Modification of nutritional values and flavor qualities of muscle of swimming crab (*Portunus trituberculatus*): Application of a dietary lipid nutrition strategy

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ABSTRACT

Lipid sources as alternatives to fish oil could alter the nutritional value and flavor quality of crab meat affecting consumer preferences. Herein, an 8-week nutritional trial was designed to investigate the effects of dietary lipid sources including fish oil (FO), krill oil (KO), palm oil, rapeseed oil, soybean oil and linseed oil on profiles of amino acids, fatty acids and volatiles in muscle of swimming crab (*Portunus trituberculatus*). Volatiles of crab muscle were characterized by headspace solid-phase microextraction and gas chromatography-tandem mass spectrometry. Results revealed that crabs fed FO and KO had significantly higher levels of protein, indispensable amino acids, eicosapentaenoic acid and docosahexaenoic acid in muscle. Principal component analysis and hierarchical cluster analysis demonstrated that muscle volatiles of crabs fed different dietary oils exhibited significant variations. Dietary FO and KO significantly increased the relative levels of 3-methylbutanal, heptanal, benzaldehyde and nonanal in muscle, which may produce more pleasant flavors.

***Keywords*:** *Portunus trituberculatus*; Lipid source; Amino acid; Fatty acid; Volatile compound; HS-SPME; GC-MS/MS

Chemical compounds studied in this article:

3-Methylbutanal (PubChem CID: 11552); Hexanal (PubChem CID: 6184); Heptanal (PubChem CID: 8130); Benzaldehyde (PubChem CID: 240); Nonanal (PubChem CID: 31289); 1-Butanol (PubChem CID: 263); 1-Octen-3-ol (PubChem CID: 18827); 2-Heptanone (PubChem CID: 8051); (3*E*,5*E*)-Octadiene-2-one (PubChem CID: 181575); Trimethylamine (PubChem CID: 1146)

1. Introduction

Fish oil (FO), containing a high content of n-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFA), especially eicosapentaenoic (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), has been traditionally recognized as the most important lipid source in commercial aquafeeds (Betancor et al., 2015). However, the annual global production of FO is insufficient to meet the rapid growth and future demand of aquaculture as these are finite resources, which inevitably results in unstable and generally increasing feed prices in the aquaculture industry (Tocher, 2015). Therefore, alternative lipid sources to FO are urgently required to satisfy the long-term sustainable development of aquaculture (Tocher, 2015). Vegetable oils (VOs) supply energy effectively with almost no restraints concerning supply but they lack EPA and DHA, which may raise nutritional issues (NRC, 2011; Turchini, Ng, & Tocher, 2010). Aside from VOs, krill oil (KO), a marine oil extracted from *Euphausia superba*, not only contains abundant n-3 LC-PUFA, but also has various natural antioxidants (e.g., astaxanthin and flavonoid) not present in FO that may stabilize EPA and DHA against oxidative damage (Tou, Jaczynski, & Chen, 2007; Turchini et al., 2010).

In the past two decades, many studies have reported the impacts of different dietary lipid sources on growth, physiology, metabolism, welfare and product quality of aquatic animals (NRC, 2011; Shu-Chien et al., 2017; Turchini et al., 2010). The quality of farmed aquatic animals greatly impacts consumer preferences and purchasing behaviors, ultimately dictating the success or failure of farming industries (Hardy & Lee, 2010). The quality of aquaculture products is determined by a combination of nutritional value (e.g., protein, amino acid, fatty acid, vitamin and mineral contents) and sensory quality (e.g., skin or fillet color, texture, flavor and odor) of the edible portion (fillet from fish or meat from crab/shrimp) (Grigorakis, 2007), which are both closely related to diet composition (Hardy & Lee, 2010). Dietary lipids can alter nutritional and sensory qualities

(especially flavor quality) as reported in fish such as *Carassius auratus gibelio* (Zhou, Han, Zhu, Yang, Jin, & Xie, 2016), *Oreochromis niloticus* (Liu et al., 2019), *Sparus aurata* (Grigorakis, Fountoulaki, Giogios & Alexis, 2009), *Tinca tinca* (Turchini, Moretti, Mentasti, Orban & Valfre, 2007), and crustaceans including *Eriocheir sinensis* (Wu, Fu, Zhuang, Wu, & Wang, 2018) and *Litopenaeus vannamei* (Zhong, Zhang, Li, Huang & Wang, 2011; Zhou, Li, Liu, Chi & Yang, 2007).

Swimming crab (*Portunus trituberculatus*), one of the most important economic marine crustacean species (Jin, Wang, Huo, Huang, Mai, & Zhou, 2015), is popular with the public and has become a distinctive food in coastal areas owing to its delicious meat, rich nutrition, unique flavor and accessibility, particularly in China (Sun, Ding, Lu, Yuan, Ma, & Zhou, 2017). In commercial production, swimming crab are fed trash fish and low-value shellfish, leading to water pollution, increased bacterial load and oxygen demand, which have negative impacts on the health and nutritional value, restricting the development of farming (Craig & Helfrich, 2009). With the increasing demand for safe, nutritious and high-quality crab, the swimming crab breeding and production industries have faced enormous pressures (Jin et al., 2015). Recently, there have been increased researches into nutritional and flavor qualities in crustaceans such as *Eriocheir sinensis* (Gu, Wang, Tao & Wu, 2013; Kong et al., 2012; Wang et al., 2016; Wu et al., 2018; Wu, Wang, Tao, & Ni, 2016; Zhuang et al., 2016), *Litopenaeus vannamei* (Mall & Schieberle, 2017), *Portunus trituberculatus* (Song, Wang, Xu, Wang & Shi, 2018) and *Scylla serrata* (Yu & Chen, 2010). However, to date, there is no information regarding the impacts of dietary lipid sources on the nutritional value and flavor quality of swimming crab. The overarching aim of the present study was to provide novel insight into the regulation of nutritional quality of crab meat through a nutritional strategy, specifically by modifying dietary lipid source.

2. Materials and Methods

2.1. Chemicals, standard compounds and reagents

Hydrochloric acid (HCl, 36~38% purity, CAS 7647-01-0), methanol (CH_3OH, ≥ 99.7% purity, CAS 67-56-1), petroleum ether (60-90 ℃, CAS 8032-32-4), potassium hydroxide (KOH, ≥ 85.0% purity, CAS 1310-58-3) and sodium chloride (NaCl, ≥ 99.5 % purity, CAS 7647-14-5) were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Amino acid mixture standard solution (Type H) and ninhydrin coloring solution (including ninhydrin reagent and buffer solution) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 2,6-Di-*tert*-butyl-4-methylphenol (BHT) was of extra-pure grade and provided by Aladdin Reagents (Shanghai, China). HPLC-grade *n*-hexane (C_6H_{14}, ≥ 97.0% purity, CAS 110-54-3) and the standard mixture of 37 fatty acid methyl esters (FAMEs) were purchased from Sigma (St. Louis, MO, USA). The chemical standards used for identification including 3-methylbutanal, hexanal, heptanal, nonanal, decanal, dodecanal, hexadecanal, benzaldehyde, benzeneacetaldehyde, 2-heptanone, 2-nonanone, 2-decanone, 2-undecanone, 3-methyl-1-butanol, 1-butanol, 1-octen-3-ol, octanol, 3-decanol, 3-undecanol, 1-hexadecanol, 4-methylphenol, butylated hydroxytoluene and 2-methylpyrazine were purchased from Sigma-Aldrich (Shanghai, China). A C_5–C_{25} *n*-alkane mixture and 2,4,6-trimethylpyridine (TMP, 99% purity, CAS 108-75-8) were also purchased from Sigma-Aldrich (Shanghai, China). Ultrapure water was produced by a laboratory water purification system (Hitech Master-S15, Shanghai, China).

2.2. Nutritional trial design

2.2.1. Animal ethics approval

All experimental procedures complied with Chinese law pertaining to research on animals. The detailed experimental protocol was approved by the Ethics-Scientific Committee for Experiments on Animals of Ningbo University and followed the Guidance of the Care and Usage of Laboratory Animals in China.

2.2.2. Experimental diets

Six isonitrogenous (crude protein, approximately 450 g/kg) and isolipidic (crude lipid, approximately 80 g/kg) experimental diets containing either fish oil (FO), krill oil (KO), palm oil (PO), rapeseed oil (RO), soybean oil (SO) and linseed oil (LO) as lipid sources were formulated to meet the nutrient requirements of swimming crab juveniles based on NRC (2011) recommendation as described previously (Jin et al., 2015). The formulation and proximate composition of six experimental diets are shown in Supplementary Table 1, and the fatty acid compositions (% total fatty acids) are shown in Supplementary Table 2. Fishmeal, soybean protein concentrate and soybean meal were used as the main protein sources, wheat flour was used as the carbohydrate source, and sodium alginate was used as a natural binder. The diets were prepared followed the process as described in detail previously (Jin et al., 2015). The experimental diets were sealed in vacuum-packed bags and stored at -20 ℃ until used in the feeding trial in order to maintain good quality.

2.2.3. Feeding trial and experimental conditions

The feeding trial was conducted in Ningbo Marine and Fishery Science and Technology Innovation Base (Ningbo, China) located at N29°39′2.19″, E121°46′27.10″. Similar sized and healthy swimming crab juveniles were obtained from a pond in Xiangshan crab field (Ningbo, China) and were acclimated in an indoor rectangular cement pool (8.5 m × 3.0 m × 1.5 m) for 7

days and fed a commercial feed (Ningbo Tech-Bank Feed Co. Ltd., Ningbo, China) containing 450 g/kg crude protein and 80 g/kg crude lipid, respectively. A total of 270 swimming crab juveniles (initial weight 5.43 ± 0.03 g) were randomly allocated to one of the six diets, then placed into 270 individual rectangular plastic baskets (35 cm × 30 cm × 35 cm) in a new cement pool (6.8 m × 3.8 m × 1.7 m). Each diet had three replicates, with each replicate consisting of 15 crabs. Fifteen plastic baskets were placed in a line next to each other in the cement pool based on the methodology described in detail previously (Sun et al., 2017). Each plastic basket had two compartments, one section filled with sand to mimic the habitat of the swimming crab whereas the other section was the feeding area. Crabs were fed the allocated experimental diet once daily at 17:00h (daily ration was 6-8 % of wet weight depending upon crab weight). The crabs were weighed every 2 weeks and the daily ration adjusted accordingly. Every morning, feces and uneaten feed were removed, and 60 % of seawater in the cement pool was exchanged daily to maintain water quality. During the experimental period of 8 weeks (from July 25th to September 11th), the seawater conditions were as follows: temperature 29.3 ℃, salinity 27.0 ± 1.5 g/L, pH 7.6 ± 0.3, ammonia and nitrogen lower than 0.05 mg/L, and dissolved oxygen higher than 6.0 mg/L as measured by YSI Proplus (YSI, Yellow Springs, OH, USA).

2.3. Sample collection and preparation

At the end of the feeding trial, a sample of approximately 3 g of fresh muscle from three crabs (1 g per crab) per replicate was dissected, collected and mixed as one sample (n = 3 per dietary treatment) in a 5 ml microfuge tube, then stored immediately at -20 ℃ prior to proximate composition, amino acid and fatty acid analyses. A larger sample of approximately 9 g of fresh muscle from nine crabs (1 g per crab) per replicate was pooled as one sample (n = 3 per dietary

148 treatment) and collected into a 10 ml microfuge tube and stored at -20 ℃ for the analysis of volatile
149 compounds. All the operations were carried out on ice.

150 *2.4. Determination of proximate composition*

151 Proximate composition of diet and crab muscle samples was determined by measuring
152 moisture, crude protein, crude lipid, and ash contents, following the procedures of the Association
153 of Official Analytical Chemists (AOAC, 2016). In brief, moisture content was determined by drying
154 the samples to a constant weight at 105 ℃. Crude protein content was measured by determining
155 nitrogen content (N × 6.25) using the Dumas combustion method with a protein analyzer (FP-528,
156 Leco, USA). Crude lipid content was determined by petroleum ether extraction using the Soxhlet
157 method (Soxtec System HT6, Tecator, Sweden). Ash content was determined using a muffle furnace
158 at 550 ℃ for 8 h. The differences in the weight of samples before and after experimental processing
159 were used to calculate the moisture, crude lipid and ash contents.

160 *2.5. Identification and quantification of amino acids*

161 Amino acid profiles of muscle samples were determined using a High-speed Amino Acid
162 Analyzer (L-8900, Hitachi High-Technologies Co., Tokyo, Japan) based on the method described
163 previously with a few modifications (Unnikrishnan & Paulraj, 2010). Briefly, samples of
164 approximately 30 mg freeze-dried muscle were weighed into a 15 ml glass thread screw neck vial
165 with 18 mm screw cap containing a translucent blue silicone septa gasket (CNW, Germany). Five
166 ml HCl (6 N) was added, the tube sealed under N_2, and immersed in a sand bath at 110 ℃ for 24 h
167 for digestion. After cooling, the digested samples were washed into a 50 ml volumetric flask using
168 ultrapure water. One ml of this solution was transferred into a 4 ml ampoule bottle (CNW,
169 Germany), evaporated to dryness in a rotary evaporator (IKA RV10, Germany), resuspended in 1 ml

HCl (0.02 N) and filtered through a 0.22 μm membrane using a hydrophilic polyether sulfone (PES) syringe filter (CNW, Germany) to remove any residue and impurity. Finally, 20 μl of the solution was used for amino acid determination. The packed column was Hitachi ion-exchange resin 2622 (4.6 mm × 60 mm, particle size 5 μm) and ninhydrin coloring solution was the reactive reagent for the detection of amino acids. Results were expressed as g/100 g dry matter with all determinations performed in triplicate, with the coefficient of variation within 1.0 %.

2.6. Identification and relative quantification of fatty acids

2.6.1. Preparation of fatty acid methyl esters (FAMEs)

The fatty acid compositions of diets and crab muscle samples were determined according to the methods described by Zuo with minor modifications after preliminary tests to ensure that all fatty acids were esterified using the following procedures (Zuo, Ai, Mai, & Xu, 2013). All solvents contained 0.005% (w/v) 2,6-di-*tert*-butyl-4-methylphenol (BHT) to prevent the oxidation of PUFA. Diets samples (approximately 100 mg) and muscle samples (approximately 120 mg) were thawed at 4 ℃, then added to a 12 ml glass screwed tube with a lid containing a teflon gasket. Three ml KOH-CH_3OH (1 N) was added and samples incubated in a water bath at 75 ℃ for 20 min. After cooling, 3 ml HCl-CH_3OH (2 N) was added and the mixture incubated in a water bath at 75 ℃ for a further 20 min. Finally, 1 ml *n*-hexane was added to the above mixture, shaken vigorously for 1 min, 1 ml ultrapure water added to promote layer separation, and the supernatant filtered through a 0.22-μm ultrafiltration membrane (Millipore, MA, USA) and collected into a clean ampoule bottle. The FAMEs solution in the ampoule was reduced to dryness at 50 ℃ using a Termovap sample concentrator (MIULAB NDK200-1N, Hangzhou, China), and the FAMEs resuspended in 500 μL *n*-hexane and stored at -20 ℃ until analysis by gas chromatography-mass spectrometry (GC-MS).

192 *2.6.2. Gas chromatography-mass spectrometry (GC-MS) analysis*

193 FAMEs were separated and analyzed on a gas chromatograph mass spectrometer (GC-MS,
194 Agilent 7890B-5977A, Agilent Technologies, CA, USA) fitted with a fused-silica ultra-inert
195 capillary column (DB-WAX, 30 m × 250 μm i.d., film thickness 0.25 μm, Agilent J & W Scientific,
196 CA, USA), with the following temperature program and column conditions: initial temperature
197 100 ℃, increasing at 10 ℃/min up to 200 ℃, held at 200 ℃ for 5 min, then 2 ℃/min to 230 ℃ and
198 held at 230 ℃ for 10 min, with a final ramp from 230 to 240 at 10 ℃/min. The injection
199 temperature was set at 250 ℃, the interface temperature was set to 240 ℃, and the ion source
200 temperature was adjusted to 230 ℃. Highly pure helium (99.999 %) was used as the carrier gas
201 with a constant flow rate of 1.0 ml/min. 0.5 μL of sample was injected in a 1:20 split ratio by
202 auto-sampler. The acquisition of mass spectra data was carried out in full-scan mode (mass range
203 m/z 40-500). Fatty acids were identified using retention times of standards by comparing the mass
204 spectra with a commercially available standard library (National Institute of Standards and
205 Technology Mass Spectral Library 2011). Results were calculated using the peak area ratio and
206 presented as relative percentages of each fatty acid (% total fatty acids).

207 *2.7. Identification and relative quantification of volatile compounds*

208 *2.7.1. Extraction of volatile compounds using HS-SPME*

209 Volatile compounds of muscle samples were extracted using headspace solid-phase
210 microextraction (HS-SPME) according to the previous method with minor modifications (Silva,
211 Valente, Castro-Cunha, Bacelar, & De Pinho, 2012). Immediately before analysis, in order to
212 facilitate the release of the volatile compounds, muscle samples were thawed at 4 ℃ for 20 min,
213 then minced and mixed, and subjected to HS-SPME. For quantitative determination,

2,4,6-trimethylpyridine (TMP) was used as an internal standard. Briefly, three pooled muscle samples, each consisting of nine crabs, were analysed for volatile compounds from each replicate (n = 3 per dietary treatment). The mixed muscle samples (9 g) were weighed, placed into a 20 ml headspace vial (CNW, Germany) with 18 mm magnetic screw cap containing a translucent blue silicone septa gasket. 5 ml saturated NaCl solution, 10 µL 2,4,6-trimethylpyridine solution (100 ppm) and a stir bar were placed in the headspace vial, and the vial placed in a water bath at 60 ℃. Muscle samples were mixed for 30 min with continuous magnetic stirring at 500 rpm by a magnetic stirrer (C-MAG HS7, IKA, Germany). Finally equilibrated for 5 min at 60 ℃. The volatile compounds were extracted from muscle samples using HS-SPME equipped with a divinylbenzene/carboxen/polymethylsiloxane 50/30 µm fiber (1 cm, DVB/CAR/PDMS, gray, Supelco, PA, USA) which was heated in the GC injector port at 250 ℃ for 45 min. The extraction lasted for 30 min at 60 ℃. Then the analytes desorbed at 250 ℃ for 2 min in the injection port of the gas chromatograph.

2.7.2. Gas chromatography-tandem mass spectrometry (GC-MS/MS) analysis

The separation and detection of volatile compounds was performed by gas chromatography-tandem mass spectrometry GC-MS/MS (Agilent 7890B-7000C, GC-QQQ-MS, Agilent Technologies, CA, USA) equipped with a Vocol fused-silica capillary column (60 m × 0.32 mm i.d., 0.25 µm film thickness; Supelco, PA, USA). The oven temperature program and column conditions were as follows: initial temperature of 35 ℃ for 2 min, before increasing at 15 ℃/min up to 125 ℃, held at 125 ℃ for 1 min, then increasing at 2 ℃/min to 200 ℃ and held at 200 ℃ for 12 min. The carrier gas was 99.999 % highly pure helium, at a constant flow of 2.25 ml/min. The injector temperature was set at 210 ℃, and injection performed in split-less mode. The mass

spectrometer was operated in the electron impact (EI) mode at an ionizing voltage of 70 eV with an ion source temperature of 220 ℃. The acquisition and processing of mass spectra data were performed in scanning mode with a mass range from m/z 45 to 500 by Agilent MassHunter workstation (B.07.00, Agilent Technologies, CA, USA). Volatile compounds were identified qualitatively by comparison with the retention indices (RI), the mass spectra of standard compounds and NIST14.L mass spectral library (National Institute of Standards and Technology 14.L, USA) with an acceptance criterion of a score match above 85 %. The RI values were calculated using the carbon numbers of *n*-alkanes (Sigma-Aldrich Chemical Co., USA) via the equation of Van Den Dool & Kratz (1963) at the same chromatography conditions. The relative concentration (ng/g) of each volatile compound was quantified by calculating the peak area ratio of each compound with that of the internal standard.

Conc (ng/g) = Peak area ratio (compound/TMP) × 1 μg (TMP) / 9 g (crab muscle samples)

2.8. Statistical analysis

All the experimental analyses were performed in triplicate and the results are presented as means ± SEM (n=3). All the data were first tested to confirm normal distribution and homogeneity of variance. Differences between mean values were analyzed by one-way analysis of variance (ANOVA), using Tukey's multiple range post hoc test using SPSS 22.0 software (Chicago, USA). The results were considered to be statistically significant at $P < 0.05$. The principal component analysis (PCA) of volatile compounds detected from muscle was carried out to understand the communalities and discrepancies among diets formulated with different lipid sources by reducing the number of dimensions without much loss of information using SIMCA-P+ software (Version 11.0.0.0, Umetrics AB, Malmo, Sweden). Hierarchical cluster analysis (HCA) was conducted to

analyze the relationship between the volatile compounds and different samples using Pearson correlation and average clustering algorithm after log2 transformation. A heat map was also used for visualizing complex data sets (volatile compounds) organized as Pearson correlation matrices. Hierarchical cluster analysis and heat map visualization were performed using the online program ImageGP, a free online platform for data analysis (http://www.ehbio.com/ImageGP/index.php/).

3. Results and discussion

3.1. Proximate composition of crab muscle

The proximate composition (g/kg wet weight) of the muscle of swimming crab juveniles fed different dietary lipid sources is shown in Supplementary Table 3. Moisture and ash contents of juvenile swimming crab muscle were not affected by dietary lipid sources ($P > 0.05$). However, crabs fed the FO and KO diets had significantly higher contents of protein in muscle than those fed the other diets ($P < 0.05$). Crabs fed the SO diet had a significantly higher level of lipid in muscle than those fed the other diets ($P < 0.05$), with the lowest muscle lipid level found in crabs fed the RO diet. In general, the proximate composition of swimming crab muscle in the present study was similar to the values obtained in previous studies with the same species (Han, Wang, Hu, Li, Jiang, & Wang, 2015; Jin et al., 2015). The proximate composition of the edible portion indicated the nutritional quality of crustaceans as food for human consumers (Vijayavel & Balasubramanian, 2006). The results of the present study indicated that dietary soybean oil promoted lipid accumulation in the muscle to some extent whereas dietary marine oils (FO and KO) increased the muscle protein content of swimming crab. It was reported that *L. vannamei* fed a diet with 1% conjugated linoleic acid (CLA, a group of geometric and positional isomers of 18:2n-6) replacing fish oil significantly increased muscle lipid content (Zhong et al., 2011). Another study found tail

muscle of *L. vannamei* fed a diet supplemented with pollack fish oil had the highest crude protein content, similar to the result in the present study (Zhou et al., 2007). However, the precise mechanisms by which n-3 LC-PUFA (particularly EPA and DHA) affect the muscle protein content and act on muscle protein synthesis process are not entirely clear. One possible mechanism may be through the rapamycin (TOR) signaling pathway, which regulates cell growth and metabolism in response to nutrients (Laplante & Sabatini, 2012). Protein synthesis and accumulation in muscle requires much expenditure of energy and the mechanistic target of rapamycin complex 1 (mTORC1), one major branch of the TOR signaling network, senses the energy status of a cell through AMP-activated protein kinase (AMPK) which is activated under low cellular energy. When AMPK is activated, many energetically demanding processes, like protein synthesis, are down-regulated, while, β-oxidation of fatty acids is stimulated to produce more energy in order to maintain cellular energy homeostasis (Wullschleger, Loewith, & Hall, 2006). The characteristic fatty acids from different dietary lipid sources may impact the AMPK signaling pathway and alter the TOR signaling pathway, which in turn might result in the change of protein anabolism. However, in-depth studies are required to clarify the relationship between dietary lipid sources and muscle protein content in swimming crab. Generally, the relationship between the lipid and moisture contents in the muscle follows a negative correlation (Ljubojevic et al., 2013), although this was not the case in the present study. This may be due to the significant change of protein content in muscle among crabs fed the different feeds, which in turn may make the inverse relationship less pronounced.

3.2. Identification and quantification of amino acids

The amino acid composition of muscle of juvenile swimming crab fed different lipid sources

are presented in Table 1. A total of 17 amino acids were detected in the crab muscle including 10 indispensable amino acids (IAA) and 7 dispensable amino acids (DAA), with high amounts of glutamic acid (Glu), followed by arginine (Arg) > glycine (Gly) > aspartic acid (Asp) > proline (Pro) > lysine (Lys) > alanine (Ala) > leucine (Leu). Therefore, the predominant IAA in crab muscle were Arg, Lys, and Leu, and those amongst the DAA were Glu, Gly, Asp and Pro. This was consistent with previous research in swimming crab where the contents of each amino acid were generally similar to the levels found in the present study (Jin et al., 2015). The IAA/TAA ratio is also an important reference index for evaluating the nutritional value of protein in aquatic products, and it is generally agreed that the ideal IAA/TAA is approximately 0.4 in high-quality proteins (WHO, FAO, UNU, 2007). In the present study, the ratio of IAA/TAA of swimming crab muscle ranged from 0.46 to 0.48, which indicated that the muscle supplies high-grade protein for human consumption. Additionally, it was shown that the amounts of most amino acids in muscle were significantly affected by dietary lipid sources ($P < 0.05$). Crabs fed the KO diet had a significantly higher content of TAA ($P < 0.05$), followed by crabs fed the FO and LO diets, with similar trends observed for IAA and DAA levels. In addition, compared to crabs fed the other diets, crabs fed the diet containing KO had significantly higher contents of functional amino acids (e.g., Glu, Gly and Lys), which are good for human health (Wu, 2013). In conclusion, dietary FO and KO increased the IAA contents of swimming crab muscle, with dietary KO supplementation leading to higher contents of some functional amino acids. Functional amino acids could participate in the transport of fatty acids, activate the oxidation of long-chain fatty acids, and inhibit fatty acid synthesis (Wu, 2013). Conversely, the metabolism of fatty acid leads to the production of many intermediates like acetyl-CoA that could regulate the metabolism of amino acids (Newgard, 2012). Further investigation is required to demonstrate the relationship between fatty acids and amino acids,

specifically in regards to the shared metabolite intermediates.

3.3. Identification and relative quantification of fatty acids

The fatty acid profiles (% total fatty acids) of muscle of swimming crab juveniles fed different lipid sources are shown in Table 2. A total of twenty-one fatty acids were detected and identified with the predominant fatty acids being palmitic acid (PA, 16:0), stearic acid (SA, 18:0), oleic acid (OA, 18:1n-9), linoleic acid (LA, 18:2n-6), α-linolenic acid (ALA, 18:3n-3), EPA and DHA. Significant differences were observed for most fatty acids in muscle of swimming crab fed the different dietary lipid sources ($P < 0.05$). For instance, crabs fed FO and KO showed significantly higher percentages of EPA, DHA, n-3 PUFA and n-3 LC-PUFA in muscle than those fed VOs ($P < 0.05$). In contrast, crabs fed diet PO had significantly higher percentages of PA and saturated fatty acids (SFA), whereas muscle of crabs fed the RO diet had significantly higher levels of OA and monounsaturated fatty acids (MUFA). Crabs fed diet SO had significantly higher proportions of LA and n-6 PUFA, and crabs fed diet LO had highest ALA contents compared to crabs fed the other diets ($P < 0.05$). In summary, the fatty acid composition of the crab muscle clearly reflected the fatty acid composition of the experimental diets and, thus, the characteristic fatty acids in each diet were reflected in similarly higher proportions of these fatty acids in the crab muscle. Similar results have been observed in previous studies on crustaceans fed different dietary lipid sources (Han et al., 2015; Shu-Chien et al., 2017; Zhou et al., 2007). Dietary supplementation with either FO or KO increased the levels of the beneficial n-3 LC-PUFA, particularly EPA and DHA, while the lack of these fatty acids in the muscle of crabs fed VOs reduced the health value of crab meat. It is known that dietary n-3 LC-PUFA help to mitigate the effects of various diseases, and also can promote ongoing health and vitality of human consumers (Larsen, Eilertsen, & Elvevoll, 2011; Tou et al.,

2007). In the present study, the muscle fatty acids showed that crabs fed dietary FO and KO had a significantly higher ratio of n-3 LC-PUFA/n-6 PUFA in the muscle than crabs fed any of the VO. Some dietary n-6 PUFA (e.g., LA, 18:2n-6) could also lead to an increase in pro-inflammatory mediators through the metabolic conversion of 18:2n-6 to arachidonic acid (20:4n-6) as well as oxidation of low density lipoprotein (LDL), which may lead to some adverse health effects (Larsen et al., 2011). In conclusion, the high levels of n-3 LC-PUFA (mainly EPA and DHA) in the muscle of swimming crab fed dietary FO and KO provides potential health benefits to human consumers of crab.

3.4. Volatile compounds of crab muscle

3.4.1. Identification and relative quantification of volatile compounds

The identification and relative quantification (ng/g) of volatile compounds detected in muscle of swimming crab are summarized in Tables 3 and 4, respectively. Forty-nine volatile compounds, including 11 aldehydes, 8 ketones, 2 esters, 9 alcohols, 2 alkenes, 8 alkanes, 3 aromatics, 3 amines and 3 additional compounds, dimethyl sulfide (sulfur compound), 2-methylpyrazine (pyrazine compound) and 2-acetylthiazole (thiazole compound), were identified in crab muscle samples by HS-SPME-GC-MS/MS, some of which were also identified in the meat of other crabs such as *E. sinensis* (Wang et al., 2016) and *S. serrata* (Yu & Chen, 2010). The common volatile compounds included 3-methylbutanal, hexanal, heptanal, benzaldehyde, nonanal, decanal, pentadecanal, hexadecanal, 2,3-pentanedione, 2-heptanone, 2-nonanone, 1-octen-3-ol, 1-octanol, 3-decanol and 3-undecanol. In the present study, aldehydes and alcohols were the main volatile compounds detected, containing approximately 500 ng/g volatile compounds detected, and up to approximately 800 ng/g in crabs fed diet KO. Aldehydes were known to be the dominant volatile components

contributing to the flavor of crab meat due to their high content and low odor thresholds (Wang et al., 2016). In the present study, total aldehydes including 3-methylbutanal, hexanal, heptanal, benzaldehyde and nonanal ranged from 260 ng/g volatile compounds identified in crabs fed the SO diet to 666 ng/g in crabs fed the KO diet. In the present study, the relative content of nonanal was the highest in muscle of crabs fed diets FO and KO (197 ng/g and 196 ng/g, respectively), and significantly higher than in crabs fed any of the VO diets that ranged from 34 ng/g to 108 ng/g ($P < 0.05$). Nonanal has a strong flavor and imparts a meaty and grassy aroma to crab meat (Zhuang et al., 2016). The relative contents of 3-methylbutanal, a compound that conferred a strong aroma of green grass and vegetables (Wang et al., 2016), were also higher in the muscle of crabs fed diets FO and KO (110 ng/g and 154 ng/g, respectively). Furthermore, hexanal which conferred a grassy and fatty odor to the crab muscle (Zhuang et al., 2016), was present in significantly higher contents in crab fed diet KO ($P < 0.05$). Another aldehyde, benzaldehyde, an aromatic compound with a bitter and almond odor, was present in highest levels in crabs fed diet KO ($P < 0.05$), followed by FO-fed crabs. The aforementioned aldehydes had a synergistic effect as well as a strong flavor even under trace conditions, which contributed to the formation of flavors in the crab muscle (Song et al., 2018).

Alcohols, the second largest group among the volatile compounds in crab muscle, included mainly 3-methyl-1-butanol, 1-butanol, 1-octen-3-ol and 1-hexadecanol, and in total represented from 214 ng/g (LO diet) to 285 ng/g (SO diet) of all volatile compounds. In contrast to aldehydes, the total relative contents of alcohols in crabs fed diets containing VO, other than PO and LO, were generally significantly higher than in crabs fed the marine oils ($P < 0.05$). However, 1-octen-3-ol, the alcohol detected at the highest relative content in swimming crab muscle was present in muscle

of crabs fed the FO and KO diets at significantly higher levels than in muscle of crabs fed the other diets ($P < 0.05$). This alcohol compound contributed to the grassy odor of crab meat and it was the primary volatile odor-active alcohol in many aquatic animal products including clam, crab and oyster (Zhuang et al., 2016). In contrast, 3-methyl-1-butanol was described as conferring a balsamic aroma (Mu, Wei, Yi, Shentu, Zhang, & Mai, 2017), but no significant differences were obtained in the relative content of this compound among crab fed the different diets ($P > 0.05$).

Ketones were the third largest group of volatiles representing approximately 150 ng/g volatile compounds, and mainly included 2,3-pentanedione, 2-heptanone, 2,3-octanedione, 2-nonanone and (3*E*,5*E*)-octadiene-2-one. The relative content of (3*E*,5*E*)-octadiene-2-one was significantly higher ($P < 0.05$) in crab fed diet FO, and this could confer a milky and candy odor to the crab meat (Gu et al., 2013). Sulfur- and nitrogen-containing compounds were considered as vital odor-active components (Zhuang et al., 2016). In the present study, two sulfur-containing compounds (dimethyl sulfide and 2-acetylthiazole) and four nitrogen-containing compounds (trimethylamine, octodrine, amphetamine and 2-methylpyrazine) were detected in crab muscle. Trimethylamine conferred a typical odor of fish and amines in many aquatic products (Zhuang et al., 2016), and high levels of trimethylamine in seafood conferred a strong fish flavor, unpopular with the public, whereas low levels of trimethylamine produced a more pleasant crustacean-like odor (Wang et al., 2016). Interestingly, the relative content of trimethylamine was lowest in crabs fed diets FO and KO. In conclusion, swimming crab fed diets FO and KO had higher relative levels of volatiles promoting the green grass, sweet and fatty odors and lower relative levels of the fishy odor, which may be suited to the tastes of the general public.

3.4.2. Principal component analysis (PCA) of volatile compounds

Principal component analysis (PCA) was applied to provide an overall picture of the distribution of the 49 volatile compounds in muscle of swimming crab fed the different dietary lipid sources (Figure 1A and 1B). PCA is an unsupervised technique for classifying sample groups based on the inherent similarity or dissimilarity of their chemical information without prior knowledge of sample classes. The first two principal components (PCs) accounted for 54.86 % of the variation (Figure 1A; 41.38 % and 13.48 % of the total variance, respectively). The profiles of the volatile compounds were grouped into three clusters: cluster 1 (FO and KO groups), cluster 2 (PO and RO groups), and cluster 3 (SO and LO groups), which indicated that the volatile compounds in muscle of crabs fed FO and KO diets have much more similarity, while those fed VO diets showed more differences to the marine oil diets. As observed in Figure 1A, the three clusters were clearly separated, which meant that the volatile compounds of muscle of crab fed the different lipid sources could be distinctly distinguished. The PCA loading plot revealed the compounds responsible for the separation between samples (Figure 1B). Thus, 2-heptanone, hexanal, heptanal, benzaldehyde, 3-methylbutanal, 2,3-pentanedione, (3*E*,5*E*)-octadiene-2-one, nonanal, 1-octen-3-ol, 3-methyl-1-butanol and 2,3-octanedione were on the right side of PC1. These volatile compounds were correlated with the muscle samples from crabs fed the marine oil diets, FO and KO. Butylated hydroxytoluene, 2-acetylthiazole, 1-butanol, 1-octanol, amphetamine and octodrine were highly correlated with the muscle samples from crabs fed diets PO and RO.

3.4.3. Hierarchical cluster analysis (HCA) of volatile compounds

Hierarchical cluster analysis (HCA) was performed and shown in Figure 2 via heat map visualization and, based on the dendrogram, the diet groups could also be grouped into three clusters, reflecting the information shown in the PCA diagram (Figure 1A). Combining the

information in Figures 1A and 2, it was shown that the distance between Clusters 1 and 2 was shorter than the distance between Clusters 1 and 3. The volatile compounds detected in crab muscle were themselves grouped into two main clusters (Figure 2). Cluster I showed that some volatiles such as nonanal, 3-methylbutanal, 2,3-pentanedione, 1-octen-3-ol, heptanal, hexanal, benzaldehyde and (3*E*,5*E*)-octadiene-2-one were higher in crabs fed diets FO and KO. On the other hand, Cluster II was mainly divided into two subgroups. Subgroup I of Cluster II included volatiles present at high concentrations in muscle of crabs fed diets SO and LO such as decanal, dodecanal, undecane, tridecane and nonadecane. Whereas subgroup II of Cluster II included volatile compounds found at high concentrations in muscle of crabs fed diets PO and RO, including 1-butanol, 1-octanol, octodrine and amphetamine.

4. Conclusions

In conclusion, the results of the present study showed that feeding swimming crab with diets supplemented with marine oils, fish and krill oil, increased the protein and IAA contents of crab muscle. Furthermore, feeding swimming crab with diets containing krill oil may lead to a higher muscle contents of functional amino acids such as glutamic acid, glycine and lysine. The FO and KO diets also contributed to higher relative contents of n-3 LC-PUFA, particularly EPA and DHA, in the crab muscle, which enhanced their nutritional value from a health of human consumers point of view. In addition, as indicated by the analysis of volatile compounds, the muscle of swimming crab fed diets FO and KO may have a more pleasant flavor than those fed VO diets. These findings not only showed how dietary manipulation can contribute towards the nutritional values and flavor qualities of swimming crab, but also provided scientific evidence and novel insight into the

457 modulation of nutritional quality through a dietary strategy.

458

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469

470 **Declaration of interest**

471 All the authors declare that they have no conflict of interest that could have appeared to
472 influence the work reported in this paper.

473

474 **Appendix A. Supplementary data**

475 Supplementary Table 1. Formulation and proximate composition of experimental diets.

476 Supplementary Table 2. Fatty acid composition (% total fatty acids) of the experimental diets.

Supplementary Table 3. Proximate composition (% wet weight) in muscle of juvenile swimming crab fed different dietary lipid sources (n=3).

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593

Table legends

Table 1. Amino acid composition (g/100g dry matter) of muscle of juvenile swimming crab (*Portunus trituberculatus*) fed different dietary lipid sources.

Table 2. Fatty acid composition (% total fatty acids) of muscle of juvenile swimming crab (*Portunus trituberculatus*) fed different dietary lipid sources.

Table 3. Volatile compounds identified in muscle of juvenile swimming crab fed different dietary lipid sources.

Table 4. Relative concentration (ng/g) of volatile compounds in muscle of juvenile swimming crab fed different dietary lipid sources.

Figure legends

Fig 1. Principal component analysis (PCA) score plot (A) and loading plot (B) based on volatile compound compositions of juvenile swimming crab muscle fed different dietary lipid sources.

Fig 2. Hierarchical cluster analysis (HCA) and heat map visualization of samples and volatile compounds of muscle of juvenile swimming crab fed different dietary lipid sources. The color box for each compound in the heatmap indicates the abundance of the compound and represent the fold-change according to the scale on the right: red for higher levels; green for lower levels. The scale in the color bar is logarithm to base 2 of the ratio of the respective abundances to the average abundance of the compounds in the six treatments. Color spots before the compound names indicates the chemical family of each compound: red, aldehydes; yellow, ketone; blue, ester; green, alcohol; purple, alkene; orange, alkane; grey, aromatic; dark blue, amine; black, other.

616

Table 1. Amino acid composition (g/100g dry matter) of muscle of juvenile swimming crab (*Portunus trituberculatus*) fed different dietary lipid sources

Amino acid	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
Tyrosine	1.94 ± 0.03	1.93 ± 0.06	1.96 ± 0.05	1.97 ± 0.04	1.98 ± 0.03	1.93 ± 0.04
Lysine	5.05 ± 0.05^{b}	5.06 ± 0.09^{b}	4.33 ± 0.08^{a}	4.39 ± 0.06^{a}	4.36 ± 0.10^{a}	4.43 ± 0.06^{a}
Valine	2.35 ± 0.07^{a}	2.37 ± 0.08^{a}	2.77 ± 0.05^{b}	2.74 ± 0.13^{b}	2.32 ± 0.05^{a}	2.24 ± 0.05^{a}
Methionine	0.99 ± 0.02^{a}	1.11 ± 0.04^{ab}	1.23 ± 0.07^{b}	1.39 ± 0.12^{c}	1.08 ± 0.02^{ab}	1.10 ± 0.03^{ab}
Leucine	4.08 ± 0.06^{a}	4.14 ± 0.05^{a}	4.30 ± 0.06^{ab}	4.48 ± 0.11^{b}	4.58 ± 0.21^{b}	4.05 ± 0.05^{a}
Isoleucine	2.24 ± 0.02	2.10 ± 0.08	2.16 ± 0.04	2.24 ± 0.03	2.25 ± 0.05	2.09 ± 0.09
Phenylalanine	2.21 ± 0.04^{a}	2.24 ± 0.05^{a}	2.35 ± 0.08^{ab}	2.49 ± 0.10^{b}	2.24 ± 0.05^{a}	2.16 ± 0.06^{a}
Histidine	1.25 ± 0.04	1.33 ± 0.09	1.20 ± 0.03	1.25 ± 0.03	1.21 ± 0.03	1.35 ± 0.07
Arginine	6.30 ± 0.22^{b}	6.35 ± 0.15^{b}	6.12 ± 0.05^{b}	5.63 ± 0.08^{a}	6.30 ± 0.10^{b}	6.84 ± 0.08^{c}
Threonine	2.40 ± 0.07^{b}	2.44 ± 0.06^{b}	2.34 ± 0.06^{ab}	2.14 ± 0.11^{a}	2.36 ± 0.04^{ab}	2.32 ± 0.04^{ab}
IAA [1]	28.93 ± 0.13^{b}	29.08 ± 0.04^{b}	28.76 ± 0.13^{a}	28.74 ± 0.03^{a}	28.67 ± 0.07^{a}	28.71 ± 0.10^{a}
Alanine	4.61 ± 0.12^{b}	4.62 ± 0.06^{b}	4.10 ± 0.07^{a}	4.52 ± 0.13^{ab}	4.42 ± 0.08^{ab}	4.42 ± 0.05^{ab}
Glycine	5.59 ± 0.09^{b}	5.91 ± 0.05^{c}	5.42 ± 0.18^{b}	4.73 ± 0.10^{a}	5.51 ± 0.09^{b}	5.32 ± 0.13^{b}
Serine	2.19 ± 0.03	2.22 ± 0.07	2.09 ± 0.07	2.13 ± 0.04	2.14 ± 0.07	2.17 ± 0.04

Proline	5.44 ± 0.12^{b}	5.34 ± 0.10^{b}	4.93 ± 0.10^{a}	5.15 ± 0.05^{ab}	4.88 ± 0.07^{a}	5.63 ± 0.04^{c}
Glutamic acid	9.58 ± 0.19^{a}	10.60 ± 0.08^{b}	9.83 ± 0.06^{a}	9.60 ± 0.06^{a}	9.61 ± 0.12^{a}	9.77 ± 0.11^{a}
Aspartic acid	5.37 ± 0.08^{ab}	5.62 ± 0.07^{b}	5.00 ± 0.01^{a}	5.17 ± 0.03^{a}	5.35 ± 0.04^{ab}	5.39 ± 0.08^{ab}
Cysteine	0.48 ± 0.02	0.48 ± 0.04	0.54 ± 0.04	0.46 ± 0.02	0.54 ± 0.04	0.48 ± 0.02
DAA [2]	33.25 ± 0.12^{b}	34.79 ± 0.14^{c}	31.91 ± 0.13^{a}	31.76 ± 0.10^{a}	32.45 ± 0.24^{a}	33.19 ± 0.16^{b}
TAA [3]	62.20 ± 0.12^{c}	63.88 ± 0.17^{d}	60.66 ± 0.13^{a}	60.50 ± 0.08^{a}	61.12 ± 0.23^{b}	61.90 ± 0.24^{c}
IAA/TAA [4]	0.47 ± 0.00	0.46 ± 0.00	0.47 ± 0.00	0.48 ± 0.00	0.47 ± 0.00	0.46 ± 0.00

619 Data are presented as means ± SEM (n = 3). Values in the same row with different superscripts are significantly different ($P < 0.05$). FO, fish oil; KO,

620 krill oil; PO, palm oil; RO, rapeseed oil; SO, soybean oil; LO, linseed oil.

621 [1] IAA: indispensable amino acids.

622 [2] DAA: dispensable amino acids.

623 [3] TAA: total amino acids.

624 [4] IAA/TAA: the ratio of indispensable amino acids to total amino acids.

625

626 **Table 2.** Fatty acid composition (% total fatty acids) of muscle of juvenile swimming crab (*Portunus trituberculatus*) fed different dietary lipid sources.

Fatty acid	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
14:0	1.67 ± 0.07^{a}	1.85 ± 0.03^{a}	3.57 ± 0.05^{b}	1.60 ± 0.02^{a}	1.57 ± 0.02^{a}	1.66 ± 0.07^{a}
16:0	19.80 ± 0.55^{b}	18.91 ± 0.35^{ab}	20.67 ± 0.14^{c}	18.64 ± 0.30^{ab}	17.74 ± 0.52^{a}	17.34 ± 0.81^{a}
18:0	14.12 ± 0.16^{ab}	14.30 ± 0.45^{ab}	16.30 ± 0.24^{b}	13.40 ± 0.52^{a}	13.55 ± 0.70^{a}	14.85 ± 0.33^{ab}
20:0	0.66 ± 0.01^{b}	0.63 ± 0.01^{a}	0.68 ± 0.00^{b}	0.67 ± 0.01^{b}	0.69 ± 0.02^{b}	0.67 ± 0.01^{b}
22:0	0.57 ± 0.02	0.53 ± 0.02	0.53 ± 0.01	0.56 ± 0.01	0.55 ± 0.02	0.56 ± 0.02
24:0	0.41 ± 0.02	0.40 ± 0.03	0.40 ± 0.02	0.42 ± 0.01	0.40 ± 0.01	0.39 ± 0.01
SFA [1]	37.23 ± 0.65^{b}	36.62 ± 0.72^{b}	42.15 ± 0.33^{c}	35.29 ± 0.79^{a}	34.50 ± 0.59^{a}	35.47 ± 0.77^{a}
16:1n-7	1.13 ± 0.15^{c}	1.04 ± 0.04^{c}	0.90 ± 0.04^{b}	0.62 ± 0.03^{a}	0.73 ± 0.03^{ab}	0.71 ± 0.01^{ab}
18:1n-9	17.33 ± 0.47^{a}	17.79 ± 0.48^{a}	17.23 ± 0.29^{a}	22.83 ± 0.70^{b}	17.05 ± 0.57^{a}	16.52 ± 0.32^{a}
20:1n-9	1.49 ± 0.03^{b}	1.41 ± 0.02^{ab}	1.51 ± 0.03^{b}	1.48 ± 0.04^{b}	1.32 ± 0.02^{a}	1.36 ± 0.03^{a}
22:1n-9	0.13 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.14 ± 0.02	0.09 ± 0.01	0.10 ± 0.00
MUFA [2]	20.08 ± 0.33^{a}	20.34 ± 0.48^{a}	19.74 ± 0.27^{a}	25.07 ± 0.41^{b}	19.19 ± 0.31^{a}	18.69 ± 0.31^{a}
18:2n-6	16.72 ± 0.36^{a}	18.25 ± 0.18^{b}	17.83 ± 0.27^{ab}	18.61 ± 0.32^{b}	22.64 ± 0.09^{c}	19.55 ± 0.42^{b}
20:2n-6	1.65 ± 0.06^{a}	2.19 ± 0.20^{b}	1.75 ± 0.14^{a}	2.51 ± 0.11^{b}	2.79 ± 0.21^{b}	2.58 ± 0.18^{b}
20:3n-6	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
20:4n-6	1.63 ± 0.09^{a}	2.25 ± 0.17^{b}	1.80 ± 0.13^{a}	2.54 ± 0.14^{b}	2.95 ± 0.14^{b}	2.66 ± 0.14^{b}

22:5n-6	0.16 ± 0.02	0.13 ± 0.01	0.15 ± 0.01	0.14 ± 0.01	0.13 ± 0.01	0.14 ± 0.02
n-6 PUFA [3]	20.21 ± 0.44^{a}	22.88 ± 0.18^{ab}	21.59 ± 0.18^{ab}	23.85 ± 0.21^{b}	28.57 ± 0.08^{c}	24.98 ± 0.57^{b}
18:3n-3	0.92 ± 0.06^{a}	1.20 ± 0.06^{a}	1.10 ± 0.02^{a}	1.38 ± 0.09^{a}	1.32 ± 0.11^{a}	4.51 ± 0.33^{b}
18:4n-3	0.52 ± 0.01	0.49 ± 0.01	0.47 ± 0.02	0.50 ± 0.02	0.51 ± 0.03	0.48 ± 0.03
20:3n-3	0.36 ± 0.02^{a}	0.36 ± 0.01^{a}	0.35 ± 0.01^{a}	0.36 ± 0.02^{a}	0.41 ± 0.03^{a}	0.75 ± 0.04^{b}
20:5n-3	8.33 ± 0.05^{b}	7.96 ± 0.09^{b}	5.45 ± 0.08^{a}	5.56 ± 0.24^{a}	5.74 ± 0.12^{a}	5.87 ± 0.32^{a}
22:5n-3	0.52 ± 0.06	0.57 ± 0.04	0.54 ± 0.02	0.55 ± 0.05	0.54 ± 0.05	0.53 ± 0.05
22:6n-3	8.78 ± 0.25^{b}	8.98 ± 0.15^{b}	5.47 ± 0.14^{a}	5.38 ± 0.22^{a}	5.64 ± 0.13^{a}	5.74 ± 0.23^{a}
n-3 PUFA [4]	19.43 ± 0.27^{c}	19.56 ± 0.34^{c}	13.38 ± 0.13^{a}	13.73 ± 0.49^{a}	14.16 ± 0.24^{a}	17.88 ± 0.20^{b}
n-3 LC-PUFA [5]	17.99 ± 0.25^{b}	17.87 ± 0.38^{b}	11.81 ± 0.12^{a}	11.85 ± 0.55^{a}	11.79 ± 0.27^{a}	12.89 ± 0.39^{a}
n-3 PUFA/n-6 PUFA [6]	0.96 ± 0.02^{d}	0.85 ± 0.02^{c}	0.62 ± 0.01^{ab}	0.58 ± 0.03^{ab}	0.50 ± 0.01^{a}	0.72 ± 0.01^{b}

Data are presented as means ± SEM (n = 3). Values in the same row with different superscripts are significantly different ($P < 0.05$). Some fatty acids, found in only trace amounts or not detected, such as 8:0, 12:0, 13:0, 15:0, 14:1n-7, 18:3n-6 and 20:5n-6 were not listed in Table. 2. FO, fish oil; KO, krill oil; PO, palm oil; RO, rapeseed oil; SO, soybean oil; LO, linseed oil.

[1] SFA: saturated fatty acids.

[2] MUFA: mono-unsaturated fatty acids.

[3] n-6 PUFA: n-6 polyunsaturated fatty acids.

[4] n-3 PUFA: n-3 polyunsaturated fatty acids.

[5] n-3 LC-PUFA: n-3 long chain poly-unsaturated fatty acid.

[6] n-3 PUFA/n-6 PUFA: the ratio of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.

636 **Table 3.** Volatile compounds identified in muscle of juvenile swimming crab fed different dietary lipid sources.

Volatile compound	RI [1]	Identification [2]
Aldehydes (11)		
3-Methylbutanal	655	MS, S, RI
Hexanal	802	MS, S, RI
Heptanal	903	MS, S, RI
Benzaldehyde	962	MS, S, RI
Benzeneacetaldehyde	1048	MS, S, RI
Nonanal	1106	MS, S, RI
Decanal	1207	MS, S, RI
Dodecanal	1403	MS, S, RI
Tetradecanal	1604	MS, RI
Pentadecanal	1702	MS, RI
Hexadecanal	1821	MS, S, RI
Ketones (8)		
2,3-Pentanedione	696	MS, RI
2-Heptanone	887	MS, S, RI
2,3-Octanedione	985	MS, RI
2-Nonanone	1094	MS, S, RI

(3*E*,5*E*)-Octadiene-2-one	1097	MS, RI
2-Decanone	1196	MS, S, RI
2-Undecanone	1298	MS, S, RI
6,10-Dimethyl-(5E,9)-Undecadien-2-one	1460	MS, RI
Esters (2)		
Acetic acid butyl ester	820	MS, RI
Dibutyl phthalate	1453	MS, RI
Alcohols (9)		
3-Methyl-1-butanol	730	MS, S, RI
1-Butanol	870	MS, S, RI
1-Octen-3-ol	977	MS, S, RI
2-Ethyl-1-hexanol	1030	MS, RI
1-Octanol	1062	MS, S, RI
3-Decanol	1198	MS, S, RI
3-Undecanol	1297	MS, S, RI
1-Hexadecanol	1489	MS, S, RI
2-Hexyl-decan-1-ol	1501	MS, RI
Alkenes (2)		
1,3-Cyclooctadiene	1075	MS, RI

(7Z)-Hexadecene	1473	MS, RI
Alkanes (8)		
Undecane	1100	MS, S, RI
Pentylcyclohexane	1134	MS, S, RI
Dodecane	1200	MS, S, RI
Tridecane	1300	MS, S, RI
Tetradecane	1400	MS, S, RI
Octadecane	1800	MS, S, RI
Nonadecane	1900	MS, S, RI
Pentacosane	2500	MS, S, RI
Aromatics (3)		
4-Methylphenol	1070	MS, S, RI
2-Methyl-naphthalene	1288	MS, RI
Butylated hydroxytoluene	1510	MS, S, RI
Amines (3)		
Trimethylamine	566	MS, RI
Octodrine	1921	MS, RI
Amphetamine	1120	MS, RI
Other (3)		

Dimethyl sulfide	520	MS, RI
2-Methylpyrazine	803	MS, S, RI
2-Acetylthiazole	1028	MS, RI

[1] RI = retention indices calculated.

[2] Identification based on RI (retention indices), S (standard) and MS (mass spectrometry). MS, mass spectrum comparison using NIST14.L mass spectral libraries (https://www.sisweb.com/manuals/nist.htm).

641 **Table 4.** Relative concentration (ng/g) of volatile compounds in muscle of juvenile swimming crab fed different dietary lipid sources.

Volatile compound	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
Aldehydes (11)						
3-Methylbutanal	110.47 ± 10.42^{c}	153.71 ± 6.66^{d}	59.29 ± 4.59^{b}	40.75 ± 4.42^{a}	32.07 ± 4.09^{a}	37.95 ± 2.17^{a}
Hexanal	49.76 ± 1.75^{a}	82.19 ± 7.12^{b}	33.45 ± 5.75^{a}	31.05 ± 2.59^{a}	40.29 ± 1.96^{a}	36.21 ± 2.44^{a}
Heptanal	81.95 ± 1.80^{b}	93.88 ± 6.62^{b}	37.36 ± 3.16^{a}	35.42 ± 1.73^{a}	45.64 ± 4.39^{a}	43.10 ± 4.12^{a}
Benzaldehyde	62.32 ± 5.80^{b}	81.18 ± 9.19^{c}	31.43 ± 3.56^{a}	30.36 ± 4.12^{a}	34.94 ± 5.82^{a}	37.27 ± 3.51^{a}
Benzeneacetaldehyde	20.01 ± 2.89^{b}	14.18 ± 2.06^{ab}	10.63 ± 1.40^{a}	13.69 ± 3.85^{ab}	26.33 ± 2.99^{c}	28.64 ± 2.13^{c}
Nonanal	197.38 ± 22.08^{c}	196.36 ± 11.73^{c}	107.82 ± 6.04^{b}	72.97 ± 9.63^{ab}	36.54 ± 4.84^{a}	34.13 ± 3.92^{a}
Decanal	12.51 ± 3.58^{a}	12.12 ± 2.62^{a}	17.79 ± 2.40^{ab}	11.52 ± 1.86^{a}	23.51 ± 2.70^{b}	22.23 ± 1.73^{b}
Dodecanal	12.75 ± 4.49^{a}	10.37 ± 1.04^{a}	9.84 ± 1.50^{a}	13.74 ± 1.44^{a}	19.09 ± 3.06^{ab}	27.18 ± 3.09^{b}
Tetradecanal	7.92 ± 1.78^{b}	8.71 ± 0.58^{b}	10.76 ± 1.35^{b}	2.50 ± 0.88^{a}	7.92 ± 1.34^{b}	18.29 ± 3.01^{c}
Pentadecanal	6.20 ± 2.80^{a}	7.40 ± 1.02^{a}	6.75 ± 1.23^{a}	4.14 ± 1.08^{a}	9.51 ± 1.46^{a}	19.83 ± 2.52^{b}
Hexadecanal	3.82 ± 1.27^{a}	6.07 ± 1.10^{a}	5.82 ± 0.35^{a}	4.36 ± 1.25^{a}	8.37 ± 1.44^{a}	20.67 ± 2.83^{b}
Total	565.07 ± 10.77^{c}	666.16 ± 17.06^{d}	330.92 ± 6.51^{b}	260.50 ± 11.07^{a}	284.21 ± 8.85^{ab}	325.49 ± 4.08^{b}
Ketones (8)						

2,3-Pentanedione	54.53 ± 7.84^{b}	98.61 ± 10.91^{c}	43.85 ± 2.66^{ab}	35.27 ± 7.88^{ab}	20.08 ± 2.79^{a}	27.37 ± 1.90^{ab}
2-Heptanone	23.58 ± 5.69^{ab}	28.44 ± 5.64^{b}	11.07 ± 2.11^{a}	17.91 ± 3.73^{ab}	21.96 ± 3.97^{ab}	23.51 ± 2.72^{ab}
2,3-Octanedione	35.47 ± 9.33	34.71 ± 10.85	20.48 ± 4.34	32.99 ± 3.16	30.42 ± 4.74	26.47 ± 3.15
2-Nonanone	18.24 ± 3.27^{b}	10.91 ± 1.61^{ab}	6.91 ± 2.27^{a}	11.32 ± 1.20^{ab}	17.78 ± 2.59^{b}	14.37 ± 1.58^{ab}
(3*E*,5*E*)-Octadiene-2-one	52.73 ± 4.95^{c}	30.76 ± 4.95^{b}	22.87 ± 5.68^{ab}	17.42 ± 2.48^{a}	16.11 ± 2.67^{a}	18.00 ± 4.04^{a}
2-Decanone	5.01 ± 1.69^{a}	6.40 ± 1.84^{a}	11.93 ± 1.91^{b}	9.26 ± 1.36^{b}	16.90 ± 2.06^{c}	15.56 ± 2.94^{c}
2-Undecanone	6.41 ± 1.39^{ab}	4.89 ± 0.45^{a}	6.85 ± 2.75^{ab}	8.99 ± 1.73^{ab}	12.73 ± 2.56^{b}	12.16 ± 2.92^{b}
6,10-Dimethyl-(5E,9)-Undecadien-2-one	7.98 ± 1.32^{a}	9.45 ± 1.23^{a}	7.48 ± 2.02^{a}	9.02 ± 0.45^{a}	14.57 ± 2.03^{b}	8.11 ± 2.33^{a}
Total	203.95 ± 14.08^{b}	224.18 ± 10.19^{b}	131.45 ± 2.31^{a}	142.18 ± 10.55^{a}	150.54 ± 6.25^{a}	145.56 ± 8.98^{a}
Esters (2)						
Acetic acid butyl ester	17.81 ± 4.19^{a}	14.97 ± 1.61^{a}	17.43 ± 3.78^{a}	18.47 ± 2.41^{a}	28.74 ± 2.25^{b}	22.17 ± 3.66^{ab}
Dibutyl phthalate	9.50 ± 2.15^{ab}	4.35 ± 0.74^{a}	10.60 ± 1.65^{ab}	14.75 ± 3.77^{b}	27.11 ± 2.65^{c}	28.82 ± 2.54^{c}
Total	27.30 ± 5.85^{b}	19.33 ± 0.89^{a}	28.02 ± 5.02^{b}	33.21 ± 4.90^{b}	55.85 ± 2.08^{c}	50.99 ± 6.20^{c}
Alcohols (9)						
3-Methyl-1-butanol	31.84 ± 3.06	45.01 ± 4.10	39.11 ± 5.63	31.94 ± 6.57	35.95 ± 7.00	28.70 ± 3.67
1-Butanol	22.05 ± 3.18^{a}	20.63 ± 1.22^{a}	32.52 ±5.70^{b}	32.20 ± 4.33^{b}	27.90 ± 2.36^{ab}	25.18 ± 1.85^{ab}
1-Octen-3-ol	94.49 ± 8.02^{c}	95.60 ± 10.35^{c}	57.84 ± 5.33^{ab}	68.10 ± 6.84^{b}	50.05 ± 2.33^{ab}	32.07± 4.08^{a}
2-Ethyl-1-hexanol	4.07 ± 0.65^{a}	1.00 ± 0.26^{a}	6.60 ± 2.31^{a}	16.25 ± 1.61^{b}	31.79 ± 2.95^{c}	17.61 ± 1.82^{b}
1-Octanol	19.44 ± 7.16^{a}	18.81 ± 4.42^{a}	27.83 ± 3.99^{ab}	34.50 ± 2.94^{b}	30.84 ± 3.60^{ab}	24.91 ± 1.60^{ab}

3-Decanol	17.06 ± 7.00^{ab}	9.24 ± 0.85^{a}	8.93 ± 1.18^{a}	17.39 ± 5.40^{ab}	29.54 ± 3.62^{b}	17.51 ± 1.05^{ab}
3-Undecanol	6.22 ± 1.95^{ab}	1.55 ± 0.30^{a}	7.59 ± 1.73^{ab}	9.89 ± 0.88^{b}	19.66 ± 4.34^{c}	19.11 ± 1.06^{c}
1-Hexadecanol	46.27 ± 5.39^{b}	33.63 ± 3.82^{ab}	39.10 ± 2.89^{ab}	44.84 ± 3.71^{b}	38.99 ± 2.64^{ab}	24.23 ± 1.98^{a}
2-Hexyl-decan-1-ol	1.86 ± 0.67^{a}	5.44 ± 2.15^{ab}	5.57 ± 1.67^{ab}	8.72 ± 0.62^{b}	20.11 ± 5.18^{c}	24.32 ± 2.11^{c}
Total	243.31 ± 31.73^{ab}	230.90 ± 13.29^{a}	225.09 ± 6.95^{a}	263.82 ± 3.45^{ab}	284.83 ± 12.62^{b}	213.63 ± 2.64^{a}
Alkenes (2)						
1,3-Cyclooctadiene	8.47 ± 3.93^{a}	12.29 ± 2.70^{ab}	10.53 ± 3.34^{ab}	10.00 ± 0.60^{ab}	13.94 ± 2.58^{ab}	18.59 ± 1.71^{b}
(7Z)-Hexadecene	4.26 ± 1.66^{a}	7.32 ± 1.13^{ab}	10.91 ± 4.13^{b}	7.11 ± 1.52^{ab}	12.92 ± 2.43^{b}	21.27 ± 1.58^{c}
Total	12.73 ± 5.55^{a}	19.61 ± 3.80^{ab}	21.44 ± 5.21^{ab}	17.11 ± 1.51^{ab}	26.86 ± 4.07^{b}	39.86 ± 3.28^{c}
Alkanes (8)						
Undecane	2.66 ± 0.73^{a}	5.32 ± 1.64^{b}	4.43 ± 0.79^{b}	13.68 ± 2.77^{bc}	15.55 ± 2.59^{c}	19.42 ± 2.32^{c}
Pentylcyclohexane	3.46 ± 1.53^{a}	3.01 ± 0.67^{a}	5.42 ± 1.78^{ab}	6.36 ± 1.16^{ab}	8.93 ± 2.52^{b}	19.38 ± 1.32^{c}
Dodecane	5.06 ± 2.29^{a}	7.61 ± 1.23^{ab}	9.23 ± 2.72^{ab}	3.94 ± 1.51^{a}	9.80 ± 1.38^{ab}	15.18 ± 2.41^{b}
Tridecane	3.59 ± 1.20^{a}	4.43 ± 0.79^{a}	5.72 ± 1.10^{a}	7.32 ± 0.37^{a}	12.96 ± 1.98^{b}	13.33 ± 2.56^{b}
Tetradecane	3.82 ± 1.00^{a}	2.72 ± 0.23^{a}	6.18 ± 1.22^{ab}	9.16 ± 1.47^{b}	7.86 ± 2.19^{ab}	10.95 ± 1.87^{b}
Octadecane	3.91 ± 0.71^{a}	4.18 ± 0.60^{a}	8.12 ± 1.04^{ab}	9.33 ± 1.06^{b}	8.22 ± 2.28^{ab}	10.22 ± 1.37^{b}
Nonadecane	6.32 ± 1.49^{a}	7.12 ± 1.61^{a}	9.56 ± 1.51^{a}	6.18 ± 1.65^{a}	16.17 ± 2.40^{b}	15.92 ± 2.47^{b}
Pentacosane	6.78 ± 1.22^{a}	6.83 ± 1.96^{a}	9.26 ± 1.50^{ab}	12.12 ± 1.36^{ab}	16.99 ± 2.84^{b}	13.30 ± 2.40^{ab}
Total	35.59 ± 6.73^{a}	41.22 ± 3.96^{a}	57.90 ± 6.31^{b}	68.09 ± 5.32^{b}	96.48 ± 14.60^{c}	117.68 ± 6.72^{d}

Aromatics (3)						
4-Methylphenol	13.29 ± 2.11^{ab}	7.29 ± 2.29^{a}	15.69 ± 4.70^{ab}	16.12 ± 1.61^{b}	25.35 ± 3.35^{c}	21.45 ± 2.04^{bc}
2-Methyl-naphthalene	6.33 ± 0.61^{a}	4.77 ± 1.16^{a}	25.15 ± 1.93^{c}	13.50 ± 2.67^{b}	15.53 ± 2.97^{b}	19.63 ± 0.86^{bc}
Butylated hydroxytoluene	28.33 ± 4.15^{c}	7.41 ± 0.95^{a}	26.03 ± 3.06^{c}	24.37 ± 2.55^{bc}	13.56 ± 1.70^{ab}	19.96 ± 1.36^{b}
Total	47.96 ± 5.64^{b}	19.47 ± 3.53^{a}	66.86 ± 4.65^{c}	54.00 ± 3.51^{bc}	54.44 ± 4.56^{bc}	61.04 ± 0.37^{bc}
Amines (3)						
Trimethylamine	5.55 ± 1.91^{a}	4.44 ± 0.87^{a}	6.74 ± 1.33^{ab}	9.79 ± 2.34^{ab}	7.30 ± 1.40^{ab}	11.38 ± 3.29^{b}
Octodrine	4.86 ± 2.14^{a}	4.02 ± 1.99^{a}	10.39 ± 1.81^{b}	10.05 ± 1.04^{b}	7.95 ± 1.68^{ab}	8.81 ± 1.11^{ab}
Amphetamine	6.76 ± 1.34^{a}	3.35 ± 0.99^{a}	9.55 ± 2.16^{b}	14.88 ± 0.87^{c}	10.63 ± 0.99^{b}	10.56 ± 1.92^{b}
Total	17.17 ± 5.16^{ab}	11.81 ± 3.56^{a}	26.69 ± 4.66^{bc}	34.71 ± 2.61^{c}	25.88 ± 1.47^{bc}	30.74 ± 2.08^{c}
Other (3)						
Dimethyl sulfide	18.76 ± 3.65^{b}	9.05 ± 2.85^{a}	7.50 ± 2.26^{a}	6.36 ± 2.07^{a}	15.45 ± 2.87^{ab}	13.46 ± 3.41^{ab}
2-Methylpyrazine	23.90 ± 2.36^{c}	7.63 ± 3.34^{ab}	3.91 ± 1.53^{a}	16.02 ± 2.00^{bc}	13.51 ± 3.02^{b}	17.56 ± 1.54^{bc}
2-Acetylthiazole	14.26 ± 2.00^{bc}	5.65 ± 2.09^{a}	10.21 ± 1.87^{ab}	19.01 ± 2.38^{c}	8.61 ± 1.05^{ab}	13.99 ± 3.33^{bc}
Total	56.91 ± 7.90^{b}	22.33 ± 8.17^{a}	21.63 ± 5.57^{a}	41.39 ± 6.42^{ab}	37.58 ± 5.21^{ab}	45.01 ± 6.80^{b}

642 Data are presented as means ± SEM (n = 3). Values in the same row with different superscripts are significantly different ($P < 0.05$).

643 FO, fish oil; KO, krill oil; PO, palm oil; RO, rapeseed oil; SO, soybean oil; LO, linseed oil.

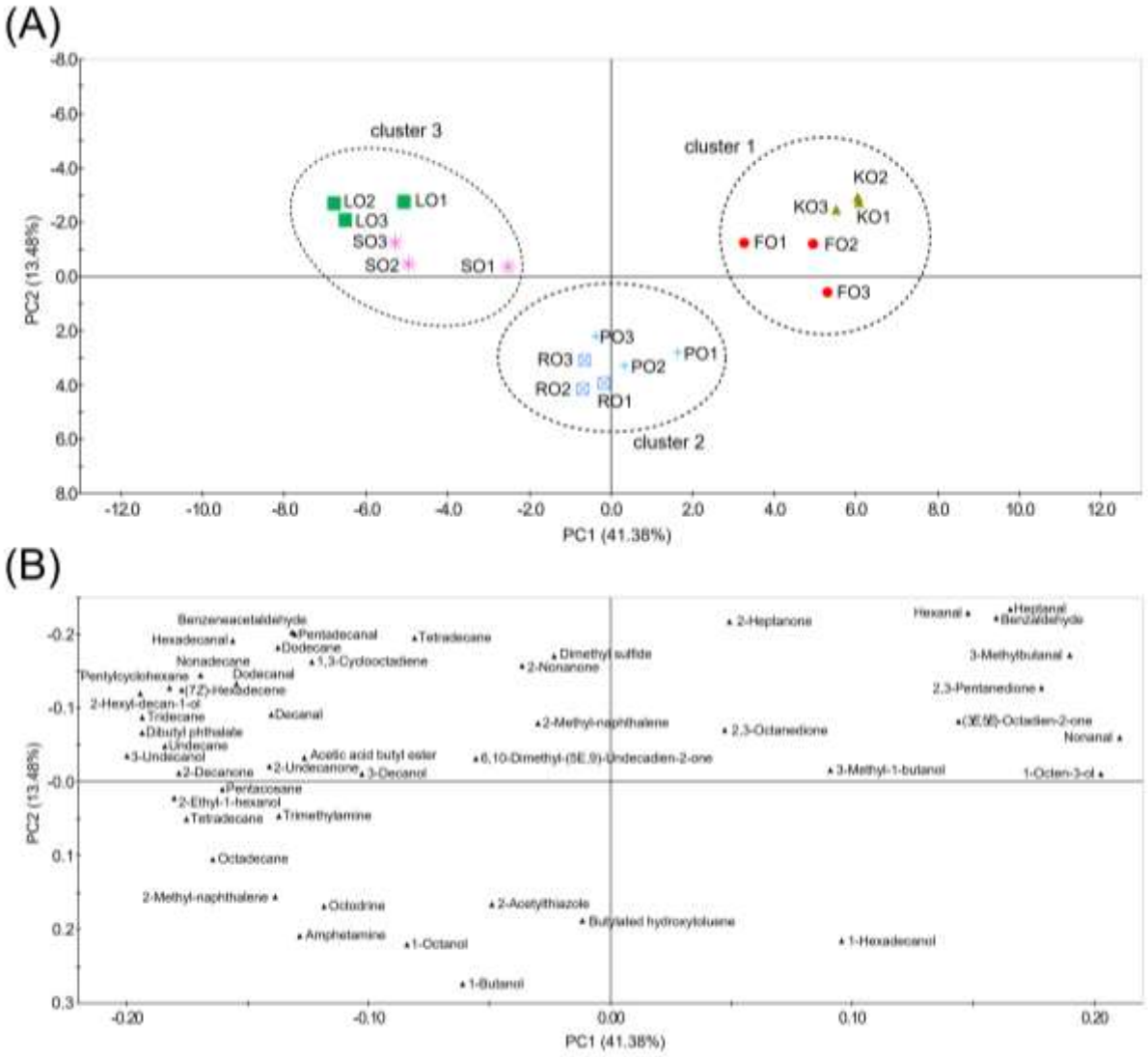

Fig 1. Principal component analysis (PCA) score plot (A) and loading plot (B) based on volatile compound compositions of juvenile swimming crab muscle fed different dietary lipid sources.

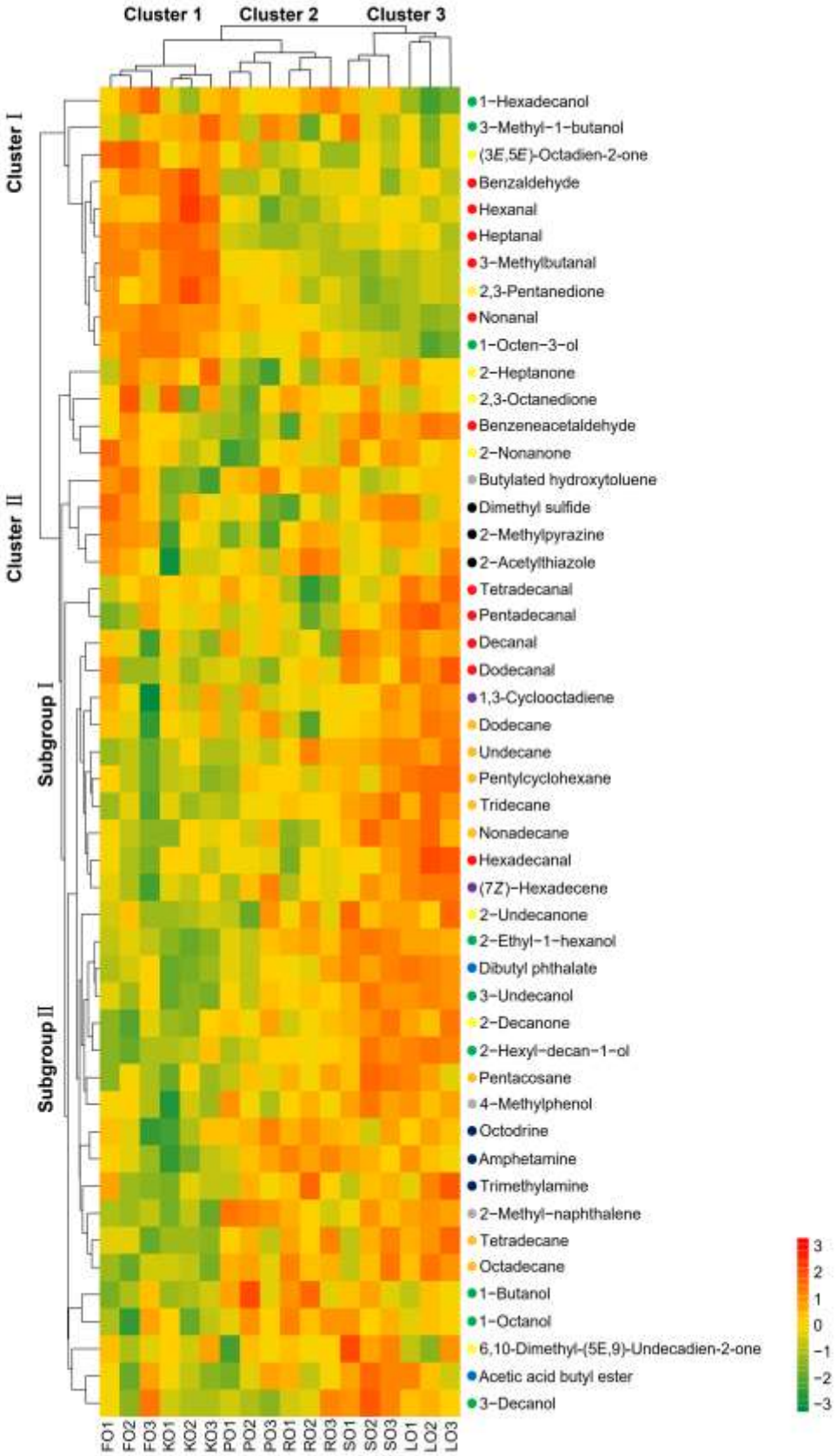

Fig 2. Hierarchical cluster analysis (HCA) and heat map visualization of samples and volatile compounds of muscle of juvenile swimming crab fed different dietary lipid sources. The color box for each compound in the heatmap indicates the abundance of the compound and represent the fold-change according to the scale on the right: red for higher levels; green for lower levels. The

scale in the color bar is logarithm to base 2 of the ratio of the respective abundances to the average abundance of the compounds in the six treatments. Color spots before the compound names indicates the chemical family of each compound: red, aldehydes; yellow, ketone; blue, ester; green, alcohol; purple, alkene; orange, alkane; grey, aromatic; dark blue, amine; black, other.

SUPPLEMENTARY TABLE 1

Formulation and proximate composition of experimental diets (dry matter basis)

Ingredients (g/kg)	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
Fishmeal [1]	150.0	150.0	150.0	150.0	150.0	150.0
Soybean protein concentrate [1]	260.0	260.0	260.0	260.0	260.0	260.0
Soybean meal [1]	200.0	200.0	200.0	200.0	200.0	200.0
Krill meal [1]	30.0	30.0	30.0	30.0	30.0	30.0
Wheat flour [1]	235.0	235.0	235.0	235.0	235.0	235.0
Fish oil [2]	20.0					
Krill oil [2]		20.0				
Palm oil [2]			20.0			
Rapeseed oil [2]				20.0		
Soybean oil [2]					20.0	
Linseed oil [2]						20.0
Soybean lecithin [3]	30.0	30.0	30.0	30.0	30.0	30.0
Vitamin premix [4]	10.0	10.0	10.0	10.0	10.0	10.0
Mineral premix [5]	15.0	15.0	15.0	15.0	15.0	15.0
$Ca(H_2PO_4)_2$	15.0	15.0	15.0	15.0	15.0	15.0
Choline chloride	3.0	3.0	3.0	3.0	3.0	3.0
Sodium alginate	32.0	32.0	32.0	32.0	32.0	32.0
Proximate composition (g/kg)						
Crude protein	464.2	467.0	466.1	465.2	466.8	467.0
Crude lipid	78.0	78.1	77.9	77.9	78.4	78.0
Moisture	125.9	129.3	126.1	120.4	127.6	129.0
Ash	95.4	96.6	95.4	96.0	95.9	95.4

[1] Fishmeal (dry matter, g/kg): crude protein 734.8, crude lipid 125.4; Soybean protein concentrate (dry matter, g/kg): crude protein 681.2, crude lipid 4.3; Soybean meal (dry matter, g/kg): crude protein 535.7, crude lipid 16.2; Krill meal (dry matter, g/kg): crude protein 650.3, crude lipid 65.1; Wheat flour (dry matter, g/kg): crude protein 153.2, crude lipid 7.9. These ingredients were purchased from Ningbo Tech-Bank Feed Co., Ltd. (Ningbo, China).

[2] FO (fatty acids, % TFA): SFA 31.91, MUFA 27.63, 18:2n-6 4.15, 18:3n-3 1.13, EPA 11.21, DHA 11.64; KO (fatty acids, % TFA): SFA 35.29, MUFA 22.37, 18:2n-6 4.42, 18:3n-3 1.98, EPA 17.26, DHA 12.64; PO (fatty acids, % TFA): 16:0, 39.48, SFA 45.75, MUFA 37.18, 18:2n-6 15.46, 18:3n-3 0.34; RO (fatty acids, % TFA): SFA 9.11, 18:1n-9, 57.42, 18:2n-6 20.17, 18:3n-3 8.58; SO (fatty acids, % TFA): SFA 15.98, MUFA, 29.54, 18:2n-6 47.55, 18:3n-3 5.34; LO (fatty acids, % TFA): SFA 12.43, MUFA, 20.68, 18:2n-6 16.47, 18:3n-3 48.81; Fish oil, krill oil, palm oil, and linseed oil were purchased from Ningbo Tech-Bank Feed Co., Ltd. (Ningbo, China), Kangjing Marine Biotechnology Co., Ltd. (Qingdao, China), Longwei grain oil industry Co., Ltd. (Tianjin, China) and Longshang farm agricultural development Co., Ltd. (Gansu, China), respectively. Rapeseed oil and soybean oil both obtained from Yihai Kerry Co., Ltd. (Shanghai, China).

[3] Soybean lecithin was purchased from Ningbo Tech-Bank Feed Co., Ltd. Ningbo, China. Acetone insoluble ≥ 60%; Acid value ≤ 35 mg KOH/g; Ether-insoluble matter ≤ 1%.

[4] Vitamin premix supplied the diet with (g/kg premix): retinyl acetate, 2,500,000 IU; cholecalciferol, 500,000 IU; all-rac-a-tocopherol, 25,000 IU; menadione, 5.63; thiamine, 11.25; riboflavin, 9.5; ascorbic acid, 95; pyridoxine hydrochloride, 10; cyanocobalamin, 0.02; folic acid, 2; biotin, 0.375; nicotinic acid, 37.5; D-Ca pantothenate, 21.5; inositol, 80; antioxidant, 0.5; corn starch, 696.775.

[5] Mineral mixture (g/kg premix): $FeC_6H_5O_7$, 4.57; $ZnSO_4 \cdot 7H_2O$, 9.43; $MnSO_4 \cdot H_2O$ (99%), 4.14; $CuSO_4 \cdot 5H_2O$ (99%), 6.61; $MgSO_4 \cdot 7H_2O$ (99%), 238.97; KH_2PO_4, 233.2; NaH_2PO_4, 137.03; $C_6H_{10}CaO_6 \cdot 5H_2O$ (98%), 34.09; $CoCl_2 \cdot 6H_2O$ (99%), 1.36.

SUPPLEMENTARY TABLE 2

Fatty acid composition (% total fatty acids) of the experimental diets

Fatty acid	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
14:0	6.58	8.54	2.76	1.37	1.68	1.72
16:0	18.47	22.78	29.42	10.27	12.84	11.20
18:0	5.42	4.37	4.86	2.43	4.56	5.31
20:0	0.52	0.42	0.64	0.54	0.61	0.56
22:0	0.24	0.25	0.38	0.44	0.43	0.49
24:0	0.31	0.22	0.39	0.37	0.42	0.30
SFA [1]	31.54	36.58	38.45	15.42	20.54	19.58
16:1n-7	4.13	3.87	2.87	2.04	2.31	1.99
18:1n-9	22.44	16.44	24.16	38.45	18.51	17.02
20:1n-9	1.78	0.59	0.95	1.24	0.49	0.54
22:1n-9	0.37	0.57	0.22	0.34	0.36	0.47
MUFA [2]	28.72	21.47	28.2	42.07	21.67	20.02
18:2n-6	13.34	15.31	19.47	24.52	42.93	21.56
20:2n-6	0.84	0.50	0.27	0.22	0.32	0.42
20:3n-6	0.26	0.28	0.29	0.24	0.31	0.28
20:4n-6	0.96	0.43	0.43	0.73	0.51	0.69
22:5n-6	0.12	0.08	0.12	0.14	0.16	0.15
n-6 PUFA [3]	15.52	16.60	20.58	25.85	44.23	23.10
18:3n-3	4.39	5.84	4.65	6.47	5.80	27.73
18:4n-3	0.88	1.12	0.51	0.54	0.47	0.54
20:3n-3	0.24	0.35	0.22	0.27	0.16	0.15
20:5n-3	7.94	8.71	3.23	3.41	3.26	3.85
22:5n-3	0.42	0.35	0.18	0.16	0.13	0.24
22:6n-3	7.42	7.54	3.19	3.29	3.18	3.69
n-3 PUFA [4]	21.29	23.91	11.98	14.14	13.00	36.20
n-3 LC-PUFA [5]	16.02	16.95	6.82	7.13	6.73	7.93

n-3 PUFA/n-6 PUFA [6]	1.37	1.44	0.58	0.55	0.29	1.57

Some fatty acids, of which the contents are minor, trace amount or not detected, such as 8:0, 12:0, 13:0, 15:0, 14:1n-7, 18:3n-6 and 20:5n-6 were not listed in the Supplementary Table 2. FO, fish oil; KO, krill oil; PO, palm oil; RO, rapeseed oil; SO, soybean oil; LO, linseed oil.

[1] SFA, saturated fatty acids.

[2] MUFA, mono-unsaturated fatty acids.

[3] n-6 PUFA, n-6 polyunsaturated fatty acids.

[4] n-3 PUFA, n-3 polyunsaturated fatty acids.

[5] n-3 LC-PUFA, n-3 long chain poly-unsaturated fatty acid.

[6] n-3 PUFA/n-6 PUFA: the ratio of n-3 polyunsaturated fatty acids to n-6 polyunsaturated fatty acids.

SUPPLEMENTARY TABLE 3

Proximate composition (% wet weight) in muscle of juvenile swimming crab fed different dietary lipid sources (n=3)

Parameter	Dietary lipid sources					
	FO	KO	PO	RO	SO	LO
Moisture	79.38 ± 0.30	79.30 ± 0.28	79.74 ± 0.35	79.78 ± 0.43	79.42 ± 0.33	79.51 ± 0.30
Protein	16.72 ± 0.04^{c}	16.78 ± 0.07^{c}	16.31 ± 0.05^{a}	16.33 ± 0.05^{a}	16.44 ± 0.08^{b}	16.50 ± 0.11^{b}
Lipid	0.69 ± 0.04^{b}	0.68 ± 0.02^{b}	0.64 ± 0.05^{b}	0.57 ± 0.03^{a}	0.83 ± 0.05^{c}	0.64 ± 0.00^{b}
Ash	3.21 ± 0.04	3.24 ± 0.03	3.31 ± 0.05	3.32 ± 0.04	3.31 ± 0.04	3.25 ± 0.03

Data are presented as the mean ± SEM (n = 3). Values in the same line with different superscripts are significantly different ($P < 0.05$). FO, fish oil; KO, krill oil; PO, palm oil; RO, rapeseed oil; SO, soybean oil; LO, linseed oil.