Effect of Compound Ratio of Oyster and Acetes chinensis on Degree of Hydrolysis, Sensory and Flavor Quality of Hydrolysate

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Abstract. In order to obtain high quality flavor base materials for aquatic products, oyster and *Acetes chinensis* were hydrolyzed by compound protease (trypsin and flavourzyme). Under the same enzymolysis conditions (enzymolysis time 2 h, pH 8.0, temperature 55 ℃), the effects of oyster and *Acetes chinensis* compound ratio on the degree of hydrolysis, sensory score and volatile components of the hydrolysate were investigated. The results showed that under the same enzymolysis conditions, the quality of the enzymolysis solution was the best when the ratio of oyster and *Acetes chinensis* was 5:1. The degree of hydrolysis was 24.98%, and the sensory score was 67.7. Nineteen compounds were identified from the volatile flavor components, including four aldehydes (16.79 %), two alcohols (36.68 %), two esters (0.42 %), four ketones (2.24 %), six hydrocarbons (2%), one heterocyclic classes (0.2 %).

Keywords: oyster; *Acetes chinensis*; degree of hydrolysis; sensory; volatile components.

1. Introduction

Acetes chinensis is also known as shrimp rice and sea rice[1]. It is a typical plankton and valuable marine fishery resources. *Acetes chinensis* is abundant in the shallow waters of China's coastal areas. Fresh *Acetes chinensis* was not easy to be preserved, so the traditional *Acetes chinensis* products mainly supplied in the market are dried skin, sauce and oil [2].

Oyster is the largest cultured shellfish in the world and also an important cultured shellfish in China. Oyster meat is rich in protein and amino acids, meanwhile its nutritional value is very high, known as marine milk[3].At present, the traditional products of oyster on the market in China are mainly fresh food, dried products or condiments.Overall, the utilization of oyster related by products is low[4]. Therefore, how to improve the added value of oyster products has gradually attracted the attention of many scholars and has gradually become a research hotspot.

With the concept of great health deeply rooted in the hearts of the people and rapid economic development, single condiments can no longer meet people's nutritional and taste needs. Producing enzymatic products by deep enzymatic hydrolysis of proteins is an important method for preparing flavor condiments. The main products of deep enzymatic hydrolysis contain a large number of amino acids and small molecule peptide, which are commonly used in the preparation of condiments[5].Using modern biotechnology can significantly improve the quality and nutritional value of high-end compound condiments[6].

In order to improve the utilization rate of *Acetes chinensis* and oyster, give full play to the nutritional value of them, and make the flavor more prominent, different proportions of oyster and *Acetes* chinensis were compounded to explore the best proportion.Through the deep enzymatic hydrolysis of proteins, the flavor of *Acetes chinensis* and oyster were stimulated, which were more prominent and the nutritional value was more easily absorbed. The compound development of oyster and *Acetes chinensis* can help promote the sustainable and healthy development of the processing industry of them and the innovation of their related industries, which provide theoretical reference

and practical basis for the development and utilization of oyster and *Acetes chinensis* and other related products[7].

2. Materials and methods

2.1. Materials and instruments

Oyster, purchased in raoping, guangdong, oyster meat was packed in a sealed bag and frozen at-18 ℃, defrost it before use ;*Acetes chinensis*, purchased in Guangzhou Longdong Shangshe market, *Acetes* chinensis was packed in a sealed bag and frozen at -18 ℃, defrost it before use ;trypsin (4000 μ/g), flavourzyme (5000 μ/g), both food grade, Nanning Pangbo Bioengineering Co., Ltd. ;sucrose, edible salt, monosodium glutamate, quinine hydrochloride and citric acid : all food grade ;sodium hydroxide, concentrated sulfuric acid, hydrochloric acid and other reagents were analytically pure.

TLE104E analytical balance, Metler Toledo International Trade (Shanghai) Co., Ltd. ;s220 pH meter, Metler Toledo Instruments (Shanghai) Co., Ltd. ; GL-21M desktop high-speed freezing centrifuge, Hunan Xiangyi Laboratory Development Co., Ltd. ; TS-110×50 water bath oscillation incubator, Shanghai Tiancheng Experimental Instrument Manufacturing Co., Ltd. ; DKZ-3B constant temperature water bath, Shanghai Yi Heng Science Instrument Co., Ltd. ; GCMS-QP2010 Ultra Gas Chromatography-Mass Spectrometry, Japan Shimadzu Production Institute ; KS-1053 multifunctional machine, Guangzhou Qihe Electric Appliance Co., Ltd. ; K9840, Kjeldahl nitrogen analyzer, Jinan Haineng Instrument Co., Ltd. .

2.2. Method

2.2.1. Enzymatic hydrolysis process

The oyster meat and *Acetes chinensis* frozen at -18 ℃ were transferred to room temperature for thawed and cleaned as required. The oyster meat and *Acetes chinensis* were mixed according to the mass ratios of 1:1,2:1,3:1,4 :1 and 5:1. The ratio of solid to liquid was 1:1. After mixing evenly, the mixture was poured into a large beaker. After shaking well, 1 mol / L NaOH solution or 1 mol / L HCL was adjusted to pH 8.0, and trypsin and flavourzyme were added. The amount of enzyme added was 0.7 % of the solid of aquatic products, and the ratio was 3:1. After shaking well, it was placed at 55 ℃ for 2 h, pH 8.0, and pH was adjusted every hour. After enzymolysis, it was kept in a boiling water bath for 15 min for enzyme inactivation. Then, it was placed at room temperature for cooling. After cooling, it was centrifuged at 8000 r / min for 10 min. After centrifugation, the supernatant was taken and frozen at -18 ℃[4].

2.2.2. Determination of degree of hydrolysis

Total nitrogen content was determined according to GB5009.5-2016 protein determination-Key type nitrogen determination method. Amino acid nitrogen content was determined by formaldehyde titration[8]. The degree of hydrolysis was calculated according to this formula:

Degree of hydrolysis % = amino acid nitrogen content / total nitrogen content × 100%

2.2.3. Sensory evaluation

Seven students were selected for sensory evaluation in the sensory evaluation room. Sensory evaluators were trained with five basic tastes before sensory evaluation to preliminarily understand the gradient changes of various tastes. The standards for training were sweet (1 % sucrose), salty (0.35 % edible salt), umami (0.2 % monosodium glutamate), bitter (0.02 g/L quinine hydrochloride), and sour (0.04 % citric acid). Each standard was diluted with four gradient concentrations (0, 2, 4, 6, 8). The evaluator took 5 mL of the sample solution to be tested from low to high concentrations and put it into the mouth. The sample solution stayed in the mouth for 20 ~ 25 s, then spitted out, and the sensory results were recorded[9].

2.2.4. Analysis of volatile flavor components

2.2.4.1. Flavor extraction

3 mL hydrolysate was weighed and placed in the headspace extraction bottle. After equilibrium at 55℃for 10 min, the extraction head was inserted into the headspace bottle. After adsorption for 1 h, the extraction head was pulled out for volatile components detection.

2.2.4.2. Component identification

Chromatographic conditions : unsplit stream sampling, high purity helium (99.999 %) as carrier gas, flow rate of 0.8 mL / min. Furnace program : column initial temperature was 35℃, maintained for 2 min, increased to 40℃ at 4℃/min, maintained for 2 min, then increased to 100℃ at 5℃/min, maintained for 10 min, and then increased to 230℃ at 10℃/min, maintained for 10 min. The injector temperature was 230℃.The desorption temperature was controlled at 230℃. Mass spectrometer conditions were: ionization mode: EI, electron energy 70 eV , ion source temperature: 200℃, mass scan range: 35-500 m/z[10].

2.2.5. Data analysis

All experiments were carried out three times, and the experimental data were averaged. The statistical software Excel2010 was used for statistical analysis.

3. Results and analysis

3.1. Degree of hydrolysis of different proportions of oyster and Acetes chinensis

The degree of hydrolysis of hydrolysates obtained by enzymatic hydrolysis of oyster and *Acetes chinensis* at different ratios was different. Under the same enzymolysis time and enzymolysis conditions, it can be seen from Figure 1 that the degree of hydrolysis of enzymatic hydrolysate of *Acetes chinensis* changed with different proportions of oyster and *Acetes chinensis*, and the change curve was not linear.

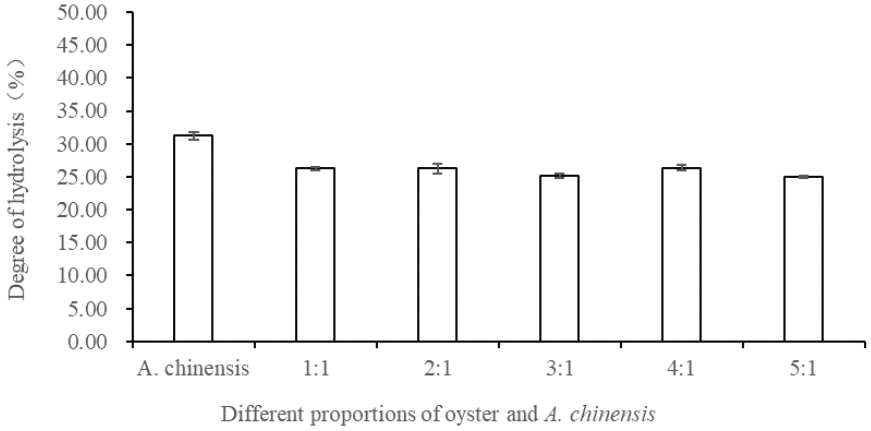


Fig. 1 Degree of hydrolysis of oyster and *Acetes chinensis* in different proportions

The degree of hydrolysis of hydrolysates obtained by hydrolysis of *Acetes chinensis*, oyster and *Acetes chinensis* at the ratio of 1:1, 2:1, 3:1, 4:1 and 5:1 were 31.20 %, 26.23 %, 26.23 %, 25.21 %, 26.34 % and 24.98 %, respectively.The degree of hydrolysis of oyster and *Acetes chinensis* at the ratio of 5:1 was the lowest, and that of *Acetes chinensis* was the highest.With the increase of oyster meat, the proportion of oyster and *Acetes chinensis* decreased gradually from 1:1 to 5:1, but the degree of hydrolysis was not much difference.

3.2. Sensory evaluation of different proportions of oyster and Acetes chinensis

The flavor condiments is composed of free amino acids and small molecular polypeptides to form a round and full taste[5].The flavor condiments not only has a special rich flavor, but also has a delicious taste [11].In sensory evaluation, umami represents better sensory quality, while bitter represents worse.

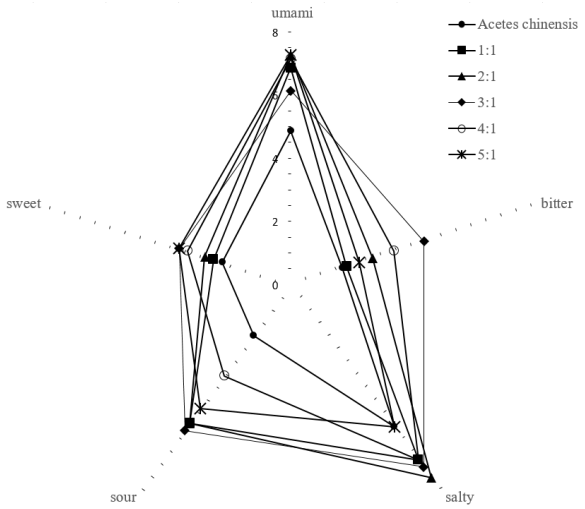


Fig. 2 Radar chart of taste characteristics of six enzymatic hydrolysates

From figure 2, the umami evaluation of enzymatic hydrolysates of *Acetes chinensis*, 1:1,2:1,3:1,4:1 and 5:1 were 4.86, 6.86, 7.28, 6.14, 7.14 and 7.28, respectively. The umami of enzymatic hydrolysates of *Acetes chinensis* was weak. The salty taste scores of hydrolysates of *Acetes chinensis*, 1:1,2:1,3:1,4:1 and 5:1 were 5.57, 6.86, 7.57, 7.14, 6.86 and 5.57, respectively. The salty taste scores of hydrolysates of *Acetes chinensis* and 5:1 were the same, and the salty taste was the lowest in the six hydrolysates. The bitterness scores of enzymatic hydrolysates of *Acetes chinensis*, 1:1,2:1,3:1,4:1 and 5:1 were 1.71, 1.86, 2.71, 4.43, 3.43 and 2.28, respectively. The bitterness of enzymatic hydrolysates of *Acetes chinensis* was lowest. The sweet, sour, umami, salty and bitter taste scores of hydrolysates of oyster and *Acetes chinensis* at 5:1 were 3.71, 4.86, 7.28, 5.57 and 2.28, respectively. Among the six sensory evaluations, the overall sensory evaluation of the hydrolysates of oyster and *Acetes chinensis* at 5:1 was better.

3.3. Analysis of volatile flavor components of oyster and Acetes chinensis

The total ion chromatograms of volatile flavor components in enzymatic hydrolysates of *Acetes chinensis*, oyster and *Acetes chinensis* 5:1 were shown in Figure 3 ~ 4. The quantitative results of each volatile flavor component were shown in Table 1.After analysis, compounds with matching degree greater than or equal to 80 were retained. A total of 30 compounds were identified in the two hydrolysates, and 19 and 19 compounds were identified respectively when the ratio of *Acetes chinensis* alone, oyster and *Acetes chinensis* was 5:1.

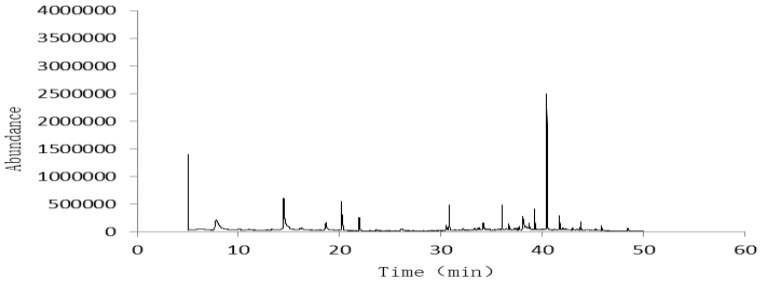


Fig. 3 Total ion flow chromatogram of volatile flavor components in enzymatic hydrolysate of *Acetes chinensis*

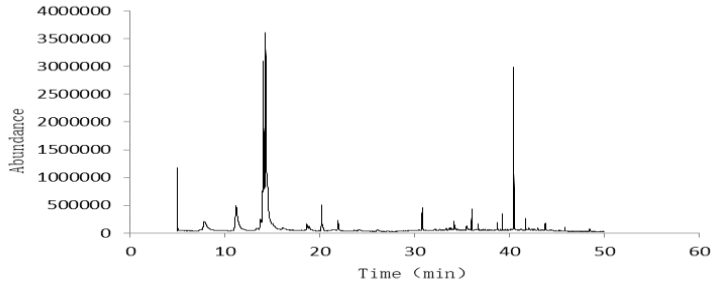


Fig. 4 Total ion flow chromatogram of volatile flavor components in in 5:1 enzymatic hydrolysate of oyster *Acetes chinensis*

Table 1 The species and relative content of volatile flavor compounds in hydrolysates

Category	Compound name	Relative content/%	
		Acetes chinensis	5:1
Aldehydes	Benzaldehyde	—	3.88
	Octanal	3.42	1.02
	4-Heptenal, (Z)	—	10.9
	2-Hexene, 3,5,5-trimethyl	—	0.99
Alcohols	1-hexanol, 2-ethyl	4.14	0.36
	1-Octen-3-ol	—	36.32
	3-t-Butyl-oct-6-en-1-ol	9.17	—
	4-Octen-3-ol, 2,2-dimethyl	0.99	—
Esters	Isopropyl palmitate	0.56	0.18
	Phthalic acid, diisobutyl ester	1.29	0.24
	Tetracosamethyl-cyclododecasiloxane	0.63	—
Ketones	Heptan-2-one	—	0.7
	5,9-Undecadien-2-one, 6,10-dimethyl	—	0.9
	Tricyclo[4.2.1.1(2,5)]decan-9-one	11.76	—
	2-Butanone,4-(2,2-dimethyl-6-methylenecyclohexyl)	—	0.37
	4,8,12-Tetradecatrienal, 5,9,13-trimethyl	—	0.27
Hydrocarbons	Dodecane	1.04	0.28
	Eicosane	0.94	0.07
	Hexadecane	6.21	—
	Tetradecane	4.99	1.05
	Pentadecane	4.49	—
	Heneicosane	1.16	0.24
	Hexadecane, 2,6,10,14-tetramethyl	0.9	—
	Dodecane, 4,6-dimethyl	0.68	—
	1,7-Dimethyl-4-(1-methylethyl)cyclodecane	0.84	—
	Nonane, 5-methyl-5-propyl	0.96	—
	Eicosane	—	0.28
	Tricosane	—	0.08
Heterocyclic class	Morpholine, 4-octadecyl	0.16	—
	Cedrol	—	0.2

The main volatile components of enzymatic hydrolysate of *Acetes chinensis* were octanal,1-hexanol, 2-ethyl,3-t-Butyl-oct-6-en-1-ol,4-Octen-3-ol, 2,2-dimethyl,Isopropyl palmitate,Phthalic acid, diisobutyl ester.The specific volatile components of oyster and *Acetes chinensis* hydrolysates at 5:1 were Benzaldehyde,Octanal,4-Heptenal, (Z),2-Hexene, 3,5,5-trimethyl,1-hexanol, 2-ethyl,1-Octen-3-ol,Isopropyl palmitate,Phthalic acid, diisobutyl ester,Heptan-2-one,5,9-Undecadien-2-one,

6,10-dimethyl,2-Butanone,4-(2,2-dimethyl-6-methylenecyclohexyl),4,8,12-Tetradecatrienal, 5,9,13-trimethyl,Dodecane,Eicosane,Tetradecane,Heneicosane,Eicosane ,Tricosane and Cedrol.

Table 2 The category of volatile flavor compounds in hydrolysates

Category	*Acetes chinensis*		5:1	
	Relative content(%)	Number (kind)	Relative content(%)	Number (kind)
Aldehydes	3.42	1	16.79	4
Alcohols	14.3	3	36.68	2
Esters	2.48	3	0.42	2
Ketones	11.76	1	2.24	4
Hydrocarbons	22.21	10	2	6
Heterocyclic class	0.16	1	0.2	1
Total	54.33	19	58.33	19

As can be seen from table 2, twenty compounds were identified in the enzymatic hydrolysate of *Acetes chinensis*, among which hydrocarbons were the highest, accounting for 22.21 % of the total volatile components, followed by alcohols and ketones, 14.3 % and 11.76 %, respectively, and aldehydes were 3.42 %. In addition, a small amount of esters and heterocyclic compounds were also detected. It mainly included 1 aldehyde (3.42 %), 3 alcohols (14.3 %), 3 esters (2.48 %), 1 ketone (11.76 %), 10 hydrocarbons (22.21 %), 1 heterocyclic classes (0.16 %). It can be inferred that hydrocarbons, alcohols and ketones are the main volatile flavor compounds of hydrolysis products of *Acetes* chinensis.

19 compounds were identified by 5:1 hydrolysate of oyster and *Acetes chinensis*. Alcohols and aldehydes were the most abundant volatile flavor compounds, accounting for 36.68 % and 16.79 %, respectively. Hydrocarbons accounted for 2 % of the volatile components. It contained four aldehydes (16.79 %), two alcohols (36.68 %), two esters (0.42 %), four ketones (2.24 %), six hydrocarbons (2%), one heterocyclic classes (0.2 %). Alcohols, aldehydes and hydrocarbons together constituted the main volatile flavor components of the hydrolysis products of mixed oyster and *Acetes chinensis* at 5:1.

4. Conclusions

In this paper, oyster and *Acetes chinensis*s were used as raw materials for enzymatic hydrolysis. Trypsin and flavourzyme were used to hydrolyze oyster and *Acetes chinensis*s. The degree of hydrolysis of *Acetes chinensis* was the highest. However, with the increase in the amount of oyster meat and the decrease in the amount of *Acetes chinensis*, the degree of hydrolysis of the data was not significantly different. The overall sensory evaluation of the hydrolysates in the six hydrolysates was better, showing strong umami, salty, weak sweet and bitter taste. The volatile components of the enzymatic hydrolysates with the ratio of oyster to *Acetes chinensis* of 5:1 were analyzed. A total of 30 compounds were identified in the two hydrolysates, and 19 and 19 compounds were identified respectively when the ratio of *Acetes chinensis* alone, oyster and *Acetes chinensis* was 5:1.

5. Outlook

The sensory evaluation of enzymatic hydrolysates with oyster and *Acetes chinensis* ratio of 5:1 was carried out. The hydrolysate showed a compound enzymatic ratio of strong flavor, obvious salty flavor, and weak sweetness and bitterness. However, there are still many problems worthy of further discussion and in depth study. For example, there is no specific umami component of the complex hydrolysate, nor quantitative analysis of beneficial volatile flavor components.

Acknowledgments

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