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# SHORT COMMUNICATION

# Effects of low dietary fish meal on the volatile compounds in muscle of large yellow croaker *Larimichthys crocea*

**Hua Mu[1,*], Zehong Wei[1,*], Lina Yi[1], Jikang Shentu[2], Wenbing Zhang[1] & Kangsen Mai[1]**

[1]The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture; The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, China

[2]Ningbo Ocean and Fisheries Research Institute, Ningbo, China

**Correspondence:** W Zhang, The Key Laboratory of Aquaculture Nutrition and Feeds, Ministry of Agriculture; The Key Laboratory of Mariculture, Ministry of Education; Ocean University of China, Qingdao 266003, China. E-mail: wzhang@ouc.edu.cn

*These authors contributed equally to this work.

## Introduction

Volatile profiles, which are responsible for the overall aroma of fish products, can contribute to positive sensory attributes and also produce 'off-flavours'. These compounds in raw fish can be generated using many processes such as microbial enzymatic breakdown and lipid degradation (enzymatic and oxidative) (Kawai 1996). The volatiles that conduce to the aroma of fish can be determined and used as quality indicators (Leduc, Tournayre, Kondjoyan, Mercier, Malle, Kol, Berdagué & Duflos 2012). Headspace solid-phase microextraction (HS-SPME) commonly used in determination of volatile compounds is an extraction technique that integrates sampling, isolation and concentration in one step (Ouyang & Pawliszyn 2008). In recent years, the technique of HS-SPME combined with gas chromatography–mass spectrometry (GC-MS) has been widely used in flavour analysis of aquatic products (Zimba, Schrader, Hyldig, Strobel & Jörgensen 2012; Zhou, Han, Zhu, Yang, Jin & Xie 2015).

Large yellow croaker *Larimichthys crocea* (LYC) is a highly appreciated and famous local mariculture fish species in China. With 148 616 metric tons of production in 2015 (China Fishery Statistical Yearbook 2016), it is now the first major mariculture fish species in China. Compared with the wild, however, farmed LYC showed the fattier body, whiter skin colour, softer muscle, higher fishy odour and odour intensity and lower taste feeling. These changes result in lower market price and poorer consumer acceptability of farmed LYC. Frank, Poole, Kirchhoff and Forde (2009) had found that the wild barramundi *Lates calcarifer* was more intensively associated with prawn odour, while the farmed barramundi was more strongly related to fishy and earthy odour. Furthermore, it has been reported that the volatile profiles can be changed by rearing conditions, diets, handling conditions, storage methods and fish size (Hallier, Prost & Serot 2005; Giogios, Grigorakis & Kalogeropoulos 2013; Moreira, Soares, Valente, Castro-Cunha, Cunha & de Pinho 2014). In respect of the dietary factors, for example, it has been found that dietary protein sources significantly affected the perception of the typical odour and colour (white) of gilthead sea bream (*Sparus aurata*) muscle (Matos, Gonçalves, Bandarra, Colen, Nunes, Valente, Dinis & Dias 2012). Previous studies also concluded that partial inclusion of meat meal and vegetable oils in diets slightly changed the flavour scores of organoleptic evaluation of barramundi and gilthead sea bream respectively (Williams, Paterson, Barlow, Ford & Roberts 2003; Izquierdo, Montero, Robaina, Caballero, Rosenlund & Ginés 2005).

Fish meal (FM) was used as the main protein source in compound aquafeed for mariculture fish. However, increasing demand, uncertain availability and high price of FM with the expansion of aquaculture made it essential to search alternative

protein sources. During the past decade, a great deal of researches has been devoted to reduce the need for FM in aquafeeds, by means of using alternative protein sources including plant proteins (e.g. soya bean meal, peanut meal) and animal proteins (e.g. meat and bone meal) (Moreira *et al.* 2014). Replacement of FM in diets for LYC has been studied with respect to the growth, digestive enzyme activity and digestive tract histology (Zhang, Zhang, Mai & Sun 2012). However, no information is available on the effects of feeding plant proteins or reducing dietary FM content on muscle quality (e.g. volatile compounds and texture) of LYC.

Given all of above, the aims of this study were to evaluate possible effects of low dietary FM content on volatile compounds in muscle of LYC, and to compare the differences in volatiles between the flesh of wild and farmed LYC. Moreover, to screen the potential and sensitive volatile indicators in muscle, the volatile profile characteristics of LYC muscle samples were statistically interpreted by principle component analysis (PCA).

## Materials and methods

### Feeding trial

*Experimental diets*

Two isonitrogenous (44% crude protein) and isolipidic (12% crude lipid) diets were formulated to contain two levels of FM (44% and 25% respectively). The diet with 44% of FM was named as CF. The other diet was named as LF. Ingredients and proximate composition of the experimental diets are given in Table 1.

*Feeding trial*

The feeding trial was carried out in Xiangshan of Ningbo, Zhejiang Province, China. Large yellow croakers were obtained from a local commercial hatchery. At the beginning of the feeding trial, fish were fasted for 24 h and weighed. Fish of similar size (189.87 ± 0.89 g) were randomly distributed into six sea cages (1.5 × 1.5 × 2 m) at a density of 38 fish per cage. Each diet was assigned to triplicate cages. Fish were hand-fed to apparent satiation twice (05:00 and 17:30) daily. The feeding trial lasted for 82 days.

### Sample collecting

At the end of the feeding trial, fish were not fed for 24 h. Total number and weight of fish in each cage were determined. The final body weights of LYC in the CF and LF group were found as 267.47 ± 5.88 g and 251.46 ± 3.92 g respectively. Two fish per cage were randomly selected, packed in polythene bags, transported in insulated and sealed boxes containing enough ice packs to laboratory. Within 24 h, the fish were manually dissected, eviscerated, beheaded, peeled, filleted and washed with tap water as soon as arrival at the laboratory. The left boneless fillet of each fish was used to determined volatiles. After being

**Table 1** Formulation and proximate compositions of the experimental diets (% dry matter)

| Ingredients | Control diet (CF) | Low fish meal diet (LF) |
|---|---|---|
| Fish meal* | 44 | 25 |
| Soya bean meal* | 0 | 25 |
| Wheat meal* | 35 | 26 |
| Fish oil | 4.5 | 6 |
| Soya bean lecithin | 2.5 | 2.5 |
| Vitamin premix† | 2 | 2 |
| Mineral premix‡ | 2 | 2 |
| Choline chloride | 0.2 | 0.2 |
| Attractant§ | 1.5 | 1.5 |
| Mould inhibitor¶ | 0.1 | 0.1 |
| Ethoxyquine | 0.05 | 0.05 |
| Amino acid premix | 5.14 | 6.5 |
| Microcrystalline cellulose | 3.01 | 3.15 |
| Proximate analyses | | |
| Moisture | 5.33 | 5.32 |
| Crude lipid | 12.76 | 12.82 |
| Crude protein | 44.40 | 43.56 |

*Fish meal, obtained from Qingdao Great Seven Bio-Tech Co., Ltd. Shandong Province, China: crude protein, 74.31% dry matter, crude lipid, 8.98% dry matter; soya bean meal, obtained from Qingdao Great Seven Bio-Tech Co., Ltd. Shandong Province, China, crude protein, 57.40% dry matter, crude lipid, 1.70% dry matter; wheat meal, obtained from Qingdao Great Seven Bio-Tech Co., Ltd. Shandong Province, China, crude protein, 17.39% dry matter, crude lipid, 1.47% dry matter.

†Vitamin premix (mg $kg^{-1}$ or g $kg^{-1}$ diet): thiamine, 25 mg; riboflavin, 45 mg; pyridoxine HCl, 20 mg; vitamin $B_{12}$, 10 mg; vitamin $K_3$, 10 mg; inositol, 800 mg; pantothenic acid, 60 mg; niacin acid, 200 mg; folic acid, 20 mg; biotin, 60 mg; retinol acetate, 32 mg; cholecalciferol, 5 mg; α-tocopherol, 240 mg; ascorbic acid, 2000 mg; wheat middling, 16.473 g.

‡Mineral premix (mg $kg^{-1}$ or g $kg^{-1}$ diet): $Na_2SeO_3$, 20 mg; $Ca(IO_3)_2{\cdot}6H_2O$, 60 mg; $CoCl_2{\cdot}6H_2O$, 50 mg; $CuSO_4{\cdot}5H_2O$, 10 mg; $FeSO_4{\cdot}H_2O$, 80 mg; $ZnSO_4{\cdot}H_2O$, 50 mg; $MnSO_4{\cdot}H_2O$, 45 mg; $MgSO_4{\cdot}7H_2O$, 1200 mg; $Ca(H_2PO_4)_2{\cdot}H_2O$, 10 g; NaCl, 100 mg; Microcrystalline cellulose, 8.485 g.

§Attractant: glycine and betaine (1:2).

¶Mould inhibitor: contained 50% calcium propionic acid and 50% fumaric acid.


 

processed, each muscle sample was packed in polythene bags and stored at −20°C prior to further analysis.

Four wild LYC (296.15 ± 6.55 g) were obtained at Xiangshan bay of Ningbo, Zhejiang Province, China, and were performed in the same process with the farmed LYC, including package, transport and other treatments in laboratory.

### Chemicals

Propanal, 3-methyl-butanal, pentanal, decanal, undecanal, dodecanal, 3-methyl-1-butanol, 1-pentanol, 1-hexanol, 1-undecanol, acetone, 2,3-pentanedione, cyclohexanone, 2-undecanone, decane, undecane, dodecane, *p*-xylene, *o*-xylene, *m*-xylene, hexadecanoic acid, octadecanoic acid and 2,4,6-trimethyl-pyridine standards were purchased from Sigma-Aldrich. All reagents used in this work were of analytical reagent grade.

### Analysis of volatile compounds

#### *Sample preparation*

Frozen fillets were thawed at 4°C just before analysis. The fillets were minced and mixed respectively. Five grams of fillets and 0.25 g of sodium chloride were weighted and placed into a 15-mL headspace vial. Then, 33 μL of an aqueous solution of 91.82 μg $mL^{-1}$ of 2,4,6-trimethyl-pyridine was added as an internal standard (IS). Headspace vial was sealed with a screw-cap fitted with a septum.

#### *Headspace solid-phase microextraction*

The manual HS-SPME device (Supelco Inc., Bellefonte, PA, USA) was used for extraction of volatiles from the fish muscle. It is equipped with a 50/30 μm divinyl benzene–carboxen–polydimethylsiloxane fibre (DVB/CAR/PDMS; Supelco Inc.) which was conditioned in the GC injection port prior to use according to the manufacturer's instructions. The sample in vial was equilibrated at 60°C for 20 min followed by HS-SPME exposure at the same temperature. The volatile profiles in the headspace were absorbed onto SPME fibres for 40 min.

#### *GC-MS analysis of volatile compounds*

After the headspace collection of volatiles, GC-MS analysis was performed with a GCMS-QP2010 (Shimadzu, Japan), which was equipped with a Rxi-1MS capillary column (30 m × 0.25 mm id, 0.25 μm film thickness; Shimadzu, Japan). HS-SPME fibres were thermally desorbed into the GC injector for 5 min. The injector temperature was set at 250°C, and injection was performed in splitless mode. The carrier gas was helium, at a constant flow of 1.5 mL $min^{-1}$. The oven temperature was held at 35°C for 3 min and then programmed at 10°C $min^{-1}$ to 200°C, and when reached, increased to 260°C at 20°C $min^{-1}$ and maintained at 260°C for 8 min. The mass spectrometer was conducted in the electron impact mode with a source temperature of 230°C and an ionizing voltage of 70 eV. The spectra acquisition was performed in scanning mode with a scan range from $m/z$ 30 to 500 amu.

Volatile compounds were identified by comparing their retention times with those of authentic compounds analysed under the same conditions. The comparison of MS fragmentation pattern with those of pure compounds and mass spectra database search were performed using the National Institute of Standards and Technology (NIST) MS08 spectral database, considering similarity values higher than 80%. Semiquantitative determinations were performed using 2,4,6-trimethyl-pyridine as an internal standard. The concentrations of volatiles in the samples were calculated in μg $g^{-1}$ muscle by comparing the peak area of each compound with that of the internal standard.

#### *Statistical analysis*

The differences in the volatile profiles in muscle among the wild and the two groups of farmed LYC were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range test. The level of significance was chosen at $P < 0.05$ and data are expressed as mean values ($n = 4$) accompanied by the standard errors of means. Above data analyses were performed using SPSS 17.0 for windows.

The volatile profiles in the fillets were also analysed using PCA to understand the similarities and dissimilarities among variables. The analysis was carried out using the software of SIMCA-P 11.5 (Umetrics AB, Malmö, Sweden).

## Results and discussion

Table 2 lists the 42 volatiles detected in the muscle of LYC, in which, 40, 39 and 28 volatile compounds were found in the LF group, CF group and wild group respectively. All these volatiles can be divided into eight groups, including alcohols,

aldehydes, ketones, hydrocarbons, aromatics, acids, esters and miscellaneous compounds. Aldehydes, hydrocarbons and aromatics were the major volatiles, while the amounts of acids and miscellaneous compounds were the minors. Most of the identified compounds, in the present study, have already been reported in several fish species, such as Senegalese sole (*Solea senegalensis* Kaup, 1858) and turbot (*Psetta maxima*) (Moreira *et al.* 2014; Xu, Liu, Jiang, Zhang, Li, Zhu & Li 2014).

**Volatile compounds in muscle of farmed LYC**

*Aldehydes*

In total, nine aldehydes were identified in muscle of farmed LYC (Table 2). Fish fed the LF diet had significant lower concentrations of propanal, hexanal and nonanal than that in the CF group ($P < 0.05$). There were no significant differences in the levels of heptanal, benzaldehyde, octanal, decanal, undecanal and dodecanal between the two farmed LYC groups ($P > 0.05$). Previous study in Senegalese sole showed that the decrease in dietary FM content from 51.5% to 5.5% significantly decreased the amounts of decanal, (E)-2-octenal and benzaldehyde in muscle (Moreira *et al.* 2014). In the present study, the aldehydes which made differences between the two farmed fish groups generally belong to *n*-alkanals. All *n*-alkanals are usually generated from polyunsaturated fatty acid (PUFA). Alkanals are more derived from n-6 or n-9 PUFA or monounsaturated fatty acid (MUFA). For example, the precursors of propanal in fish flesh are mainly linolenic acid (n-3 PUFA) (Varlet, Prost & Serot 2007). Most aldehydes have been identified as giving low odour threshold values. Hexanal was present at a higher content which was found above its odour threshold value (4.5–5 μg $kg^{-1}$ (Frank *et al.* 2009)) and could be considered as an odour-active compound in LYC muscle.

*Alcohols*

A total of six alcohols were detected in muscle of farmed LYC (Table 2). Among these alcohols, the levels of 1-penten-3-ol, (Z)-2-penten-1-ol and 1-pentanol were found significantly lower in the LF group than the CF group ($P < 0.05$). There were no significant differences in the amounts of 1-heptanol, 1-octanol and undecanol between the two farmed fish groups ($P > 0.05$) (Table 2). Moreira *et al.* (2014) indicated that the total replacement of FM by plant protein sources reduced the concentrations of 1-penten-3-ol and 1-octen-3-ol in Senegalese sole muscle. In general, volatile alcohols are minor contributors to food flavour due to their higher odour thresholds, unless they are present at high amounts or are unsaturated (Tanchotikul & Hsieh 1989). It was showed that the concentrations of 1-penten-3-ol, (Z)-2-penten-1-ol and 1-pentanol were relatively higher among alcohols identified in the muscle of LYC (Table 2). The 1-penten-3-ol has been reported to be produced from the oxidation of eicosapentaenoic acid (EPA) by 15-lipoxygenase and hydroperoxide lyases (Kawai 1996). It has been mentioned as the most distinct volatile characterizing gilthead sea bream, Senegalese sole (Grigorakis, Taylor & Alexis 2003; Silva, Valente, Castro-Cunha, Bacelar & de Pinho 2012). Plastic, green, solvent, vegetal, burnt and meaty are some odour descriptors used to define the aroma of 1-penten-3-ol in different literature studies (Girard & Durance 2000; Frank *et al.* 2009; Giri, Osako & Ohshima 2010). The (Z)-2-penten-1-ol with an odour of grilled hazel nut (Prost, Serot & Demaimay 1998) was suggested as a main volatile alcohol in sockeye and pink salmon and turbot (Prost *et al.* 1998; Girard & Durance 2000).

*Ketones*

Five and four ketones were identified in fillets of LYC fed the LF and CF diet respectively (Table 2). Compared with the fish fed the CF diet, the fish fed the LF diet had significant lower levels of 2-hexanone and 2,3-octanedione and higher content of cyclohexanone ($P < 0.05$). In the present study, cyclohexanone was detected only in the LF group. Moreira *et al.* (2014) reported that a total replacement of dietary FM with plant protein significantly decreased the concentration of 3-octanone in Senegalese sole muscle. There were no significant differences in the amounts of 6-methyl-5-hepten-2-one and 2-undecanone between the two farmed fish groups ($P > 0.05$) (Table 2). In previous study, low dietary FM content also had no significant effects on the content of 2-undecanone in flesh (Silva *et al.* 2012). The 2-undecanone with a relatively lower odour threshold value (5.5 μg $kg^{-1}$ (Giri *et al.* 2010)) has tallow, musty, fruity, floral and woody flavour (Tanchotikul & Hsieh 1989; Prost *et al.* 1998; Giri *et al.* 2010). It has been described as a main aroma component in turbot (Xu *et al.* 2014). Ketones may be produced by thermal oxidation/degradation of PUFA, amino acid degradation, microbial oxidation or Maillard

  

**Table 2** Volatile compounds (μg $g^{-1}$ muscle) in muscle of large yellow croaker including the farmed fish fed low fish meal diet (LF), farmed fish fed control diet (CF) and wild fish (WF), and odour descriptors of some volatiles given by the literature

| Code* | Volatile compounds | LF | CF | WF | Odour descriptors† |
|---|---|---|---|---|---|
| a | *Aldehydes (9)* | | | | |
| a1 | Propanal | 0.10 ± 0.04[a] | 0.32 ± 0.05[b] | 0.38 ± 0.07[b] | Acetaldehyde-like, pungent[G,H] |
| a2 | Hexanal | 0.35 ± 0.06[a] | 0.96 ± 0.11[b] | 1.03 ± 0.17[b] | Garlic, fresh, green, grassy, pungent, tallow, fat, green beans, fishy[A,C,E,G,H] |
| a3 | Heptanal | 0.09 ± 0.02[a] | 0.26 ± 0.05[a] | 0.99 ± 0.29[b] | Green, floral, fatty, pungent, fishy, dry fish, citrus fruit, nutty, chocolate[A,G,H,K,M] |
| a4 | Benzaldehyde | 0.44 ± 0.10[b] | 0.27 ± 0.12[ab] | nd[a] | Almond, fruity, creamy, nutty[F,G] |
| a5 | Octanal | 0.06 ± 0.01[a] | 0.11 ± 0.02[a] | 0.84 ± 0.08[b] | Sweet, orange, floral, spicy, citrus, green, fatty[A,G,J] |
| a6 | Nonanal | 0.14 ± 0.03[a] | 0.41 ± 0.05[b] | 0.41 ± 0.08[b] | Geranium, raw fish, plastic, marine, citrus, green, fatty[A,G,H,J] |
| a7 | Decanal | 0.11 ± 0.01[b] | 0.14 ± 0.03[b] | nd.[a] | Marine, cucumber, floral, fat, orange peel, tallow, green[E,J] |
| a8 | Undecanal | 0.02 ± 0.00[b] | 0.02 ± 0.00[b] | nd.[a] | Minty, fruity[L] |
| a9 | Dodecanal | 0.02 ± 0.00[b] | 0.03 ± 0.01[b] | nd.[a] | Lily, fat, citrus[E] |
| | Sub-total | 1.33 ± 0.12[a] | 2.51 ± 0.20[b] | 3.39 ± 0.30[c] | |
| b | *Alcohols (7)* | | | | |
| b1 | 3-methyl-1-Butanol | nd[a] | nd[a] | 0.19 ± 0.03[b] | Balsamic, bitter, chocolate[H,M] |
| b2 | 1-Penten-3-ol | 0.05 ± 0.01[a] | 0.17 ± 0.03[b] | 0.14 ± 0.04[ab] | Plastic, green, pungent, solvent, vegetal, burnt, meaty[A,D,G,H] |
| b3 | 1-Pentanol | 0.06 ± 0.00[a] | 0.19 ± 0.03[b] | 0.13 ± 0.04[ab] | Mushroom, earthy, green, wax[A,H] |
| b4 | (Z)-2-Penten-1-ol | 0.07 ± 0.00[a] | 0.22 ± 0.03[b] | 0.18 ± 0.04[b] | Grilled hazel nut[I] |
| b5 | 1-Heptanol | 0.02 ± 0.00[b] | 0.03 ± 0.01[b] | nd[a] | Green, savoury, fermented, fresh, nutty[A,C,H] |
| b6 | 1-Octanol | 0.01 ± 0.00[b] | 0.01 ± 0.00[b] | nd[a] | Fatty, green, floral[H,G] |
| b7 | Undecanol | 0.02 ± 0.00[b] | 0.03 ± 0.00[b] | nd[a] | |
| | Sub-total | 0.22 ± 0.01[a] | 0.66 ± 0.07[b] | 0.60 ± 0.04[b] | |
| c | *Ketones (6)* | | | | |
| c1 | 2,3-Pentanedione | nd.[a] | nd.[a] | 0.20 ± 0.06[b] | Sweet, buttery, caramel, fruity[G,J] |
| c2 | 2-Hexanone | 0.07 ± 0.01[a] | 0.26 ± 0.02[b] | 0.18 ± 0.04[b] | Green, fruity, floral[B] |
| c3 | Cyclohexanone | 0.22 ± 0.05[b] | nd.[a] | nd.[a] | |
| c4 | 2,3-Octanedione | 0.02 ± 0.00[a] | 0.05 ± 0.00[b] | 0.07 ± 0.01[b] | savoury, cooked[A] |
| c5 | 6-methyl-5-Hepten-2-one | 0.03 ± 0.00[b] | 0.02 ± 0.00[b] | nd.[a] | Citrus fruit, green, sweet, fruity[C,H] |
| c6 | 2-Undecanone | 0.01 ± 0.00[b] | 0.01 ± 0.00[b] | nd.[a] | Tallow, musty, fruity, floral, woody[F,H,I] |
| | Sub-total | 0.34 ± 0.05 | 0.33 ± 0.03 | 0.43 ± 0.04 | |
| d | *Hydrocarbons (10)* | | | | |
| d1 | Hexane | 0.26 ± 0.11[a] | 0.68 ± 0.20[ab] | 1.11 ± 0.26[b] | |
| d2 | Undecane | 0.02 ± 0.00[a] | 0.07 ± 0.03[ab] | 0.16 ± 0.05[b] | |
| d3 | Dodecane | 0.04 ± 0.00[a] | 0.23 ± 0.06[ab] | 0.29 ± 0.07[b] | Cheese[I] |
| d4 | 1-Tridecene | 0.01 ± 0.00[b] | 0.02 ± 0.00[b] | nd.[a] | |
| d5 | Tridecane | 0.03 ± 0.00 | 0.08 ± 0.01 | 0.10 ± 0.03 | |
| d6 | Tetradecane | 0.05 ± 0.01 | 0.10 ± 0.02 | 0.11 ± 0.04 | |
| d7 | Pentadecane | 0.29 ± 0.03[b] | 0.30 ± 0.06[b] | 0.05 ± 0.01[a] | |
| d8 | Hexadecane | 0.03 ± 0.01[b] | 0.03 ± 0.01[b] | nd.[a] | |
| d9 | Heptadecane | 0.21 ± 0.04[b] | 0.24 ± 0.07[b] | 0.02 ± 0.00[a] | |
| d10 | 2,6,10,14-Tetramethyl-Pentadecane | 0.04 ± 0.00[b] | 0.03 ± 0.01[ab] | 0.02 ± 0.00[a] | Floral, woody, green, cooked[F,L] |
| | Sub-total | 0.97 ± 0.09 | 1.78 ± 0.32 | 1.86 ± 0.37 | |
| e | *Aromatics (6)* | | | | |
| e1 | Toluene | 0.04 ± 0.01[a] | 0.32 ± 0.09[ab] | 0.40 ± 0.12[b] | Plastic[F] |
| e2 | Ethylbenzene | 0.12 ± 0.02[a] | 0.37 ± 0.11[a] | 0.95 ± 0.13[b] | Ethereal, floral, concrete-like[F,H] |
| e3 | Styrene | 0.09 ± 0.01[a] | 0.21 ± 0.05[ab] | 0.43 ± 0.14[b] | Plastic[F] |
| e4 | *p*-Xylene | 0.09 ± 0.01[a] | 0.22 ± 0.01[b] | 0.34 ± 0.05[c] | Pungent, phenolic[F] |
| e5 | *o*-Xylene | 0.12 ± 0.01[a] | 0.31 ± 0.09[ab] | 0.65 ± 0.19[b] | Geranium, oily, pungent[F,H] |
| e6 | *m*-Xylene | 0.02 ± 0.00[a] | 0.12 ± 0.04[ab] | 0.21 ± 0.07[b] | Phenolic, plastic[F,K] |
| | Sub-total | 0.49 ± 0.03[a] | 1.55 ± 0.15[b] | 2.40 ± 0.31[c] | |

**Table 2** (continued)

| Code* | Volatile compounds | LF | CF | WF | Odour descriptors† |
|---|---|---|---|---|---|
| f | *Acids (1)* | | | | |
| f1 | Hexadecanoic acid | $0.01 \pm 0.00^{ab}$ | $0.03 \pm 0.01^{b}$ | nd.[a] | Fresh, fruity[L] |
| | Sub-total | $0.01 \pm 0.00^{ab}$ | $0.03 \pm 0.01^{b}$ | nd.[a] | |
| g | *Esters (2)* | | | | |
| g1 | Butyl propanoate | $0.03 \pm 0.01^{b}$ | $0.03 \pm 0.01^{b}$ | nd.[a] | |
| g2 | Diethyl phthalate | $0.24 \pm 0.02^{b}$ | $0.62 \pm 0.08^{c}$ | $0.01 \pm 0.00^{a}$ | |
| | Sub-total | $0.27 \pm 0.02^{b}$ | $0.65 \pm 0.08^{c}$ | $0.01 \pm 0.00^{a}$ | |
| h | *Miscellaneous compounds (1)* | | | | |
| h1 | 1-methylene-1H-Indene | $0.05 \pm 0.01^{b}$ | $0.04 \pm 0.01^{ab}$ | $0.02 \pm 0.00^{a}$ | |
| | Total | $3.67 \pm 0.17^{a}$ | $7.55 \pm 0.24^{b}$ | $8.71 \pm 0.41^{c}$ | |

*Compound code as it appears in Table 3 and Figure 1A. nd.: not detected; tr.: traces (<0.01 μg $g^{-1}$muscle).

†Different superscript uppercase are literature citations, in which the odour descriptors of volatile compounds were described.

[A]Frank *et al.* (2009); [B]Grigorakis *et al.* (2003); [C]Hallier *et al.* (2005); [D]Kawai (1996); [E]Varlet *et al.* (2007); [F]Tanchotikul and Hsieh (1989); [G]Girard and Durance (2000); [H]Giri *et al.* (2010); [I]Prost *et al.* (1998); [J]Cayhan and Selli (2010); [K]Le Guen, Prost and Demaimay (2001); [L]Selli, Rannou, Prost, Robin and Serot (2006); [M]Cadwallader, Tan, Chen and Meyers (1995).

All the odour descriptors for each of volatile detected in large yellow croaker muscle were integrated aroma according to different published studies.

Data within the same row with different superscript lowercase letters are significantly ($P < 0.05$) different.

reaction (Josephson & Lindsay 1986; Alasalvar, Taylor & Shahidi 2005). In general, carbonyls (aldehydes and ketones) contribute to the overall fresh-like odours more than their corresponding alcohols because of their lower threshold values (Josephson & Lindsay 1986).

*Hydrocarbons*

Hydrocarbons including alkenes and alkanes detected in fillets of the two farmed fish groups both were 10 (Table 2). There were no significant differences in the levels of hydrocarbons between the CF and LF groups ($P > 0.05$). Similarly, the decrease in dietary FM content from 61% to 4% had no significant effect on the content of a certain alkene (limonene) in Senegalese sole muscle (Silva *et al.* 2012). Most alkanes identified in the present study also were detected in turbot (Xu *et al.* 2014).

*Aromatics*

Six aromatics were detected in fillets of the two farmed LYC groups (Table 2). Compared with the CF group, a significant lower content of *p*-xylene was found in the LF group ($P < 0.05$) (Table 2). Silva *et al.* (2012) found that the decrease in dietary FM content from 61% to 15% significantly increased the concentration of one aromatic (ethylbenzene) in Senegalese sole muscle. There were no significant differences in the levels of toluene, ethylbenzene, styrene, *o*-xylene and m-xylene in fillets between the two farmed fish groups ($P > 0.05$). Ethylbenzene has been described as having ethereal, floral and concrete-like aroma (Tanchotikul & Hsieh 1989; Giri *et al.* 2010). This compound also has been detected in flesh of various fish species, such as farmed meagre *Argyrosomus regius*, gilthead sea bream (Grigorakis *et al.* 2003; Giogios *et al.* 2013).

*Acids*

Only one acid was identified in fillets of the two groups of farmed LYC (Table 2). Although the fish fed the LF diet had a relatively lower content of hexadecanoic acid, the difference between these two farmed fish groups was not significant ($P > 0.05$). This acid also has been found in farmed meagre (Giogios *et al.* 2013).

*Esters*

Two kinds of esters were detected in fillets of the two groups of farmed LYC (Table 2). The content of diethyl phthalate was significantly lower in the LF group ($P < 0.05$). There was no significant difference in the concentration of butyl propanoate in fillets between the LF and CF groups ($P > 0.05$).

*Miscellaneous compounds*

There was only one kind of miscellaneous compound identified in muscle of farmed LYC (Table 2). The concentration of the 1-methylene-1H-indene had no significant difference between the two groups ($P > 0.05$).

  

**Comparison of volatiles in muscle between the farmed and wild LYC**

*Aldehydes*

In total, nine and five aldehydes were identified in fillets of the farmed and wild LYC respectively (Table 2). Benzaldehyde, decanal, undecanal and dodecanal were identified only in the two farmed fish groups. Compared with the wild LYC, farmed fish had significantly higher levels of decanal, undecanal and dodecanal and lower amounts of heptanal and octanal ($P < 0.05$) (Table 2). Heptanal and octanal were also found in muscle of various fish species, such as meagre, Senegalese sole (Giogios *et al.* 2013; Moreira *et al.* 2014). Heptanal has been considered as contributing to green, floral, fatty, pungent, fishy and dry fish aroma (Girard & Durance 2000; Frank *et al.* 2009; Giri *et al.* 2010). Octanal has been detected as having sweet, orange, floral, spicy, citrus odour (Girard & Durance 2000; Frank *et al.* 2009). Meanwhile, the levels of propanal, hexanal and nonanal were significantly lower, and the content of benzaldehyde was significantly higher in the LF group than the wild group ($P < 0.05$) (Table 2). Wild barramundi muscle also had a significantly lower content of benzaldehyde than that of the farmed fish (Frank *et al.* 2009), which is in agreement with the present results. However, no significant differences were found in the levels of propanal, hexanal, benzaldehyde and nonanal in the flesh between the CF and wild group ($P > 0.05$) (Table 2).

*Alcohols*

Six and four alcohols were found in fillets of the farmed and wild LYC respectively. The 3-methyl-1-butanol was identified only in the wild group (Table 2). Similar study showed that wild gilthead sea bream also had a relatively higher concentration of 3-methyl-1-butanol than that of the farmed one (Grigorakis *et al.* 2003). This alcohol with a low odour threshold value of 4 μg $kg^{-1}$ has been characterized by a balsamic aroma (Giri *et al.* 2010). The 1-heptanol, 1-octanol and undecanol were only detected in the farmed LYC (Table 2). Meanwhile, the wild LYC had a significantly higher content of (Z)-2-penten-1-ol ($P < 0.05$). It is in agreement with the result observed in wild and farmed turbot (Prost *et al.* 1998). The 1-octanol has been considered as having fatty and green aroma (Giri *et al.* 2010). However, no significant differences were obtained in the content of (Z)-2-penten-1-ol in muscle between the fish fed the CF diet and the wild fish, as well as the levels of 1-penten-3-ol and 1-pentanol between the farmed and wild fish groups ($P > 0.05$) (Table 2).

*Ketones*

There were five, four and three ketones identified in fillets of the fish in the LF, CF and wild groups respectively (Table 2). The 2,3-pentanedione was identified only in the wild LYC. It was also found in the flesh of farmed and wild sea bream (Alasalvar *et al.* 2005) and was detected with a sweet and buttery odour in sockeye and pink salmon (Girard & Durance 2000). The 6-methyl-5-hepten-2-one and 2-undecanone were detected only in the farmed LYC. Meanwhile, the levels of 2-hexanone and 2,3-octanedione were significantly lower in the fish fed the LF diet than those in the wild group ($P < 0.05$) (Table 2). However, no significant differences were found in the levels of 2-hexanone and 2,3-octanedione between the CF and wild group ($P > 0.05$). The 2,3-octanedione has been detected in various fish species, such as sea bream, turbot (Xu *et al.* 2014; Parlapani, Verdos, Haroutounian & Boziaris 2015).

*Hydrocarbons*

In total, 10 and eight kinds of hydrocarbons were detected in muscle of farmed and wild LYC respectively (Table 2). The 1-tridecene and hexadecane were identified only in the two farmed fish groups. Meanwhile, the amounts of pentadecane and heptadecane were significantly higher in the two farmed fish groups than the wild one ($P < 0.05$) (Table 2). Similarly, compared with the wild barramundi, the farmed fish also had significantly higher concentrations of some alkenes in muscle, including (*E*,*E*)-2,4-octadiene, 1,4,9-decatriene (Frank *et al.* 2009). In addition, the wild group had significantly higher levels of hexane, undecane and dodecane, while lower content of 2,6,10,14-tetramethylpentadecane than the LF group ($P < 0.05$) (Table 2). However, no significant differences were observed in the amounts of above four hydrocarbons between the CF and wild groups ($P > 0.05$). And there were no significant differences in the levels of tridecane and tetradecane between the farmed and wild groups ($P > 0.05$) (Table 2). Prost *et al.* (1998) reported that wild and farmed turbot also had similar concentration of dodecane which was described as imparting a cheese aroma.

*Aromatics*

There were six aromatics identified both in muscle of farmed and wild LYC respectively (Table 2). The wild group had significantly higher levels of ethylbenzene and *p*-xylene ($P < 0.05$). In addition, the amounts of toluene, styrene, *o*-xylene and *m*-xylene were also significantly lower in the LF group than the wild group ($P < 0.05$). However, no significant differences were observed in the levels of these above four aromatics between the CF and wild groups ($P > 0.05$) (Table 2). Grigorakis *et al.* (2003) found that compared with farmed gilthead sea bream, wild fish had a higher content of *p*-xylene, while a lower concentration of styrene and *o*-xylene in the fillets.

*Acids*

Hexadecanoic acid identified only in the two farmed LYC (Table 2).

*Esters*

Two and one esters were detected in the farmed and wild LYC respectively (Table 2). In all the fish muscle, butyl propanoate was only detected in the farmed group. Meanwhile, the content of diethyl phthalate was significantly higher in the farmed fish than that of the wild one ($P < 0.05$).

*Miscellaneous compounds*

There was only one miscellaneous compound (i.e. 1-methylene-1H-Indene) was found in muscle of farmed and wild LYC (Table 2). The content of 1-methylene-1H-indene was significantly higher in the LF group than the wild group ($P < 0.05$). However, no significant differences were observed in the concentration of this compound in the muscle between the wild and CF groups ($P > 0.05$).

**Principal component analysis (PCA)**

A PCA was used for a simplified view of the relationship among the volatile profiles identified in the muscle of LYC. Three factors explained 78.5% of total variance. The representation of the variables and samples applying the first two PCs is shown in Figure 1a,b. The first principal component (PC1), which explained 51.0% of total variance, was positively correlated to ethylbenzene, *p*-xylene, 2,3-octanedione, etc., and negatively related to 6-methyl-5-hepten-2-one, undecanal, pentadecane, etc. The second principal component (PC2), which accounted for 19.9% of total variance, was positively related to 1-penten-3-ol, 1-pentanol, (Z)-2-penten-1-ol, etc., and negatively related to 3-methyl-1-butanol, octanal, 2,3-pentanedione, etc.

The PC score plot (Fig. 1b) shows that there were three separate groups of points, corresponding to different muscle samples of LYC. Obviously, PC1 separated all the fish muscle. The muscle of wild fish tended to place in the positive PC1, being characterized by higher levels of the associated variables. In contrast, all the farmed fish were located in the negative PC1, being characterized by higher amounts of the related variables. Consequently, muscle volatiles of the wild fish were separated from the farmed fish in Figure 1b, showing that they had different volatile profiles. Frank *et al.* (2009) also indicated that different flavour sensory attributes contributed to the separation between farmed barramundi and wild counterparts on PC1. However, it is obvious that compared with the fish fed the LF diet, the fish in the CF group were located closer to the wild fish in the score plot (Figure 1b). Therefore, there were more similar volatile profiles between the wild fish and the fish fed the CF diet. This further indicated that lower dietary FM content could affect the volatile compounds in the muscle of LYC. There are 14 compounds whose percentage accounted by PC1 was greater than 60.0% shown in Table 3. The top 10 important loadings were ethylbenzene, octanal, heptanal, *p*-xylene, 6-methyl-5-hepten-2-one, 3-methyl-1-butanol, *o*-xylene, 2,3-octanedione, undecanal and pentadecane. These volatiles contributed greatly to the differences in the characteristics of volatile profiles in the fillets of three fish groups and could be considered as potential and sensitive indicators which could differentiate the muscle volatile composition of LYC in the present study.

From the above results, it was found that PC1 discriminated the fillets of three different fish groups in the present study. After that, a new PCA model for each two muscle samples was separately conducted to discover potential and sensitive volatiles which could distinguish the muscle of every two fish groups. Figure 2 shows the score plots after PCA of different variables in the muscle of each two fish groups separately by PC1 and PC2. The corresponding loading plots were not given, but the most important loadings and the percentage accounted by PC1 after PCA are shown in Table 3.

Figure 2a shows the score plot of volatiles in muscle of the two farmed LYC groups. The first two principal components of the PCA explained 65.2% of total variance. PC1 explained 46.2% of

  

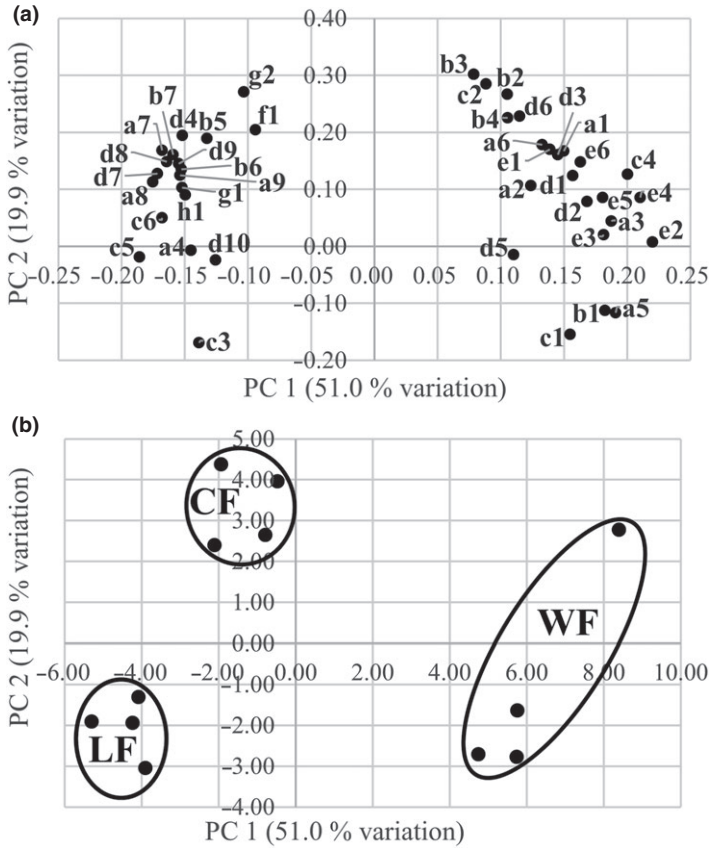


**Figure 1** Loading plot (a) for the principal component analysis carried out with the volatile compounds (code as indicated in Table 2) in all muscle samples of large yellow croaker, corresponding to the score plot in (b). LF, muscle of fish fed the low fish meal diet. CF, muscle of fish fed the control diet. WF, muscle of wild fish.

total variance, whereas PC2 accounted for 19.0%. It can be found from Figure 2a that PC1 separated the fillets of the two farmed fish groups. The CF group was placed in the positive PC1, while the LF group was located in the negative PC1 (Fig. 2a). Similarly, Moreira *et al.* (2014) observed a clear separation of muscle volatile composition of Senegalese sole in PCA between fish fed a diet contained 100% plant protein sources and fish fed a diet included 100% FM. There were 17 compounds whose percentage accounted by PC1 were greater than 60.0% (Table 3). The top 10 important loadings were *p*-xylene, 1-pentanol, propanal, 2,3-octanedione, tetradecane, hexanal, 1-penten-3-ol, cyclohexanone, diethyl phthalate and 2-hexanone. These volatiles contributed greatly to the differences in the characteristics of volatile profiles in muscle of the two groups of farmed fish and could be considered as the potential and sensitive indicators, which could differentiate the muscle of the CF-fed fish from the LF-fed fish. Most of these potential and sensitive volatiles were aldehydes, ketones and alcohols, which arose from lipoxygenase action on various fatty acids in fish muscle. Therefore, low dietary FM content could change the fatty acid composition and/or metabolism in fish fillets, which was suggested in rainbow trout by Timm-Heinrich, Eymard, Baron, Nielsen and Jacobsen (2013).

Figure 2b shows the score plot of volatiles in fillets of LYC fed the LF diet and the wild fish. The first two principal components of the PCA explained 85.4% of the total data variability. PC1 explained 74.1% of total variance, whereas PC2 accounted for 11.3%. In Figure 2b, PC1 separated the flesh of the two fish groups. The wild fish were situated in the positive zone of PC1, while in the negative part was the LF group. There were 37 compounds whose percentage explained by PC1 was greater than 60.0% in Table 3. The top 10 important loadings were decanal, dodecanal, diethyl phthalate, 1-tridecene, ethylbenzene, pentadecane, octanal, *p*-xylene, 2,3-octanedione and 1-heptanol. These compounds contributed greatly to the differences in the characteristics of volatile profiles in muscle of the two fish groups and could be considered as potential sensitive markers, which could differentiate the fillets of the wild fish from the fish fed the LF diet in the present study.

**Table 3** Results of the principal component analysis on the volatile compositions in different muscle samples of large yellow croaker showing the most important loadings and the percentage variance accounted for by the first principal components (PC1)

| All samples (PC1) | | LF versus CF (PC1) | |
|---|---|---|---|
| Compound code (14)* | $R^2_x$ (>60%) | Compound code (17)* | $R^2_x$ (>60%) |
| e2 | 0.850 | e4 | 0.884 |
| a5 | 0.816 | b3 | 0.872 |
| a3 | 0.786 | a1 | 0.846 |
| e4 | 0.777 | c4 | 0.838 |
| c5 | 0.772 | d6 | 0.818 |
| b1 | 0.745 | a2 | 0.785 |
| e5 | 0.729 | b2 | 0.775 |
| c4 | 0.705 | c3 | 0.762 |
| a8 | 0.687 | g2 | 0.758 |
| d7 | 0.659 | c2 | 0.755 |
| d2 | 0.633 | e6 | 0.735 |
| c6 | 0.631 | a6 | 0.724 |
| a7 | 0.630 | b4 | 0.696 |
| d8 | 0.603 | e3 | 0.661 |
| | | a3 | 0.657 |
| | | d3 | 0.639 |
| | | a5 | 0.631 |
| **CF versus WF (PC1)** | | **LF versus WF (PC1)** | |
| Compound code (19)* | $R^2_x$ (> 60%) | Compound code (37)* | $R^2_x$ (> 60%) |
| a5 | 0.914 | a7 | 0.948 |
| b6 | 0.855 | a9 | 0.944 |
| a8 | 0.831 | g2 | 0.942 |
| d4 | 0.820 | d4 | 0.896 |
| b7 | 0.812 | e2 | 0.888 |
| g2 | 0.801 | d7 | 0.859 |
| a7 | 0.800 | a5 | 0.851 |
| b1 | 0.792 | e4 | 0.848 |
| d7 | 0.783 | c4 | 0.847 |
| d8 | 0.766 | b5 | 0.844 |
| c5 | 0.739 | d8 | 0.837 |
| e2 | 0.732 | a8 | 0.833 |
| a9 | 0.709 | c5 | 0.833 |
| b5 | 0.703 | d9 | 0.830 |
| d9 | 0.669 | b7 | 0.821 |
| c6 | 0.654 | d10 | 0.815 |
| f1 | 0.612 | c6 | 0.799 |
| g1 | 0.609 | e5 | 0.793 |
| c1 | 0.605 | b1 | 0.792 |
| | | a3 | 0.759 |
| | | c3 | 0.758 |
| | | a1 | 0.738 |
| | | d3 | 0.736 |
| | | e1 | 0.735 |
| | | c2 | 0.729 |
| | | e6 | 0.728 |
| | | h1 | 0.711 |
| | | g1 | 0.705 |

**Table 3** (continued)

| CF versus WF (PC1) | | LF versus WF (PC1) | |
|---|---|---|---|
| Compound code (19)* | $R^2_x$ (> 60%) | Compound code (37)* | $R^2_x$ (> 60%) |
| | | d1 | 0.702 |
| | | a4 | 0.694 |
| | | d2 | 0.687 |
| | | a6 | 0.675 |
| | | b4 | 0.651 |
| | | a2 | 0.649 |
| | | b6 | 0.647 |
| | | b2 | 0.639 |
| | | e3 | 0.617 |

*Compound code as indicated in Table 2. $R^2_x$, variance explained. LF, muscle of fish fed the low fish meal diet. CF, muscle of fish fed the control diet. WF, muscle of wild fish.

The score plot of volatiles of the CF and wild group is shown in Figure 2c. The first two principal components of the PCA accounted for 67.2% of the total data variability. PC1 explained 47.3% of total variance, whereas PC2 accounted for 19.8%. Figure 2c shows that PC1 discriminated fillets of the two fish groups. The wild group was positioned in the positive PC1, while the CF group was placed in the negative PC1. Nineteen volatile compounds whose percentage explained by PC1 greater than 60.0% were obtained (Table 3). The top 10 important loadings were octanal, 1-octanol, undecanal, 1-tridecene, undecanol, diethyl phthalate, decanal, 3-methyl-1-butanol, pentadecane and hexadecane. These substances contributed greatly to the differences in the characteristics of volatile profiles in muscle of those two fish groups and could be deemed to be potential sensitive indicators, which could distinguish the muscle of the wild fish from the CF-fed fish. Many studies have shown that the contents of 20:4 (n-6), 20:5 (n-3), 22:6 (n-3), n-3 PUFA and the ratio of n-3 to n-6 PUFA were higher in wild fish muscle than that of the farmed one. Meanwhile, the farmed fish had higher levels of 18:2 (n-6) and n-6 PUFA in muscle than that of the wild one (Fuentes, Fernández-Segovia, Serra & Barat 2010; Lenas, Chatziantoniou, Nathanailides & Triantafillou 2011). These fatty acids, which were presented at different levels in the farmed and wild fish, were mostly the precursors of many volatile carbonyls and alcohols. This could lead to the different volatile profiles between the farmed and wild fish, which had been found in wild and farmed turbot by Prost *et al.* (1998).


 

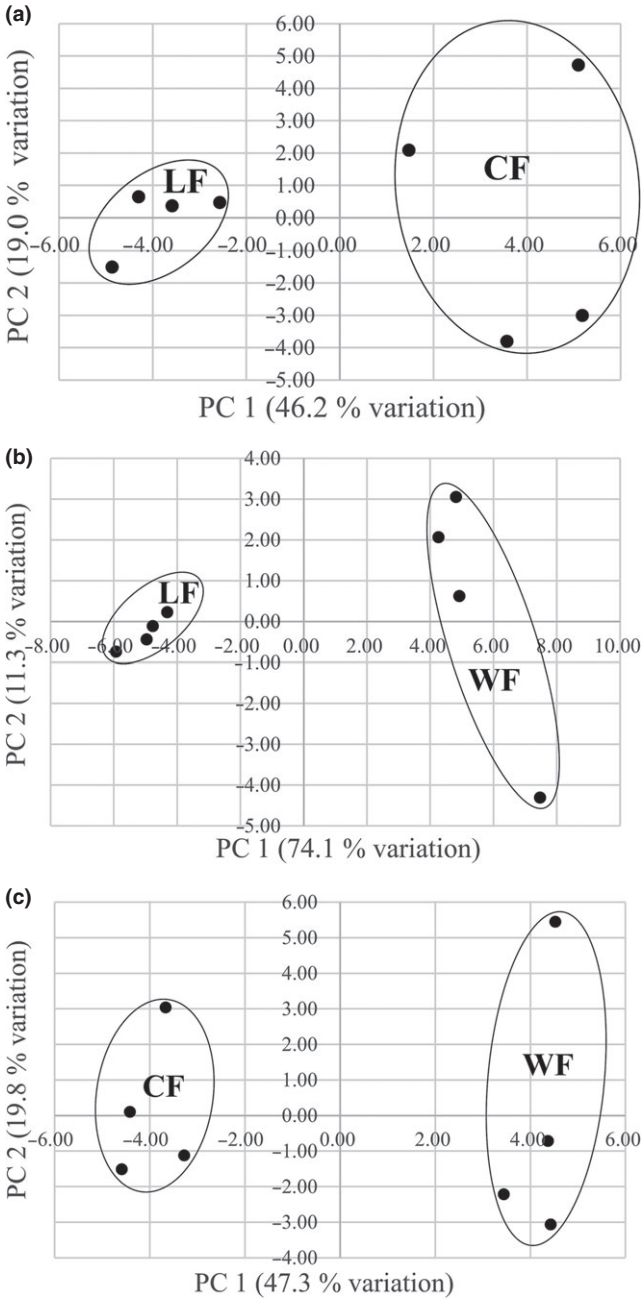


**Figure 2** Score plots for the principal component analysis carried out with the volatile compounds in muscle of large yellow croaker fed the low fish meal diet and control diet (a), large yellow croaker fed the low fish meal diet and wild fish (b), large yellow croaker fed the control diet and wild fish (c) respectively. LF, muscle of fish fed the low fish meal diet. CF, muscle of fish fed the control diet. WF, muscle of wild fish.

## Conclusion

In conclusion, 42 volatiles were detected in muscle of LYC. These volatiles were divided into eight groups, including nine aldehydes, seven alcohols, six ketones, 10 hydrocarbons, six aromatics, one acid, two esters and one miscellaneous compound. Fish in the LF group had significantly lower amounts of total aldehydes, alcohols, esters and aromatics in than the CF group. Compared with the wild fish, the farmed fish had significantly lower amounts of total aldehydes and aromatics, while significantly higher content of total esters. In addition, the fish fed the LF diet had significantly lower content of total alcohols, while significantly higher level of miscellaneous compound than the wild. According to the PCA, it was found that some volatiles could be considered as sensitive indicators to

classify muscle samples of LYC. Ethylbenzene, octanal, etc. were sensitive indicators for the muscle of three fish groups. *P*-xylene, 1-pentanol, etc. could distinguish the muscle of the two farmed fish groups. Sensitive indicators which could discriminate the muscle between the fish fed the LF diet and the wild fish were decanal, dodecanal, etc. Octanal, 1-octanol, etc. could differentiate the muscle of the fish fed the CF diet from the wild fish.

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**Keywords:** large yellow croaker, muscle, volatile compounds, principal component analysis, fish meal