

CHANGES IN LIPID CONTENT WITH ROASTING TEMPERATURE OF LARGE YELLOW CROAKER (*LARIMICHTHYS CROCEA*) ROE

L. ZHANG¹, M. ZHANG¹, X. YANG^{*2}, L. CHEN^{1,3,4}, W. CHENG^{1,3,4} and P. LIANG^{*1,3,4}

¹College of Food Science, Fujian Agriculture and Forestry University, Fuzhou 350002, PR China

²Key Laboratory of Aquatic Product Processing, Ministry of Agriculture and Rural Affairs; National R&D Center for Aquatic Product Processing; South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, PR China

³Engineering Research Centre of Fujian-Taiwan Special Marine Food Processing and Nutrition Ministry of Education, Fuzhou 350002, PR China

⁴Key Laboratory of Marine Biotechnology of Fujian Province, Fuzhou 350002, PR China

*Corresponding author: yxqgd@163.com and liangpeng137@sina.com

ABSTRACT

This study aims to clarify the changes with roasting temperature in the lipid content of ready-to-eat large yellow croaker (*Larimichthys crocea*) roe product. Almost all the lipid class/species showed the same trend with the increasing temperature. Except for some minor differences, the relative amounts of lipids decreased with temperature increase from 0°C (control group, raw roe) to 100°C; increased with further temperature increase to 120°C, at which the amount was maximum; and then decreased with further temperature increase to 180°C. Finally, 120°C was selected as the optimal processing temperature, which may result in a better appearance and a high lipid quality, indicating its potential application value. This study also enhances the understanding of lipid profile in fish roe and demonstrates the applicability of the lipidomic method in aquatic food production.

Keywords: *Larimichthys crocea* roe, phospholipid molecular species, lipid content, roasting temperature

1. INTRODUCTION

Fish roe is known for its high nutritional value. Its products have been consumed as caviar (from sturgeon) and caviar substitutes (from other species); other product forms include whole skeins and formulations with oils and cheese bases, in salted or smoked forms, and the international and domestic markets of these products continue to increase (BLEDSOE *et al.*, 2003). In recent years, the valorization of roe has received much attention because of its gigantic yield and health benefits. Some reports have shown that fish roe is available at lower prices. It can be incorporated in various food preparations to combat protein malnutrition and is an interesting source for supplementing the human diet with marine lipids (MAHMOUD *et al.*, 2008; BALASWAMY *et al.*, 2009; SALIU *et al.*, 2019). In addition, the valorization of roe by encouraging professional exploitation (for fillets, caviar, or nutritional supplement production) can also lower the pressure on the aquatic ecosystem (SALIU *et al.*, 2017). The large yellow croaker (*Larimichthys crocea*) is a major commercial marine fish in the south of China, with a total production of approximately 180,000 tons in 2017 (CHEN *et al.*, 2018). However, its roe is usually large and has an unattractive appearance. During the processing of *L. crocea*, its roe is a major by-product, which is usually discarded as waste. This is an important commercial loss and an environmental problem from the fish industry. The *L. crocea* roe has been verified to contain large amounts of n-3 polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic acids (EPA, C20:5 n-3) and docosahexaenoic acids (DHA, C22:6 n-3) as known, which can help prevent the incidence of coronary heart diseases, inflammatory and autoimmune disorders, and cancers (WANG *et al.*, 2008; ROSA *et al.*, 2012; OZOGUL *et al.*, 2007). The predominant phospholipids (PLs) in *L. crocea* roe are phosphatidylcholine (PC) and phosphatidylethanolamine (PE), as determined in our previous study (LIANG *et al.*, 2017a), which corresponds with other reports on fish roe (HAYASHI *et al.*, 1999; SHIRAI *et al.*, 2006).

Phospholipids are major polar lipid components, and they serve as building blocks for cell membranes and have important physiological and biological functions in almost all known living beings (BURRI *et al.*, 2012; SUZUMURA, 2005). The diversity of PL molecule species can be ascribed to the number of carbons and double bonds in the fatty acid moiety and the moiety locations on the glycerol backbone with one headgroup, which are the sn-1 and/or sn-2 position and sn-3 position of PLs. Marine PLs have also been confirmed capable of reducing inflammatory reactions (DEUTSCH, 2007) and preventing colon cancer growth induced by chemicals *in vitro* (HOSSAIN *et al.*, 2009). Moreover, our previous study identified that the PLs with docosahexaenoic acid (DHA) from the *L. crocea* roe had beneficial effects on the lipid metabolism of hyperlipidemic mice (LIANG *et al.*, 2017b).

The changes in the PLs and other lipid components can be affected by the storage time and freezing/cooking temperatures, which causes autoxidation, hydrolytic decomposition, lipid browning, lipid-protein copolymerization reactions, and lipolysis or enzymatic degradation in the food products (IGENE *et al.*, 1981; LEE *et al.*, 1976; WANG *et al.*, 2011). Additionally, the PL loss can be due to different mechanisms of heat transfer, which cause cell rupture (MONDY *et al.*, 1977). Furthermore, the PL quantity can affect the flavor and nutritional quality in the food matrix (DE LIMA *et al.*, 2008). However, the changes in the total lipid content and PL molecular species with high temperatures are still not clear for the *L. crocea* roe.

Recently, the shotgun lipidomic approach was developed to replace the traditional methods (i.e., thin-layer chromatography) for monitoring the molecular compositions and

abundances of individual lipid species from unfractionated lipid extracts more rapidly and with higher sensitivity (WANG *et al.*, 2011). For the comprehensive analysis of lipid structures, the developed technology of reversed-phase ultra-performance liquid chromatography (UPLC) coupled with electrospray ionization–quadrupole–time-of-flight–mass spectrometry (UPLC-ESI-Q-TOF-MS) possesses superior separation ability, higher resolution, greater sensitivity, and faster speed (WANG *et al.*, 2011; BASCONCILLO *et al.*, 2009; LAAKSONEN *et al.*, 2006; YAN *et al.*, 2010; ZHAO *et al.*, 2014). Herein, we assumed that the changes in PLs and other lipid components can be confirmed via shotgun lipidomics.

This study aimed to identify the relative changes in lipid content, especially the PL molecular species, with the roasting temperature in the ready-to-eat *L. crocea* roe product and determine the appropriate roasting temperature that can keep the best lipid compositions of the roe. The study clarifies the value of the *L. crocea* roe products and broadens the comprehensive utilization of the roe. Moreover, the application of lipidomics on the roasting processing of aquatic foods is shown to be beneficial for evaluating the changes in lipid compositions. This study can also promote the lipidomics method application in aquatic food production.

2. MATERIALS AND METHODS

2.1. Materials and reagents

The *L. crocea* roe was provided by Fujian Yuehai Aquatic Food Ltd. (Ningde City, Fujian Province). The roe was mixed and kept under refrigeration (0–4°C) for less than 24 h before analysis in the lab of Aquatic Food Products Processing at Fujian Agriculture and Forestry University.

In total, 10 lipid standards PC (17:0), lysophosphatidylcholine (LPC; 15:0/0:0), phosphatidylglycerol (PG; 15:0/15:0), PC (15:0/15:0), PE (15:0/15:0), sphingomyelin (SM; d18:1/17:0), phosphatidylserine (PS; 17:0/17:0), ceramides (Cer; d18:1/17:0), diacylglycerol (DG; 17:0/0:0/17:0), and triglyceride (TG; 15:0/15:0/15:0) were purchased from Avanti Polar Lipids (Alabaster, Alabama, US). High-performance liquid chromatography (HPLC)-grade isopropanol (IPA) and methanol were purchased from Merck (Darmstadt, Germany). Other HPLC-grade compounds, acetonitrile (ACN), formic acid, ammonium formate, leucine-enkephalin, and sodium formate, were purchased from Thermo Fisher Scientific (Shanghai, China).

2.2. Sample preparation

The fresh *L. crocea* roe was first powderized. This was achieved by drying using a vacuum freeze-dryer (True Ten Industrial Co., Ltd. Taichung, Taiwan) and filtering through an 80-mesh sieve. Then, a certain amount of water (1:2 w/w) was added into the *L. crocea* roe powder. The mixture was evenly stirred and moved onto a plate. A specific shape (1×4×4 cm) of the mixture was cut after extrusion and molding. Afterward, the mixture was moved into a commercial microwave oven (Newsail NS-X4, Henan, China). The roasting temperatures were 100, 120, 140, 160, and 180°C, and the roasting time was 20 min. Finally, the finished ready-to-eat product was prepared. After the product was cooled, it was vacuum-packaged and stored in a freezer (-20°C).

The finished product was added to 1.4 mL of IPA in a 2 mL centrifuge tube, vortex-mixed for 1 min, and sonicated for 10 min. The samples were kept in a freezer (-20°C) for 1 h and then freeze-centrifuged at 14,000 g for 10 min. The supernatant was collected, and 1 mL was filtered into UPLC vials through a 0.22 µm organic filter membrane. The samples were kept in a freezer (-20°C) for later analysis.

2.3. PL molecular species analysis via UPLC-Q-TOF-MS

2.3.1 UPLC parameters

The UPLC system was equipped with a C₁₈CSH column (1 × 50 mm, 1.7 µm; Waters Ltd., Elstree, U.K.). The mass spectrometry (MS) method of the Xevo G2-S Q-TOF (Waters Ltd., Manchester, U.K.) was implemented to improve the isotopic distribution and mass accuracy and to reduce the high ion intensities. In total, 2 µL of the samples was injected onto a C₁₈CSH column at 55°C. The mobile-phase flow rate was set as 400 µL/min. The mobile phases were as follows: (A) ACN/H₂O (60%/40%), including 10 mM ammonium formate and 0.1% formic acid; (B) IPA/ACN (90%/10%), including 10 mM ammonium formate and 0.1% formic acid. The gradient profile was as presented in Table 1.

Table 1. The gradient profile of UPLC.

Time (min)	% A	% B	Curve
Initial	60.0	40.0	Initial
2.0	57.0	43.0	6
2.1	50.0	50.0	1
12.0	46.0	54.0	6
12.1	30.0	70.0	1
18.0	1.0	99.0	6
18.1	60.0	40.0	6
20.0	60.0	40.0	6

Note: “6” means linear increasing, “1” means rapid increasing

2.3.2 Q-TOF-MS parameters

For both positive-ion and negative-ion modes, the MS parameters were as follows: capillary voltage of 3 kV, cone voltage of 25 V, ESI source temperature of 120°C, desolvation temperature of 500°C, desolvation gas flow of 800 L/h, and cone gas flow of 50 L/h. The mass spectra were acquired over m/z 50 to 2000. Leucine enkephalin (m/z 556.2771 in ESI⁺, m/z 554.2615 in ESI⁻) was continuously infused at 30 µL/min and used as the lock mass.

2.4. Statistical analysis

All analyses were conducted in triplicate, and the results were indicated as mean \pm standard deviation. The means and standard deviations were calculated using the SPSS statistical software (version 19.0, SPSS). The software was used to perform a one-way analysis of variance and Tukey's honest significant difference test at a 95% confidence level ($P < 0.05$) to identify differences among groups. A statistical *t*-test model was applied for comparative analysis involving different groups.

MassLynx software version 4.1 was used for the MS data acquisition and analysis. All lipid profile data were first standardized and normalized and then subjected to principal component analysis using SIMCA-P 13.0. Heatmaps of lipids data were created using the R software.

3. RESULTS AND DISCUSSION

3.1. Effect of roasting temperature on *L. crocea* roe appearance

Consumers usually assess the quality of a food product by its appearance, which is also important to evaluate how well a product is cooked. Fig. 1 shows the appearances of the prepared *L. crocea* roe product under different roasting temperatures. The appearance changed gradually (yellow-brown-deep brown) with increase in the temperature, from 0°C (control group) to 180°C. A similar finding has been reported for soybeans (YOSHIDA *et al.*, 2003). NAKAMURA *et al.* (2011) reported that the color change during grilling consisted of four steps: (1) protein denaturation, (2) water evaporation, (3) browning reaction, and (4) carbonization reaction. According to MATSUDA *et al.* (2013), fish fillets began to darken during grilling when the temperature was close to 150°C under radiant (far-infrared radiation) heating. In the present study, the *L. crocea* roe product started to darken at 140°C and exhibited an attractive appearance at 120°C.

Meanwhile, cyclic compounds (e.g., some aldehydes, pyrazines) can be formed to release a fragrant odor during roasting. The brown pigments from the *L. crocea* roe product could also degrade under very high temperatures. The lipids inside may deteriorate through hydrolysis and oxidation (FRITSCH, 1981). Furthermore, PEs have been reported to be related to lipid browning deterioration, which may cause a brown color (LEE *et al.*, 1976). The PE in the *L. crocea* roe could be responsible for the browning at high temperatures through hydrolysis and oxidation.

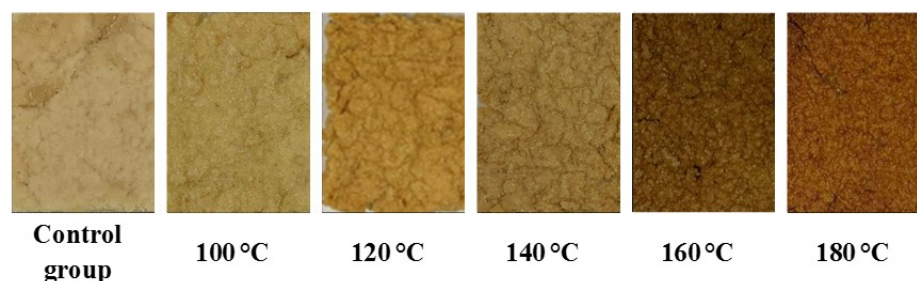


Figure 1. Appearances of the *L. crocea* roe at different roasting temperatures.

3.2. Lipid contents of *L. crocea* roe at different roasting temperatures

The lipid profile was identified and separated using UPLC-ESI-Q-TOF-MS. More analysis details can be seen in our previous paper (LIANG *et al.*, 2018).

The species of 167 PCs, 105 PEs, 17 PIs, 26 PGs, 78 PSs, 55 PAs, 12 CLs, 17 SMs, 27 Cers, 10 MGs, 181 DGs, and 248 TGs were detected in the *L. crocea* roe product at different roasting temperatures.

3.2.1 Effects of roasting temperature on relative content of PL

Fig. 2 displays the relative content variations of different lipid classes at different roasting temperatures. Almost all lipid classes exhibited the same trend. Generally, their quantities decreased with temperature increase from 0 to 100°C, increased with further temperature increase to 120°C, at which the amounts were largest; and reduced again with temperature increase from 120°C to 180°C; however, PI, PG, PS, and CL exhibited some minor differences. PI and PG increased with temperature increase from 0 to 100 to 120°C. PI remained constant from 100°C to 160°C, with the largest amount at 160°C, and it decreased slightly from 160°C to 180°C. However, PG decreased gradually with temperature increase from 120°C to 180°C. PS and CL remained stable from 0 to 100°C, and the rest exhibited the same trend.

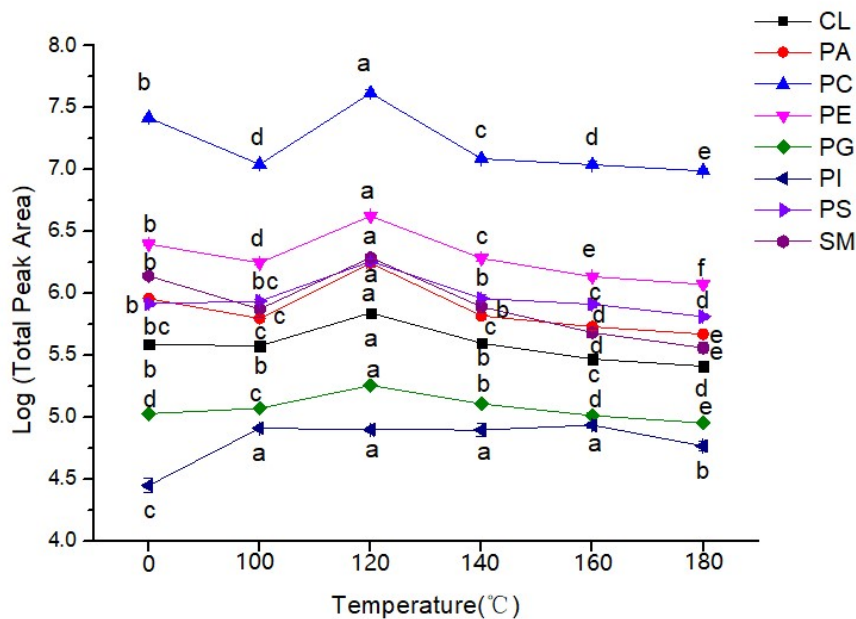


Figure 2. Effects of roasting temperature on the relative content of PLs in *L. crocea* roe.

Note: The same letters in the same column means that no significant difference exists between the amounts at the significance level of $P < 0.05$, and vice versa.

The variable importance in projection (VIP) values were adopted for the selected lipid classes that changed significantly. The VIP method is usually used to identify the variables ($VIP > 1$) in the orthogonal projections to latent structures discriminant analysis. According

to their VIP values (VIP > 1), 56 PCs, 24 PEs, 17 PSs, 6 CLs, 5SMs, 4 PAs, 3 PGs, and 3 PIs were selected for analysis among the detected lipid molecular species.

All lipid classes except PI showed the highest value at 120°C. VUJASINOVIC *et al.* (2012) determined that the total PL content increased from 0 to 130°C (0, 90, 110, and 130°C) in roasted pumpkin oil. CLARK *et al.* (1991) reported that a high phosphorus content was obtained through the preheating process at 130°C in crude soybean oil extracted from fine flour. They suggested that PLs had better solubility in the hot oil, and PLs may be released from cell membranes. However, to the best of our knowledge, this study is the first to identify 120°C as the temperature at which the highest concentration of most lipid classes was found. Further research is needed to obtain the underlying reason for this. Perhaps, this temperature allowed the release of more lipids, which could not be extracted using the abovementioned method.

Apart from the temperature of 120°C, the PL class gradually declined from 0 to 180°C, except for PI, which agrees with the report on safflower seeds (LEE *et al.*, 2004), in which temperatures of 0, 140, 160, and 180°C were considered using an electric oven. With increasing temperature, PC, PE, and PA were found to decrease, while PI increased. In the current study, the PL decomposition or formation through a reaction with protein or carbohydrate may explain the PL reduction after the roasting treatment (YOSHIDA *et al.*, 2005). ABOU-GHARBIA *et al.* (2000) determined that PC, PI, PE, and PS in sesame oil decreased with roasting treatment (200°C) and steaming (100°C), while PA and LPC increased.

In this study, PI exhibited a distinctive trend, increasing with temperature increase from 0-100°C; this may be because it had the highest saturated fatty acid content among the PLs of the *L. crocea* roe product. The decrease in PE may be related to the PI increase; that is, PE transformed to PI with the increasing temperature (LEE *et al.*, 2004). PC can be obtained via subsequent methylation of the amine by S-adenosyl methionine from PE. SM is the only lipid belonging to the sphingolipids class and the PL class. It was formed via the transfer of phosphorylcholine from PC to Cer via SM synthase (MERRILL, 2011). Therefore, it is possible that some PEs were transferred to PCs and that some PCs were transferred to SMs. Similarly, PS is formed when PA reacts with serine, and PE is formed when PA reacts with ethanolamine (AMBROSEWICZ-WALACIK *et al.*, 2015). However, it is not clear if the reaction can occur in the *L. crocea* roe product at high temperatures. Further study is needed to explore this in more detail.

However, the compositions of each PL species are different in different food matrices; for example, marine PLs contain more n-3 PUFAs. Normally, the PLs, which contained more unsaturated fatty acids, were easily oxidized. A considerable loss has been detected for the PL molecular species containing more than four double bonds (YOSHIDA *et al.*, 2001a). We analyzed the relative content changes in the PCs with VIP of more than four, and the results are illustrated in Fig. 3. The relative contents of these nine PCs displayed the same change trend as the PC total amount in Fig. 2. Almost all the nine analyzed PCs contained unsaturated fatty acids in their sn-2 positions except for one PC (0:0/16:0), and six of them consisted of C20:5 omega-3 and C22:6 omega-3, which influenced the omega-3/omega-6 ratio, meaning that they were easily oxidized in the oven. Similarly, the PEs, PGs, and PSs with the variable of VIP > 1 all contained an unsaturated fatty acid at their sn-1, sn-2 positions, or both.

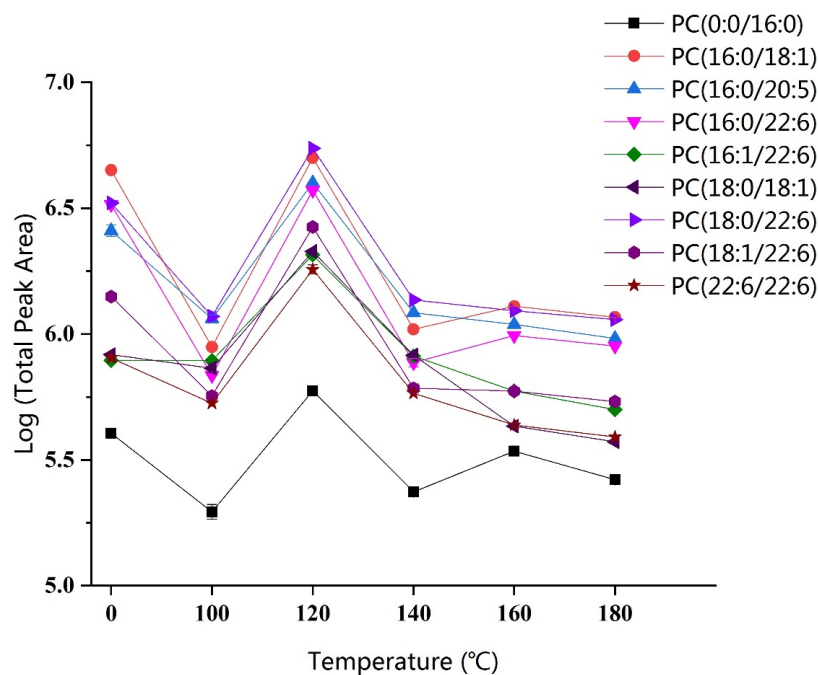


Figure 3. Effects of roasting temperatures on the relative content of PCs in the *L. crocea* roe. Note: The same letters in the same column means that no significant difference exists between the amounts at the significance level of $P < 0.05$, and vice versa.

3.2.2 Effect of roasting temperature on the relative contents of other lipids

A characteristic pattern of TGs exists in almost every type of oil or other food matrices. The pattern is determined by the abundances of different TG molecular species. YOSHIDA *et al.* (2001b) and COSSIGNANI *et al.* (1998) determined that the TG fraction decreased, while DG and MG increased over time in the microwave roasting for sunflower and olive oil.

In this study, 181 DGs, 248 TGs, 10 MGs, and 27 Cers were detected in the *L. crocea* roe at different roasting temperatures. According to Fig. 4, the relative contents of TGs, DGs, and MGs decreased with temperature increase from 0 to 100°C, which corresponds to the findings of previous studies, but the values increased again with temperature increase from 100 to 120°C and reached the highest point at 120°C, which is not mentioned in the other reports. YOSHIDA *et al.* (2001b) only tested the TG loss at 98, 137, 164, and 172°C; they did not know if the TGs changed at 120°C in sunflower oil; however, the TG content at 137°C was less than that at 172°C, which disagrees with this study results. In this study, the DGs slightly increased with temperature increase from 140 to 180°C, possibly due to the TG decomposition; however, the MGs decreased, which may be because the temperature was still insufficient for the TG and DG decomposition to MGs. This inference needs further verification. Cer remained stable from 0 to 100°C, and the rest exhibited the same trend.

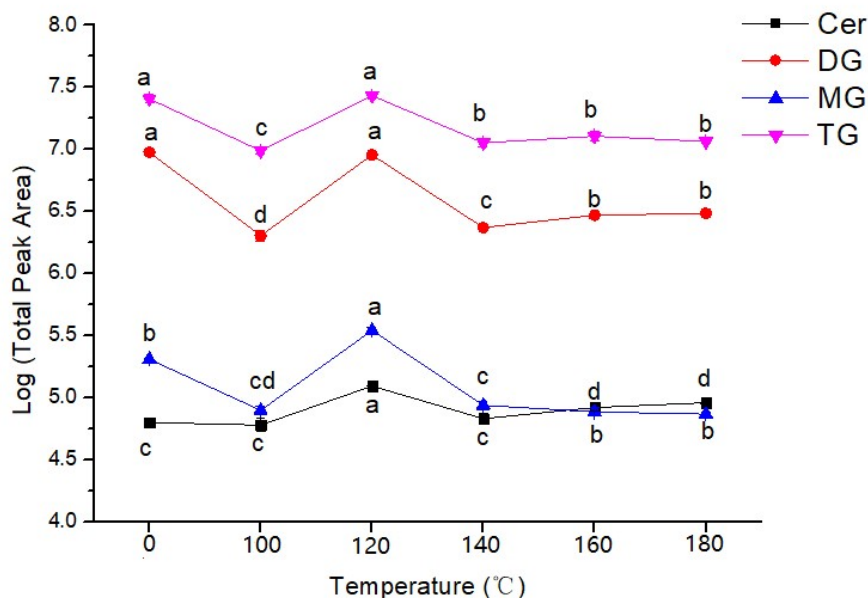


Figure 4. Effects of roasting temperature on the relative amount of glycerol lipids (GLs) and Cer in *L. crocea* roe.

Note: The same letters in the same column means that there is no significant difference between the amounts at the significance level of $P < 0.05$, and vice versa.

According to their VIP values ($VIP > 1$), 4 Cers, 84 TGs, 31 DGs, and 2 MGs were detected in the lipid profile. However, for further analysis, three TGs were selected based on their VIP values ($VIP > 3$); the compounds were TG (16:0/16:0/18:1), TG (16:0/16:1/16:1), and TG (16:0/16:1/18:1) with 53, 51, and 53 carbon numbers (CN), respectively. They all contained an unsaturated fatty acid linked at the sn-3-position. YOSHIDA *et al.* (2001a) reported that an unsaturated fatty acid linked at the sn-2-position of glycerol moiety in TGs helped to keep the moiety more stable than the same unsaturated fatty acid at the sn-1 or sn-3 positions. However, this finding disagrees with the results by LIU *et al.* (2017), who studied TGs with 51–56 CN, which were stable than TGs with 26–48 CN.

3.2.3 Heatmap analysis of the lipid profile data

A heatmap was adopted to better interpret the qualitative information of lipidomics datasets using the R software (Fig. 5). The lipid molecular species in the heatmap were selected according to a combination of multidimensional and one-dimensional analyses. The changes appeared between every two neighboring groups based on the VIP value and P -value in the student's t -test ($VIP > 1$, $P < 0.05$). Green color denotes increase, and red denotes decrease. From Fig. 5, all the selected lipid molecular species followed the same trends in Figs. 2 and 3. PCs were the molecular species that changed the most between two neighboring groups.

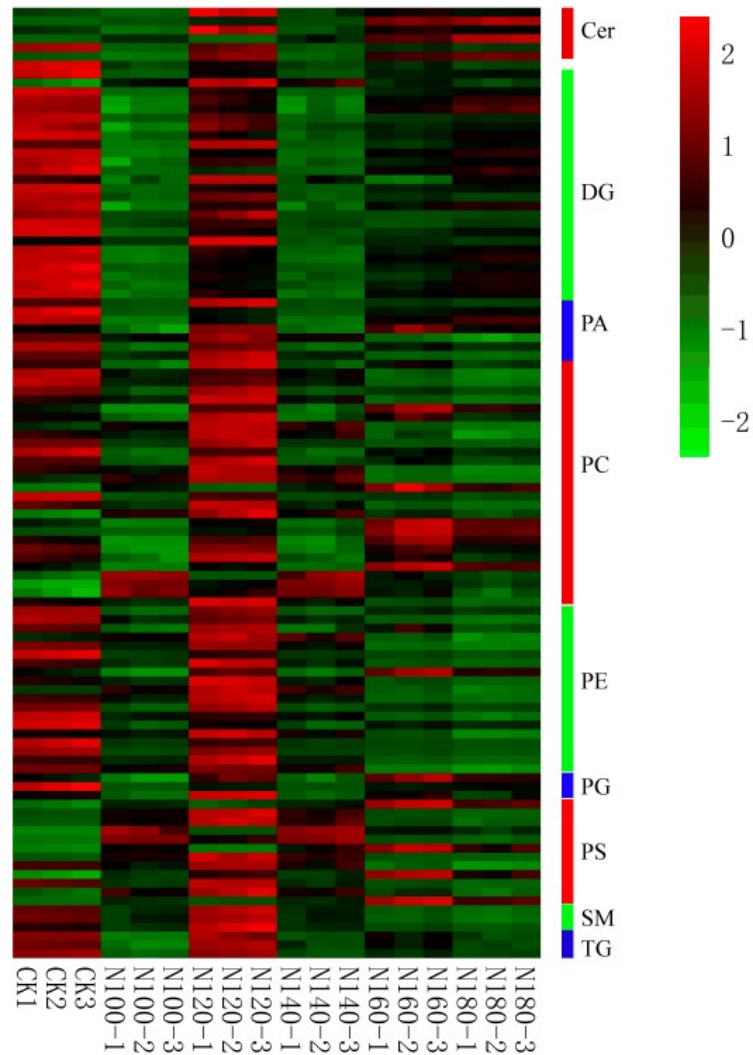


Figure 5. Heatmaps of the lipidomics dataset profiles.

Note: Red color represents an increase, and green represents a decrease. CK-1, CK-2, and CK-3 represent the first, second, and third analyses conducted using the control group, respectively; N100-1, N100-2, and N100-3 represent the first, second, and third analyses conducted using the group at 100 °C, respectively; the rest mark number is similar.

4. CONCLUSIONS

The *L. crocea* roe is a valuable byproduct that contains high amounts of valuable EPA and DHA. However, it is large and has an unattractive appearance. Discarding this roe as waste would be an important commercial loss and an environmental problem from the fish industry. This study investigated the further processing of the roe to make it more acceptable to consumers. The roe was roasted under different temperatures to determine the lipid amount changes. The relative amounts of almost all lipid classes were highest at 120°C, except for PI; meanwhile, the temperature of 120°C allowed to obtain a practically sterilized product, stable at room temperature if well packaged. No previous study has

mentioned this point before. Perhaps, this temperature is the best for processing without a significant loss of valuable PLs, as the obtained product is both of good quality and ready-to-eat. Moreover, this study clarifies the changes in lipid classes, especially PLs molecular species in the *L. crocea* roe, with temperature. Using the lipidomics method for analysis, the study also demonstrates the value of the method in the fish industry.

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ABBREVIATIONS

Phospholipid: PL
Phosphatidylcholine: PC
Phosphatidylethanolamine: PE
Phosphatidylinositol: PI
Phosphatidylglycerol: PG
Phosphatidylserine: PS
Phosphatidic acid: PA
Cardiolipin: CL
Sphingomyelin: SM
Ceramides: Cer
Diacylglycerol: DG
Triglyceride: TG
Monoacylglycerol: MG
Ultra-performance liquid chromatography–electrospray ionization–quadruple-time-of-flight–mass spectrometry: UPLC-ESI-Q-TOF-MS

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