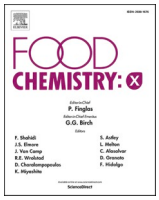
Food Chemistry: X 17 (2023) 100569

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Metabolomics investigation on the volatile and non-volatile composition in enzymatic hydrolysates of Pacific oyster (*Crassostrea gigas*)

Li Liu, Yuanhui Zhao, Shixue Lu, Yihuan Liu, Xinxing Xu *, Mingyong Zeng *

College of Food Science and Engineering, Ocean University of China, Qingdao, Shandong Province 266003, China

ARTICLE INFO

Keywords:
Oyster
Enzymatic hydrolysis
Metabolomics
Flavor formation

Chemical compounds studied in this article:
Pentanal (PubChem CID: 8063)
1-penten-3-ol (PubChem CID: 15394)
Hexanal (PubChem CID: 6184)
(*E*)-2-Pentenal (PubChem CID: 5364752)
Heptanal (PubChem CID: 8130)
(*E*)-2-Hexenal (PubChem CID: 5281168)
4-Octanone (PubChem CID: 11516)
(*E*)-4-Heptenal (PubChem CID: 5283318)
3-Octanone (PubChem CID: 246728)
Octanal (PubChem CID: 454)
Nonanal (PubChem CID: 31289)
1-Octen-3-ol (PubChem CID: 18827)
Benzaldehyde (PubChem CID: 240)
(*E*)-2-Nonenal (PubChem CID: 5283335)
(*E*, *Z*)-2,6-Nonadienal (PubChem CID: 129662528)

ABSTRACT

To investigate the differences of volatile and non-volatile metabolites between oyster enzymatic hydrolysates and boiling concentrates, molecular sensory analysis and untargeted metabolomics were employed. "Grassy," "fruity," "oily/fatty," "fishy," and "metallic" were identified as sensory attributes used to evaluate different processed oyster homogenates. Sixty-nine and 42 volatiles were identified by gas chromatography–ion mobility spectrometry and gas chromatography–mass spectrometry, respectively. Pentanal, 1-penten-3-ol, hexanal, (*E*)-2-pentenal, heptanal, (*E*)-2-hexenal, 4-octanone, (*E*)-4-heptenal, 3-octanone, octanal, nonanal, 1-octen-3-ol, benzaldehyde, (*E*)-2-nonenal, and (*E, Z*)-2,6-nonadienal were detected as the key odorants (OAV $>$ 1) after enzymatic hydrolysis. Hexanal, (*E*)-4-heptenal, and (*E*)-2-pentenal were significantly associated with off-odor, and 177 differential metabolites were classified. Aspartate, glutamine, alanine, and arginine were the key precursors affecting the flavor profile. Linking sensory descriptors to volatile and nonvolatile components of different processed oyster homogenates will provide information for the process and quality improvement of oyster products.

1. Introduction

Oysters are economically important marine shellfish. The Pacific oyster (*Crassostrea gigas*) is the main breeding species with the largest export in Shandong, China. It contains high-quality protein, vitamins, and minerals, especially taurine (Feng et al., 2022). The oysters are traditionally processed by salting, boiling, baking, and frying. High-temperature boiling is the most widely used method for developing the flavor of the basic material. Currently, enzymatic hydrolysis of aquatic products and their by-products is widely used to develop products with high added value, such as collagen peptides and antioxidant peptides (Liang, Zhang, Fu, Zhu, & Mou, 2020). Previous studies have demonstrated that oyster hydrolysates have angiotensin-converting enzyme-inhibiting, antifatigue, and aphrodisiac activities (Luo et al., 2021). However, uneven product quality and nutrient loss are caused by differences in the processing method. Exopeptidases act from the C or N terminus of a protein, while endopeptidases act by targeting peptide bonds to produce peptides and free amino acids (FAAs), which greatly changes the flavor of the product (Zhao et al., 2020). Therefore, systematic research is necessary to control the quality of products of enzymatic hydrolysis.

Shellfish hydrolysates usually exhibit strong off-odors, such as fishy odors, which limits their industrial processing. The volatile compounds (VOCs) produced by hydrolysis of shellfish products consist of unsaturated aldehydes, ketones, and alcohols (Li et al., 2021). During oyster processing, changes in odor compounds are mainly caused by protein

* Corresponding authors at: No.5 Yushan Road, Shinan District, Beijing 100083, China.
E-mail addresses: liuli19960320@163.com (L. Liu), zhaoyuanhui@ouc.edu.cn (Y. Zhao), 18266133360@163.com (S. Lu), huan1293686793@163.com (Y. Liu), xxx@ouc.edu.cn (X. Xu), mingyz@ouc.edu.cn (M. Zeng).

https://doi.org/10.1016/j.fochx.2023.100569
Received 1 October 2022; Received in revised form 15 December 2022; Accepted 6 January 2023
Available online 9 January 2023

oxidation to form amino acids, small peptides, and amines, which results in the development of odors and sensory rejection (Ma et al., 2022). Besides, lipid oxidation during enzymatic hydrolysis is also the main reason for further deterioration of the flavor of hydrolysates. A study has reported that the off-odor in oyster hydrolysates is contributed by 3-methylbutanal, hexanal, 1-penten-3-ol, 2-pentyl-furan, (*Z*)-4-heptenal, (*E, E*)-2,4-heptadienal (Liang, Zhang, Fu, Zhu, & Mou, 2020). Few studies have focused on the formation pathways of key odorants in the enzymatic hydrolysis of oyster products. Hexanal and 1-penten-3-ol are potential markers used to distinguish fresh aquatic products from processed products, which can contribute "oily" and "rancid" odors (Wu, Wang, Wang, Tian, & Zhan, 2022). Clarification of the composition and formation mechanism of the flavor of oyster hydrolysates may provide important guidance for the development of aquatic hydrolysate products. The conditions of enzymatic hydrolysis are closely associated with changes in the flavor profile, while specific odor compounds are associated with precursors. FAAs and free fatty acids are important precursors of VOCs. Changes in precursors during processing have important effects on the formation of the overall flavor (Zang, Yu, Zhang, Xu, & Xia, 2022). Many precursors and intermediate reaction products with different concentrations and odor characteristics, as well as the products formed by the corresponding reactions, are associated with flavors and specific VOCs.

Flavor precursors mainly consist of amino acids, peptides, sugars, and lipids. VOCs, including aldehydes, alcohols, ketones, hydrocarbons, and esters, are generated via many chemical reactions, such as lipid oxidation, the Strecker reaction, and the Maillard reaction (Wu, Zhan, Tang, Li, & Duan, 2022). The reaction mechanism comprises the decarboxylation and deamination of amino acids and peptides to form aldehydes, hydrogen sulfide, and benzene compounds. It has been reported that cystine and cysteine in food are very important for the formation of a "meaty" odor (Majcher & Jeleń, 2007). The Strecker reaction of phenylalanine and leucine is the main source of aldehydes, for example hexanal and heptanal, which produce sweet, flowery, grassy, and fruity flavors. A study reported that arginine, histidine, and other alkaline amino acids have a molecular correlation with the formation of 2-phenylethanol, ethyl acetate, and ethyl benzoate (Yang et al., 2022). 2-Methylbutyraldehyde and 3-methylbutyraldehyde impart cheesy and nutty odors, respectively, and are mainly produced from the amino acids leucine and isoleucine as formed by the Strecker degradation pathway and biosynthesis pathway (Xie et al., 2022). In aquatic products, lipid oxidation is also an important formation pathway of volatile flavor compounds. Free fatty acids produced by lipid degradation can produce lipid-derived compounds, such as aldehydes, ketones, acids, hydrocarbons, lactones, and furans. The oxidation reaction of unsaturated fatty acids leads to the release of long-chain aldehydes, causing the change of the fatty acid composition. Benzaldehyde, which can produce almond and nutty flavors, is produced by Strecker degradation of phenylalanine or the linolenic acid oxidation pathway (Wang & Kays, 2000). Pentanal, octanal, and nonanal have been described as contributing almond, meaty, and flowery flavors and waxy odors, and are produced by the oxidation of oleic acid (Duan, Dong, Sun, Dong, & Gao, 2021). Besides, hexanol is associated with the oxidative degradation of oleic acid, and oct-1-en-3-ol is produced by a lipase-catalyzed reaction and oxidative decomposition of polyunsaturated fatty acids (PUFAs) such as arachidonic acid (Chang, Wu, Zhang, Jin, & Wang, 2019). However, the differences of volatile and non-volatile metabolites in oyster enzymatic hydrolysates, steaming concentrates, and other processed samples are still unknown.

The objectives of the present study were: (1) to identify the sensory attributes and key volatile components in oyster samples including enzymatic hydrolysates, boiling concentrates, and raw oyster homogenates; (2) to investigate the profile of non-volatile metabolites in oyster samples using metabonomics; and (3) to predict the relationships between sensory attributes, odorants, and nonvolatile metabolites using bioinformatics analysis. This study compared the compositional differences between the processes of enzymatic hydrolysis and traditional high-temperature boiling, which has laid a foundation for expanding the range of application of enzymatic hydrolysis in aquatic products and achieving deodorization, aroma enhancement, and directional regulation of flavor.

2. Materials and methods

2.1. Materials and chemicals

Fresh oysters (*Crassostrea gigas*) were purchased from local seafood market (Qingdao, China), preserved with ice, and immediately transported to the laboratory. 2-Methylheptan-3-one (assay $\geq$ 97 %) was obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). Flavor protease (5×10^5 U/g from *Aspergillus oryzae*) was purchased from Nanning Pangbo Bio-Engineering Co., Ltd (Nanning, Guangxi, China). Deionized water was obtained from a water purifier (CR-SP412; Heal Force Bio-Meditech Co. Ltd, Shanghai, China). Standards of *cis*-3-decen-1-ol (98 %), 2-methyl-3-furanthiol (95 %), (*E, E*)-2,4-Dodecadienal (>90 %), 2,3-diethyl-5-methylpyrazine (98 %), 3-octanol (>98 %), and 2-methylpropanal (98 %) were purchased from Aladdin Bio-Chem Technology Co., Ltd (Shanghai, China). Serial *n*-alkanes (C7–C40) were obtained from Sigma-Aldrich Chemicals (St. Louis, MO, USA). All flavor standards were diluted with ultrapure water to 100 times their threshold concentrations as reference solutions. All other chemical reagents were of analytical grade and were purchased from Nanjing Reagent Corp., Ltd (Nanjing, Jiangsu, China).

2.2. Preparation of oyster samples

The edible part of the oyster was separated from the shell and homogenized by homogenizer (F6/10, Shanghai Jingxin Technology Co., Ltd, Shanghai, China) at 8000r/min for 30 s at room temperature (~20 °C). Next, flavor protease (0.3 %, w/v) was mixed with the homogenate after the addition of water (1:2, v/v). The homogenate was stirred in a shaking water bath (OLB-100C; OLABO Scientific Instrument Co., Ltd, Shandong, China) at 50 °C and 150 rpm for 4 h. Finally, the reaction was terminated by cooling in an ice bath to yield oyster enzymatic hydrolysate with low-temperature inactivation (OYH-L) and by boiling at 100 °C for 10 min to yield oyster enzymatic hydrolysate with high-temperature inactivation (OYH-H). Oyster boiling concentrates (OYB) was prepared by boiling raw oyster homogenate (OY) with water (1:2, v/v) at 100 °C for 80 min as previously reported (Liu et al., 2013). The remaining oyster meat was stored at −60 °C for further testing.

2.3. Aroma profiles evaluation

The aroma profiles were analyzed by the artificial sensory method (Xu et al., 2021). Ten trained sensory assessors (aged 22–35 years, 5 males and 5 females) were selected to perform sniffing tests. Four oyster samples (OY, OYH-L, OYH-H, and OYB) were prepared separately in 20 mL transparent vials with polytetrafluoroethylene-silicone stoppers at room temperature for 30 min. The assessors were asked to define the perceived flavor characteristics of each sample, and then all the assessors agreed on the selected characteristic flavor descriptors. The selected flavor descriptors ("grassy," "fruity," "oily/fatty," "fishy," and "metallic") and the overall score were scored on a scale of intensities from 0 (not perceivable) to 5 (extremely strong).

2.4. Determination of free amino acids

FAAs were quantified by a previously reported method with some modifications. A 2.0 g oyster sample was homogenized with 10 mL ultrapure water for 2 min at 1000 rpm for three times and then allowed to stand for 30 min. Then, the mixture was centrifuged at 10,000 rpm and 4 °C for 10 min. The above operation was repeated twice, and the

supernatants were combined in a volumetric flask (25 mL). The constant-volume solution (10 mL) was mixed with trichloroacetic acid (10 mL, 10 %, w/v). After the mixture had been allowed to stand for 1 h, centrifugation was performed for 10 min at 10000 rpm and 4 °C. The supernatant was diluted to 25 mL, and the pH was adjusted to 2.0. Finally, the solution was filtered through a 0.22 μm aqueous-phase membrane, and the FAA concentrations were determined by an amino acid analyzer (L-8900, HITACHI Limited, Kyoto, Japan). The taste properties of FAAs were determined by calculating the taste activity value (TAV) by the following equation (Yin et al., 2022):

$$\mathrm{TAV} = \frac{\text{concentration of free amino acid}}{\text{taste threshold corresponding to free amino acid}} \quad (1)$$

Compounds with a TAV of >1 were considered to be taste-active, while compounds with a TAV of <1 were thought to have little effect on taste.

2.5. Determination of free fatty acids

Lipids were extracted from oyster samples. A chloroform–methanol solution (50 mL, 2:1) was added to a 1.00 g lyophilized oyster sample, which was homogenized in an ice bath and placed at 4 °C for 2 h. The extract solution was filtered by natural filtration through medium-speed qualitative filter paper, which separates liquids from solids by the ability of the filter paper to retain solid particles. Then, an NaCl solution (5 mL, 0.9 %) was added to the filtrate, which was centrifuged at 4000 rpm and 4 °C for 10 min. Finally, the lower chloroform–lipid solution was collected and blown with nitrogen until a constant weight was reached to obtain the total lipids in the oyster samples. The lipids (1 g) were added to a solution of potassium hydroxide in methanol (0.5 mol/L, 2 mL). The lipid samples were saponified in a water bath at 60 °C for 20 min until the oil beads dissolved and were then cooled to room temperature. Next, a solution of boron trifluoride in methanol (14 %, 3 mL) was added to the saponification solution, and the lipids were esterified in a water bath at 60 °C for 20 min. The esterification solution was mixed with *n*-hexane and ultrapure water (2:1, 3 mL), and the operation was repeated three times. After blowing with nitrogen until a constant weight was reached, the fatty acids were brought to a constant volume of 10 mL and filtered through a 0.22 μm membrane for measurement. The fatty acid contents of oyster samples were determined by a GC/MS (QP2010-SE; Shimadzu, Kyoto, Japan) with an HP-5 ms chromatography column (30 m × 0.25 mm × 0.25 μm, Agilent Technologies Inc., Santa Clara, USA). The injection temperature was 250 °C, and helium was used as the carrier gas with a flow rate of 1.5 mL/min. The GC conditions were as follows: the initial temperature was 60 °C for 1 min, and the temperature was increased to 160 °C at 10 °C/min, held for 5 min, increased to 200 °C at 3 °C/min, held for 10 min, and finally increased to 280 °C at 6 °C/min and held for 5 min. The shunt ratio was 10:1 with an injection volume of 1 μL. The MS conditions were as follows: the ion source temperature was 250 °C; the electron energy was 70 eV; the mass scan range was 35–500 *m/z*; and the solvent removal time was set to 2 min (Medeiros Vicentini-Polette, Rodolfo Ramos, Bernardo Gonçalves & Lopes De Oliveira, 2021).

2.6. Identification of odor compounds by GC–MS and GC-IMS

Four oyster samples (50 g) were extracted with 150 mL CH_2Cl_2 in a separating funnel after shaking at 100 rpm for 12 h. The extracts were mixed with 10 μL 2-methylheptan-3-one (0.816 μg/μL) as an internal standard, and the mixture was subjected to solvent-assisted flavor evaporation (SAFE). Subsequently, the oyster samples were slowly concentrated to a volume of approximately 1 mL at 40 °C using a Vigreux column (50 cm × 1 cm; Ban Xia Science and Technology Development Co., Ltd, Beijing, China) after distillation and stored at −80 °C until ready for GC analysis. The four oyster samples (2 mL) and 2-methylheptan-3-one (0.1 μg/mL, 20 μL) were placed separately into 20 mL vial. A solid-phase microextraction (SPME) fiber (50/30 μm, DVB/CAR/PDMS; Supelco, Bellefonte, PA, USA) was inserted into the vial headspace and incubated at 250 rpm and 50 °C for 30 min.

Headspace solid-phase microextraction (HS-SPME) and SAFE were combined with GC–MS and GC-IMS to detect odor compounds in different oyster samples. A GC–MS (QP2010SE, Shimadzu, Kyoto, Japan) was used for the test. The gas chromatograph effluent was separated with the polar DB-WAX capillary column (J & W Scientific, Folsom, CA, USA) (30 m × 0.25 mm, 0.25 μm). The GC parameters were as follows: the initial temperature was 40 °C, which was maintained for 3 min, then increased to 200 °C at 6 °C/min, and finally increased to 250 °C at 10 °C/min and held for 10 min. The carrier gas was helium at a flow rate of 1.0 mL/min. The MS conditions were as follows: mass spectra were recorded in electron impact mode at an electron energy of 70 eV over the mass scan range of 33–300 *m/z*; the ion source temperature was 230 °C; and the detector interface temperature was 250 °C. All measurements were performed in triplicate. The MS data were analyzed using GC–MS solution software (GC–MS solution, Shimadzu, Kyoto, Japan), and chemicals were identified against the NIST 14 library. 2-Methylheptan-3-one was used as an internal standard, and the relative percentage content and retention index of each compound were calculated.

The main aroma compounds (with OAVs of >1) were quantitatively analyzed by the external standard method. The following 15 standard aroma compounds were selected for quantification: pentanal, 1-penten-3-ol, hexanal, (*E*)-2-pentenal, heptanal, (*E*)-2-hexenal, 4-octanone, (*E*)-4-heptenal, 3-octanone, octanal, nonanal, 1-octen-3-ol, benzaldehyde, (*E*)-2-nonenal, and (*E*, *Z*)-2,6-nonadienal. Selective ion monitoring mode (SIM) was used for accurate quantification. The standard solutions were diluted by a factor of 1000 in *n*-hexane, which was followed by five concentration gradient dilutions to give mixed stock solutions. Adsorption was carried out under the same conditions as the abovementioned SPME. Standard curves were plotted (Table 2) by plotting the response rates of the standard compounds and internal standards against their respective concentrations. All analyses were repeated in triplicate.

Oyster samples (2 mL) were separately added to a vial (20 mL) and incubated in an autosampler (CTC-PAL, CTC Analytics AG, Zwingen, Switzerland) at 250 rpm and 50 °C for 30 min. After incubation, the SPME fiber with headspace gas of each oyster sample (500 μL) was injected into a gas chromatograph (Agilent Technologies, Inc., Palo Alto, CA, USA) with an MXT-WAX column (15 m × 0.53 mm i.d.; G.A.S., Dortmund, Germany). Nitrogen was used as the carrier gas with the following specific flow program: the flow rate was initially 2 mL/min for 2 min, then 10 mL/min for 10 min, 100 mL/min for 20 min, and finally 150 mL/min for 25 min. For IMS (FlavorSpec®, G.A.S., Dortmund, Germany), the drift tube temperature was 45 °C, and the drift gas was high-purity nitrogen at a constant flow rate (150 mL/min). All tests were performed in triplicate. A fingerprint map based on the analytical spectra was generated and processed using Laboratory Analytical Viewer software (G.A.S., Dortmund, Germany).

2.7. Identification by untargeted metabolomics using ultra-performance liquid chromatography–Q Exactive Orbitrap mass spectrometry

A lyophilized oyster sample (100 mg) was mixed with L-2-chlorophenylalanine (800 μL, 0.02 mg/mL in methanol/water = 4:1, v/v) in a 2 mL centrifuge tube. The mixture was ground at 50 Hz and −10 °C for 6 min using a frozen-tissue grinder and was then extracted at 40 kHz and 5 °C for 30 min. The sample was centrifuged (13,000 × *g*, 4 °C for 15 min) after storage at −20 °C for 30 min, and the supernatant was obtained for subsequent analysis. In addition, 20 μL of the supernatant of each sample was collected, and the supernatants were mixed to give a quality control sample. Moreover, 2 μL of the supernatant from each oyster sample was injected into an ultra-performance liquid chromatography (UPLC)-Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with an ACQUITY UPLC HSS T3 column (100

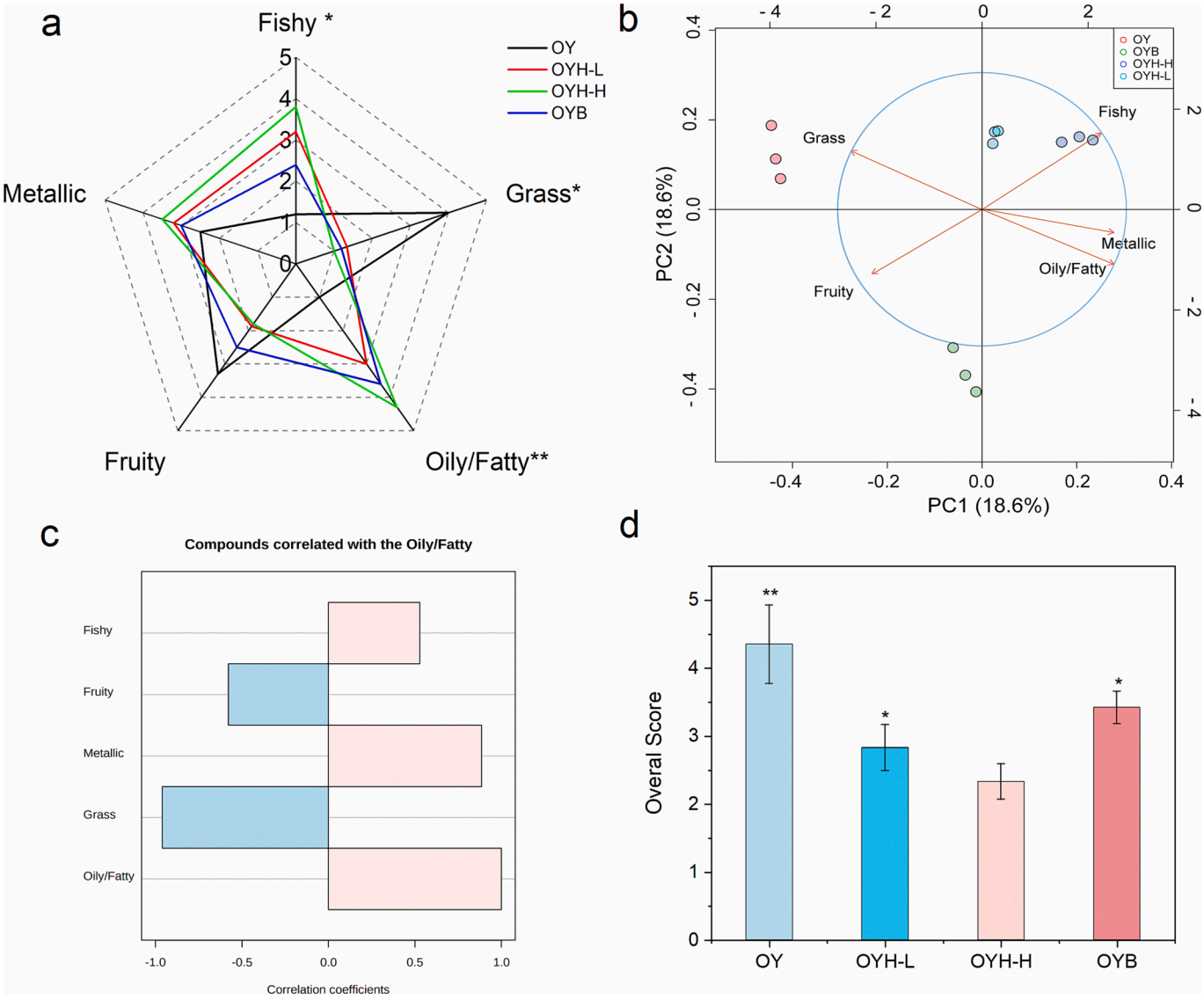


Fig. 1. Sensory evaluation of different oyster samples. (a) Intensity values of five odor attributes. (b) Principal component Biplot of intensity values. (c) Correlation analysis of different odor attributes. (d) Overall odor score. (c) Principal component Biplot of intensity values. OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates. The data are expressed as the means ± standard deviations (n = 3). *, $p < 0.05$; **, $p < 0.005$.

× 2.1 mm, 1.8 μm; Waters Corporation, Milford, MA, USA). The UPLC mobile phase was prepared as follows: mobile phase A was composed of acetonitrile and water (5:95, v/v) with 1 % formic acid, whereas acetonitrile, isopropanol, and water (47.5:47.5:5) with 1 % formic acid made up mobile phase B. The gradient elution procedure was as follows: 0–0.1 min, 100 % A, 0 % B; 0.1–2.0 min, 95 % A, 5 % B; 2.0–9.0 min, 75 % A, 25 % B; 9.0–13.0 min, 0 % A, 100 % B; and 13.0–13.1 min, 100 % A, 0 % B. The flow rate was 0.40 mL/min, and the column temperature was 40 °C. As for the MS conditions, the electrospray ionization probe was heated in positive/negative ion-switching mode, and the capillary temperature was 320 °C. In positive-ion mode, the electrospray voltage was 3500 V, which was converted to 2800 V in negative-ion mode. The gas flow rates were 40 Arb for sheath gas and 10 Arb for auxiliary gas, and the scan range was 70–1050 *m/z*. The resolution of full-scan spectra (Full MS) was 70000, and that of fragment spectra (MS2) was 17500.

2.8. Data analysis

The results were analyzed using SPSS 17.0 statistical software (SPSS, Inc., Chicago, IL, USA). Partial least squares regression (PLSR) was performed using Unscrambler X 10.4 software (CAMO ASA, Oslo, Norway). Metabolomics data were analyzed on the free online platform Majorbio Cloud Platform (https://www.majorbio.com). Metabolic pathways were designed according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/kegg). All data were expressed as the mean ± standard deviation. Least significant differences ($p < 0.05$) between the treatments were accepted.

3. Results and discussion

3.1. Sensory analysis by free-choice profiling

Samples of the processed oyster homogenates subjected to different treatments were evaluated by the group of trained sensory assessors via a free-choice sensory test. “Grassy,” “fruity,” “oily/fatty,” “fishy,” and “metallic” were selected as the main flavor attributes perceived in the different oyster samples. Research via free-choice analysis usually focuses on a single type of attribute, such as odor (Xu et al., 2021). The

Table 1
Taste activity values (TAVs) of free amino acids and taste attributes (+pleasant, − unpleasant).

FAAs	Taste attribute	Taste threshold (mg/100 g)	OY		OTH-L		OTH-H		OYG	
			TAVs	content	TAVs	content	TAVs	content	TAVs	content
Asp	Umami (+)	100	1.17 ± 0.0007	117.83 ± 3.24^{a}	0.67 ± 0.005	67.67 ± 1.86^{b}	0.46 ± 0.19	46.04 ± 1.35^{c}	1.76 ± 0.005	176.25 ± 2.21^{b}
Glu	Umami (+)	30	1.97 ± 0.074	59.26 ± 2.05^{c}	1.16 ± 0.018	34.94 ± 1.43^{a}	1.47 ± 0.12	44.21 ± 1.47^{b}	4.67 ± 0.023	140.14 ± 1.27^{d}
Thr	Sweet (+)	260	0.35 ± 0.00007	91.55 ± 0.81^{c}	0.98 ± 0.0065	256.74 ± 5.21^{a}	0.89 ± 0.006	232.09 ± 3.10^{a}	0.63 ± 0.00048	127.26 ± 1.36^{b}
Ser	Sweet (+)	150	0.61 ± 0.0012	91.71 ± 1.21^{d}	2.10 ± 0.00021	315.04 ± 2.76^{a}	1.74 ± 0.0018	261.18 ± 1.58^{b}	1.20 ± 0.00023	180.06 ± 1.06^{c}
Gly	Sweet (+)	130	1.80 ± 0.006	234.03 ± 1.04^{d}	3.78 ± 0.0056	491.42 ± 3.41^{a}	2.47 ± 0.008	321.13 ± 1.75^{c}	1.01 ± 0.0062	131.64 ± 2.37^{b}
Ala	Sweet (+)	60	1.60 ± 0.038	96.12 ± 0.83^{a}	0.96 ± 0.0072	57.44 ± 2.23^{c}	0.76 ± 0.0076	45.27 ± 0.78^{d}	1.31 ± 0.0053	78.41 ± 1.13^{b}
Arg	Sweet/bitter (+)	50	0.86 ± 0.17	43.72 ± 0.69^{a}	0.51 ± 0.00023	25.53 ± 0.32^{d}	0.57 ± 0.083	28.48 ± 0.72^{c}	0.61 ± 0.087	30.52 ± 1.43^{b}
Pro	Sweet/bitter (+)	300	3.14 ± 0.0008	941.82 ± 3.68^{b}	1.02 ± 0.06	305.16 ± 6.71^{c}	0.78 ± 0.00037	233.25 ± 4.87^{d}	3.67 ± 0.007	1101.43 ± 4.24^{a}
Tyr	Bitter (+)		–	176.87 ± 1.03^{d}	–	2624.72 ± 5.03^{b}	–	3112.47 ± 4.24^{a}	–	134.62 ± 6.12^{c}
Val	Bitter/sweet (−)	40	1.16 ± 0.0004	46.38 ± 0.71^{c}	2.66 ± 0.0014	129.64 ± 2.14^{b}	3.24 ± 0.032	106.37 ± 1.06^{a}	–	nd
Met	Bitter/sweet/ sulfurous (−)	30	0.09 ± 0.00002	2.74 ± 0.12^{c}	1.17 ± 0.00012	27.33 ± 0.45 ^{a}	0.91 ± 0.00063	35.14 ± 0.83^{b}	–	nd
Ile	Bitter (−)	90	0.81 ± 0.00005	72.85 ± 0.74^{d}	2.67 ± 0.0047	176.36 ± 1.04 ^{a}	1.96 ± 0.0036	240.31 ± 1.72^{b}	1.08 ± 0.002	97.23 ± 0.83^{c}
Leu	Bitter (−)	190	1.08 ± 0.0008	205.16 ± 1.04^{c}	4.48 ± 0.12	851.23 ± 6.43^{b}	5.13 ± 0.0058	976.45 ± 3.12^{a}	1.25 ± 0.008	237.54 ± 1.84^{c}
Phe	Bitter (−)	90	0.915 ± 0.06	82.36 ± 2.06^{b}	0.11 ± 0.0006	9.86 ± 0.08^{d}	0.22 ± 0.026	20.21 ± 1.28^{c}	0.93 ± 0.06	84.09 ± 2.62^{a}
Lys	Bitter/sweet (−)		–	348.64 ± 2.32^{d}	–	4371.43 ± 7.35^{a}	–	4519.72 ± 8.15^{b}	–	466.27 ± 5.23^{c}
His	Bitter (−)	20	0.83 ± 0.0072	19.38 ± 0.83	–	nd	0.19 ± 0.00004	3.81 ± 0.021	3.75 ± 0.13^{c}	16.62 ± 0.62
UAA (Umami amino acid)				177.09 ± 1.45^{d}		90.25 ± 3.14^{a}		90.25 ± 2.01^{b}		316.39 ± 1.98^{c}
SAA (Sweet amino acid)				513.41 ± 2.86^{d}		1119.44 ± 4.12^{a}		859.67 ± 2.34^{b}		517.37 ± 4.21^{c}
BAA (Bitter amino acid)				1939.92 ± 8.23^{d}		8521.26 ± 7.37^{a}		9272.40 ± 6.29^{b}		2168.32 ± 7.42^{c}
Total FAAs				2530.42 ± 8.23^{d}		10372.77 ± 7.37^{a}		10792.35 ± 6.29^{b}		3602.08 ± 7.42^{c}

Data are mean ± standard deviation (n = 3). Different letters within a row indicate significant difference ($p < 0.05$). Nd: not detected.
OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates.

odor attributes and overall evaluation of the different processed oyster homogenates were estimated on a scale from 0 to 5 (Fig. 1). As shown in Fig. 1a, the "fishy" and "oily/fatty" attributes of enzymatic hydrolysates were stronger in comparison with those of raw oyster homogenates, whereas the "fishy" and "metallic" were increased during high temperature inactivation enzyme. Moreover, the "fruity" attribute of boiling concentrates was more pronounced, while the reverse was perceived in the case of the "fishy" attribute, in comparison with enzymatic hydrolysates. The most obvious change during enzymatic hydrolysis was the conversion of proteins into peptides and FAAs, which was prone to oxidation. Malondialdehyde (MDA) is one of the primary aldehydes produced by lipid oxidation (Sajib & Undeland, 2020). MDA can further go on non-enzymatic browning reaction with amino acids to generate unpleasant aldehydes or degrade into acetaldehyde and formic acid during high temperature inactivation, causing an increase in the intensity of the "fishy" attribute (Wen et al., 2019). Summarily, the "oily/fatty" and "fishy" attributes were the main flavor attribute after enzymatic hydrolysis.

Principal component analysis (PCA) showed the degrees of correlation between different processed oyster homogenates and odor attributes (Fig. 1b). The odor of raw oyster homogenates was mainly contributed by the "grassy" attribute, while the "fishy" attribute became the main contributor to the odor after enzymatic hydrolysis, and the "fishy" attribute was stronger after high temperature inactivation enzyme. The characteristic odor of boiling concentrates was contributed by the "fruity" and "oily/fatty" attributes. The "fishy" attribute produced after enzymatic hydrolysis was selected as a designated factor for correlation analysis (Fig. 1c). The results demonstrated that this odor attribute was positively correlated with the "metallic" and "oily/fatty" attributes. "Grassy," which was one of the principal attributes contributing to the pleasant odor of raw oyster homogenates, was negatively correlated with the "fishy" attribute. Hence, enzymatic hydrolysis could change the sensory profile of oyster, leading to the increase of off-odor, while pleasant sensory attributes were maintained after traditional high-temperature boiling.

Evaluation of the overall flavor is beneficial for indicating product quality and optimizing product processing. As shown in Fig. 1d, the overall odor scores of the enzymatic hydrolysates were lower than that of raw oyster homogenates, remarkably, whereas the score was lower after high temperature inactivation enzyme. The boiling concentrates had a higher score than the oyster hydrolysates. This was consistent with the results of earlier studies, which proved that the off-odors of aquatic products increased in intensity during enzymatic hydrolysis (Ruan et al., 2022). High temperatures can promote oxidation and thus result in an increase in the contents of FAAs and fatty acids, which are important precursors of odor compounds. Processing of aquatic products promoted the conversion of lipids into hydroperoxides under the action of Lipoxygenase (LOX), and were further cut into volatile compounds by

Table 2
Concentration and odor activity values of key odorants.

Key aroma-active compounds[a]	Odor threshold in water[b] (mg/g)	Concentration (mg/L)				OAV				Linear equation	R^2	Quota selected ion (*m/z*)
		OY	OTH-L	OTH-H	OYB	OY	OTH-L	OTH-H	OYB			
Pentanal	0.012	0	0.4 ± 0.13	0.46 ± 0.22	0.81 ± 0.15	0	33	38	67	y = 0.195x + 0.0102	R^2 = 0.991	44/58/19
1-Penten-3-ol	1.2	2.18 ± 0.75[a]	1.19 ± 0.32[c]	1.40 ± 0.83[b]	1.21 ± 0.24[c]	1	<1	1	1	y = 11.908x + 0.1274	R^2 = 0.9933	57/29/27
Hexanal	0.073	0.05 ± 0.02[d]	0.63 ± 0.16[b]	0.42 ± 0.32[c]	1.1 ± 0.17[a]	<1	8	5	15	y = 7.0919x + 0.974	R^2 = 0.9912	44/56/41
(*E*)-2-Pentenal	0.98	1.82 ± 0.23[a]	1.39 ± 0.42[b]	0.72 ± 0.13[c]	0.66 ± 0.15[c]	1	1	<1	<1	y = 10.136x + 1.5683	R^2 = 0.9908	55/84/83
Heptanal	0.028	6.48 ± 1.26[a]	1.06 ± 0.31[b]	0.40 ± 0.18[d]	0.71 ± 0.12[c]	231	37	14	25	y = 12.504x − 1.943	R^2 = 0.9915	70/41/44
(*E*)-2-Hexenal	0.4286	0.036 ± 0.014[c]	1.48 ± 0.43[a]	0.59 ± 0.25[b]	0.04 ± 0.03[c]	<1	3	1	<1	y = 6.8613x + 11.858	R^2 = 0.9932	41/42/83
4-Octanone	0.041	0.34 ± 0.14[d]	0.83 ± 0.12[b]	1.48 ± 0.11[a]	0.59 ± 018[c]	8	20	36	14	y = 5.976x + 6.945	R^2 = 0.9919	43/57/71
(*E*)-4-Heptenal	0.01	0.087 ± 0.02[c]	0.1 ± 0.06[b]	0.23 ± 0.03[a]	0.07 ± 0.04[c]	8	10	23	7	y = 7.3069x + 0.2364	R^2 = 0.9947	41/68/55
3-Octanone	0.0214	0.28 ± 0.23	0.34 ± 0.10	1.27 ± 0.08	0	13	15	59	0	y = 15.162x + 0.5274	R^2 = 0.9928	43/57/72
Octanal	0.0069	5.6 ± 1.04[a]	1.41 ± 0.64[c]	2.88 ± 0.07[b]	1 ± 0.06[d]	811	204	417	144	y = 7.6629x + 5.6143	R^2 = 0.9907	43/41/56
Nonanal	0.02	0.26 ± 0.07[c]	0.49 ± 0.15[b]	2.03 ± 012[a]	0.51 ± 0.22[b]	13	24	101	25	y = 4.8202x + 3.3223	R^2 = 0.9915	57/41/43
1-Octen-3-ol	0.025	8.00 ± 1.57[a]	2.91 ± 0.82[b]	1.62 ± 0.36[c]	2.64 ± 0.41[b]	320	116	64	105	y = 4.3925x + 22.752	R^2 = 0.9902	57/43/72
Benzaldehyde	0.75089	0.43 ± 0.12[d]	3.85 ± 0.74[c]	4.78 ± 0.86[b]	6.24 ± 0.94[a]	<1	5	6	8	y = 0.7947x + 35.488	R^2 = 0.9918	77/51//74
(*E*)-2-Nonanal	0.00019	0.31 ± 0.04	0.22 ± 0.09	1.18 ± 0.25	0	1631	1158	6210	0	y = 1.1403x + 1.9612	R^2 = 0.9959	43/55/70
(*E*, *Z*)-2,6-Nonadienal	0.00069	0.61 ± 0.13[c]	0.76 ± 014[b]	9.77 ± 1.18[a]	0.64 ± 0.08[c]	884	1101	14,159	927	y = 1.28x + 5.3498	R^2 = 0.9988	41/70/27

Data are mean ± standard deviation (n = 3). Different letters within a row indicate significant difference ($p < 0.05$). Nd: not detected.
OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates.

hydroperoxide lyase (HPL) (Buchhaupt, Guder, Etschmann, & Schrader, 2012). Ketones and aldehydes were reported to be responsible for the "fishy" attribute in aquatic products as a result of protein degradation (Luo et al., 2022). Therefore, it was necessary to analysis the correlation of key odor compounds and metabolites, which can preferably explain the differences in the perception of flavor attributes between different processed oyster homogenates.

3.2. Analysis of free amino acids correlated with the overall flavor

FAAs are precursors of odor compounds, which are important for the complex synthesis of compounds and the overall aroma. The FAAs present in processed oyster homogenates subjected to different treatments are listed in Table 1. Sixteen amino acids were detected. Glycine, proline, leucine, and lysine were the main amino acids in raw oyster homogenates and accounted for 8.11–37.22 % of total amino acids, which was consistent with previous reports (Jiang, Liu, Xu, Zeng, & Zhao, 2019). The TAVs of these four FAAs were greater than 1, and these were therefore considered to be active FAAs in different processed oyster homogenates. In comparison with raw oyster homogenates, the contents of sweet amino acids and bitter amino acids (BAAs) increased significantly after enzymatic hydrolysis. The content of umami amino acids (UAAs) decreased from 177.09 to 90.25 mg/100 g, while the UAA content of boiling concentrates was nearly double that of raw oyster homogenates. Remarkably, the BAA content was increased in high temperature inactivation enzyme ($p < 0.05$). The increase of amino acids and small peptides was the main feature of enzymatic hydrolysis, which could be deaminated and decarboxylated to form branched aldehydes and ketones and further yield acid compounds by the Strecker reaction. A study confirmed that hydrophobic amino acids (such as isoleucine, tyrosine, phenylalanine, and tryptophan) could increase the bitterness of a hydrolysate (Su et al., 2021). Moreover, the structure of proteins was changed by high-temperature inactivation: phenylalanine, histidine, and tyrosine sites were exposed, and small peptides were further hydrolyzed into FAAs, which resulted in an increase in the intensities of sensory attributes (Zhang, Zhang &Wang, 2016). These results indicated that total amount of amino acids increased and the concentration changed, bitterness increased and umami decreased after enzymatic hydrolysis, while traditional high-temperature boiling could enhance umami.

Glutamic acid and aspartic acid were the two UAAs that contributed to the associated flavor characteristics. The contents of these two FAAs significantly decreased after enzymatic hydrolysis (from 1.16 and 1.47 to 0.46 and 0.67 mg/g, respectively), while a different trend was exhibited in boiling concentrates. The leucine content of the oyster hydrolysates was approximately-four times than the other two treatments. A previous study reported that some aldehydes (such as 3-methylbutanal) were derived from leucine via oxidative deamination and decarboxylation and were associated with acorn-like, salty, and cheesy aroma (Domínguez et al., 2019). Thus, more amino acids and small peptides were released after enzymatic hydrolysis, resulting in the increase of health benefits. The off- odor was more obvious with the composition and content changes of protein metabolites, which were the important precursors of key odor compounds.

3.3. Effect of fatty acid composition on flavor

Fatty acids, such as linoleic, linolenic, stearic, and arachidonic acids, act as important precursors of flavor compounds by generating various hydroperoxides after oxidation reaction. Fresh oysters have a large content of PUFAs, especially eicosapentaenoic acid (EPA; 20:5n − 3) and docosahexaenoic acid (DHA; 22:6n − 3), which are beneficial for human

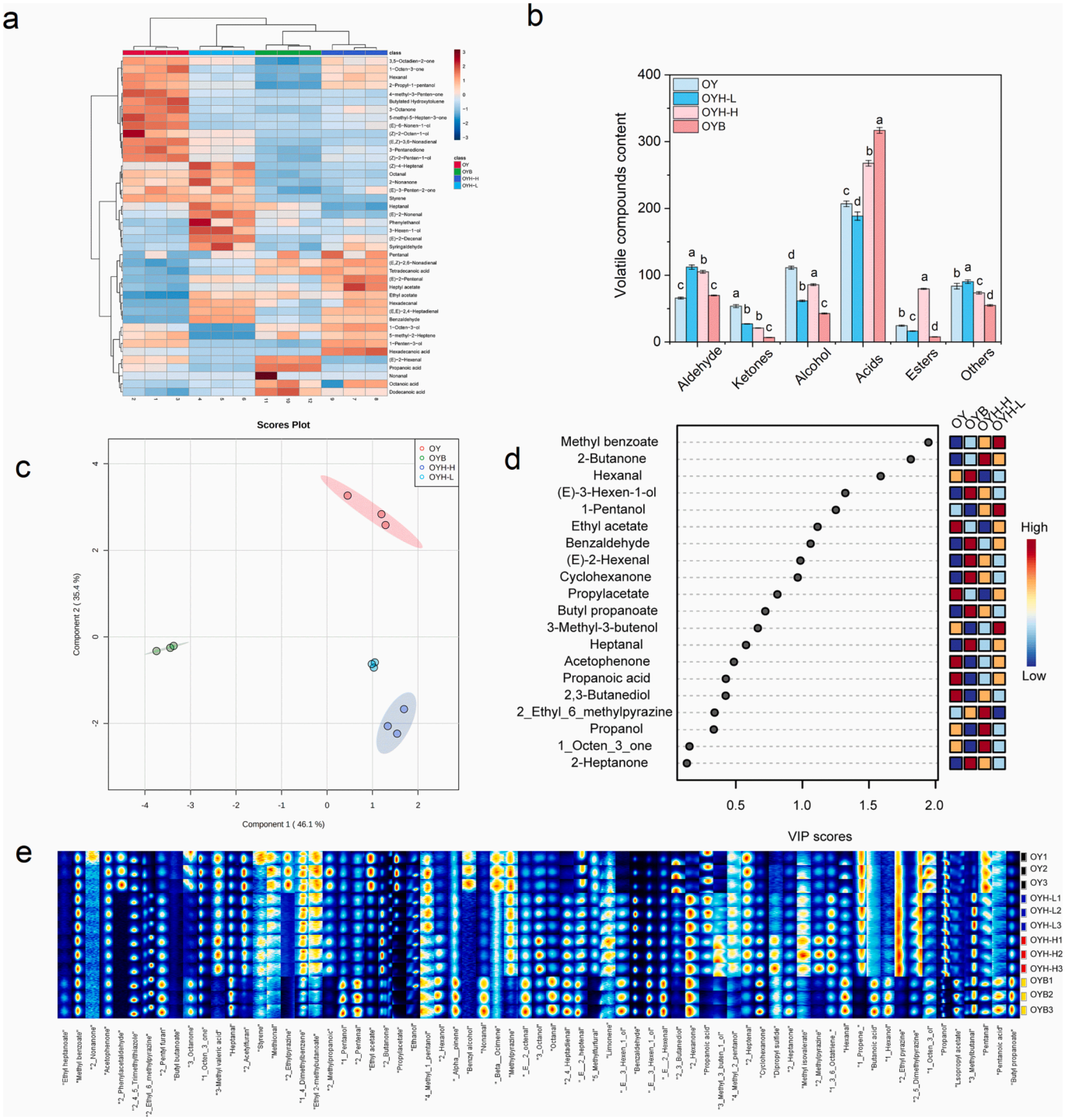


Fig. 2. Cluster heat map of volatile compounds based on GC–MS (a) and concentration diagram based on species (b). PLS-DA score diagram based on odor information (69) of IMS signal intensity (c), VIP score (d), and gallery plot of the volatile compounds (e). OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates. The data are expressed as the means ± standard deviations (n = 3).

health and disease treatment. In this study, 22 fatty acids were identified in different oyster samples, including 10 saturated fatty acids (SFAs), 5 monounsaturated fatty acids (MUFAs), and 7 PUFAs (Table S1). The content of PUFAs in raw oyster homogenates was significantly higher than those of SFAs and MUFAs ($p < 0.05$), which was consistent with previously reported results (Gao et al., 2021). In this study, EPA (20:5) and DHA (22:6) were the most abundant fatty acids, with contents of approximately 4.460 and 4.627 mg/g, respectively, in raw oyster homogenate, followed by arachidonic acid (20:3), palmitic acid (16:0), oleic acid (18:1), linoleic acid (18:2), and myristic acid (14:0). The DHA content decreased to 2.083 mg/g after high-temperature inactivation because of the high-temperature treatment, while the DHA content was unchanged during low-temperature inactivation. The contents of EPA in different samples were found to exhibit similar trends. A previous study reported that DHA and EPA were the most abundant fatty acids in oysters, followed by palmitic acid and oleic acid, and were easily

oxidized during processing. The degree of oxidation was affected by the temperature and enzymes (Solomando, Antequera, & Perez-Palacios, 2020). The results showed that a large amount of unsaturated fatty acids was oxidized after enzymatic hydrolysis, which was aggravated by high temperature inactivation.

Enzymatic oxidation and autooxidation of oleic acid (18:1, *cis*-9) and linoleic acid (18:2, *cis*-9,12) have been widely explained (Xiao et al., 2022). The arachidonic acid content increased from 2.171 to 3.734 mg/g after enzymatic hydrolysis but exhibited a different tendency in boiling concentrates (Table S1). Lipolysis is the first step in the conversion of lipids into flavor compounds catalyzed by lipase and the production of large amounts of free fatty acids (Huang, Li, Huang, Li, & Sun, 2014). The presence of these unsaturated fatty acids (UFAs) can affect the quality and flavor profile of foods because of oxidation reactions (Ding et al., 2020). Some unsaturated aldehydes, ketones, and alcohols could be produced via the oxidation of oleic and linoleic acids: for instance, heptanal, nonanal, and octanal, which could contribute greatly to the odor of aquatic products and provide "fishy" and "oily/fatty" attributes. Therefore, the fat was degraded to a lot of free fatty acids during enzymatic hydrolysis, while the abundant fatty acids were oxidized as precursors to produced more off-odor, especially in high-temperature inactivation. Changes in nonvolatile precursors of key odor compounds and their relationships with odor formation need to be further determined and discussed.

3.4. SPME/SAFE-GC–MS analysis of key odorants

HS-SPME-GC–MS and SAFE-GC–MS were used to analyze volatile compounds (VOCs) in different processed oyster homogenates. Forty-two VOCs were identified by SAFE-GC–MS, and these can be divided into aldehydes (14), ketones (8), alcohols (7), acids (5), esters (2) and others (6) (Fig. 2a and 2b). Specifically, a total of 31 and 39 compounds were found in raw and processed homogenates, respectively. The main volatile components in oyster homogenates were organic acids, occupying 37.93 % and 47.98 % (raw/processed) of the total volatile flavor compounds in content. The relative percentage of aldehydes in content was in the range of 12.05 %–22.61 %. Alcohols ranged from 20.39 % to 8.56 % showing a declining trend during processing. A total of 15 odor compounds were detected through GC–MS, many of which were aldehydes (12) for their low odor threshold and high concentration in oyster homogenates. The contents of aldehydes, aromatic compounds, acids, and esters increased significantly ($p < 0.05$) while those of ketones, alcohols, and alkenes exhibited a downward trend as detected by SAFE-GC–MS after enzymatic hydrolysis. These results indicated that enzymatic hydrolysis had an effect on the composition of VOCs in processed oyster homogenates subjected to different treatments, which was consistent with the results of SPME-GC–MS (Fig. S1b).

A PCA plot of VOCs showed that different oyster samples were clearly classified according to the first two principal components, which had a cumulative contribution of 77 % (Fig. S1c). The overall odors of oyster enzymatic hydrolysates were similar and clearly differentiated from those of other treatments. Heat map clustering analysis was applied to further understand the differences in flavor between raw oyster homogenates and enzymatic hydrolysates. The VOCs were identified as variables using the concentrations estimated by SAFE-GC–MS, and the frequency distribution of each substance in the different processed oyster homogenates is shown in Fig. 2a. As the squared Euclidean distance increases, the samples can be divided into three categories, namely, high-temperature processed homogenates, low-temperature processed homogenates, and raw oyster homogenates. The intensities of each corresponding VOC determined by this method and SPME-GC–MS were similar (Fig. S1a), which resulted from the low sensitivity to short-chain alcohols and fatty acids (Schranz, Lorber, Klos, Kerschbaumer, & Buettner, 2017). Therefore, there were obvious flavor differences between oyster homogenate and enzymatic hydrolysates. The types and intensity of VOCs were changed after enzymatic hydrolysis, while the concentration and contribution to the overall flavor needed to be further determined quantitatively.

Short-chain aldehydes normally have a "fresh, grassy, green" scent and as carbon atoms add up, they may give the impression of being intense as "greasy and fatty". The hexanal ("grass, cucumber") content gradually decreased with the progress of enzymatic hydrolysis, especially after inactivation, but the content in boiling concentrates exhibited an increasing trend. Oyster high-temperature inactivation homogenates had higher contents of benzaldehyde ("almonds"), (*E*)-2-decenal ("peanut, fatty"), and (*E*, *Z*)-2,6-nonadienal ("fatty, almond") than low-temperature inactivation, which was also derived from the thermal oxidation of UFAs, like the degradation of linoleic acid. Aldehydes were produced in large quantities with the increase in the intensity of heating after enzymatic hydrolysis, which resulted in changes in the flavor profile (Liu, Shen, Xiao, Jiang, & Shi, 2022). The concentration of 1-octen-3-ol (mushroom) was highest in oyster high-temperature inactivation homogenates, while the content of 1-penten-3-ol ("fatty, mushroom") was significantly lower after low-temperature inactivation than those in other treatments ($p < 0.05$). 2-Nonanone and 3-octanone were the volatile components of methyl/ethyl ketones, which were mainly associated with non-enzymatic browning reaction and contributed specific "chocolate" and "tobacco" aroma attributes (Belleggia et al., 2020). The acid content in oyster enzymatic hydrolysates was higher than that in raw oyster homogenates. A similar phenomenon also occurred in boiling concentrates, which had the highest contents of caproic acid and palmitic acid. Enzymatic hydrolysis promoted the decomposition of fats with the emergence of off-odors, which had a negative impact on the aroma. This indicated that high-temperature inactivation was more conducive to increasing the contents of saturated alcohols, which caused off-odors in aquatic product hydrolysates (Gao, Xia, Li, & Liu, 2020).

The OAV represents the contribution of a compound to the overall odor, and compounds with an OAV of >1 are generally considered to be odor-active compounds (Tan, Wang, Zhan, & Tian, 2022). Fifteen key odor compounds (with OAV of >1) present in higher concentrations were detected and quantitatively analyzed in combination with the quantitative external standard method. According to the GC–MS results, the most important odor compounds were aldehydes (11), ketones (2), and alcohols (2). As shown in Table 2, the highest OAV of (*E*, *Z*)-2,6-nonadienal was 14159, which was one of the main unsaturated aldehydes in oysters that contributed to the formation of off-odors. The second highest OAV was that of (*E*)-2-nonenal, which contributed the "cardboard" odor and is one of the main aroma compounds in beer (Yang et al., 2021). The OAVs of (*E*, *Z*)-2,6-nonadienal and (*E*)-2-nonanal increased significantly after enzymatic hydrolysis in comparison with raw oyster homogenates, in particular after high-temperature inactivation, which resulted in a deterioration in flavor. Moreover, the OAVs of 3-octanone and 4-octanone increased after enzymatic hydrolysis to 59 and 36, respectively, which contributed to the oxidized and oily odors after enzymatic hydrolysis. The metabolic pathway leading to the formation of these ketones might involve the reduction of aldehydes and dehydrogenation reactions. (1)-Penten-3-ol and (*E*)-2-hexenal were essential for the "green" and "floral" odors and had low OAVs after enzymatic hydrolysis, indicating the change of flavor profile after enzymatic hydrolysis. Combined the HS-SPME and SAFE-GC–MS results, hexanal, octanal, nonanal, (*E*)-2-decenal, (*E*, *Z*)-2,6-nonadienal, benzaldehyde, 1-octen-3-ol, 1-penten-3-ol, 2-nonanone, and 3-octanone were believed to be the main odor compounds which have the key sensory attributes of "fishy", "oily", "grass", "fruity" and "metallic" in oyster homogenates. Compounds only presented in oyster enzymatic hydrolysates could be indicators for estimating the enzymatic hydrolysis like (*E*, *Z*)-2,6-nonadienal and benzaldehyde.

3.5. GC-IMS analysis of volatile flavor compounds

The fingerprint spectra of VOCs detected by GC-IMS are shown in

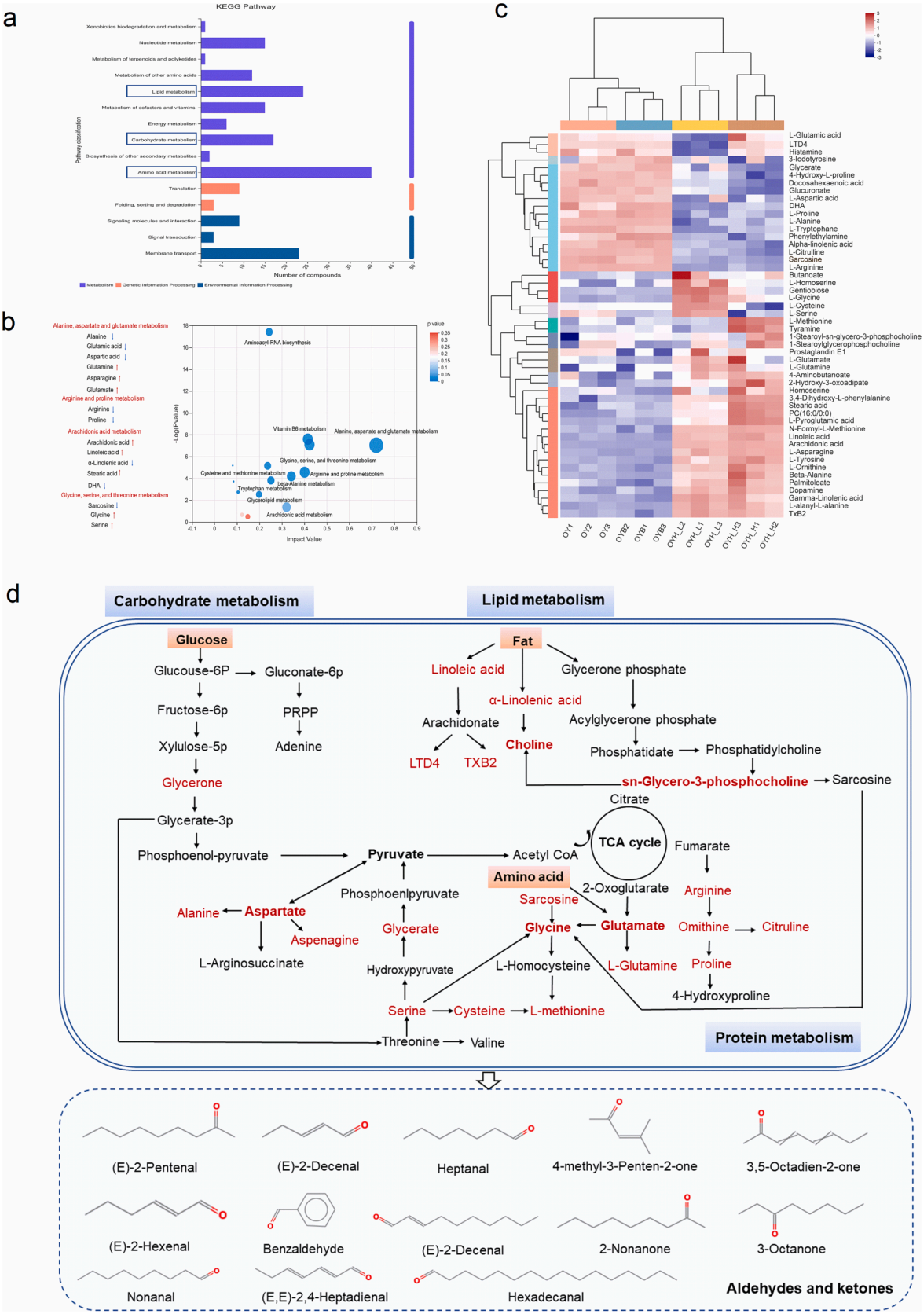


Fig. 3. KEGG pathway classification of annotated differential compounds (a), pathway prediction of differential metabolites based on KEGG analysis (b), heat map of 50 differential metabolites based on VIP score (c), and proposed formation pathway of amino acid, fat and carbohydrate metabolism to form flavor compounds (d). OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates.

Fig. 2e. A total of 69 VOCs were identified, namely, 12 aldehydes, 9 ketones, 4 olefins, 11 alcohols, 3 acids, 10 esters, 7 aromatic compounds, 2 furans, 6 pyrazines, and 5 others. The contents of five aldehydes and five ketones increased significantly after enzymatic hydrolysis. For example, the content of 2-butaone increased by a factor of 3 in oyster enzymatic hydrolysates in comparison with raw oyster homogenates, which resulted in "apple" and "peach" attributes at low concentrations, while "rubbery" and "nutty" odors arose at high concentrations (Fan, Schneider, & Sarnoski, 2022). The presence of C7-C11 unsaturated aldehydes and C8 ketones was mostly responsible for unpleasant attributes, such as rancid, fusty, oxidized, and muddy (Zhang et al., 2020). The intensities of the signals due to flavor compounds were analyzed using a partial least squares discriminant analysis model. As shown in Fig. 2c, there were significant differences between the flavors of different oyster samples, among which oyster enzymatic hydrolysates had similar overall flavors. The variable importance in projection (VIP) scores of VOCs further revealed differences in their contributions across different processed oyster homogenates (Fig. 2d). Nine VOCs were considered to contribute significantly (VIP score >1), and the overall aroma was contributed by (*E*)-3-hexen-1-ol, 2-butaone, hexanal, and methyl benzoate, which shown with sensory attribute of green, fruity, floral, fatty, and aldehydic (Cerreta & Furton, 2015).

Unlike GC–MS, IMS obtained information from product ions and was more sensitive to short-chain alcohols, furans, and acids. Li, Dong, Jiang, Qi, & Lin (2022) used GC-IMS to investigate differences in flavor components of sea cucumber soaked with different seasonings. The concentration of 1-octene-3-one, 3-methyl-2-butanol displayed a decreasing trend after soaking, while the concentrations of linalool, furan, and other representative VOCs increased. Chen et al. (2022) determined that nonanal, benzaldehyde, hexanal, and heptanal, which had low odor thresholds, were the odor-active compounds in hydrolysates of sea cucumber. According to the results of GC-IMS, more VOCs were detected and the flavor difference between oyster enzymatic hydrolysates and raw oyster homogenate was well proved in PLS-DA. Meanwhile, the peculiar smell increases after high-temperature inactivation.

3.6. Analysis of differential metabolites in processed oyster homogenates

Untargeted metabolomics methods can be used to infer the identities of precursors of volatile flavor compounds to reveal their relationships with metabolites. According to the Human Metabolome Database classification of metabolites, the proportions of organic acids and their derivatives were 26.24 % and 34.53 %, respectively (Fig. S2a). Forty-eight metabolites were associated with amino acids, short peptides, and their analogs, and 37 metabolites were produced by degradation and oxidation of lipids. Partial metabolites ($p < 0.05$, VIP score >1.0) were selected as potential candidate differential compounds. A total of 134 and 43 differential metabolites were identified in negative-ion and positive-ion modes, respectively. The KEGG classification of compounds was based on the level of biological function at which metabolites were involved. Fifty differential metabolites were found in different processed oyster homogenates (Fig. S2b). These were divided into six categories of organic compounds and were mainly composed of bioactive peptides, lipids, organic acids, carbohydrates, and nucleotides (Fig. S2c).

KEGG pathways were annotated, and amino acids, lipids, and carbohydrates were found to be metabolites with significantly different abundances (Fig. 3a). Different metabolites, such as amino acids (aspartic acid, tyrosine, alanine, and phenylalanine), lipids (phospholipids and creatine), and some sugars (glucose, maltose, etc.) were detected in different oyster samples in previous study (Wang et al., 2022). As shown in Fig. 3b, 15 metabolic pathways were identified in the KEGG enrichment results. Most of these pathways were involved in amino acid metabolism, which suggested that amino acids were the main cause of the changes in volatile flavor compounds in oyster enzymatic hydrolysates. The production of metabolites was mainly caused by alanine, aspartate and glutamate metabolism and linoleic acid metabolism. Moreover, metabolic pathways such as glycine, serine and threonine metabolism, arginine and proline metabolism, vitamin B6 metabolism, and arachidonic acid metabolism were significantly enriched. A heat map showed that a limited number of metabolites were significantly enriched after enzymatic hydrolysis, such as linoleic acid, stearic acid, glycine, L-methionine, tyrosine, and glutamate. Conversely, the contents of sarcosine, L-aspartic acid, histidine, leukotriene D4, YXB2, and DHA decreased gradually (Fig. 3c). Glutamic acid and aspartic acid are among the main contributors to the formation of the flavor of fresh aquatic products and account for 14.56–20.51 % of FAAs (Liu et al., 2021). Fig. 3b shows that aspartic acid and glutamic acid were transformed into asparagine and glutamine, respectively, after enzymatic hydrolysis, and the fresh flavor disappeared gradually. Besides, the content of sarcosine also decreased after enzymatic hydrolysis and was lower after high-temperature inactivation. This resulted from the fact that sarcosine was an intermediate in the metabolism of glycine and choline and was transformed into glycine, which played an important role in protein oxidation and biological metabolism (Cappello et al., 2018). Benzaldehyde was the product of the oxidation of linoleic acid, which was consistent with the results of GC-IMS and GC–MS. Carboxylic acids were the products of the oxidation of fatty acids. Therefore, protein degradation (alanine, aspartate and glutamate metabolism) and lipid oxidation (linoleic acid metabolism) were main metabolic pathway for different processed oyster homogenates. Vitamin B6 was closely associated with protein metabolism, participated in amino acid conversion and decarboxylation, and, in the form of coenzymes, was involved in the conversion of cysteine and methionine. The content of metabolites of stearic acid and linoleic acid in oyster high-temperature inactivation homogenates was significantly higher than low-temperature inactivation treatment. Oxidized linoleic and oleic acids were metabolized to produce (*E*)-2-heptenal and (*E*, *E*)-2,4-decadienal, and other carbonyl compounds, which were considered unpleasant attributes of off-odor. In addition, 2-hydroxy-3-oxoadipate, which was an intermediate in gluconeogenesis, was also present among the differential metabolites. It could combine with pyruvate to promote fatty acid oxidation and thus result in changes in the flavor profile. The results indicated that non-enzymatic browning reaction were taken place (the reaction of amino acids and lipid oxidation products to produce aldehydes), causing different flavor profile of oyster homogenates and the off-odor produce after enzymatic hydrolysis (Lu, Nielsen, Baron, Diehl, & Jacobsen, 2013). Thus, lipid oxidation, protein degradation, and carbohydrate metabolism were the main factors affecting metabolites in oysters during enzymatic hydrolysis and inactivation, which could be the precursors to regulate and control the flavor formation (Ma et al., 2022).

In the process of enzymatic hydrolysis and inactivation, a large number of bioactive peptides may be generated, which can also participate in further metabolic pathways associated with flavor formation. The flavor formation pathways of some annotated differential metabolites are shown in Fig. 3d and mainly comprised fatty acid metabolism, amino acid metabolism, and glycolysis. In the amino acid metabolism pathway, the alanine content increased after enzymatic hydrolysis, and this could be converted into octanal via Strecker amino acid degradation. The content of aspartic acid decreased while the content of asparagine increased, which resulted in the conversion of asparagine into oxaloacetic acid by transaminase and further reaction to produce ketones. In addition, VOCs were closely associated with the oxidation of UFAs such as linoleic acid. The high content of phenylalanine present in oyster enzymatic hydrolysates could be degraded to benzaldehyde. As shown in Fig. 3d, based on the correlation analysis of precursor and volatile compounds, octanal and nonanal were produced by oxidation of linoleic and stearic acids (Huang et al., 2022); (*E,E*)-2, 4-decenal, (*E*)-2-heptenal were generated by oxidation of 1-*sn*-glycerol-3-phosphocholine (Li et al., 2022); (*E,Z*)-2,6-nonadienal were came from the metabolism of aspartic acid, arginine, proline, and alpha-linolenic acid (Hou et al., 2020). Pyruvate was an important intermediate product in the flavor formation network of oyster hydrolysate. Aldehydes, diacetyl,

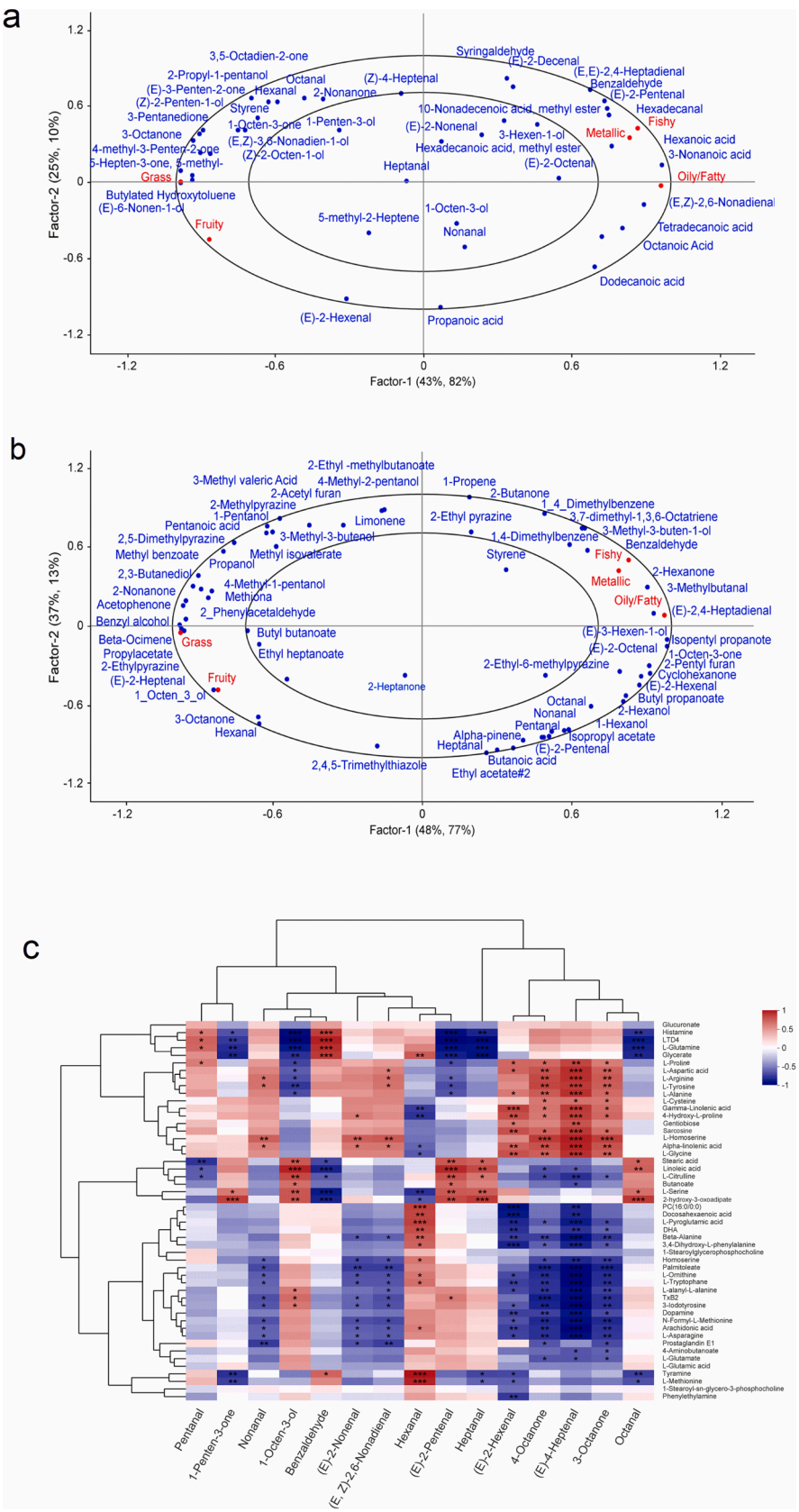


Fig. 4. Partial least squares regression (PLSR) correlation loading diagram. (a) The X-relative percentage of 42 odors detected by GC–MS and the Y-intensity values of 5 odor attributes; (b) X-signal intensity of 68 volatiles detected by GC-IMS and Y-intensity value of 5 odor attributes. (c) The heat map of Pearson correlation analysis results between differential metabolites (top 30) and aldehydes and ketones. OY: raw oyster homogenate; OYH-L: enzymatic hydrolysate with low-temperature inactivation; OYH-H: enzymatic hydrolysate with high-temperature inactivation; OYB: oyster boiling concentrates. The correlation coefficient ($-1 \leq r \leq 1$) is presented in different colors. Levels of significance are shown as follows: $*0.01 < p \leq 0.05$; $**0.001 < p \leq 0.01$; $***p \leq 0.001$. The list of compounds is as described in Table. 2.

and other flavor compounds can be generated in metabolic pathways of organic acids. According to non-target metabolism results, asparagine, alanine, glutamine, serine, threonine, arginine, proline, 1-*sn-glycero*-3-phosphocholine, and linoleic acid were identified as the main metabolites in oyster enzymatic hydrolysates, which were the key precursors of odor compounds like octanal, nonanal (*E,Z*)-2,6-nonadienal, (*E,E*)-2, 4-decenal, (*E*)-2-heptenal, and benzaldehyde in different processed oyster homogenates. Metabolites only presented in oyster enzymatic hydrolysates could be indicators for off-odor regulation of enzymatic hydrolysis such as asparagine, glutamine, serine, and linoleic.

3.7. Correlation analysis of sensory attributes, key odorants, and differential metabolites

To further investigate the correlations between VOCs, sensory attributes, and differential metabolites, two PLSR models were established, and a correlation heat map was generated (Fig. 4). As seen in Fig. 4a, 68 % of the cross-validation variance in the X and Y variables was well explained by principal components 1 and 2. Aldehydes and ketones had important effects on these properties because of their proximity to the external cycle; examples included benzaldehyde, (*E*)-2-hexenal, octanal, (*E*)-2-pentenal, 3-octanone, and 4-methyl-3-penten-2-one. Studies showed that additive or synergistic effects occurred in interactions of compounds with similar structures (Niu, Zhu, & Xiao, 2020). A total of 85 % of the explained variance is represented by the X and Y variables in Fig. 4b. The distribution of the five attributes is the same as in Fig. 4a, but in contrast more odors are located near the outer ellipse. A large number of VOCs were associated with the "grassy," "fishy," and "oily/fatty" attributes, which indicated strong correlations, while the "metallic" and "fruity" attributes had fewer VOCs around them. Odor compounds exhibited interactions and compounds of similar structures could be additive or synergistic.

A number of 15 VOCs with OAV >1 and 50 major differential metabolites (with VIP scores of >1) were selected. The Pearson method was used to analyze the correlations between key volatiles and differential metabolites (Fig. 4c). Asparagine, sarcosine, and arginine were positively correlated with most VOCs, while tyrosine, alanine, and glutamine were negatively correlated. The flavor profile of different processed oyster homogenates was formed by a mixture of various FAAs after enzymatic hydrolysis. Stearic acid and linoleic acid had strong positive correlations with hexanal, heptanal, octanal, and 1-octene-3-ol. 1-Octene-3-ol was produced by the reaction of alkoxy radicals with fatty acid molecules during lipid oxidation (Xiang, Jin, Gouda, Jin, & Ma, 2019). Therefore, asparagine, sarcosine, arginine, tyrosine, alanine, glutamine, stearic acid and linoleic acid were important precursors of odor compounds in oyster processed homogenates. The content and structure changes of precursors affect the generation of off-odor after enzymatic hydrolysis. Furthermore, glycogen was also an important precursor of volatile flavor compounds. Glucose and galactose were positively correlated with some volatile flavor compounds, and glucose could be used as a precursor in the Maillard reaction to affect the flavor of oysters. A standardized system should be established for further verification of the flavor formation pathways of the odorants, including (*E,Z*)-2,6-nonadienal, (*E*)-2-nonenal, (*E,E*)-2,4-decenal, (*E*)-2-heptenal and others, detected in our study. This study is to be considered as a first step toward a comprehensive investigation revealing the mechanisms of flavor change in different processed oyster homogenates from the perspectives of volatile odorants and non-volatile metabolites.

4. Conclusion

In summary, aldehydes and ketones were the main compounds identified in GC–MS analysis, whereas in contrast alcohols and esters were identified in GC-IMS analysis. Fifteen compounds (mainly aldehydes) were considered to be the main key odor compounds in different processed oyster homogenates. The contents of benzaldehyde, nonanal, (*E,Z*)-2,6-nonadienal, 3-octanone, and 4-octanone significantly increased after enzymatic hydrolysis. These aldehydes and ketones were positively correlated with asparagine, alanine, glutamine, serine, threonine, arginine, proline, 1-*sn-glycero*-3-phosphocholine, and linoleic acid which suggested that amino acids and fatty acids were important precursors of flavor compounds. According to the sensory qualities and flavor stability, high-temperature inactivation was the main reason for the increase in off-odors in oyster hydrolysate. However, the mechanisms and methods that controlling enzyme action, kinetic factors, and critical steps in the formation and deterioration of flavor during the enzymatic hydrolysis process are still unknown. The key precursors of flavor compounds were identified, which has laid a solid foundation for subsequent research into flavor regulation. Further investigations that take these factors into account need to be carried out to promote the application of enzymatic hydrolysate with high quality in oyster processing.

CRediT authorship contribution statement

Li Liu: Methodology, Software, Writing – original draft. **Yuanhui Zhao:** Data curation. **Shixue Lu:** Formal analysis. **Yihuan Liu:** Investigation, Validation, Visualization. **Xinxing Xu:** Project administration, Resources, Supervision, Funding acquisition. **Mingyong Zeng:** Project administration, Resources, Supervision, Funding acquisition.

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.

Data availability

No data was used for the research described in the article.

Acknowledgements

This work was supported by the financial support from National key R & D Program of China (2018YFD0901003), National key R & D Program of China (2018YFD0700900), Natural Science Foundation of Shandong Province (ZR2019MC014), and Science and Technology Support Program of North Jiangsu Province (SZ-SQ2021018).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100569.

References

Belleggia, L., Aquilanti, L., Ferrocino, I., Milanović, V., Garofalo, C., Clementi, F., … Osimani, A. (2020). Discovering microbiota and volatile compounds of surströmming, the traditional Swedish sour herring. *Food Microbiology, 91*, Article 103503. https://doi.org/10.1016/j.fm.2020.103503

Buchhaupt, M., Guder, J. C., Etschmann, M. M. W., & Schrader, J. (2012). Synthesis of green note aroma compounds by biotransformation of fatty acids using yeast cells coexpressing lipoxygenase and hydroperoxide lyase. *Applied Microbiology and Biotechnology, 93*(1), 159–168. https://doi.org/10.1007/s00253-011-3482-1

Cappello, T., Giannetto, A., Parrino, V., De Marco, G., Mauceri, A., & Maisano, M. (2018). Food safety using NMR-based metabolomics: Assessment of the Atlantic bluefin tuna, Thunnus thynnus, from the Mediterranean Sea. *Food and Chemical Toxicology, 115*, 391–397. https://doi.org/10.1016/j.fct.2018.03.038

Cerreta, M. M., & Furton, K. G. (2015). An assessment of detection canine alerts using flowers that release methyl benzoate, the cocaine odorant, and an evaluation of their behavior in terms of the VOCs produced. *Forensic Science International, 251*, 107–114. https://doi.org/10.1016/j.forsciint.2015.03.021

Chang, C., Wu, G. C., Zhang, H., Jin, Q. Z., & Wang, X. G. (2019). Deep-fried flavor: Characteristics, formation mechanisms, and influencing factors. *Critical Reviews in Food Science and Nutrition, 60*, 1–19. https://doi.org/10.1080/10408398.2019.1575792

Chen, C., Yuan, J. J., Yu, H. Y., Wang, B., Huang, J., Yuan, H. B., … Tian, H. X. (2022). Characterization of metabolic pathways for biosynthesis of the flavor compound 3-methylbutanal by Lactococcus lactis. *Journal of Dairy Science, 105*(1), 97–108. https://doi.org/10.3168/jds.2021-20779

Domínguez, R., Purriños, L., Pérez-Santaescolástica, C., Pateiro, M., Barba, F., Tomasevic, I., Campagnol, P., & Lorenzo, J. M. (2019). Characterization of volatile compounds of dry-cured meat products using HS-SPME-GC/MS technique. *Food Analytical Methods*, 12 (6):1-22, doi: 1-22 10.1007/s12161-019-01491-x.

Ding, A., Zhu, M., Qian, X., Shi, L., Huang, H., Xiong, G., … &Wang, L. (2020). Effect of fatty acids on the flavor formation of fish sauce. *LWT- Food Science and Technology, 134*, 110259. https://doi.org/10.1016/j.lwt.2020.110259

Duan, Z., Dong, S. G., Sun, Y. X., Dong, Y. W., & Gao, Q. F. (2021). Response of Atlantic salmon (Salmo salar) flavor to environmental salinity while culturing between freshwater and seawater. *Aquaculture, 530*, Article 735953. https://doi.org/10.1016/j.aquaculture.2020.735953

Fan, Y., Schneider, K. R., & Sarnoski, P. J. (2022). Determining spoilage of whiteleg shrimp (Litopanaeus vannemei) during refrigerated storage using colorimetric strips. *Food Chemistry: X, 14*, Article 100263. https://doi.org/10.1016/j.fochx.2022.100263

Feng, C. S., Tian, L., Jiao, Y. D., Tan, Y. Q., Liu, C., Luo, Y. K., & Hong, H. (2022). The effect of steam cooking on the proteolysis of pacific oyster (*Crassostrea gigas*) proteins: Digestibility, allergenicity, and bioactivity. *Food Chemistry, 379*, Article 132160. https://doi.org/10.1016/j.foodchem.2022.132160

Gao, J. X., Zhang, Y. Y., Huang, X. H., Liu, R., Dong, X.-P., Zhu, B.-W., & Qin, L. (2021). Comparison of amino acid, 5′-nucleotide and lipid metabolism of oysters (*Crassostrea gigas* Thunberg) captured in different seasons. *Food Research International, 147*, Article 110560. https://doi.org/10.1016/j.foodres.2021.110560

Gao, P., Xia, W. S. X., Li, X. Z., & Liu, S. Q. (2020). Optimization of the Maillard reaction of xylose with cysteine for modulating aroma compound formation in fermented tilapia fish head hydrolysate using response surface methodology. *Food Chemistry, 331*, Article 127353. https://doi.org/10.1016/j.foodchem.2020.127353

Hou, Z., Sun, A., Wei, Q., He, C., Song, L., Shao, X., Li, Y., Wang, M., Mei, T., Zhang, Y., Shang, H., Sun, Z., &Chen, J. (2020). Metabolomics study on improvement of mild cognitive impairment by SCPE: a UPLC-Q/TOF-MS mass spectrometric study. In: *Research Square*, PPR211344 doi: 10.21203/rs.3.rs-70833/v1.

Huang, Q., Dong, K., Wang, Q., Huang, X., Wang, G., An, F., … Luo, P. (2022). Changes in volatile flavor of yak meat during oxidation based on multi-omics. *Food Chemistry, 371*, Article 131103. https://doi.org/10.1016/j.foodchem.2021.131103

Huang, Y., Li, H., Huang, T., Li, F., & Sun, J. (2014). Lipolysis and lipid oxidation during processing of Chinese traditional smoke-cured bacon. *Food Chemistry, 149*, 31–39. https://doi.org/10.1016/j.foodchem.2013.10.081

Jiang, S. S., Liu, L., Xu, J. J., Zeng, M. Y., & Zhao, Y. H. (2019). Amino acid composition and digestibility of Pacific oyster (Crassostrea gigas) proteins isolated from different parts. *LWT-Food Science and Technology, 116*, Article 108591. https://doi.org/10.1016/j.lwt.2019.108591

Li, X. P., Liu, Y. W., Wang, Y. Y., Wang, J. X., Xu, Y. X., Yi, S. M., … Li, J. R. (2021). Combined ultrasound and heat pretreatment improve the enzymatic hydrolysis of clam (Aloididae aloidi) and the flavor of hydrolysates. *Innovative Food Science & Emerging Technologies, 67*, Article 102596. https://doi.org/10.1016/j.ifset.2020.102596

Li, X. R., Dong, Y. F., Jiang, P. F., Qi, L. B., & Lin, S. Y. (2022). Identification of changes in volatile compounds in sea cucumber Apostichopus japonicus during seasonings soaking using HS-GC-IMS. *LWT-Food Science and Technology, 154*, Article 112695. https://doi.org/10.1016/j.lwt.2021.112695

Li, X., Xie, W., Bai, F., Wang, J., Zhou, X., Gao, R., … Zhao, Y. (2022). Influence of thermal processing on flavor and sensory profile of sturgeon meat. *Food Chemistry, 374*, Article 131689. https://doi.org/10.1016/j.foodchem.2021.131689

Liang, S. Q., Zhang, T., Fu, X. D., Zhu, C. L., & Mou, H. J. (2020). Partially degraded chitosan-based flocculation to achieve effective deodorization of oyster (*Crassostrea gigas*) hydrolysates. *Carbohydrate Polymers, 234*, Article 115948. https://doi.org/10.1016/j.carbpol.2020.115948

Liu, C. S., Ji, W. Z., Jiang, H. Z., Shi, Y. H., He, L., Gu, Z. F., & Zhu, S. T. (2021). Comparison of biochemical composition and non-volatile taste active compounds in raw, high hydrostatic pressure-treated and steamed oysters (*Crassostrea hongkongensis*). *Food Chemistry, 344*, Article 128632. https://doi.org/10.1016/j.foodchem.2020.128632

Liu, J. Y., Shen, S. Y., Xiao, N. Y., Jiang, Q. Q., & Shi, W. Z. (2022). Effect of glycation on physicochemical properties and volatile flavor characteristics of silver carp mince. *Food Chemistry, 386*, Article 132741. https://doi.org/10.1016/j.foodchem.2022.132741

Liu, S., Wang, S. J., Fang, Y. W., Ming-Sheng, L., Jiao, Y. L., & Chen, Y. (2013). Functional and Antioxidant Properties of Protein Hydrolysates from Pacific Oyster (*Crassostrea gigas*) Thunberg Fermented by Mold Aspergillus oryzae. *Fisheries Science, 11*, 641–645.

Lu, F. S. H., Nielsen, N. S., Baron, C. P., Diehl, B. W. K., & Jacobsen, C. (2013). Impact of primary amine group from aminophospholipids and amino acids on marine phospholipids stability: Non-enzymatic browning and lipid oxidation. *Food Chemistry, 141*(2), 879–888. https://doi.org/10.1016/j.foodchem.2013.03.063

Luo, X., Liu, W., Zhong, H., Yan, Y., Feng, F., & Zhao, M. (2021). Synergistic effect of combined oyster peptide and ginseng extracts on anti-exercise-fatigue and promotion of sexual interest activity in male ICR mice. *Journal of Functional Foods, 86*(12), Article 104700. https://doi.org/10.1016/j.jff.2021.104700

Luo, X. Y., Xiao, S. T., Ruan, Q. F., Gao, Q., An, Y. Q., Hu, Y., & Xiong, S. B. (2022). Differences in flavor characteristics of frozen surimi products reheated by microwave, water boiling, steaming, and frying. *Food Chemistry, 372*, Article 131260. https://doi.org/10.1016/j.foodchem.2021.131260

Ma, T. T., Wang, Q., Wei, P. Y., Zhu, K. X., Feng, A. G., He, Y. F., … Li, C. (2022). EGCG-gelatin biofilm improved the protein degradation, flavor and micromolecule metabolites of tilapia fillets during chilled storage. *Food Chemistry, 375*, Article 131662. https://doi.org/10.1016/j.foodchem.2021.131662

Majcher, M. A., & Jeleń, H. H. (2007). Effect of cysteine and cystine addition on sensory profile and potent odorants of extruded potato snacks. *Journal of Agricultural and Food Chemistry, 55*(14), 5754–5760. https://doi.org/10.1021/jf0703147

Medeiros Vicentini-Polette, C., Rodolfo Ramos, P., Bernardo Gonçalves, C., & Lopes De Oliveira, A. (2021). Determination of free fatty acids in crude vegetable oil samples obtained by high-pressure processes. *Food Chemistry: X, 12*, Article 100166. https://doi.org/10.1016/j.fochx.2021.100166

Niu, Y. W., Zhu, Q., & Xiao, Z. B. (2020). Characterization of perceptual interactions among ester aroma compounds found in Chinese Moutai Baijiu by gas chromatography-olfactometry, odor Intensity, olfactory threshold and odor activity value. *Food Research International, 131*, Article 108986. https://doi.org/10.1016/j.foodres.2020.108986

Schranz, M., Lorber, K., Klos, K., Kerschbaumer, J., & Buettner, A. (2017). Influence of the chemical structure on the odor qualities and odor thresholds of guaiacol-derived odorants, Part 1: Alkylated, alkenylated and methoxylated derivatives. *Food Chemistry, 232*, 808–819. https://doi.org/10.1016/j.foodchem.2017.04.070

Ruan, L., Ju, Y., Zhan, C., & &Hou, L. (2022). Improved umami flavor of soy sauce by adding enzymatic hydrolysate of low-value fish in the natural brewing process. *LWT-Food Science and Technology, 155*, 112911. https://doi.org/10.1016/j.lwt.2021.112911

Sajib, M., & Undeland, I. (2020). Towards valorization of herring filleting by-products to silage 2.0: Effect of temperature and time on lipid oxidation and non-enzymatic browning reactions. *LWT-Food Science and Technology, 127*, Article 109441. https://doi.org/10.1016/j.lwt.2020.109441

Solomando, J. C., Antequera, T., & Perez-Palacios, T. (2020). Lipid digestion and oxidative stability in ω-3-enriched meat model systems: Effect of fish oil microcapsules and processing or culinary cooking. *Food Chemistry, 328*(1), Article 127125. https://doi.org/10.1016/j.foodchem.2020.127125

Su, Y. J., Chen, Z., Li, J. H., Chang, C. H., Gu, L. P., & Yang, Y. J. (2021). Characterization of salted egg yolk flavoring prepared by enzyme hydrolysis and microwave irradiation. *Food Chemistry, 338*, Article 127913. https://doi.org/10.1016/j.foodchem.2020.127913

Tan, F. L., Wang, P., Zhan, P., & Tian, H. L. (2022). Characterization of key aroma compounds in flat peach juice based on gas chromatography-mass spectrometry-olfactometry (GC-MS-O), odor activity value (OAV), aroma recombination, and omission experiments. *Food Chemistry, 366*, Article 130604. https://doi.org/10.1016/j.foodchem.2021.130604

Wang, Y., & Kays, S. J. (2000). Contribution of volatile compounds to the characteristic aroma of baked 'Jewel' sweetpotatoes. *Journal of the American Society for Horticultural Science, 125*(5), 638–643. https://doi.org/10.21273/jashs.125.5.638

Wang, Z., Zhang, Y., Yao, D., Zhao, Y., Tran, N. T., Li, S., … &Aweya, J. J. (2022). Metabolic reprogramming in crustaceans: A vital immune and environmental response strategy. *Reviews in Aquaculture, 14*(3), 1094–1119. https://doi.org/10.1111/raq.12640

Wen, R. X., Hu, Y. Y., Zhang, L. Z., Wang, Y., Chen, Q., & Kong, B. H. (2019). Effect of NaCl substitutes on lipid and protein oxidation and flavor development of Harbin dry sausage. *Meat Science, 156*, 33–43. https://doi.org/10.1016/j.meatsci.2019.05.011

Wu, W. D., Zhan, J. L., Tang, X. Y., Li, T., & Duan, S. N. (2022). Characterization and identification of pork flavor compounds and their precursors in Chinese indigenous pig breeds by volatile profiling and multivariate analysis. *Food Chemistry, 385*, Article 132543. https://doi.org/10.1016/j.foodchem.2022.132543

Wu, T., Wang, M., Wang, P., Tian, H., & Zhan, P. (2022). Advances in the formation and control methods of undesirable flavors in fish. *Foods, 11*(16), 2504.

Xiang, X. L., Jin, G. F., Gouda, M., Jin, Y. G., & Ma, M. H. (2019). Characterization and classification of volatiles from different breeds of eggs by SPME-GC–MS and chemometrics. *Food Research International, 116*, 767–777. https://doi.org/10.1016/j.foodres.2018.09.010

Xiao, Y., Liu, H., Du, P. X., Liang, X. Q., Li, H. F., Lu, Q., … Chen, X. (2022). Impact of different cooking methods on the chemical profile of high-oleic acid peanut seeds. *Food Chemistry, 379*, Article 131970. https://doi.org/10.1016/j.foodchem.2021.131970

Xie, Q., Xu, B., Xu, Y., Yao, Z., Zhu, B., Li, X., & Sun, Y. (2022). Effects of different thermal treatment temperatures on volatile flavour compounds of water-boiled salted duck after packaging. *LWT- Food Seience and Technology, 154*, Article 112625. https://doi.org/10.1016/j.lwt.2021.112625

Xu, X. X., Bi, S., Lao, F., Chen, F., Liao, X. J., & Wu, J. H. (2021). Comprehensive investigation on volatile and non-volatile metabolites in broccoli juices fermented by animal- and plant-derived Pediococcus pentosaceus. *Food Chemistry, 341*, Article 128118. https://doi.org/10.1016/j.foodchem.2020.128118

Yang, M., Zhai, X. D., Huang, X. W., Li, Z. H., Shi, J. Y., Li, Q., … Battino, M. (2021). Rapid discrimination of beer based on quantitative aroma determination using colorimetric sensor array. *Food Chemistry, 363*(2), Article 130297. https://doi.org/10.1016/j.foodchem.2021.130297

Yang, Y. J., Ai, L. Z., Mu, Z. Y., Liu, H. D., Yan, X. N., Ni, L., … Xia, Y. J. (2022). Flavor compounds with high odor activity values (OAV > 1) dominate the aroma of aged Chinese rice wine (Huangjiu) by molecular association. *Food Chemistry, 383*, Article 132370. https://doi.org/10.1016/j.foodchem.2022.132370

Yin, M., Matsuoka, R., Yanagisawa, T., Xi, Y., Zhang, L., & Wang, X. (2022). Effect of different drying methods on free amino acid and flavor nucleotides of scallop (patinopecten yessoensis) adductor muscle. *Food Chemistry, 396*, Article 133620. https://doi.org/10.1016/j.foodchem.2022.133620

Zang, J. H., Yu, D. W., Zhang, P., Xu, Y. S., & Xia, W. S. (2022). The key enzymes and flavor precursors involved in formation of characteristic flavor compounds of low-salt fermented common carp (*Cyprinus carpio L.*). *LWT-Food Science and Technology, 154*, Article 112806. https://doi.org/10.1016/j.lwt.2021.112806

Zhang, Q., Ding, Y. C., Gu, S. Q., Zhu, S. C., Zhou, X. X., & Ding, Y. T. (2020). Identification of changes in volatile compounds in dry-cured fish during storage using HS-GC-IMS. *Food Research International, 137*(4), Article 109339. https://doi.org/10.1016/j.foodres.2020.109339

Zhao, D., Li, H., Huang, M. X., Wang, T. L., Hu, Y., Wang, L. P., … Zhou, G. H. (2020). Influence of proteolytic enzyme treatment on the changes in volatile compounds and odors of beef longissimus dorsi. *Food Chemistry, 333*(10), Article 127549. https://doi.org/10.1016/j.foodchem.2020.127549

Zhang, L., Zhang, F., & Wang, X. (2016). Changes of protein secondary structures of pollock surimi gels under high-temperature (100 °C and 120 °C) treatment. *Journal of Food Engineering, 171*, 159–163. https://doi.org/10.1016/j.jfoodeng.2015.10.025