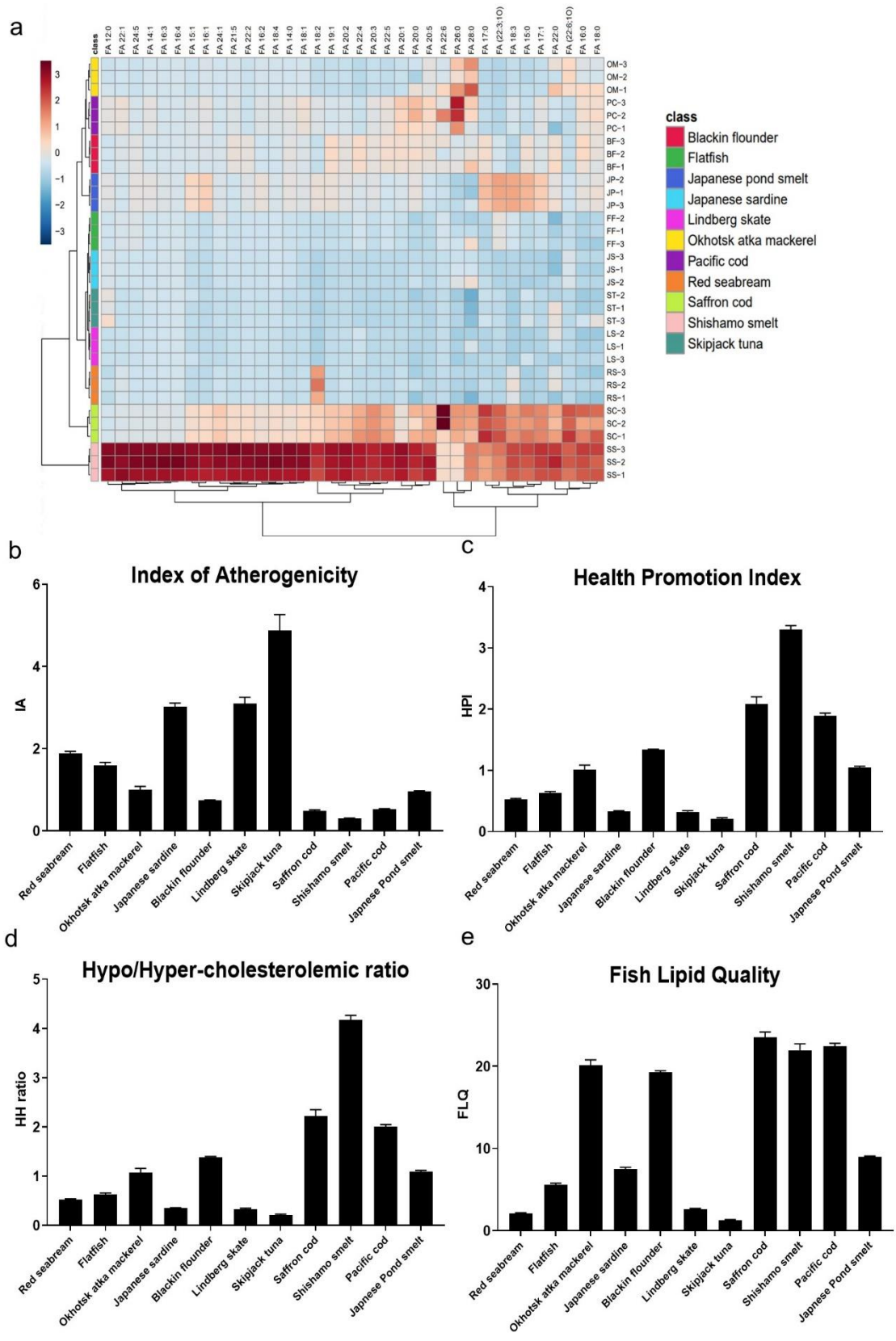

a
class
FA 12:0
FA 22:1
FA 24:5
FA 14:1
FA 16:3
FA 16:4
FA 15:1
FA 16:1
FA 24:1
FA 21:5
FA 22:2
FA 16:2
FA 18:4
FA 14:0
FA 18:1
FA 18:2
FA 19:1
FA 20:2
FA 22:4
FA 20:3
FA 22:5
FA 20:1
FA 20:0
FA 20:5
FA 22:6
FA 26:0
FA 28:0
FA 17:0
FA (22:3;1O)
FA 18:3
FA 15:0
FA 17:1
FA 22:0
FA (22:6;1O)
FA 16:0
FA 18:0
OM−3
OM−2
OM−1
PC−3
PC−2
PC−1
BF−3
BF−2
BF−1
JP−2
JP−1
JP−3
FF−2
FF−1
FF−3
JS−3
JS−1
JS−2
ST−2
ST−1
ST−3
LS−2
LS−1
LS−3
RS−3
RS−2
RS−1
SC−3
SC−2
SC−1
SS−3
SS−2
SS−1
3
2
1
0
−1
−2
−3
class
Blackin flounder
Flatfish
Japanese pond smelt
Japanese sardine
Lindberg skate
Okhotsk atka mackerel
Pacific cod
Red seabream
Saffron cod
Shishamo smelt
Skipjack tuna
b
Index of Atherogenicity
IA
6
4
2
0
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japnese Pond smelt
c
Health Promotion Index
HPI
4
3
2
1
0
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japnese Pond smelt
d
Hypo/Hyper-cholesterolemic ratio
HH ratio
5
4
3
2
1
0
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japnese Pond smelt
e
Fish Lipid Quality
FLQ
30
20
10
0
Red seabream
Flatfish
Okhotsk atka mackerel
Japanese sardine
Blackin flounder
Lindberg skate
Skipjack tuna
Saffron cod
Shishamo smelt
Pacific cod
Japnese Pond smelt

Fig 4

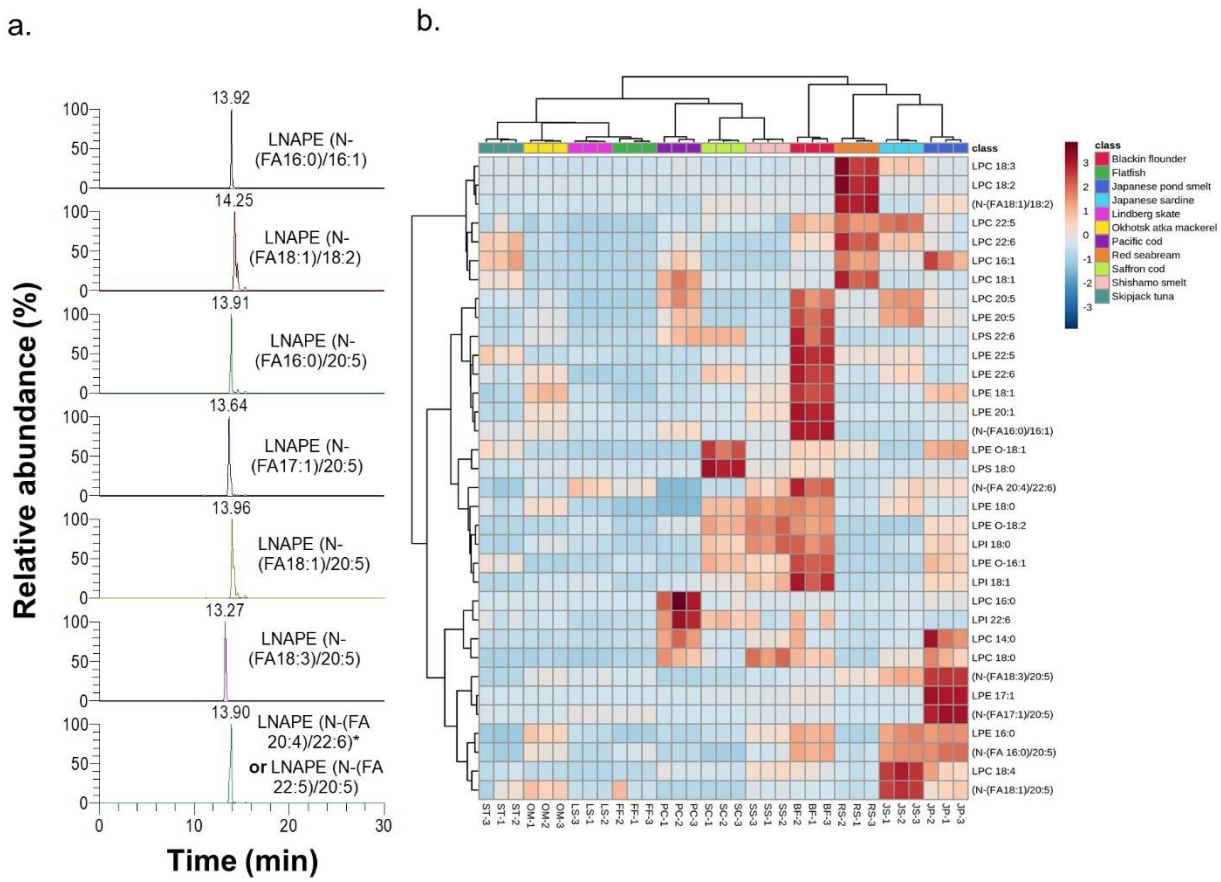

a.
b.
Relative abundance (%)
Time (min)
13.92
LNAPE (N-(FA16:0)/16:1)
14.25
LNAPE (N-(FA18:1)/18:2)
13.91
LNAPE (N-(FA16:0)/20:5)
13.64
LNAPE (N-(FA17:1)/20:5)
13.96
LNAPE (N-(FA18:1)/20:5)
13.27
LNAPE (N-(FA18:3)/20:5)
13.90
LNAPE (N-(FA 20:4)/22:6)*
or LNAPE (N-(FA 22:5)/20:5)
class
Blackin flounder
Flatfish
Japanese pond smelt
Japanese sardine
Lindberg skate
Okhotsk atka mackerel
Pacific cod
Red seabream
Saffron cod
Shishamo smelt
Skipjack tuna
LPC 18:3
LPC 18:2
(N-(FA18:1)/18:2)
LPC 22:5
LPC 22:6
LPC 16:1
LPC 18:1
LPC 20:5
LPE 20:5
LPS 22:6
LPE 22:5
LPE 22:6
LPE 18:1
LPE 20:1
(N-(FA16:0)/16:1)
LPE O-18:1
LPS 18:0
(N-(FA 20:4)/22:6)
LPE 18:0
LPE O-18:2
LPI 18:0
LPE O-16:1
LPI 18:1
LPC 16:0
LPI 22:6
LPC 14:0
LPC 18:0
(N-(FA18:3)/20:5)
LPE 17:1
(N-(FA17:1)/20:5)
LPE 16:0
(N-(FA 16:0)/20:5)
LPC 18:4
(N-(FA18:1)/20:5)

Fig 5

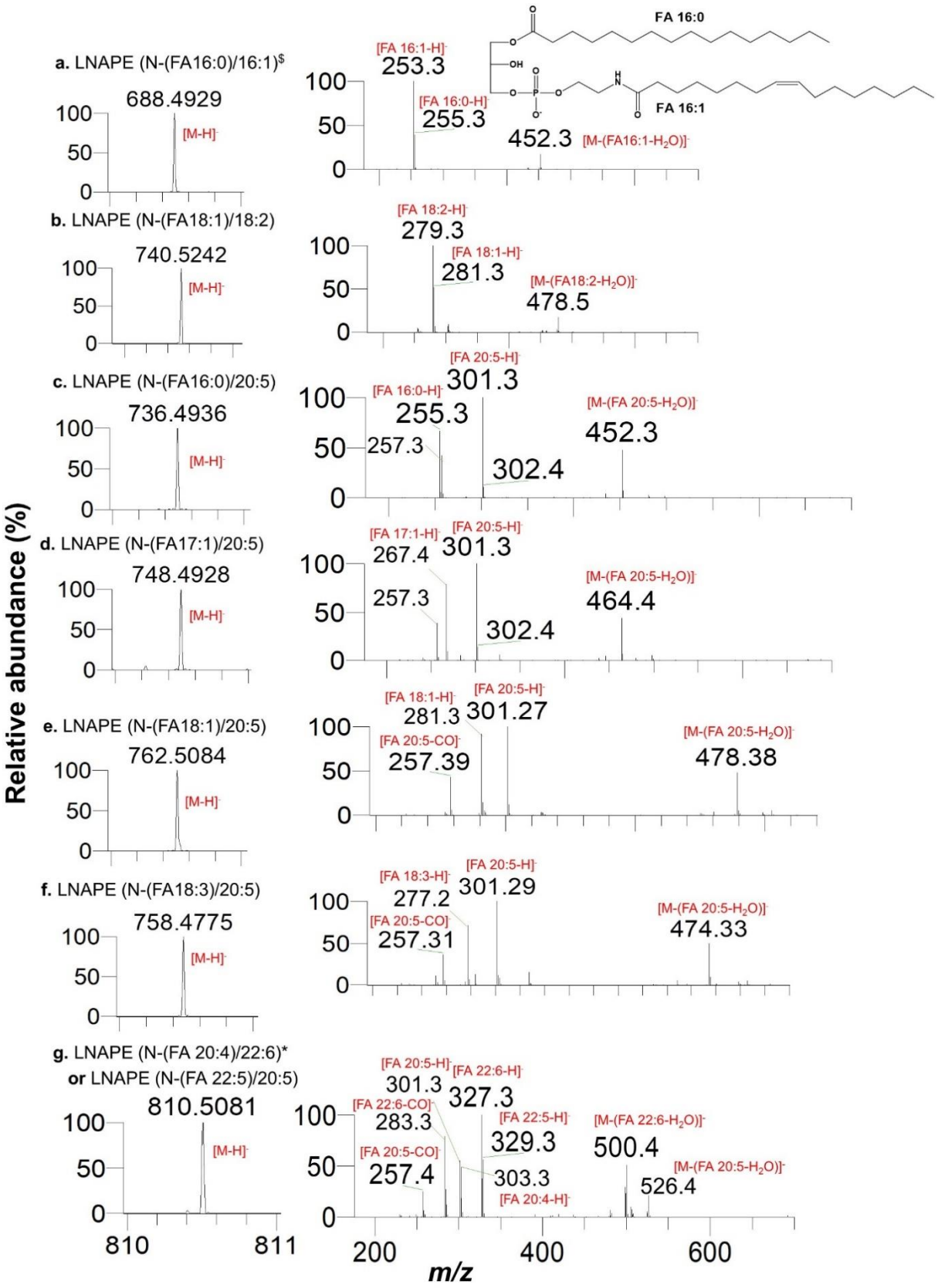
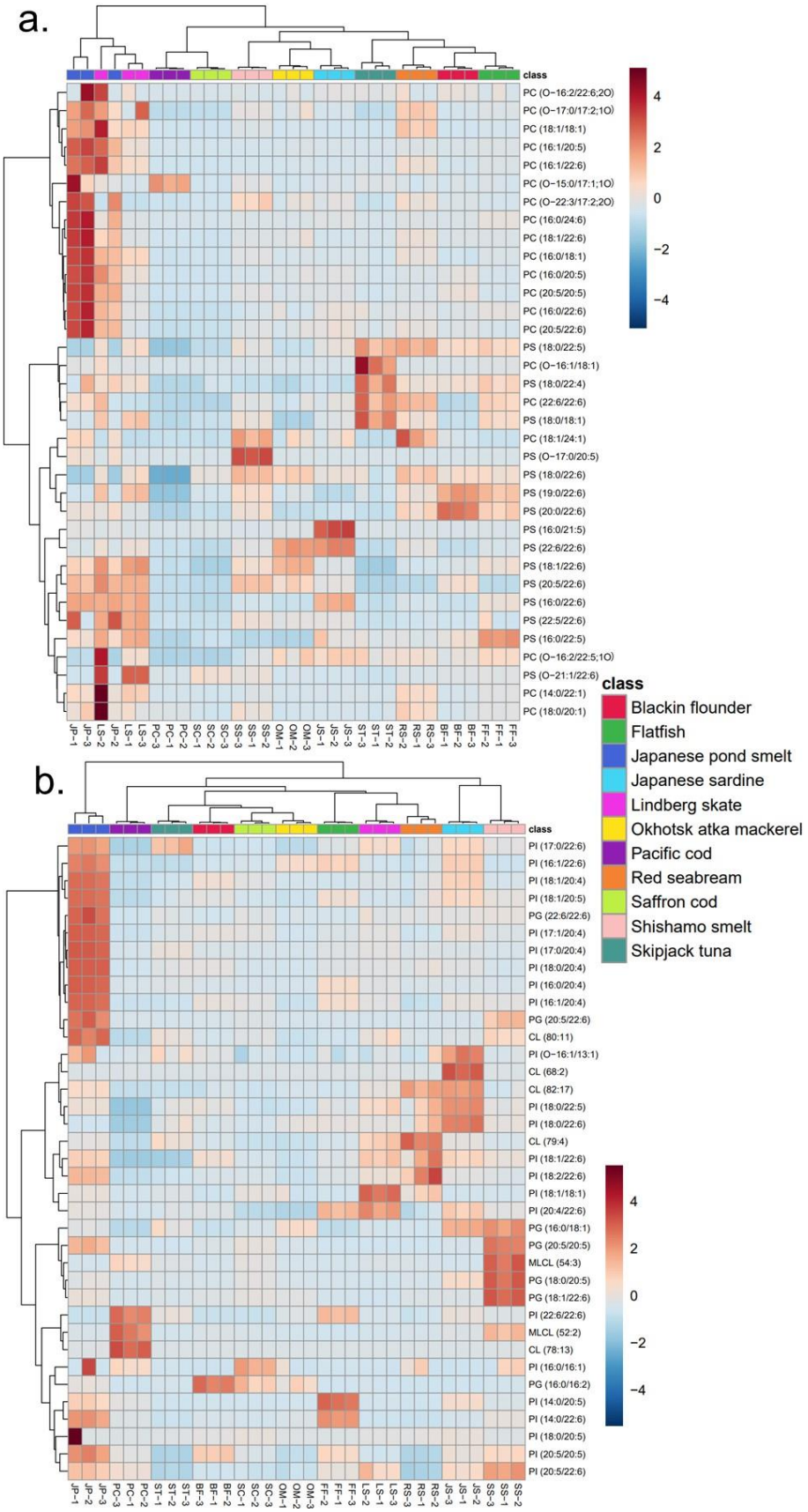
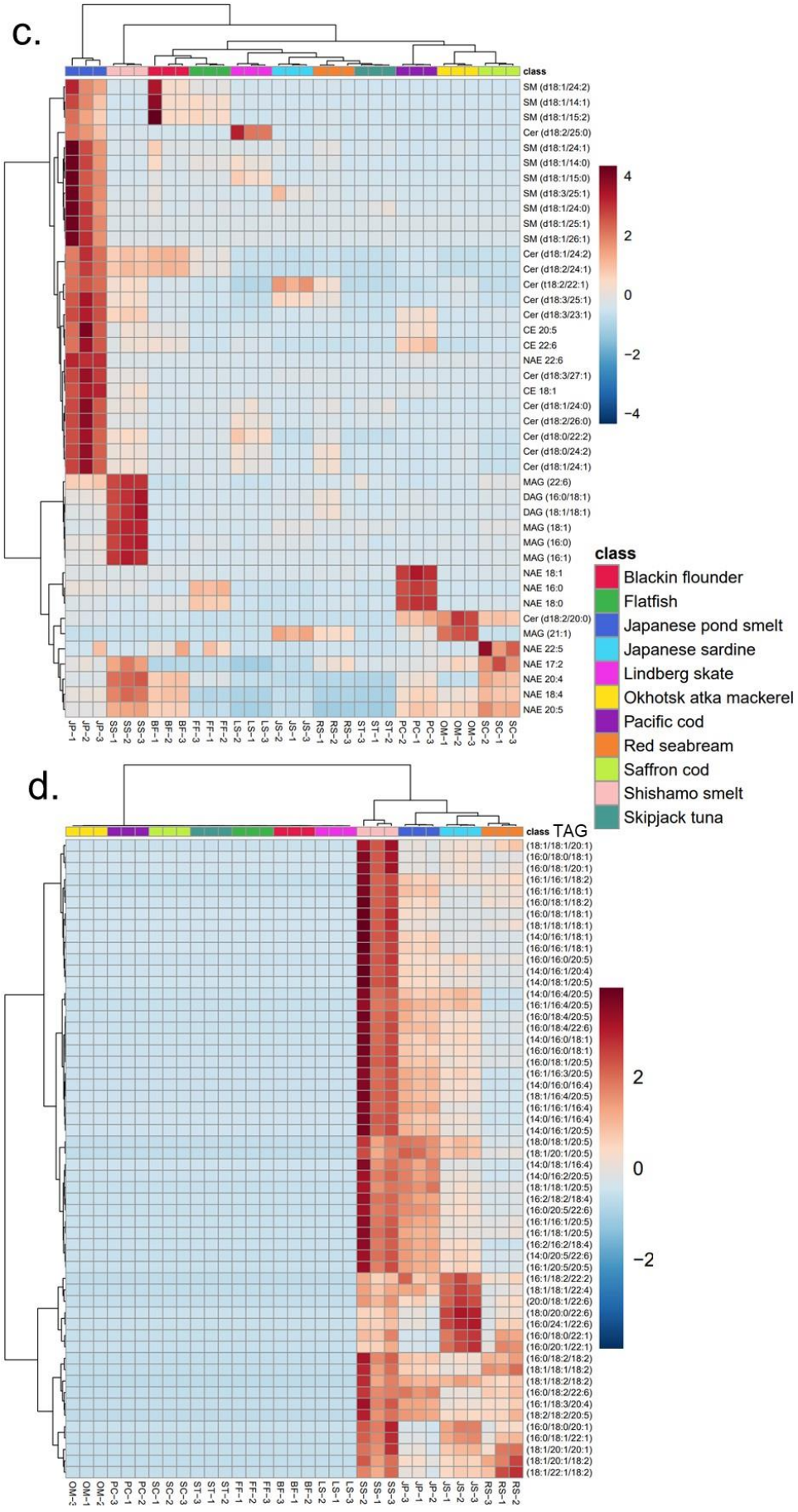

a. LNAPE (N-(FA16:0)/16:1)$
688.4929
[M-H]-
[FA 16:1-H]-
253.3
[FA 16:0-H]-
255.3
452.3
[M-(FA16:1-H2O)]-
FA 16:0
FA 16:1
b. LNAPE (N-(FA18:1)/18:2)
740.5242
[M-H]-
[FA 18:2-H]-
279.3
[FA 18:1-H]-
281.3
[M-(FA18:2-H2O)]-
478.5
c. LNAPE (N-(FA16:0)/20:5)
736.4936
[M-H]-
[FA 20:5-H]-
301.3
[FA 16:0-H]-
255.3
257.3
302.4
[M-(FA 20:5-H2O)]-
452.3
d. LNAPE (N-(FA17:1)/20:5)
748.4928
[M-H]-
[FA 17:1-H]-
267.4
[FA 20:5-H]-
301.3
257.3
302.4
[M-(FA 20:5-H2O)]-
464.4
e. LNAPE (N-(FA18:1)/20:5)
762.5084
[M-H]-
[FA 18:1-H]-
281.3
[FA 20:5-H]-
301.27
[FA 20:5-CO]-
257.39
[M-(FA 20:5-H2O)]-
478.38
f. LNAPE (N-(FA18:3)/20:5)
758.4775
[M-H]-
[FA 18:3-H]-
277.2
[FA 20:5-H]-
301.29
[FA 20:5-CO]-
257.31
[M-(FA 20:5-H2O)]-
474.33
g. LNAPE (N-(FA 20:4)/22:6)*
or LNAPE (N-(FA 22:5)/20:5)
810.5081
[M-H]-
[FA 20:5-H]-
301.3
[FA 22:6-H]-
327.3
[FA 22:6-CO]-
283.3
[FA 22:5-H]-
329.3
[M-(FA 22:6-H2O)]-
500.4
[FA 20:5-CO]-
257.4
303.3
[FA 20:4-H]-
[M-(FA 20:5-H2O)]-
526.4
Relative abundance (%)
m/z

Fig 6


a.
class
PC (O−16:2/22:6;2O)
PC (O−17:0/17:2;1O)
PC (18:1/18:1)
PC (16:1/20:5)
PC (16:1/22:6)
PC (O−15:0/17:1;1O)
PC (O−22:3/17:2;2O)
PC (16:0/24:6)
PC (18:1/22:6)
PC (16:0/18:1)
PC (16:0/20:5)
PC (20:5/20:5)
PC (16:0/22:6)
PC (20:5/22:6)
PS (18:0/22:5)
PC (O−16:1/18:1)
PS (18:0/22:4)
PC (22:6/22:6)
PS (18:0/18:1)
PC (18:1/24:1)
PS (O−17:0/20:5)
PS (18:0/22:6)
PS (19:0/22:6)
PS (20:0/22:6)
PS (16:0/21:5)
PS (22:6/22:6)
PS (18:1/22:6)
PS (20:5/22:6)
PS (16:0/22:6)
PS (22:5/22:6)
PS (16:0/22:5)
PC (O−16:2/22:5;1O)
PS (O−21:1/22:6)
PC (14:0/22:1)
PC (18:0/20:1)
JP−1 JP−3 LS−2 JP−2 LS−1 LS−3 PC−3 PC−1 PC−2 SC−1 SC−2 SC−3 SS−3 SS−1 SS−2 OM−1 OM−2 OM−3 JS−1 JS−2 JS−3 ST−3 ST−1 ST−2 RS−2 RS−1 RS−3 BF−1 BF−2 BF−3 FF−2 FF−1 FF−3
4
2
0
−2
−4
b.
class
PI (17:0/22:6)
PI (16:1/22:6)
PI (18:1/20:4)
PI (18:1/20:5)
PG (22:6/22:6)
PI (17:1/20:4)
PI (17:0/20:4)
PI (18:0/20:4)
PI (16:0/20:4)
PI (16:1/20:4)
PG (20:5/22:6)
CL (80:11)
PI (O−16:1/13:1)
CL (68:2)
CL (82:17)
PI (18:0/22:5)
PI (18:0/22:6)
CL (79:4)
PI (18:1/22:6)
PI (18:2/22:6)
PI (18:1/18:1)
PI (20:4/22:6)
PG (16:0/18:1)
PG (20:5/20:5)
MLCL (54:3)
PG (18:0/20:5)
PG (18:1/22:6)
PI (22:6/22:6)
MLCL (52:2)
CL (78:13)
PI (16:0/16:1)
PG (16:0/16:2)
PI (14:0/20:5)
PI (14:0/22:6)
PI (18:0/20:5)
PI (20:5/20:5)
PI (20:5/22:6)
JP−1 JP−2 JP−3 PC−3 PC−1 PC−2 ST−1 ST−2 ST−3 BF−3 BF−1 BF−2 SC−1 SC−2 SC−3 OM−1 OM−2 OM−3 FF−2 FF−1 FF−3 LS−2 LS−1 LS−3 RS−3 RS−1 RS−2 JS−3 JS−1 JS−2 SS−3 SS−1 SS−2
4
2
0
−2
−4
class
Blackin flounder
Flatfish
Japanese pond smelt
Japanese sardine
Lindberg skate
Okhotsk atka mackerel
Pacific cod
Red seabream
Saffron cod
Shishamo smelt
Skipjack tuna


c.
class
SM (d18:1/24:2)
SM (d18:1/14:1)
SM (d18:1/15:2)
Cer (d18:2/25:0)
SM (d18:1/24:1)
SM (d18:1/14:0)
SM (d18:1/15:0)
SM (d18:3/25:1)
SM (d18:1/24:0)
SM (d18:1/25:1)
SM (d18:1/26:1)
Cer (d18:1/24:2)
Cer (d18:2/24:1)
Cer (t18:2/22:1)
Cer (d18:3/25:1)
Cer (d18:3/23:1)
CE 20:5
CE 22:6
NAE 22:6
Cer (d18:3/27:1)
CE 18:1
Cer (d18:1/24:0)
Cer (d18:2/26:0)
Cer (d18:0/22:2)
Cer (d18:0/24:2)
Cer (d18:1/24:1)
MAG (22:6)
DAG (16:0/18:1)
DAG (18:1/18:1)
MAG (18:1)
MAG (16:0)
MAG (16:1)
NAE 18:1
NAE 16:0
NAE 18:0
Cer (d18:2/20:0)
MAG (21:1)
NAE 22:5
NAE 17:2
NAE 20:4
NAE 18:4
NAE 20:5
4
2
0
−2
−4
JP−1 JP−2 JP−3 SS−1 SS−2 SS−3 BF−1 BF−2 BF−3 FF−1 FF−2 LS−1 LS−3 JS−2 JS−1 JS−3 RS−1 RS−2 RS−3 ST−3 ST−1 ST−2 PC−2 PC−1 PC−3 OM−1 OM−2 OM−3 SC−2 SC−1 SC−3
class
Blackin flounder
Flatfish
Japanese pond smelt
Japanese sardine
Lindberg skate
Okhotsk atka mackerel
Pacific cod
Red seabream
Saffron cod
Shishamo smelt
Skipjack tuna
d.
class
TAG
(18:1/18:1/20:1)
(16:0/18:0/18:1)
(16:0/18:1/20:1)
(16:1/16:1/18:2)
(16:1/16:1/18:1)
(16:0/18:1/18:2)
(16:0/18:1/18:1)
(18:1/18:1/18:1)
(14:0/16:1/18:1)
(16:0/16:1/18:1)
(16:0/16:0/20:5)
(14:0/16:1/20:4)
(14:0/18:1/20:5)
(14:0/16:4/20:5)
(16:1/16:4/20:5)
(16:0/18:4/20:5)
(16:0/18:4/22:6)
(14:0/16:0/18:1)
(16:0/16:0/18:1)
(16:0/18:1/20:5)
(16:1/16:3/20:5)
(14:0/16:0/16:4)
(18:1/16:4/20:5)
(16:1/16:1/16:4)
(14:0/16:1/16:4)
(14:0/16:1/20:5)
(18:0/18:1/20:5)
(18:1/20:1/20:5)
(14:0/18:1/16:4)
(14:0/16:2/20:5)
(18:1/18:1/20:5)
(16:2/18:2/18:4)
(16:0/20:5/22:6)
(16:1/16:1/20:5)
(16:1/18:1/20:5)
(16:2/16:2/18:4)
(14:0/20:5/22:6)
(16:1/20:5/20:5)
(16:1/18:2/22:2)
(18:1/18:1/22:4)
(20:0/18:1/22:6)
(18:0/20:0/22:6)
(16:0/24:1/22:6)
(16:0/18:0/22:1)
(16:0/20:1/22:1)
(16:0/18:2/18:2)
(18:1/18:1/18:2)
(18:1/18:2/18:2)
(16:0/18:2/22:6)
(16:1/18:3/20:4)
(18:2/18:2/20:5)
(16:0/18:0/20:1)
(16:0/18:1/22:1)
(18:1/20:1/20:1)
(18:1/20:1/18:2)
(18:1/22:1/18:2)
2
0
−2
OM−3 OM−1 OM−2 PC−3 PC−1 PC−2 SC−1 SC−2 SC−3 ST−3 ST−1 ST−2 FF−1 FF−2 FF−3 BF−3 BF−1 BF−2 LS−2 LS−1 LS−3 SS−2 SS−1 SS−3 JP−3 JP−1 JP−2 JS−1 JS−2 JS−3 RS−3 RS−1 RS−2

Graphical Abstract

634