LWT - Food Science and Technology 134 (2020) 110259

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of fatty acids on the flavor formation of fish sauce

Anzi Ding [a], Meng Zhu [a,b], Xiaoqing Qian [a,b], Liu Shi [a], Huang Huang [b], Guangquan Xiong [a], Jun Wang [b], Lan Wang [a,*]

[a] Institute of Agricultural Products Processing and Nuclear- Agricultural Technology, Hubei Academy of Agricultural Sciences / Farm Products Processing Research Subcenter of Hubei Innovation Center of Agriculture Science and Technology, Wuhan, 430064, China
[b] College of Biological Engineering and Food, Hubei University of Technology, Wuhan, 430068, China

ARTICLE INFO

Keywords:
Fish sauce
Fatty acid
Flavor
Correlation analysis
Oxidation pathway

ABSTRACT

An appropriate fish model helps track changes of lipid and fatty acid during fermentation. Here we selected three freshwater fish (*Culter alburnus, Carassius auratus* and *Ietalurus Punetaus*) as the fermentative materials of fish sauce due to their significantly different contents of lipid and unsaturated fatty acid. The electronic nose showed that the flavor profiles of sauce samples prepared with different fish were dramatically different. A total of 71 volatile compounds of fish sauce were detected by solid-phase microextraction-gas chromatography-mass spectrometry (SPME-GC/MS). Then nine key flavor compounds, including 3-methylbutanal, 3-methyl-1-butanol, 3-(methylthio)propanal, 1-octen-3-ol, phenylacetaldehyde, nonanal, dimethyl trisulfide, decanal and hexanol, were screened based on odor activity values. According to correlation analysis between fatty acid profiles and fish sauce aroma, possible pathways of oleic acid (C18:1) and linoleic acid (C18:2) oxidation were suggested: oleic acid undergone the auto-oxidation to mainly form 10- hydroperoxide and 11- hydroperoxide, and (or) secondary form 8-hydroperoxide, then converted to nonanal, octanal and decanal; linoleic acid undergone the enzymatic oxidation to form 10-L(S)-hydroperoxy-cis-9, trans-11-octadecadieuoic acid, then converted to 1-octen-3-ol.

1. Introduction

Fish sauce, a traditional fermented condiment, is popular in Southeast and East Asian countries due to its special aroma and taste (Yoshikawa et al., 2010). In China, fish sauce is mainly produced along the southeast coastline. The production processing of traditional fish sauce usually includes spontaneous fermentation of low-value aquatic species in the presence of high-concentration salt for at least 6 months. Therefore, the quality, especially flavor, of fish sauce is quite diverse due to different compositions of raw material, fermentation condition and fermentation process.

Flavor is an important indicator to evaluate the quality of fish sauce, which is formed by the degradation of proteins and lipids in fish via various biochemical metabolic pathways under synergistic actions among halophilic microorganisms and enzymes (Anupam Giri, Osako, Okamoto, & Ohshima, 2010). It has been reported that the evolution of volatile components, such as aldehydes, alcohols and ketones, largely depends on fatty acid oxidation and lipolysis during fermentation (Qian Chen, Kong, Han, Xia, & Xu, 2017; Yunzi Feng et al., 2013). In addition, aroma substances were remarkably influenced by lipid contents in raw materials (Y. Feng et al., 2014; Gambacorta et al., 2009; X.-L. Gao et al., 2010; Xu et al., 2018). Different fish species, such as Nile tilapia, anchovy, grass carp and eel, had been used as raw materials for fish sauce, and their aroma components were quite different (L. Chen et al., 2020; P. Gao, Li, Xia, Xu, & Liu, 2020; B. Wang, Hu, & Tong, 2020'). Microorganisms were also reported to play diverse roles during lipid production from fermentation (Hierro, de la Hoz, & Ordóñez, 1999; Lorenzo, Gómez, & Fonseca, 2014). Besides, the team of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences has focused their attention on the relationship between microbial succession and its metabolites and the flavor of fish sauce in recent years (Li et al., 2018; Y. Wang et al., 2019; Yueqi. Wang, Li, Yan, & Li, 2018; Y. Wang et al., 2020). However, little attention has been paid to the role of fatty acids in flavor fermentation of fish sauce.

Freshwater fish are abundant in China, with total output ranking first in the world for more than two decades. During the preliminary screening of raw materials for fish sauce fermentation, *Culter alburnus, Carassius auratus* and *Ietalurus Punetaus* were selected as the mode

* Corresponding author. Wuhan, 430064, China.
E-mail address: lilywang_2016@163.com (L. Wang).

https://doi.org/10.1016/j.lwt.2020.110259
Received 3 July 2020; Received in revised form 15 September 2020; Accepted 18 September 2020
Available online 23 September 2020

species due to large diversity among their lipid contents. Therefore, the objective of this study was to investigate the effect of lipid composition on the development of volatile compounds in fish sauce made from *Culter alburnus, Carassius auratus* and *Ietalurus Punetaus*, and explore the mechanism of flavor compound generation during fermentation. This study may serve as a new theory for the production of high-quality fish sauce.

2. Materials and methods

2.1. Materials and reagents

Fresh and living *Culter alburnus*, *Carassius auratus* and *Ietalurus Punetaus* were purchased from Baishazhou aquatic product market (Wuhan, China) in April 2018, and transported to the laboratory within 1 h. They were selected as raw materials in this study with an average weight of 50 ± 13 g, 500 ± 70 g and 1500 ± 240 g, respectively. After been sacrificed, scaled, gilled, gutted and rinsed, fish muscles were stored at −18 °C for 4 h and then ground with a JR130 frozen meat grinder (Shenyang Yishi Food Machinery Co., Ltd., Shenyang, China). Afterward, the ground fish was vacuum packaged with plastic bags and stored at −18 °C until further use.

Aspergillus oryzae (GIM 3.31) was obtained from Guangdong culture collection center (Guangzhou, China). Bran, salt and flour were purchased from a local market (Wuhan, China). 2-methyl-3-heptanone was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals and solvents were analytical grade and obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

2.2. Proximate and fatty acid composition determination

Moisture, ash and crude protein were determined according to standard AOAC methods (AOAC, 2006). To determine moisture content, 3.0 g of each sample was placed on an aluminum dish (55 × 35 mm, Shanghai Leigu Instrument Co., Ltd, Shanghai, China), spread evenly across the plate and oven-dried at 105 °C for 24 h. Crude protein was determined by the Kjeldahl method (Kjeltec Auto 1100 Analyzer, Shandong Hanon Instruments Co., Ltd., Dezhou, China). Ash content was determined by placing 3.0 g of each sample in a crucible and incineration in an SGM.s80/13T muffle furnace (Sigma High Temperature Technology Group Co., Ltd., Henan, China) at 550 °C for 24 h.

The lipid extraction was modified based on the methods of Folch, Lees, and Sloane (1957) and Wang (Yueqi. Wang et al., 2018). 5.0 g of ground fish muscles were placed in a 50 mL polypropylene centrifuge tube, with 15 mL chloroform/methanol (2:1, v/v) with 0.01 g/100g butylated hydroxytoluene (BHT) added. Then the tube was closed with a stopper and ground twice with a homogenizer at 10000 rpm for 15 s in an ice bath. The corresponding solvent was added to obtain a total volume of 30 mL and the mixture was filtered after 1 h standing. The filtrate with a 0.2-fold volume of 0.85 g/100g salines was centrifuged at 3000 r/min for 15min. The lipid solution in the bottom layer was dried with a nitrogen flow until a constant weight and the crude lipid was obtained.

$$\text{Crude lipid content (g/100g)} = (M\text{-}M_0)/m \times 100$$

Where M, M_0 and m stand for the total weight of crude lipid and centrifuge tube, the weight of empty centrifuge tube, and the weight of the fish muscles.

The extracted crude lipid was transferred into a 50 mL polypropylene centrifuge tube, with 0.5 mL methanol/NaOH (0.5 mol/L) and 2.5 mL hexane(chromatographic grade) added. The tube was closed with a stopper and shaken with a vortex mixer at 2500 rpm for 5 min and then centrifuged at 4000 r/min for 10 min. The transparent liquid in the top layer was extracted for analysis by GC (7890A, Agilent Technologies Co. Ltd., CA, USA).

Separation was performed with a DB-FFAP column (30 m × 0.25 μm, 0.25 μm, Agilent Technologies Co. Ltd., CA, USA). The volume of each sample was 2.0 μL. Helium was used as the carrier gas with a flow rate of 1.5 mL/min, and the split ratio was 1:20. Analytical conditions of GC were as follows: the initial temperature was 150 °C, ramped to 210 °C at 10 °C/min, holding for 7 min, and then raised to 230 °C at a rate of 20 °C/min, holding for 6 min. Injection temperature was 280 °C and the ion source temperature was set at 250 °C. Identification of fatty acids was carried out by comparing the retention time with standard compounds (Sigma-Aldrich Co., St. Louis, MO, USA). Undecanoic acid methyl ester was used as an internal standard to quantify the contents of the fatty acid methyl ester assuming the detector response to each fatty acid was identical.

2.3. Preparation of fish sauce samples

Fish sauce samples were prepared according to the methods by Anupam. Giri, Osako, and Ohshima (2010) with modifications. Mixture of bran, wheat flour and distilled water were blended with a ratio of 4:1:5 (w/w/w), sterilized at 121 °C for 15 min and cooled to 25 °C. *Aspergillus oryzae* (0.05 g/100g, w/w) was incubated in the above medium with a relative humidity of 90% at 30 °C for 44 h. During *koji* fermentation, the medium was remixed once at 16 h. After the incubation, the preparation of *koji* was completed when the color of culture turned to greenish-yellow. The ground fish muscles were thawn with running tap water, and thoroughly mixed with distilled water, *koji* and salt with a ratio of 1: 1.5: 0.5: 0.575 (w/w/w/w) in a 5 L beaker as the fermentation container. Then the beaker was sealed with two layers of PE film (33 ± 40 g/100g RH and 18500 ± 40 g/100g O_2GTR) to ensure that the loss rate of water was less than 5% during the whole fermentation process (30 °C, 180 d). Stirring (1000 r/min, 10 min) was performed once per day during the first 30 days, and twice per week afterward. After fermentation, the broth was first filtered with gauze (100 meshes) to obtain the raw fish sauce. Then the raw solution was boiled at 90 °C for 15 min, cooled to room temperature and centrifuged at 4000 r/min for 10 min. The clear liquid in the middle layer was collected and used as the fish sauce samples.

2.4. Electronic nose analysis

An electronic nose was used according to the methods by Zhang (Zhang et al., 2020) with some modifications. The flavor profile of fish sauce was distinguished using an electronic nose (Pen III, Airsense, Germany) with 10 different metal oxide semiconductor (MOS) sensors. 4 mL of fish sauce was placed in 30 mL headspace vial. The vial was sealed with a polytetrafluoroethylene/silicone septum cap and then incubated at 45 °C for 10min. Three biological replicates per sample were carrie out for electronic nose analysis. The headspace gas in the vial was extracted using a pump of the electronic nose system at a flow rate of 150 mL/min and evaluated with the MOS sensors. The sample inspection was held for 2 min and the signal was recorded every second. After the determination, all sensors were cleaned thoroughly for 1080s.

2.5. Detection of volatile compounds

Fish sauce sample (10 mL) was mixed with 20 μL of 2-methyl-3-heptanone (5.75 mg/L), which was an internal standard, and 1 g NaCl in a 30 mL sample bottle. The sample was heated in a water bath at 50 °C for 30 min until absorption and release of volatile compounds reached a dynamic equilibrium and then extracted with DVB/CAR/PDMS (50/30 μm) fiber for 40 min. After extraction, the fiber was inserted into the GC/MS injector for 5 min to desorb analytes and the injector port was maintained at 250 °C.

The working condition of GC-MS in this study were set according to the methods byYimdee and Wang (2015). Analysis of the volatiles was performed using the Trace GC-MS system equipped with an Ultra GC, a

Trisplus automated sampler and a quadrupole DSQ II LT (Thermo Fisher, USA). The separation was performed with a DB-5 MS column (330 m × 0.25 mm, 0.25 μm, J&W Scientific, Folsom, CA) or a DB-Wax column (30 m × 0.25 mm, 0.25 μm, J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas with a flow rate of 1.0 mL/min and no split. The analytical condition was as follows: the temperature of column was maintained at 40 °C for 4 min, ramped to 100 °C at 3 °C/min, hold for 10 min, and raised to 150 °C at a rate of 6 °C/min, holding for 8 min and then increased to 280 °C at a rate of 20 °C/min, holding for 10 min. Injection temperature was 280 °C and the ion source temperature was set at 250 °C; The emission current, ionization energy and scan range was 200 μA, 70 eV and m/z 35–500, respectively. Retention indices (RI) value were calculated using the standard C_6-C_{33} n-alkane series under the same chromatographic conditions.

$$RI = 100n + 100 \times \frac{t - t_n}{t_{n+1} - t_n} \quad (1)$$

where n and n+1 stand for the numbers of carbon atoms in n-alkanes, respectively; t stands for the retention time of the unknown substance(s), t_n and $t_n + 1$ stand for the corresponding n-alkane retention time, respectively.

2.6. Calculation of odor activity values (OAVs)

Qualitative analysis of the volatiles of fish sauce was based on retention indices in comparison to the NIST/Wiley database. Semi-quantitative analysis was carried out for all the volatile compounds according to the mass concentration of the internal standard substance and the peak area of inner standard substance and target substance. OAVs were used to evaluate the contribution of every single volatile compound to the overall aroma of samples.

$$OVA_i = C_i / T_i \quad (2)$$

Where C_i and T_i stand for the content of every single volatile compound and its corresponding odor threshold, respectively.

2.7. Statistical analysis

Data were analyzed using Excel 2013 spreadsheet software (2013, Microsoft Inc., China). The results of experiments were subjected to analysis of variance (ANOVA) using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA)and Duncan's new multiple range tests was performed to determine the significant difference between samples at $p < 0.05$. Correlation analysis was performed using cor package of R studio 3.4.3 (RStudio Inc, USA).

3. Results and discussion

3.1. Proximate composition and fatty acid composition analysis

The type and intensity of fish sauce aroma were highly related to the composition and content of protein and lipid (Xu et al., 2018). According to our previous research, *Culter alburnus, Carassius auratus* and *Italurus Punetaus* were selected from 10 spices of common freshwater fish (carp, grass carp, silver carp, bighead carp, carp, crucian carp, bream, mandarin fish, perch and catfish) as the materials of fish sauce in this study. These three fish were significantly different in protein and lipid contents, as shown in Table 1. The crude protein and fat contents of the three species ranged from 15.78% to 19.47% and 5.98%–10.83%, respectively. The highest protein and fat content was obtained by *Culter alburnus* and *Ietalurus Punetaus*, respectively. Comparing with the crude fat content detected by Wei, Li, Liao, Long, and Yang (2017), the higher fat content result in this research was possibly caused by the material and lipid extraction method differences. Whole fish (including head) and chloroform-methanol extraction were applied in this study, comparing with the fish meat and Soxhlet extraction mentioned by Wei et al. (2017).

Table 1
Contents of basic components in freshwater fish muscle (wet matter, %).

	Water	Protein	Fat	Ash content
Culter alburnus	75.06 ± 0.20a	17.47 ± 0.14a	5.98 ± 0.17c	3.48 ± 0.08b
Carassius auratus	73.45 ± 0.65b	17.70 ± 0.15a	8.03 ± 0.21b	3.95 ± 0.10a
Ietalurus Punetaus	70.76 ± 0.68c	15.78 ± 0.12b	10.83 ± 0.16a	2.45 ± 0.06c

Note: Different letters in the same row mean the significant difference ($P < 0.05$).

Table 2 shows the fatty acid profile of these three freshwater fish muscle. Thirty kinds of fatty acids, consisting of 13 saturated fatty acids (SFA), 10 polyunsaturated fatty acids (PUFA) and 7 monounsaturated fatty acids (MUFA), were identified in the three fish. SFA content ranged from 20.79% to 27.62% (*Culter alburnus* > *Carassius auratus* > *Ietalurus Punetaus*), PUFA content ranged from 28.79% to 32.33% (*Carassius auratus* > *Culter alburnus* > *Ietalurus Punetaus*), and MUFA content ranged from 42.28% to 55.56% (*Ietalurus Punetaus* > *Carassius auratus* > *Culter alburnus*). The relative contents of unsaturated fatty acids (UFA) were all above 70%. The relative abundant of UFAs in three fish in descending order were oleic acid (C18:1), linoleic acid (C18:2) and arachidonic acid (C20:4, AA). The percentages of these UFAs were similar to the results reported by Peng et al. (2009) and K. Wang et al. (2012).

Table 2
Contents of fatty acid components in freshwater fish muscle (wet matter, %).

	Culter alburnus	*Carassius auratus*	Ietalurus Punetaus
C9:0	0.03 ± 0.00a	0.03 ± 0.00a	0.02 ± 0.00a
C10:0	0.03 ± 0.00	–	–
C12:0	0.16 ± 0.00a	0.13 ± 0.01b	0.12 ± 0.00b
C13:0	1.57 ± 0.09b	2.06 ± 0.18a	1.31 ± 0.24b
C14:0	1.71 ± 0.01a	1.31 ± 0.01b	0.70 ± 0.02c
C15:0	0.17 ± 0.00b	0.42 ± 0.00a	0.11 ± 0.00c
C16:0	16.62 ± 0.03a	15.84 ± 0.03b	13.75 ± 0.43c
C17:0	0.22 ± 0.00b	0.48 ± 0.00a	0.16 ± 0.02c
C18:0	5.94 ± 0.35a	4.20 ± 0.01b	3.89 ± 0.01c
C20:0	0.27 ± 0.00b	0.14 ± 0.00c	0.28 ± 0.01a
C22:0	0.08 ± 0.00c	0.14 ± 0.00a	0.11 ± 0.00b
C23:0	0.13 ± 0.01a	0.09 ± 0.00b	0.09 ± 0.00b
C24:0	0.64 ± 0.01a	0.59 ± 0.02b	0.24 ± 0.01c
ΣSFA	27.62 ± 0.30a	25.43 ± 0.17b	20.79 ± 0.65c
C14:1	0.04 ± 0.00b	0.37 ± 0.00a	0.03 ± 0.01b
C15:1	0.02 ± 0.00b	0.19 ± 0.00a	0.02 ± 0.00b
C16:1	4.18 ± 0.04a	3.47 ± 0.01b	1.61 ± 0.20c
C17:1	0.31 ± 0.01a	0.25 ± 0.22a	0.14 ± 0.08a
C18:1	37.02 ± 2.75b	34.86 ± 0.90b	49.33 ± 0.70a
C20:1	1.35 ± 0.00c	2.50 ± 0.02a	2.08 ± 0.02b
C22:1	0.46 ± 0.00c	0.54 ± 0.01b	1.08 ± 0.03a
ΣMUFA	42.28 ± 0.33b	43.32 ± 0.66b	55.56 ± 0.90a
C18:2	20.34 ± 0.07b	19.72 ± 0.37c	20.95 ± 0.13a
r-C18:3	0.31 ± 0.00c	0.57 ± 0.02a	0.49 ± 0.01b
a-C18:3	2.33 ± 0.02b	2.90 ± 0.24a	2.61 ± 0.04 ab
C20:2	0.66 ± 0.02c	0.74 ± 0.01b	1.13 ± 0.01a
a-C20:3	0.84 ± 0.01c	1.13 ± 0.01b	1.29 ± 0.06a
C20:4	0.87 ± 0.01b	2.79 ± 0.05a	0.84 ± 0.12b
r-C20:3	0.13 ± 0.01b	0.16 ± 0.02 ab	0.16 ± 0.01a
C20:5	1.38 ± 0.01a	1.18 ± 0.04b	0.15 ± 0.01c
C22:2	0.02 ± 0.00b	–	0.09 ± 0.01a
C22:6	4.11 ± 0.02a	3.14 ± 0.08b	1.08 ± 0.12c
ΣPUFA	30.97 ± 0.11b	32.33 ± 0.50a	28.79 ± 0.30c

Note: Relative amount, the percentage of each compound area to total area of identified compounds; "-", Not detected. Different letters in the same row mean the significant difference ($P < 0.05$).

3.2. Flavor profile analysis by electronic nose

The MOS sensors in the electronic nose are applied to distinguish the flavor profile of fish sauce samples by simulating the human olfactory system. The principal component analysis (PCA) from the electronic nose is shown in Fig. 1A. The two most significant principal components, PC1 and PC2, explained 99.91% of total system variance (91.4% and 8.51%, respectively), which were high enough to represent variables. It's noticed that the flavor profiles of each fish sauce sample were highly overlapped. Besides, the spatial regions showed that the electronic nose detection could clearly distinguish the flavor profile of sauce samples. Response data from 10 sensors are shown in Fig. 1B. The substances detected by sensors S6 (methyl-sensitive), and S7 and S9 (sulfur-sensitive) might contribute most to the odor different of fish sauces.

3.3. Identification of volatile compounds by SPME-GC/MS

Table 3 displays the concentration of volatile compounds of fish sauce obtained by SPME-GC/MS. Seventy-one volatile compounds from fish sauce samples were identified by mass spectra and retention indices

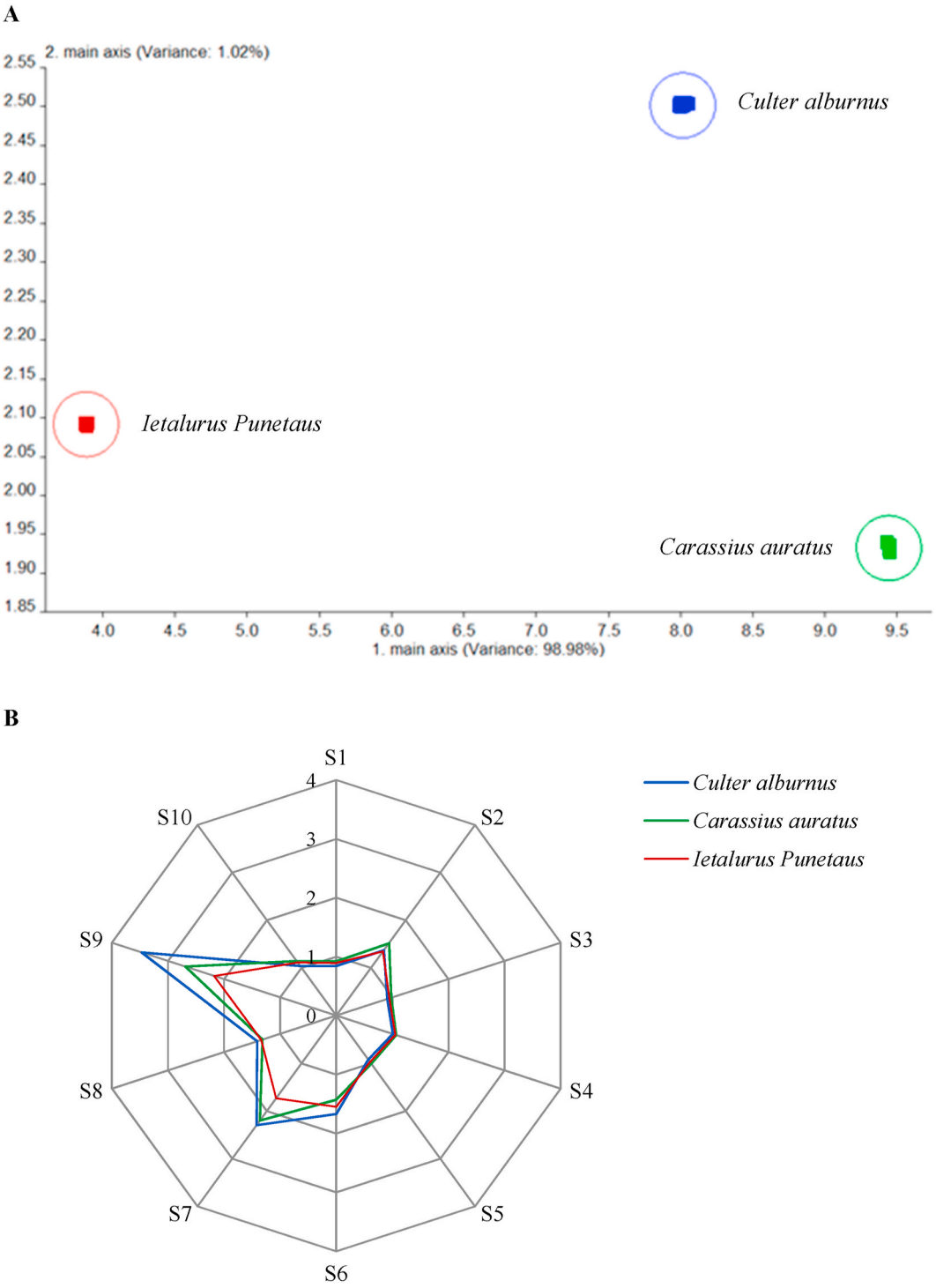

Fig. 1. Principal component analysis (PCA) (A) and Radar map (B) of three kinds of fish sauce. S1: aromatic compounds, S2: broad range, S3: ammonia used as an aromatic compound sensor, S4: hydrogen, S5: aromatic-aliph, S6: broad methane, S7: sulfate-organic, S8: broad alcohol, S9: sulfate-chloride, and S10: methane-aliph.

Table 3
Volatile compounds identified in different fish sauce.

No.	Compounds	Retention indices		Concentrations (μg/kg)						Identification
				Culter alburnus Basilewsky		*Carassius auratus*		Ietalurus Punetaus		
		DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	
Acids										
1	Acetic acid	<800	1427	18.59 ± 1.78	196.63 ± 14.36	15.33 ± 1.33	260.94 ± 23.58	9.72 ± 0.74	367.15 ± 27.98	MS、RI
2	Propanoic acid		1521	–	–	–	0.93 ± 0.08	–	2.46 ± 0.20	MS、RI
3	2-Methylpropanoic acid		1550	–	10.60 ± 0.92	–	20.25 ± 1.63	–	37.69 ± 3.00	MS、RI
4	4-Hydroxybutanoic acid	915	1588	–	–	0.55 ± 0.06	4.60 ± 0.46	–	–	MS
5	3-Methylbutanoic acid	855	1650	–	73.04 ± 5.32	11.35 ± 0.84	104.78 ± 9.02	7.89 ± 0.56	133.70 ± 14.40	MS、RI
6	2-Methylbutanoic acid	871		4.20 ± 0.38	–	8.17 ± 0.60	–	2.36 ± 0.24	–	MS、RI
7	Pentanoic acid		1719	–	1.36 ± 0.13	5.91 ± 0.45	1.93 ± 0.18	0.67 ± 0.05	–	MS、RI
8	Hexanoic acid	983	1826	0.37 ± 0.03	3.27 ± 0.31	–	11.51 ± 0.98	–	5.12 ± 0.44	MS、RI
9	Heptanoic acid	1078	1934	0.10 ± 0.00	–	0.47 ± 0.04	1.85 ± 0.19	–	1.36 ± 0.11	MS、RI
10	Octoic acid	1175	2040	0.15 ± 0.01	1.19 ± 0.12	0.49 ± 0.04	2.64 ± 0.23	0.06 ± 0.00	2.71 ± 0.20	MS、RI
11	Nonanoic acid		2147	–	6.68 ± 0.54	–	22.88 ± 1.76	–	38.67 ± 2.72	MS、RI
12	Decanoic acid		2255	–	0.70 ± 0.02	–	0.48 ± 0.02	–	–	MS、RI
13	Dodecylic acid		2468	–	0.92 ± 0.05	–	0.58 ± 0.03	–	–	MS、RI
Alcohols										
14	Ethanol	<800		0.91 ± 0.06	–	–	–	–	–	MS
15	2-Methyl-1-propanol		1110	–	2.05 ± 0.12	–	2.42 ± 0.25	–	–	MS、RI
16	3-Methyl-1-butanol	752	1204	19.94 ± 1.48	74.26 ± 5.38	4.00 ± 0.31	87.60 ± 7.12	6.41 ± 0.53	148.72 ± 10.04	MS、RI
17	Pentanol		1241	–	–	–	–	1.21 ± 0.08	–	MS、RI
18	2-Heptanol		1306	–	1.92 ± 0.13	–	4.67 ± 0.42	–	4.33 ± 0.33	MS、RI
19	Hexenol		1345	–	5.81 ± 0.59	–	21.90 ± 1.78	–	5.70 ± 0.42	MS、RI
20	Heptanol		1447	–	–	–	1.35 ± 0.09	–	–	MS、RI
21	3-Octanol		1390	–	2.14 ± 0.17	–	1.91 ± 0.16	–	2.71 ± 0.23	MS、RI
22	1-Octen-3-ol		1442	–	3.28 ± 0.33	–	7.18 ± 0.43	–	13.71 ± 1.04	MS、RI
23	2-Ethyl-1-hexanol		1415	–	0.74 ± 0.06	–	–	–	–	MS、RI
24	2,3-Butanediol		1513	–	–	–	6.44 ± 0.61	–	–	MS、RI
25	1-Nonen-4-ol		1623	–	–	–	1.33 ± 0.12	–	–	MS
26	3-Methyl-4-nonanol	1097		–	–	0.33 ± 0.02	–	–	–	MS
27	Phenethyl alcohol		1879	0.29 ± 0.01	19.57 ± 1.19	1.34 ± 0.03	23.82 ± 2.14	–	39.63 ± 3.29	MS、RI
28	2-Decen-1-ol	1206		–	–	0.18 ± 0.01	–	–	–	MS
Aldehydes										
29	3-Methylbutanal	<800	919	19.68 ± 1.81	46.57 ± 5.08	12.65 ± 1.06	28.51 ± 2.73	25.53 ± 1.91	144.26 ± 10.37	MS、RI
30	Hexanal	809		–	0.94 ± 0.06	0.95 ± 0.07	0.89 ± 0.05	0.95 ± 0.06	–	MS、RI
31	(E)-2-methyl-2-butenal		1088	–	0.45 ± 0.03	–	–	–	9.43 ± 0.84	MS、RI
32	Benzaldehyde	959	1494	1.23 ± 0.10	4.52 ± 0.45	1.36 ± 0.11	6.42 ± 0.55	1.63 ± 0.13	32.98 ± 2.62	MS、RI
33	Octanal		1286	–	1.87 ± 0.15	–	2.06 ± 0.22	–	6.64 ± 0.52	MS、RI
34	Phenylacetaldehyde	1042	1609	–	4.91 ± 0.43	6.29 ± 0.62	3.32 ± 0.25	7.67 ± 0.55	32.7 ± 2.75	MS、RI
35	(E)-2-octenal	1057		–	–	0.49 ± 0.04	–	0.31 ± 0.03	–	MS、RI
36	Nonanal	1106	1383	1.02 ± 0.09	2.04 ± 0.19	0.39 ± 0.03	1.89 ± 0.16	1.09 ± 0.08	4.79 ± 0.51	MS、RI
37	Decanal	1207		0.32 ± 0.03	–	–	–	0.18 ± 0.01	–	MS、RI
38	2,5-Dimethylbenzaldehyde	1214	1771	0.56 ± 0.05	1.76 ± 0.17	0.87 ± 0.09	2.32 ± 0.24	0.86 ± 0.08	7.82 ± 0.58	MS、RI
39	2-Phenyl-2-butenal		1891	0.33 ± 0.01	–	–	–	0.20 ± 0.01	–	MS、RI

(*continued on next page*)

Table 3 (*continued*)

No.	Compounds	Retention indices		Concentrations (μg/kg)						Identification
				Culter alburnus Basilewsky		*Carassius auratus*		Ietalurus Punetaus		
		DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	DB-5 MS	DB-WAX	
40	Dodecanal	1410		–	–	–	–	0.06 ± 0.01	–	MS、RI
Sulfur-containing compounds										
41	3-(Methylthio)propanal	909		7.37 ± 0.67	–	5.43 ± 0.40	–	12.91 ± 1.35		MS、RI
42	3-(Methylthio)propanol		1692	–	22.43 ± 2.22	–	15.85 ± 1.24	–	37.92 ± 2.99	MS、RI
43	Dimethyl trisulfide	969		0.18 ± 0.01	–	–	–	0.32 ± 0.02	–	MS、RI
Furan(one)s										
44	Furfural	859	1438	–	–	–	1.38 ± 0.14	–	4.24 ± 0.35	MS、RI
45	2-Furanmethanol	868	1638	–	14.64 ± 1.15	–	27.09 ± 2.09	0.65 ± 0.05	24.08 ± 1.91	MS、RI
46	HDMF		2001	–	0.39 ± 0.04	–	0.60 ± 0.04	–	2.15 ± 0.23	MS、RI
47	HEMF		2063	–	1.12 ± 0.10	–	0.74 ± 0.04	–	2.59 ± 0.21	MS
48	DDMP		2219	–	0.50 ± 0.04	–	0.52 ± 0.05	–	–	MS
49	γ-Butyrolactone	959	1589	–	3.92 ± 0.37	–	–	–	11.50 ± 1.13	MS、RI
50	γ-Hexanolide	1054		0.13 ± 0.01	0.63 ± 0.03	0.87 ± 0.08	3.43 ± 0.24	–	–	MS、RI
51	γ-Octalactone	1257		0.17 ± 0.01	–	0.66 ± 0.02	–	0.16 ± 0.01	–	MS、RI
52	γ-Nonalactone	1362	1988	1.17 ± 0.12	2.13 ± 0.19	4.10 ± 0.37	8.84 ± 0.68	1.00 ± 0.06	5.46 ± 0.52	MS、RI
53	γ-Decalactone									
Ketones										
54	2-Heptanone	900		1.47 ± 0.12	–	–	–	0.90 ± 0.05	–	MS、RI
55	3-Octanone	985		3.48 ± 0.28	4.25 ± 0.47	–	6.22 ± 0.54	1.13 ± 0.11	7.47 ± 0.57	MS、RI
56	1-Hydroxy-propan-2-one		1301	–	–	–	2.06 ± 0.18	–	–	MS、RI
57	1-Octen-3-one	979		–	0.49 ± 0.02	0.32 ± 0.01	1.32 ± 0.11	–	5.40 ± 0.44	MS、RI
58	6-Methyl-5-hepten 2-one		1326	–	0.57 ± 0.03	–	–	–		MS、RI
59	3,5,5-Trimethyl-2-cyclohexen-1-on	1120	1567	1.45 ± 0.10	2.18 ± 0.21	0.82 ± 0.03	1.18 ± 0.09	1.29 ± 0.12	4.87 ± 0.34	MS、RI
60	2,10-Dimethyl-5,9-undecadien-2-one	1455		0.13 ± 0.00	–	–	–	0.12 ± 0.01	–	MS、RI
61	3,5-Dimethyl-4-heptanone		2033	–	0.70 ± 0.05	–	1.10 ± 0.09	–	–	MS
62	4,5-Dimethyl-1,3-dioxol-2-one		2076	–	–	–	2.57 ± 0.21	–	18.72 ± 1.30	MS
63	2,6-Di(tert-butyl)-4-hydroxy-4-methyl-2,5-cyclohexadien-1-one	1473		0.23 ± 0.01	–	–	–	–	–	MS、RI
Esters										
64	Acetic acid isopropenyl ester		823	–	3.42 ± 0.25	–	3.04 ± 0.23	–	12.06 ± 1.06	MS
65	Methyl phenylacetate	1179	1730	0.32 ± 0.03	0.99 ± 0.08	0.65 ± 0.07	1.53 ± 0.13	0.66 ± 0.05	6.65 ± 0.63	MS、RI
66	Diethyl succinate	1184		0.08 ± 0.00	–	0.08 ± 0.00	–	0.07 ± 0.00	–	MS、RI
67	2,2,4-Trimethyl-1,3-pentanedioldiisobutyrate	1598		0.42 ± 0.04	–	0.42 ± 0.04	–	0.37 ± 0.04	–	MS、RI
68	Octyl octanoate	1880		0.09 ± 0.00	–	0.35 ± 0.02	–	0.37 ± 0.02	–	MS
Others										
69	Phenol		1968	–	0.21 ± 0.01	–	0.26 ± 0.01	–	1.15 ± 0.07	MS、RI
70	2,4-Di-tert-butylphenol		2291	–	–	0.08 ± 0.00	0.45 ± 0.02	0.07 ± 0.00	–	MS、RI
71	4-Ethenyl-2-methoxy-Phenol	1316		0.32 ± 0.02	–	0.21 ± 0.01	–	0.32 ± 0.03	–	MS、RI

Note: MS: by comparison of the mass spectrum with the NIST/Wiley mass spectral library; RI: by comparison of RI (Retention indices) with RI of an authentic compound. "-", Not detected. HDMF, 4-hydroxy-2, 5-Dimethyl-3(2H)-furanone; HEMF, 4-hydroxy-5-ethyl-2-methyl-3(2H)-furanone; DDMP, 2,3-Dihydro-3,5-dihydroxy-6-methyl-4(H)-pyran-4-one.

(RI), and quantified by comparison with those of authentic standards. They consisted of 15 alcohols, 13 acids, 12 aldehydes, 10 furan heterocyclic compounds, 10 ketones, 5 esters, 3 sulfur-containing compounds and 3 uncharacterized compounds.

The content of acids was the highest in total volatile compounds, but less contributed to the overall aroma profile of fish sauce due to their high threshold values. Acetic acid was the most dominant in the tested samples, followed by 3-methylbutyric acid, 2-methylpropionic acid and nonanoic acid. In the present study, the content of acetic acid was the highest not only in acid compounds but also in all compounds. Acetic

acid was mainly produced by microbial metabolites (Wanakhachornkrai & Lertsiri, 2003), while branched-chain acids (like 3-methylbutyric acid and 2-methylpropionic acid) were primarily generated from the oxidation of corresponding aldehydes (3-methylbutanal, 2-methylpropanal) with aldehyde dehydrogenase (Bruna et al., 2001).

Alcohols contributed to the characteristic aroma of fish sauce as their varieties and low threshold values. 3-Methyl-1-butanol, phenethyl alcohol and 1-octen-3-ol were the three dominant alcohols. 3-Methyl-1-butanol was generated from isovaleraldehyde and showed the aroma of malty (Coelho et al., 2008); Phenethyl alcohol, having a floral, sweet and honey-like note, was considered as the key aroma contributor to the flavor of most fermented foods. 1-octen-3-ol, having a mushroom-like odor, was reported to be detected in fish products(Qinqin. Chen, Song, Bi, Meng, & Wu, 2018), the spores (Bull, Yong, & Wong, 1985) and mycelium of *Aspergillus oryzae* (Seo et al., 2012). Besides, 1-octen-3-ol was generally considered as the oxidative product of arachidonic acid (AA) under the action of 12-lipoxygenase (Fratini, Lois, Pazos, Parisi, & Medina, 2012; Iglesias & Medina, 2008; Shahidi, 1994) and linoleic acid under auto-oxidation(Lee & Choe, 2012).

Aldehydes were regarded as the most contributor to fish sauce due to low odor thresholds and mainly consisted of 3-methylbutanal, benzaldehyde, and phenylacetaldehyde. 3-Methylbutanal, having a malty and nutty-like note, was the most kind in aldehydes and the key group of aroma compounds in fish sauce (Anupam Giri et al., 2010; Y. Wang et al., 2019). It was reported that 3-methylbutanal was generated from the Strecker degradation and/or biosynthetic pathway of leucine (Luo et al., 2018; Murtaza, Ur-Rehman, Anjum, Huma, & Hafiz, 2014). Benzaldehyde was ubiquitous in fish products exhibited as a pleasant almond flavor, and generated from phenylalanine through the Strecker degradation pathway or linolenic acid through oxidative degradation pathway. Phenylacetaldehyde was generated from the degradation of phenylalanine through the Strecker pathway and exhibited the floral and honey aroma. Nonanal and octanal were considered as the oxidation product of oleic acid and described as the aroma of green and fatty (Van Ba, Ryu, Lan, & Hwang, 2013). In general, branched chain aldehydes and short chain aldehydes were mainly generated from branched chain amino acids, while straight chain aldehydes were mainly generated from fatty acids (Chung, Fung, & Kim, 2005; Zeng, Xia, Jiang, Xu, & Fan, 2017).

Sulfur-containing compounds were seen as key compounds in fish sauce despite the relatively low content in total volatile compounds. 3-(Methylthio)propanal, 3-(methylthio)propanol and dimethyl trisulfide were abundant in the group of the sulfur-containing compounds. And according to (Anupam Giri et al., 2010; Y. Wang et al., 2019), 3-(methylthio)propanal, having a roasted potato aroma, was generated from methionine through the Strecker degradation pathway, and might be further degraded to 3-(methylthio)propanol under the action of microorganisms.

While the rest compounds in fish sauce included furans, ketones and phenolics. Furans were generated from the Maillard reaction or thermal decomposition product of amino acids. Ketones were associated with fatty acid oxidation (Arief, Afiyah, Wulandari, & Budiman, 2016). And 4-vinylguaiacol, a kind of phenolics which was exhibited a smoky incense, was converted from ferulic acid (rich in bran) to vanillin and vanillic acid under the action of microorganisms, and further to 4-ethyl-2-methoxyphenol and 4-vinyl-2-methoxyphenol (Mathew, Abraham, & Sudheesh, 2007).

3.4. Screening of the key flavor compounds

The ultimate contribution of each volatile compound to the overall aroma characteristics of fish sauce depends on not only its concentration but also its odor threshold, which can be gauged by the OAV. The contribution of the component to the overall volatile flavor profile is increased with of the OAV. And there were 9 compounds whose OAVs were above 1 in the three fish sauce samples (Table 4), indicating a great contribution to the overall volatile flavor profile (Hoang et al., 2016). The three highest compounds were 3-methylbutanal (malty note), 3-methyl-1-butanol (malty note) and 3-(methylthio)propanal (cooked potato note), which contributed significantly to the overall aroma profile of fish sauce with an OAV higher than 10. The result was agreed with the report of fish sauce key aroma by Fukami (Fukami et al., 2002). Therefore, amino acids and fatty acids of the fish material would greatly influence the type and intensity of fish sauce aroma.

3.5. Correlation analysis of fatty acids and fish sauce aroma

The types and contents of oxidative degradation products varied with the unsaturation degree, double bond position and carbon chain length of fatty acids. Correlation analysis of three fatty acids (arachidonic acid, linocic acid and oleic acid) and four key aroma substances (1-octen-3-ol, nonanal, octanal and decanal) were shown in Fig. 2. The contents of octanal and nonanal in fish sauce showed a significant positive correlation with the content of oleic acid in raw fish ($r = 0.9899$ and 0.9755, respectively, $P < 0.05$). And there was a hugely significant positive correlation between the contents of octanal and nonanal ($r = 0.9969$, $P < 0.01$). However, the content of the decanal did not correlate with other compounds (Fig. 2A). Cao et al. (2020) have reported that oleic acid transferred to 8-hydroperoxide (8-ROOH), 9-hydroperoxide (9-ROOH), 10-hydroperoxide (10-ROOH) and 11-hydroperoxide (11-ROOH), respectively, through four auto-oxidation routes. According to Fig. 2A, the authors speculated the possible auto-oxidation pathways of oleic acid of fish sauce (Fig. 3A). During the fermentation of fish sauce, oleic acid mainly undergone the auto-oxidation to form 10-ROOH and 11-ROOH with equal probability, and (or) secondary to develop 8-ROOH. Afterward, 10-ROOH, 11-ROOH and 8-ROOH

Table 4
Analysis results of key aroma substances in different fish sauce samples.

No.	Compounds	Thresholds (μg/kg)	OAV			Odor quality
			Culter alburnus	*Carassius auratus*	Ietalurus Punetaus	
1	3-Methylbutanal	0.2[a]	232.87	142.57	721.32	Malty
2	3-Methyl-1-butanol	4[b]	18.56	21.90	37.18	Malty, rancid, pungent
3	3-(Methylthio)propanal	0.5[b]	14.74	10.86	25.81	Cooked potato
4	1-Octen-3-ol	1.5[b]	2.19	4.79	9.14	Mushroom-like, fatty
5	Phenylacetaldehyde	4[c]	1.23	0.83	8.18	Floral, honey
6	Nonanal	1.1[d]	1.86	1.72	4.35	Green, citrus-like
7	Dimethyl trisulfide	0.1[a]	1.80	–	3.23	Onion
8	Decanal	0.1[d]	3.19	1.82	–	Green, cucumber
9	Hexanol	5.7[d]	1.02	3.84	1.00	Green, fresh

Note: OAV: odor activity values.
Previously reported as thresholds in water of aroma substances([a], Feng et al., 2014; [b], Giri, Osako, Okamoto, & Ohshima, 2010; [c], Pino & Mesa, 2006; [d], Selli & Cayhan, 2009).
Dimethyl trisulfide and Decanal were detected with a DB-5 MS column, and the rest of analyzed compounds were detected by with a DB-WAX column.

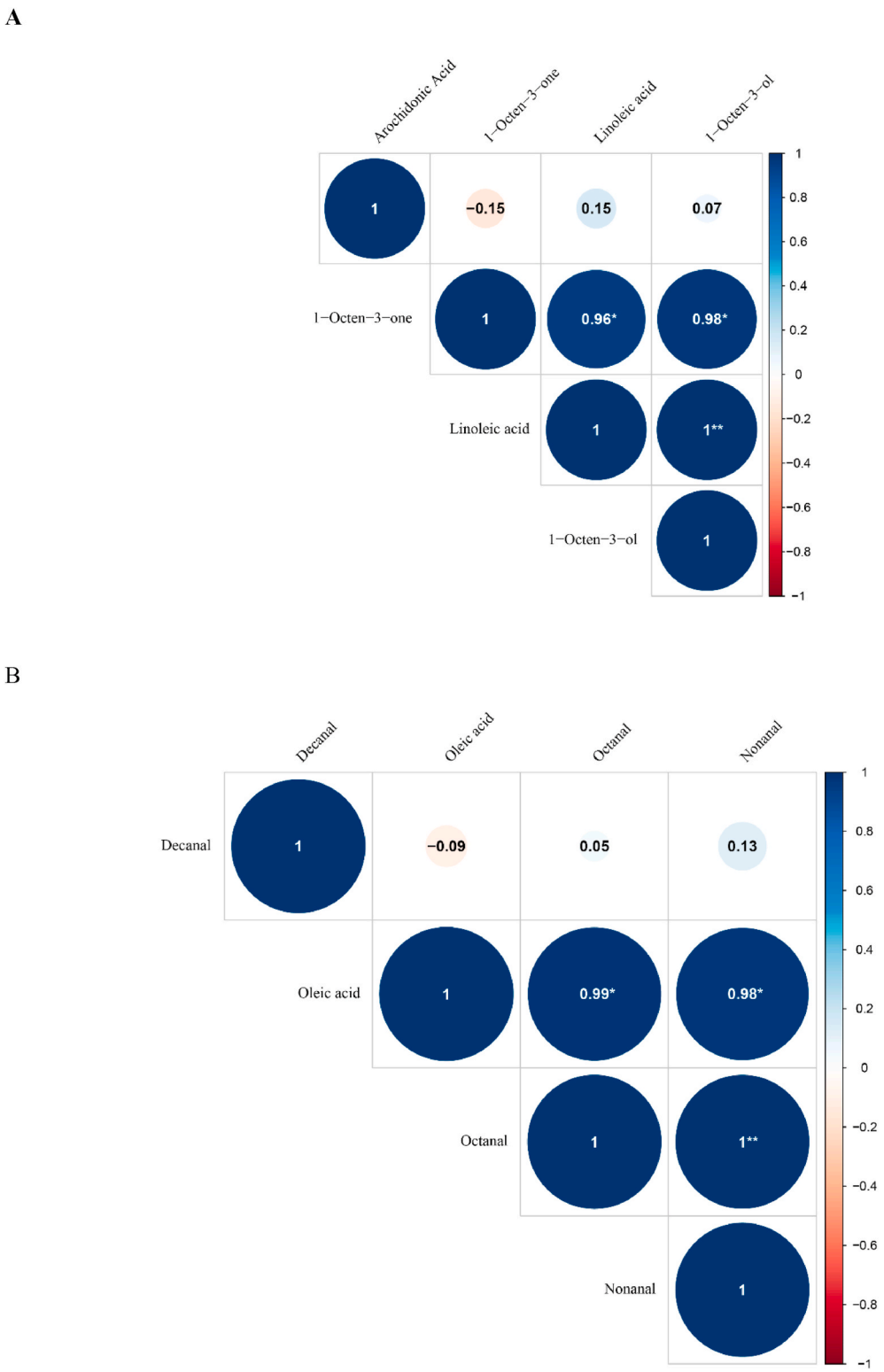

Fig. 2. Correlation analysis of fatty acids (A: arachidonic acid and linocic acid, and B: oleic acid) and key aroma of fish sauce. * means significant difference ($P < 0.05$), ** means extremely significant difference ($P < 0.01$).

subjected to homolysis and converted to nonanal, octanal and decanal.

According to Fig. 2B, there was an extremely significant correlation between the content of 1-octen-3-ol in fish sauce and the content of linoleic acid in the fish material ($r = 0.9967$, $P < 0.01$), while no correlation was noticed between 1-octen-3-ol and arachidonic acid. And the contents of 1-octen-3-one showed a significant positive correlation with the content of linoleic acid and 1-octen-3-ol in fish sauce ($r = 0.9551$ and 0.9759, respectively, $P < 0.05$). The authors speculated the possible auto-oxidation pathways of linoleic acid of fish sauce (Fig. 3B). During the fermentation of fish sauce, linoleic acid undergone the formation of 10-L(S)-hydroperoxy-cis-9, trans-11-octadecadieuoic acid (10-HPOD) under the action of lipoxygenase (EC 1.13.1.13), then hemolysis to

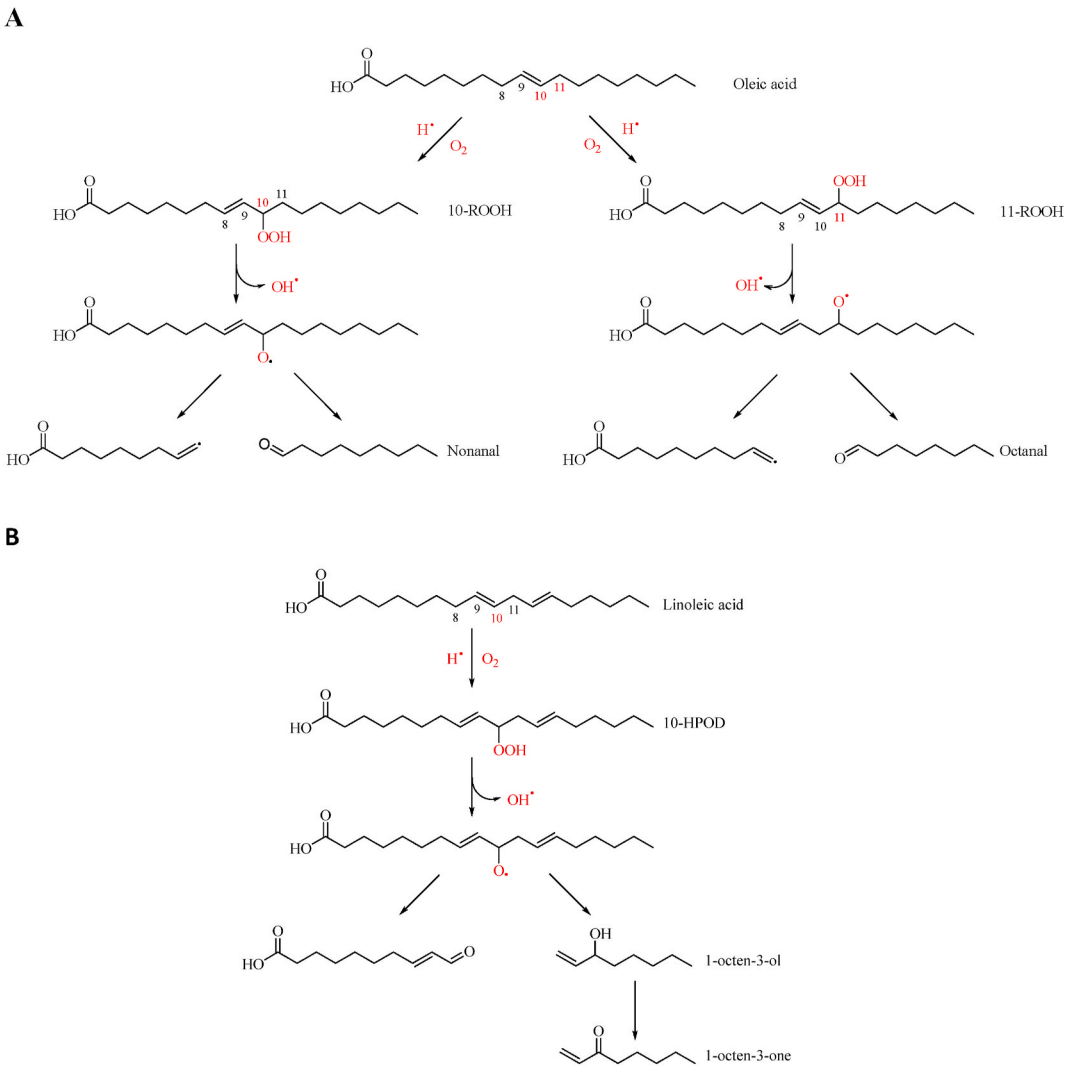

Fig. 3. Possible pathways of linocic acid (A) and oleic acid (B) during fish sauce fermentation.

produce 1-octen-3-ol, and further to oxide formation 1-octen-3-one. This speculation was consistent with the report of Wurzenberger and Grosch (1982), Assaf, Hadar, and Dosoretz (1997) and Tasaki, Kobayashi, Sato, Hayashi, and Joh (2019).

4. Conclusions

Culter alburnus, *Carassius auratus* and *Ietalurus Punetaus* were selected as the raw materials to ferment fish sauce. The aroma composition of fish sauce made from different fish species was significantly different. A total of 71 flavors compounds were found in all the three types of fish sauce using electronic nose and SPME-GC/MS, which showed a remarkable difference. Of these, 9 key flavor compounds, including 3-methylbutanal, 3-methyl butanol, 3-(methylthio)propanal, 1-octen-3-ol, phenylacetaldehyde, nonanal, dimethyl trisulfide, decanal and hexanol, made significant contributions to the aroma profile of fish sauce due to their high OAVs. Moreover, two possible pathways of oleic acid (C18:1) and linoleic acid (C18:2) oxidation were speculated based on the correlation analysis between fatty acid profiles and fish sauce aroma. Based on results obtained from this study, further work might focus on directly regulating the metabolism pathway of fatty acid. This study could provide a theoretical foundation for the production of high-quality fish sauce products.

CRediT authorship contribution statement

Anzi Ding: Writing - original draft, Conceptualization. **Meng Zhu:** Software, Validation. **Xiaoqing Qian:** Data curation. **Liu Shi:** Conceptualization, Supervision. **Huang Huang:** Data curation. **Guangquan Xiong:** Visualization. **Jun Wang:** Visualization. **Lan Wang:** Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (31871870), the China Agriculture Research System (CARS-46) and the Major Program of Technical Innovation of Hubei Province (2018ABA100).

References

AOAC. (2006). *Official method of analysis Association of Official analytical Chemists.* Washington, DC,USA.

Arief, I. I., Afiyah, D. N., Wulandari, Z., & Budiman, C. (2016). Physicochemical properties, fatty acid profiles, and sensory characteristics of fermented beef sausage by probiotics lactobacillus plantarum IIA-2C12 or lactobacillus acidophilus IIA-2B4. *Journal of Food Science, 81*(11), M2761–M2769. https://doi.org/10.1111/1750-3841.13509

Assaf, S., Hadar, Y., & Dosoretz, C. G. (1997). 1-Octen-3-ol and 13-hydroperoxylinoleate are products of distinct pathways in the oxidative breakdown of linoleic acid by Pleurotus pulmonarius. *Enzyme and Microbial Technology, 21*(7), 484–490. https://doi.org/10.1016/S0141-0229(97)00019-7

Bruna, J. M., Hierro, E. M., de la Hoz, L., Mottram, D. S., Fernández, M., & Ordóñez, J. A. (2001). The contribution of Penicillium aurantiogriseum to the volatile composition and sensory quality of dry fermented sausages. *Meat Science, 59*(1), 97–107. https://doi.org/10.1016/S0309-1740(01)00058-4

Bull, S. M., Yong, F. M., & Wong, H. A. (1985). The production of aroma by *Aspergillus oryzae* during the preparation of soy sauce *Koji*. *Food Chemistry, 17*, 251–264.

Cao, J., Jiang, X., Chen, Q., Zhang, H., Sun, H., Zhang, W.-M., et al. (2020). Oxidative stabilities of olive and camellia oils: Possible mechanism of aldehydes formation in oleic acid triglyceride at high temperature. *Lebensmittel-Wissenschaft & Technologie, 118*. https://doi.org/10.1016/j.lwt.2019.108858

Chen, Q., Kong, B., Han, Q., Xia, X., & Xu, L. (2017). The role of bacterial fermentation in lipolysis and lipid oxidation in Harbin dry sausages and its flavour development. *Lebensmittel-Wissenschaft & Technologie, 77*, 389–396. https://doi.org/10.1016/j.lwt.2016.11.075

Chen, Q., Song, J., Bi, J., Meng, X., & Wu, X. (2018). Characterization of volatile profile from ten different varieties of Chinese jujubes by HS-SPME/GC-MS coupled with E-nose. *Food Research International, 105*, 605–615. https://doi.org/10.1016/j.foodres.2017.11.054

Chen, L., Yuan, M., Zhang, S., Zhao, L., Bai, C., & Jiang, Y. (2020). Comparison of physicochemical components of fermented fish sauce from viscera of grass carp and eel. *China Condiment, 45*(2), 16–18+22.

Chung, H.-Y., Fung, P.-K., & Kim, J.-S. (2005). Aroma impact components in commercial plain sufu. *Journal of Agricultural and Food Chemistry, 53*(5), 1684–1691.

Coelho, E., Perestrelo, R., Neng, N. R., Câmara, J. S., Coimbra, M. A., Nogueira, J. M. F., et al. (2008). Optimisation of stir bar sorptive extraction and liquid desorption combined with large volume injection-gas chromatography-quadrupole mass spectrometry for the determination of volatile compounds in wines. *Analytica Chimica Acta, 624*(1), 79–89. https://doi.org/10.1016/j.aca.2008.06.032

Feng, Y., Chen, Z., Liu, N., Zhao, H., Cui, C., & Zhao, M. (2014). Changes in fatty acid composition and lipid profile during koji fermentation and their relationships with soy sauce flavour. *Food Chemistry, 158*, 438–444. https://doi.org/10.1016/j.foodchem.2014.02.147

Feng, Y., Cui, C., Zhao, H., Gao, X., Zhao, M., & Sun, W. (2013). Effect ofkojifermentation on generation of volatile compounds in soy sauce production. *International Journal of Food Science and Technology, 48*(3), 609–619. https://doi.org/10.1111/ijfs.12006

Folch, J. M. S., Lees, M. M., & Sloane, S. H. (1957). A simple method for the isolation and purification of total lipides from animal tissue. *Journal of Biological Chemistry, 226*(1), 497–509.

Fratini, G., Lois, S., Pazos, M., Parisi, G., & Medina, I. (2012). Volatile profile of Atlantic shellfish species by HS-SPME GC/MS. *Food Research International, 48*(2), 856–865. https://doi.org/10.1016/j.foodres.2012.06.033

Fukami, K., Ishiyama, S., Yaguramaki, H., Masuzawa, T., Nabeta, Y., Endo, K., et al. (2002). Identification of distinctive volatile compounds in fish sauce. *Journal of Agricultural and Food Chemistry, 50*(19), 5412–5416.

Gambacorta, G., Sinigaglia, M., Schena, A., Baiano, A., Lamacchia, C., & Pati, S. (2009). Changes in free fatty acid and diacylglycerol compounds in short-ripening dry-cured sausage. *Journal of Food Lipids, 16*, 1–18.

Gao, X.-L., Cui, C., Zhao, H.-F., Zhao, M.-M., Yang, L., & Ren, J.-Y. (2010). Changes in volatile aroma compounds of traditional Chinese-type soy sauce during moromi fermentation and heat treatment. *Food Science and Biotechnology, 19*(4), 889–898. https://doi.org/10.1007/s10068-010-0126-7

Gao, P., Li, L., Xia, W., Xu, Y., & Liu, S. (2020). Valorization of Nile tilapia (*Oreochromis niloticus*) fish head for a novel fish sauce by fermentation with selected lactic acid bacteria. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 129*. https://doi.org/10.1016/j.lwt.2020.109539

Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish *miso*, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chemistry, 120*(2), 621–631. https://doi.org/10.1016/j.foodchem.2009.10.036

Giri, A., Osako, K., Okamoto, A., & Ohshima, T. (2010). Olfactometric characterization of aroma active compounds in fermented fish paste in comparison with fish sauce, fermented soy paste and sauce products. *Food Research International, 43*(4), 1027–1040. https://doi.org/10.1016/j.foodres.2010.01.012

Hierro, E., de la Hoz, L., & Ordóñez, J. A. (1999). Contribution of the microbial and meat endogenous enzymes to the free amino acid and amine contents of dry fermented sausages. *Journal of Agricultural and Food Chemistry, 47*(3), 1156–1161. https://doi.org/10.1021/jf980834p

Hoang, N. X., Ferng, S., Ting, C.-H., Huang, W.-H., Chiou, R. Y.-Y., & Hsu, C.-K. (2016). Optimizing the initial moromi fermentation conditions to improve the quality of soy sauce. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 74*, 242–250. https://doi.org/10.1016/j.lwt.2016.07.049

Iglesias, J., & Medina, I. (2008). Solid-phase microextraction method for the determination of volatile compounds associated to oxidation of fish muscle. *Journal of Chromatography A, 1192*(1), 9–16. https://doi.org/10.1016/j.chroma.2008.03.028

Lee, E., & Choe, E. (2012). Changes in oxidation-derived off-flavor compounds of roasted sesame oil during accelerated storage in the dark. *Biocatalysis and Agricultural Biotechnology, 1*(1), 89–93. https://doi.org/10.1016/j.bcab.2011.08.003

Li, C., Wang, Y., Li, L., Chen, S., Wu, Y., Hu, X., et al. (2018). Bacterial community succession during fermentation of Chinese fish sauce and its effect on formation. *Food Science, 39*(24), 85–92. https://doi.org/10.7506/spkx1002-6630-201824014

Lorenzo, J. M., Gómez, M., & Fonseca, S. (2014). Effect of commercial starter cultures on physicochemical characteristics, microbial counts and free fatty acid composition of dry-cured foal sausage. *Food Control, 46*, 382–389. https://doi.org/10.1016/j.foodcont.2014.05.025

Luo, J., Jiang, C., Zhao, L., Zhang, M., Wang, F., Sun, E., et al. (2018). Keto acid decarboxylase and keto acid dehydrogenase activity detected during the biosynthesis of flavor compound 3-methylbutanal by the nondairy adjunct culture *Lactococcus lactis* ssp. *lactis* F9. *Journal of Dairy Science, 101*(11), 9725–9735. https://doi.org/10.3168/jds.2018-14760

Mathew, S., Abraham, T. E., & Sudheesh, S. (2007). Rapid conversion of ferulic acid to 4-vinyl guaiacol and vanillin metabolites by *Debaryomyces hansenii*. *Journal of Molecular Catalysis B: Enzymatic, 44*(2), 48–52. https://doi.org/10.1016/j.molcatb.2006.09.001

Murtaza, M. A., Ur-Rehman, S., Anjum, F. M., Huma, N., & Hafiz, I. (2014). Cheddar cheese ripening and flavor characterization: A review. *Critical Reviews in Food Science and Nutrition, 54*(10), 1309–1321. https://doi.org/10.1080/10408398.2011.634531

Peng, D., Liu, Z., Wei, L., Li, Y., Li, B., Zhang, L., et al. (2009). Determination of ictalurus punctatus oil by GC-MS. *Food and Machinery, 25*(3), 91–95. https://doi.org/10.13652/j.issn.1003

Seo, Y.-S., Bae, H.-N., Eom, S.-H., Lim, K.-S., Yun, I.-H., Chung, Y.-H., … Kim, Y.-M. (2012). Removal of off-flavors from sea tangle (*Laminaria japonica*) extract by fermentation with *Aspergillus oryzae*. *Bioresource Technology, 121*, 475–479. https://doi.org/10.1016/j.biortech.2012.07.007

Shahidi, F. (1994). *Flavor of meat and meat products*. Berlin: Springer Science & Business Media.

Tasaki, Y., Kobayashi, D., Sato, R., Hayashi, S., & Joh, T. (2019). Variations in 1-octen-3-ol and lipoxygenase gene expression in the oyster mushroom Pleurotus ostreatus according to fruiting body development, tissue specificity, maturity, and postharvest storage. *Mycoscience, 60*(3), 170–176. https://doi.org/10.1016/j.myc.2019.02.005

Van Ba, H., Ryu, K. S., Lan, N. T. K., & Hwang, I. (2013). Influence of particular breed on meat quality parameters, sensory characteristics, and volatile components. *Food Science and Biotechnology, 22*(3), 651–658. https://doi.org/10.1007/s10068-013-0127-4

Wanakhachornkrai, P., & Lertsiri, S. (2003). Comparison of determination method for volatile compounds in Thai soy sauce. *Food Chemistry, 83*, 619–629. https://doi.org/10.1016/S0308-8146(03)00256-5

Wang, K., Han, Y., Dong, J.-g., Hao, Q., Zhao, R., & Chen, X. (2012). Comparison of fatty acids and blood indexes between wild and cultured populations of *Culter alburnus* in Xingkai Lake. *Freshwater Fisheries, 42*(3), 14–18.

Wang, B., Hu, J.-g., & Tong, G. (2020). Analysis on the dynamic changes in quality of anchovy fish sauce during low salt fermentation. *China Condiment, 45*(6), 78–82.

Wang, Y., Li, C., Li, L., Yang, X., Chen, S., Wu, Y., … Yang, D. (2019). Application of UHPLC-Q/TOF-MS-based metabolomics in the evaluation of metabolites and taste quality of Chinese fish sauce (Yu-lu) during fermentation. *Food Chemistry, 296*, 132–141. https://doi.org/10.1016/j.foodchem.2019.05.043

Wang, Y., Li, C., Yan, X., & Li, L. (2018). Effect of bacterial community and free amino acids on the metabolism of biogenic amines during fermentation of Yu-lu, a Chinese fermented fish sauce. In *Paper presented at the 2018 Annual Conference of Chinese Fisheries Society, Xi an*.

Wang, Y., Li, C., Zhao, Y., Li, L., Yang, X., Wu, Y., … Yang, D. (2020). Novel insight into the formation mechanism of volatile flavor in Chinese fish sauce (Yu-lu) based on molecular sensory and metagenomics analyses. *Food Chemistry, 323*, 126839. https://doi.org/10.1016/j.foodchem.2020.126839

Wei, Y., Li, W., Liao, J., Long, J., & Yang, C. (2017). Study on the fatty components and fatty acids in muscle of crucian carp. *Guangdong Chemical Industry, 44*(6), 36–37+49.

Wurzenberger, M., & Grosch, W. (1982). The enzymic oxidative breakdown of linoleic acid in mushrooms (Psalliota bispora). *Zeitschrift für Lebensmittel-Untersuchung und -Forschung A, 175*(3), 186–190. https://doi.org/10.1007/BF01139769

Xu, Y., Li, L., Regenstein, J. M., Gao, P., Zang, J., Xia, W., et al. (2018). The contribution of autochthonous microflora on free fatty acids release and flavor development in low-salt fermented fish. *Food Chemistry, 256*, 259–267. https://doi.org/10.1016/j.foodchem.2018.02.142

Yimdee, T., & Wang, X.-C. (2015). Comparison of odor and taste of commercial brand fish sauces from East and South East Asian countries. *International Journal of Food Properties, 19*(4), 873–896. https://doi.org/10.1080/10942912.2015.1045517

Yoshikawa, S., Kurihara, H., Kawai, Y., Yamazaki, K., Tanaka, A., Nishikiori, T., et al. (2010). Effect of halotolerant starter microorganisms on chemical characteristics of fermented Chum Salmon (*Oncorhynchus keta*) sauce. *Journal of Agricultural and Food Chemistry, 58*(10), 6410–6417. https://doi.org/10.1021/jf904548u

Zeng, X., Xia, W., Jiang, Q., Xu, Y., & Fan, J. (2017). Contribution of mixed starter cultures to flavor profile of Suanyu - a traditional Chinese low-salt fermented whole fish. *Journal of Food Processing and Preservation, 41*(5), Article e13131. https://doi.org/10.1111/jfpp.13131

Zhang, L., Liao, L., Qiao, Y., Wang, C., Shi, D., An, K., et al. (2020). Effects of ultrahigh pressure and ultrasound pretreatments on properties of strawberry chips prepared by vacuum-freeze drying. *Food Chemistry, 303*. https://doi.org/10.1016/j.foodchem.2019.125386