The Hydrolysates of Oyster and Penaeus Orientalis Can be used as High Quality Flavor Base Material

Zhao Yang[a], Siying Huang, Zidan Yan, Yongqi Lin, Tonglin Zhou, Haixia Tang, Chunlan He*

Guangdong Food and Drug Vocational College, Guangzhou, Guangdong, China

*Corresponding author e-mail: hchunlan2012@163.com, [a] yangz@gdyzy.edu.cn

Abstract. Oyster and penaeus orientalis were used as raw materials, which were enzymatically hydrolyzed in 5 different ratios, and the optimal mixture ratio was selected according to the degree of hydrolysis and sensory score. Then the volatile components and amino acid composition of the mixture were analysed. The results showed that the optimal ratio of oyster and penaeus orientalis was 5:1, the sensory score was 70.7, and the degree of hydrolysis was 24.88%. Combined with the indicators of degree of hydrolysis and sensory score, the optimal ratio of oyster and penaeus orientalis was 5:1, and 28 kinds of compounds were identified in this mixture hydrolysate.

Keywords: oyster; penaeus orientalis; flavor base material; hydrolysate.

1. Introduction

People take food as the day, and taste as the first. Soy sauce, vinegar and seasoning, although common, but have involved thousands of families and become necessities of public life. Due to people's growing pursuit of a better life and gradually changing consumption awareness, the annual consumption of condiment market shows a good trend of increasing year by year. Now, seasonings can not only play the role of seasoning when cooking, but also some composite seafood seasonings can enhance the nutritional value because they contain rich nutrients. The condiment industry has also become one of the most robust growth sectors in the field of fast-selling products in China.

In recent years, the annual growth rate of fermented condiments in China is more than 15 %, which is one of the fastest growing categories in the food industry, and the growth rate of compound condiments is more than 20%. In 2016, the market sales volume of condiments has reached 150 billion yuan, the total output of condiments reached more than 13 million tons, which the yield of soy sauce reached 5 million tons. However, in the international condiment market, compound condiments have accounted for 80% of the market share, while China's compound condiments are less than 10% of the condiment market. This phenomenon also fully reflects that China ' s compound condiment market has great development space and potential.

Flavor base material is essential in the configuration of compound condiments. It is characterized by a strong umami and characteristic flavor, and can help compound condiments provide a full and mellow taste[1].After the enzymatic hydrolysis of protein by complex protease, fresh amino acids and polypeptides are produced. It is precisely because these substances give the enzymatic hydrolysis products the characteristics of delicious taste and rich taste. Protein enzymatic hydrolysis is also one of the important methods to prepare flavor base materials.

Oyster is an important food resource with low fat and high protein, ranking first in the world among cultured shellfish[2].Although oysters are delicious and nutritious, there are still few deep-processing products. In recent years, the use of biological enzymes to treat oysters and other marine products to prepare flavor peptides and bioactive peptides has become a hot research point in the deep processing of oysters and other marine products. Penaeus orientalis is also a nutrient-rich marine product. The protein content of penaeus orientalis is generally higher than that of ordinary fish, which low in fat and high in protein. It is also rich in minerals such as calcium, iron, phosphorus and vitamin A, which is in line with the dietary habits of the modern masses[3].

In this study, the advantages of oyster and penaeus orientalis were combined. Oyster and penaeus orientalis were used as raw materials, and the hydrolysis degree and sensory evaluation were used as indicators to screen the optimal ratio of hydrolysates. The volatile components of the optimal enzymatic hydrolysates were analyzed, which provided a theoretical basis for the in-depth study of the production of flavor base materials by oyster and penaeus orientalis.

2. Experimental materials and equipment

2.1. Experimental Materials and Reagents

Frozen fresh oyster (purchased in supermarket, frozen at -18℃ in the laboratory), Frozen fresh penaeus orientalis (purchased in supermarket, frozen at -18℃ in the laboratory),trypsin(enzyme activity:4000U/ g, Henan Wanbang Industrial Co., Ltd.), flavourzyme(enzyme activity:500LAPU/g, Henan Wanbang Industrial Co., Ltd.),formaldehyde(Tianjin Damao Chemical Reagent Factory).

2.2. Experimental instruments and equipment

pH meter (Mettler-Toledo Instrument (Shanghai) Co., Ltd.);thermostat water bath(Changzhou Aohua Instrument Co.,Ltd.);colorimeter (Shenzhen San Enchi Technology Co., Ltd.);analytical balance(Shimadzu Instrument (Suzhou) Co., Ltd. Model ATX224);gas chromatograph-mass spectrometer(Japan Shimadzu Production Institute GCMS-QP2010 Ultra);multifunctional machine(Guangzhou Qihe Electrical Appliances Co., Ltd.);kjeldahl apparatus(Jinan Haineng Instrument Co., Ltd.);magnetic stirrer(Aika (Guangzhou) Equipment Co., Ltd).

3. Experimental method

3.1. Enzymatic hydrolysis of oyster and penaeus orientalis

Slightly adjusted by Huang Ke-xin[4]method. The oyster meat and penaeus orientalis was thawed at room temperature and wash with running water. Oyster meat and penaeus orientalis were mixed according to the mass ratios of 1:1, 2:1, 3:1, 4:1, and 5:1. After shaken, the pH was adjusted to 8.0 with 1 mol/L NaOH solution or 1 mol/L HCL. Trypsin and flavourzyme were added at the same time. After the addition of proteinase, the mixture was quickly stirred with a glass rod to avoid agglomeration. The amount of enzyme added was 0.7 % of the solid of aquatic products, and the ratio was 3 : 1.After shaking well, the enzyme was hydrolyzed at 55 °C for 2 h, and pH was adjusted to 8.0 for per 1 h. The regulation of pH could maximize the effect of protease hydrolysis. After the end of the enzymatic hydrolysis, the enzyme was extinguished in boiling water bath for 15 min. At last, centrifuged at 8000 r/min for 10 min. After centrifugation, the supernatant was separated at -18 °C for standby.

3.2. Determination of degree of hydrolysis(DH)

According to determination of free ammonia nitrogen in oyster and penaeus orientalis hydrolysates by formaldehyde potentiometric titration. The formula of degree of hydrolysis of enzymatic hydrolysate:

$$\text{Degree of hydrolysis (DH)}=\frac{\text{Ammonia nitrogen content in supernatant}\times\text{Supernatant quality}}{\text{Total nitrogen content of raw materials}\times\text{Ingredient quality}}\times 100\%$$

3.3. Sensory evaluation of enzymatic hydrolysates

Seven sensory assessors aged 16~25 were selected to perform sensory evaluation in a sensory evaluation room with a temperature of (24 ± 1) ℃. Sensory evaluators were familiar with the flavor characteristics and sensory strength of enzymatic hydrolysates of oyster and penaeus orientalis after basic taste training before sensory evaluation. The standards for training were sweet (1% sucrose), salty (0.35% salt), umami (0.2% monosodium glutamate), bitter (0.02 g/L quinine hydrochloride), and sour (0.04% citric acid).Evaluation steps: The assessor takes 5mL of the sample solution to be tested and puts it into the mouth. The sample solution stays in the mouth for 20~25s, and then spits it out. The sensory results are recorded according to the sensory evaluation standards (see Table 1).

Table 1 Scoring standard of sensory analysis

Project	Evaluation criterion	Score
Color （30 score）	The liquid is clear and uniform without sedimentation and stratification	20-30
	The color is even, and there is a little turbidity in some parts	10-19
	Uneven color	1-9
Smell （30 score）	With the flavor of aquatic products, the aroma is harmonious and soft	20-30
	The taste of aquatic products is slightly weak without peculiar smell	10-19
	There 's no smell of aquatic products	1-9
Taste （40 score）	It has rich flavor and good taste, without sour and bitter taste	25-40
	A slight umami, general taste, slight bitter taste, no sour taste	10-24
	It has obvious sour and bitter taste.	1-9

3.4. Analysis of volatile flavor components

Extraction of volatile flavor components[5]

A total of 3 g oyster enzymatic hydrolysate was weighed and puted into a headspace extraction bottle. The cover was immediately tightened and balanced at 55 °C for 10 min. The DVB/CAR/PDMS extraction head was inserted into the headspace bottle. After adsorption for 1 h, the extraction head was pulled out for GC-MS detection.

Identification of volatile flavor components[5]

Chromatographic conditions: RTS-5MS column (30m×0.25mm, 0.25μm). The carrier gas was high purity helium (99.999%), and the flow rate was 0.8 mL/min. Heating procedure: the initial column temperature was 35 ℃, kept for 2min, then increased to 40 ℃ at 4 ℃/min, kept for 2 min, then increased to 100 ℃ at 5 ℃/min, kept for 10 min, and then increased to 230 ℃ at 10 ℃/min, kept for 10 min. The inlet temperature was 230 ℃, the desorption temperature was 230 ℃, and the desorption time was 5 min.MS conditions: Ionization mode: EI; Electron energy: 70 Ev;Ion source temperature: 200 ℃; Mass scanning range: 35-500 m/z.

The detected compounds were compared and searched through NIST11 mass spectrometry library, and the compounds with similarity greater than 80% were retained. Combined with the relevant literature, the retrieved compounds were qualitatively identified, and the relative content of each volatile flavor component was determined by area normalization method.

3.5. Data analysis

All tests were conducted for 3 times. Excel2010 and SPSS20.0 software were used for statistical analysis.

4. Results and analysis

4.1. Effect of different compounding ratios on hydrolysis degree of flavoring base materials

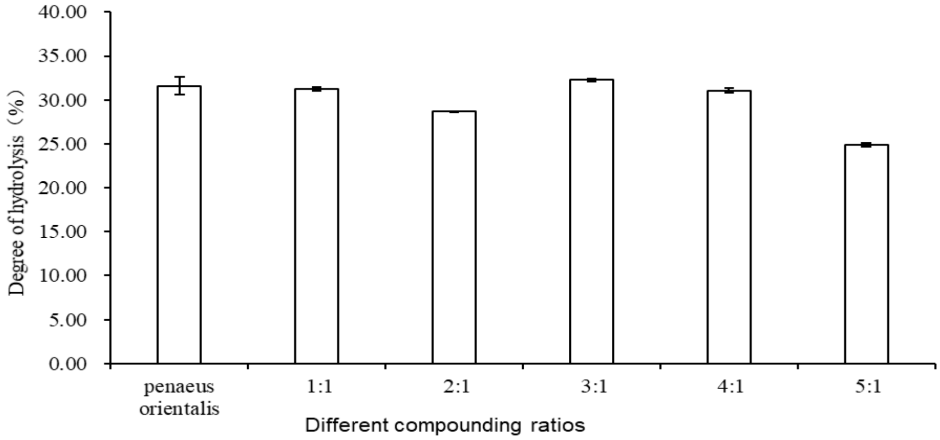

Fig. 1 Effect of different compounding ratios on degree of hydrolysis of flavoring base materials

According to figure 1, when the ratio of oyster meat to penaeus orientalis was 5:1, the minimum degree of hydrolysis was 24.88%.When the ratio of oyster meat to penaeus orientalis was 1 : 1, 2 : 1, 3 : 1 and 4 : 1, the degree of hydrolysis was 31.22 %, 28.65 %, 32.27 % and 31.08 %, respectively, and the degree of hydrolysis of penaeus orientalis alone was 31.59 %.From the perspective of analyzing the degree of hydrolysis, the mass ratio of oyster meat to penaeus orientalis was determined as 5:1.

4.2. Effects of different compounding ratios on sensory evaluation of flavoring base materials

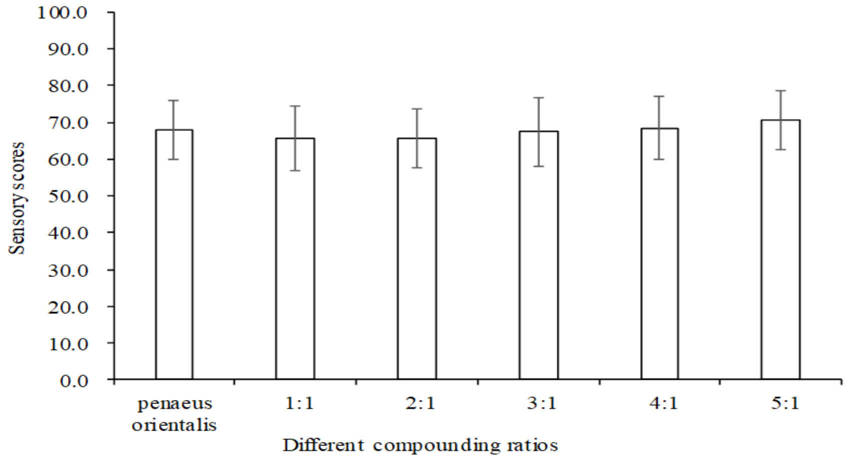

Fig. 2 Effects of different compounding ratios on sensory evaluation of flavoring base materials

Figure 2 shows that with the increase of oyster meat proportion, the sensory score increases gradually. When the ratio of oyster meat and penaeus orientalis was 1:1,2:1,3:1,4:1,5:1, the sensory score was 65.7,65.7,67.4,68.4,70.7. The sensory score of individual penaeus orientalis was 68.0.From the perspective of sensory score, the highest sensory score was obtained when the mass ratio of oyster meat to penaeus orientalis was 5:1.Sensory description of this proportion: in terms of color, the liquid was clear and uniform without precipitation and stratification; in terms of odor, it has the flavor of aquatic products, and the aroma was harmonious and soft; in terms of taste, it has rich flavor, good taste, and no sour and bitter taste.

4.3. Analysis of volatile flavor components

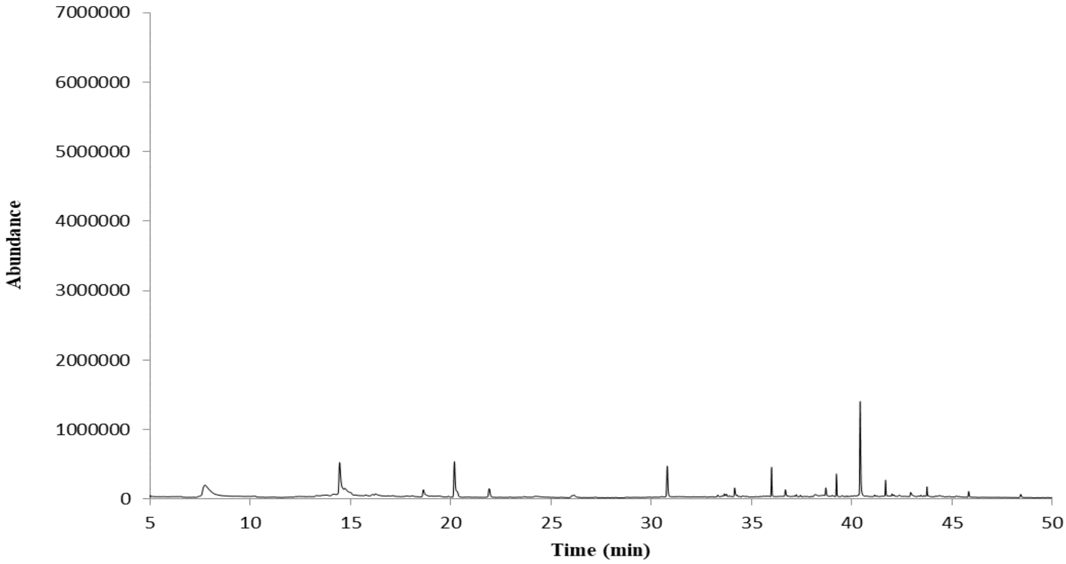

Fig.3 Total ion flow chromatogram of volatile flavor components in penaeus orientalis hydrolysates

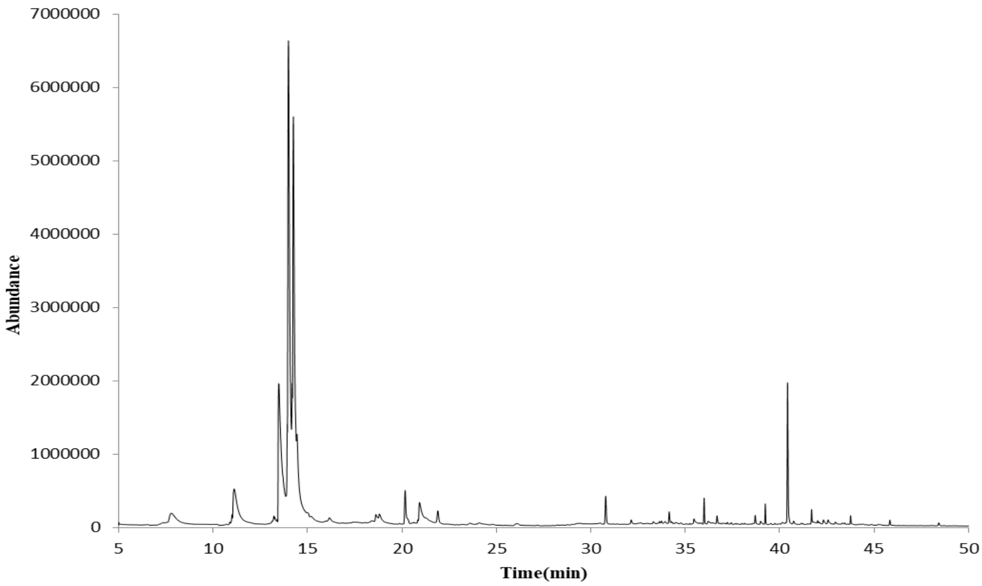

Fig.4 Total ion flow chromatogram of volatile flavor components in 5:1 hydrolysate of oyster and penaeus orientalis

The total ion flow chromatograms of volatile flavor components of penaeus orientalis and oyster with penaeus orientalis at 5:1 were shown in Fig. 3 and Fig. 4. The total ion chromatograms of the volatile flavor components of the two enzymatic products had good separation, and the peak time of the volatile components was mainly between 10-20 min and 35-45 min.

The types and relative contents of volatile flavor components of the two hydrolysates can be

Obtained from table 2.A total of 39 compounds were identified in the two hydrolysates.22 compounds were identified in hydrolysate of penaeus orientalis, and 28 compounds were identified in hydrolysate of oyster and penaeus orientalis 5:1.

According to table 2, 22 compounds were identified in penaeus orientalis hydrolysate, mainly containing 2 alcohols: 1-Hexanol, 2-ethyl(3.50%) and cyclohexanol,2-methyl-5- (1-methylethenyl)(1.41%);4 aldehydes: benzaldehyde(4.72%),pentanal (0.38%),decanal (2.28%) and cyclohexanol,2,4-bis(1,1-dimethylethyl) (0.09%);4 esters: isopropyl palmitate (1.25%),2,2,4-trimethyl-1,3-pentanediol diisobutyrate(1.80%),1,2-benzenedicarboxylic acid, bis (2-methylpropyl) ester(5.20%) and dibutyl phthalate (0.13%);1ketone:5,9-undecadien-2-one,6,10-dimethyl(6.61%);9 hydrocarbons:dodecane(0.53%),tetradecane (7.30%), pentadecane(4.68%), hexadecane(7.45%), eicosane(0.54%),heneicosane(1.52%),tricosane(1.41%),mesitylene(20.11%),hexadecane,2,6,10,14-tetramethyl(1.29%) ;2 heterocyclic and other categories:morpholine,4-octadecyl(0.47%) and tridecanedial(0.13%).

28 compounds were identified in 5:1 hydrolysate of oyster and penaeus orientalis, mainly containing 5 alcohols:3-t-butyl-oct-6-en-1-ol(0.64%), 1-hexanol, 2-ethyl(0.34%), 1-octen-3-ol(31.21%), eucalyptol(0.06%) and n-pentadecanol(0.04%);9 aldehydes:benzaldehyde(17.63%), pentanal(0.02%), nonanal(0.63%), pentadecanal(0.17%), hexadecanal(0.14%), (z)-4-heptenal(5.84%), nona-(2e, 6z)-dienal(1.46%), 9, 12-octadecadienal(0.05%) and 2-hexenal, (e)(0.09%);1ester:1, 2-benzenedicarboxylicacid, bis(2-methylpropyl)ester(0.08%);4 ketones:2-heptanone(0.34%), 2-heptanone, 6-methyl(0.05%), 2-butanone, 4-(2,2-dimethyl-6-methylenecyclohexyl) (0.09%) and 5, 9-undecadien-2-one, 6, 10-dimethyl(0.20%);7 hydrocarbons:tetradecane(0.42%), pentadecane(0.25%), hexadecane(0.27%), heneicosane(0.08%), hexadecane, 2, 6, 10, 14-tetramethyl(0.05%), 3, 5, 5-trimethyl-2-hexene(35.14%) and pentadecane, 3-methyl(0.04%) ;2 heterocyclic and other categories: pyrazine, 2, 5-dimethyl(0.22%) and morpholine, 4-octadecyl(0.07%).

There were 11 volatile flavor components in both hydrolysates, including 1 alcohol(1-hexanol, 2-ethyl), 2 aldehydes(benzaldehyde, nonanal), 1 ester(1, 2-benzenedicarboxylic acid, bis(2-methylpropyl) ester), 1 ketone (5, 9-undecadien-2-one, 6, 10-dimethyl), 5 hydrocarbons(tetradecane, pentadecane, hexadecane, heneicosane and hexadecane, 2, 6, 10, 14-tetramethyl) and 1 heterocyclic and other categories(morpholine, 4-octadecyl).

Cyclohexanol, 2-methyl-5-(1-methylethenyl), decanal, cyclohexanol, 2, 4-bis(1, 1-dimethylethyl), isopropyl palmitate, 2, 2, 4-Trimethyl-1, 3-pentanediol diisobutyrate, dibutyl phthalate, dodecane, eicosane, tricosane, mesitylene and tridecanedial were the unique volatile flavor components in penaeus orientalis hydrolysate.3-t-butyl-oct-6-en-1-ol, 1-octen-3-ol, eucalyptol, n-pentadecanol, pentanal, pentadecanal, hexadecanal, (z)-4-heptenal, nona-(2e, 6z)-dienal, 9, 12-octadecadienal, 2-hexenal, (e), 2-heptanone, 2-heptanone, 6-methyl, 2-butanone, 4-(2, 2-dimethyl-6-methylenecyclohexyl), 3, 5, 5-trimethyl-2-hexene, pentadecane, 3-methyl and pyrazine, 2, 5-dimethyl were the unique volatile flavor components in oyster and penaeus orientalis 5:1 hydrolysate.

Table 2 Types and relative contents of volatile flavor components in hydrolysate

Category	Compound	Molecular formula	Comparative content/%	
			penaeus orientalis	5:1
Alcohols	3-t-butyl-oct-6-en-1-ol	$C_{12}H_{24}O$	—	0.64
	1-hexanol, 2-ethyl	$C_8H_{18}O$	3.50	0.34
	1-octen-3-ol	$C_8H_{16}O$	—	31.21
	eucalyptol	$C_{10}H_{18}O$	—	0.06
	n-pentadecanol	$C_{18}H_{32}O$	—	0.04
	cyclohexanol,2-methyl-5-(1-methylethenyl)	$C_{10}H_{18}O$	1.41	—
Subtotal			4.91	32.29
Aldehydes	benzaldehyde	C_7H_6O	4.72	17.63
	pentanal	$C_5H_{10}O$	—	0.02
	nonanal	$C_9H_{18}O$	0.38	0.63
	pentadecanal	$C_{15}H_{30}O$	—	0.17
	hexadecanal	$C_{16}H_{32}O$	—	0.14
	decanal	$C_{10}H_{20}O$	2.28	—
	(z)-4-heptenal	$C_7H_{12}O$	—	5.84
	nona-(2e,6z)-dienal	$C_9H_{14}O$	—	1.46
	9,12-octadecadienal	$C_{18}H_{32}O$	—	0.05
	cyclohexanol,2,4-bis(1,1-dimethylethyl)	$C_{14}H_{28}O$	0.09	—
	2-hexenal, (e)	$C_6H_{10}O$	—	0.09
Subtotal			7.47	26.03
Ester	isopropyl palmitate	$C_{19}H_{38}O_2$	1.25	—
	2,2,4-trimethyl-1,3-pentanediol diisobutyrate	$C_{16}H_{30}O_4$	1.80	—
	1,2-benzenedicarboxylic acid,bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	5.20	0.08
	dibutyl phthalate	$C_{16}H_{22}O_4$	0.13	—
Subtotal			8.38	0.08
Ketone	2-heptanone	$C_7H_{14}O$	—	0.34
	2-heptanone, 6-methyl	$C_8H_{16}O$	—	0.05
	2-butanone,4-(2,2-dimethyl-6-methylenecyclohexyl)	$C_{13}H_{22}O$	—	0.09
	5,9-undecadien-2-one, 6,10-dimethyl	$C_{13}H_{22}O$	6.16	0.20
Subtotal			6.16	0.68
Hydrocarbons	dodecane	$C_{12}H_{26}$	0.53	—
	tetradecane	$C_{14}H_{30}$	7.30	0.42
	pentadecane	$C_{15}H_{32}$	4.68	0.25
	hexadecane	$C_{16}H_{34}$	7.45	0.27
	eicosane	$C_{20}H_{42}$	0.54	—
	heneicosane	$C_{21}H_{44}$	1.52	0.08
	tricosane	$C_{23}H_{48}$	1.41	—
	mesitylene	C_9H_{12}	20.11	—
	hexadecane,2,6,10,14-tetramethyl	$C_{20}H_{42}$	1.29	0.05
	3,5,5-trimethyl-2-hexene	C_9H_{18}	—	35.14
	pentadecane, 3-methyl	$C_{16}H_{34}$	—	0.04
Subtotal			44.83	36.25
heterocyclic and other categories	pyrazine, 2,5-dimethyl	$C_6H_8N_2$	—	0.22
	morpholine, 4-octadecyl	$C_{22}H_{45}NO$	0.47	0.07
	tridecanedial	$C_{13}H_{24}O_2$	0.13	—
Subtotal			0.60	0.29

Note:“—”means not detected.

5. Conclusions

With the degree of hydrolysis as the index, the optimal ratio of oyster to penaeus orientalis was 5:1, and the degree of hydrolysis was 24.88%; with the sensory score as the index, the optimal ratio of oyster to penaeus orientalis was also 5:1, and the sensory score was 70.7. The sensory description of this ratio was as follows: in terms of color, the liquid was clear and uniform without precipitation and stratification; in terms of odor, it has the flavor of aquatic products, and the aroma was harmonious and soft; in terms of taste, it has rich flavor, good taste, and no sour and bitter taste. A total of 39 compounds were identified in the two hydrolysates.22 compounds were identified in hydrolysate of penaeus orientalis, and 28 compounds were identified in hydrolysate of oyster and penaeus orientalis 5:1. There were 11 kinds of volatile flavor components in both hydrolysates, including 1 alcohol, 2 aldehydes, 1 ester, 1 ketone, 5 hydrocarbons, 1 heterocyclic and other categories.The two hydrolysates also have their own unique volatile flavor components. The hydrolysate of penaeus orientalis contain 11 unique volatile flavor components, including 1 alcohols, 2 aldehydes, 3 esters, 4 hydrocarbons and 1 heterocyclic and other categories. The hydrolysate of oyster and penaeus orientalis 5:1 contain 17 unique volatile flavor components, including 4 alcohols, 7 aldehydes, 3 ketones, 2 hydrocarbons and 1 heterocyclic and other categories. Based on good sensory characteristics and rich volatile flavor components, the hydrolysates of oyster and penaeus orientalis can be used as high quality flavor base material. It may be widely used in condiments, baked goods, flour products, etc.

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