

## Analysis of Flavor Ingredients for *Ruditapes philippinarum* by Headspace Solid-Phase Microextraction and Gas Chromatograph-Mass Spectrometer-Computer Method

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**Abstract:** To study the volatile flavor compound feature of *Ruditapes philippinarum*. The *Ruditapes philippinarum* flavor compounds were extracted with HS-SPME technology and identified combined with GC-MS. Twenty three kinds of flavor compounds were identified through the experience and the percentage composition of each flavor compound was further obtained. Conclusion: The research can provide a reference for the identification of aquatic product's flavor feature with SPME and combined with GC-MS.

**Keywords:** Gas Chromatograph-Mass Spectrometer-computer (GC-MS), Headspace Solid-Phase Microextraction (HS-SPME), *Ruditapes philippinarum*

### INTRODUCTION

*Ruditapes philippinarum* is a kind of shellfish seafood belonging to the Veneridae. It is widely distributed at the North and South Seas in China featured with rapid growth, short breeding cycle, strong adaptability (wide temperature, salt and distribution) and long survival time from the water. It is a kind of excellent shellfish suitable for the artificial high-density breeding, which is one for four major cultivated shellfishes in China. The shell length of *Ruditapes philippinarum* is 2-4 cm and it is a kind of small bivalve shellfish with wide temperature, suitable for the artificial high-density breeding. The aquaculture areas are mainly distributed at Rushan Bay, Baishan Bay, Tadao Bay and other coastal areas and they form the breeding base. Although the *Ruditapes philippinarum* is small, it is delicious and affordable. It contains 12.1 g protein, 1.6 g fat and rich calcium, phosphorus, iron and other minerals, various vitamins, amino acids and taurine in per 100 g *Ruditapes philippinarum* fresh meat, which is a kind of good nutritious food (Ji-Nian *et al.*, 2013). Most *Ruditapes philippinarum*s are fresh, it can also be made into the can and clams dry. In addition to supplying the domestic market, it is also popular in the international market. The research on the volatile flavor compounds of *Ruditapes philippinarum* can not only display the aromaticity of *Ruditapes philippinarum*, but also indicate the purity of aquatic product, therefore, it is necessary to analyze the flavor components.

Solid-phase microextraction, or SPME, is a sample preparation technique used both in the laboratory and on-site. Developed in the early 1990s at the University of Waterloo by Dr. Pawliszyn's group, it is a simple and inexpensive technique where the use of solvents is not necessary. SPME can be thought of as a very short gas chromatography column turned inside out. SPME

involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media that can be in liquid or gas phase. The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation. After extraction, the SPME fiber is transferred to the injection port of separating instruments, such as a Gas Chromatograph, where desorption of the analyze takes place and analysis is carried out (Fig. 1). In the method, the fiber head was firstly immersed in the sample solution or headspace gas for a period of time, while the solution shall be stirred to accelerate the speed to balance the two-phase (Alizadeh and Najafi, 2013). When it was balanced, the fiber head was extracted to insert into the gas chromatography vaporizer and the adsorbed component in the coating shall be extracted through the pyrolysis. After the extracted component was desorbed in the vaporizer, the chromatographic column shall be imported relying on the mobile phase to complete the whole process of extraction, separation and concentration. The SPME occupies the following advantages in the analysis of meat flavor components: sensitive, rapid, small sample consumption, simple operation, without solvent (Peruga *et al.*, 2013). The feature to directly conduct the joint usage with the gas chromatography and other modern instruments makes it occupy strong advantages on the analysis of meat flavor components. GC-MS is a technology combining the GC and MS through appropriate interface, by virtue of the computer technology to conduct the hyphenated analysis. GC-MS is the most mature bi-spectral hyphenated technique, which can be widely applied in the separation and identification of the complicated components. It is

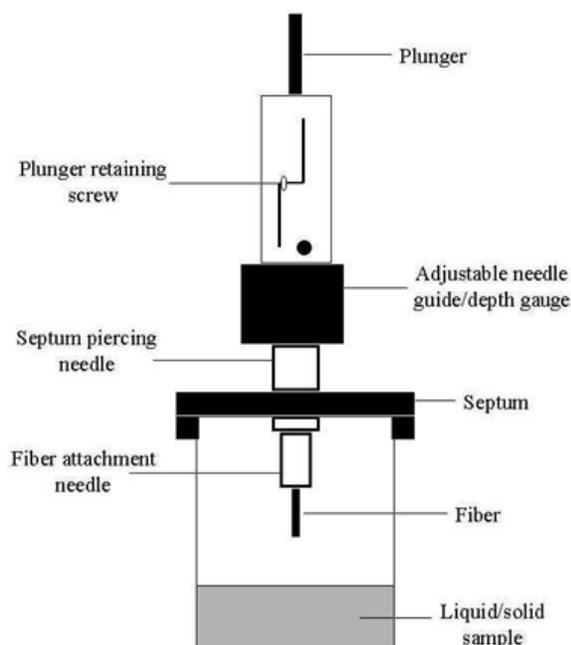


Fig. 1: The working principle of SPME

occupied with GC's high resolution and MS's high sensitivity, which is an effective tool to conduct the qualitative and quantitative research of the biological sample composition (Morán *et al.*, 2013).

The special flavor components of *Ruditapes philippinarum* were analyzed in the research with HS-SPME and GC-MS to provide the effective reference solution for the identification of aquatic product's flavor component with the method.

## MATERIALS AND METHODS

### Material and instrument:

**Sample:** *Ruditapes philippinarum*, place of origin: Weihai, Shandong, purchased in the aquaculture market; 4-methylthiazol: Shanghai Fortunebio-tech Co., Ltd., purity  $\geq 99\%$

**Instrument:** Saturn 2000 GC-MS, US Vario Company; Manual SPME sample injector, extraction head with 65,100  $\mu\text{m}$  PDMS/DVB coating (US Supelco Company); 30  $\text{m} \times 0.25 \text{ mm} \times 0.5 \mu\text{m}$  DB-5MS chromatographic column (Agilent Technologies); DS-II electric heating thermostatic water bath.

**Sample collection:** The shell of *Ruditapes philippinarum* shall be removed and washed according to the Shellfish Essence Technical Specification in the People's Republic of China (SC/T 3013-2002), took the whole cockle, after accumulated for 100 g, it shall be packaged with the silver paper and saved in  $-20^\circ\text{C}$  freezer for reservation.

**Sample handling:** After the sample was unfrozen naturally, ground the cockle into slurry at the ratio of 1:2 for cockle and deionized water, took 100mL to preheat in the thermostat water bath of  $75^\circ\text{C}$ ; Meanwhile, the sampling equipment shall be heated for reservation in the thermostat water bath of  $75^\circ\text{C}$ . After 20 min, took the sample out and added 20% sodium chloride (analytical pure) solution at  $75^\circ\text{C}$ . Adding the sodium chloride can reduce the solubility of volatile components and improve the HS-SPME sensitivity with the salting-out effect. The extraction flask shall be placed in the preheated one at  $75^\circ\text{C}$ . Stirring with the magnetic stirring rotor can improve the adsorption rate of the SPME. The adsorption head shall be inserted from the bottle cap, pushing the handle lever can extend the fiber heat from the needle. The fiber head shall be placed at the upper space of the sample (HS mode), the extraction time was 40 min, then retracted the fiber head and exited the needle from the HS bottle for the determination.

**GC-MS detection and analysis:** Gas chromatographic conditions: the carrier gas shall be high-purity He gas (purity  $>99.999\%$ ) and the column flow rate was 0.85mL/min; The sample was injected splitless; The inlet temperature was  $250^\circ\text{C}$ ; The interface temperature was  $250^\circ\text{C}$ ; The temperature programming: the column initial temperature was  $30^\circ\text{C}$  and kept for 2 min, rose from  $10^\circ\text{C}/\text{min}$  to  $100^\circ\text{C}$ , then from  $5^\circ\text{C}/\text{min}$  to  $220^\circ\text{C}$ , kept for 5 min, then rose from  $3^\circ\text{C}/\text{min}$  to  $260^\circ\text{C}$  and kept for 5 min. Mass spectrometry condition: The ion source temperature was  $250^\circ\text{C}$ ; the ionization mode was EI, electron energy was 80eV, filament emission current was 220  $\mu\text{A}$  and the range of scan quality was 30~500 (m/z) (Vandendriessche *et al.*, 2013).

**Data analysis:** The compound was determined through the NIST Library (107 k compounds) spectral library computer search carried by GC-MS combined with artificial spectrum analysis; If the matching degree is greater than 80%, the flavor components can be determined. The relative content shall be quantified with peak area normalization method. The experiment shall be conducted for five times and the average value shall be adopted.

## RESULT AND ANALYSIS

With the experimental analysis, there were 23 kinds of flavor components determined in the cockle. The total ion chromatogram obtained can be shown in Fig. 2.

The identified flavor compounds with higher content can be shown in Table 1.

The flavor compounds with higher content in *Ruditapes philippinarum* meat included esters, aldehydes, alcohols and various alkanes.

Table 1: Main flavor compound ingredient list

No.	Compound name	Molecular formula	Relative Content (%)	Matching degree (%)
1	Oxime-	C4H9NO	2.62	89
2	methoxy-phenyl-	C17H19N5	0.92	88
3	Eicosane	C20H42	1.24	94
4	Pentadecane	C15H32	3.63	92
5	Heptadecane, 2-methyl-	C27H42N2O5S	0.89	90
6	Hexadecanoic acid, methyl ester	C17H34O2	1.56	92
7	2-Propenoic acid, 3-phenyl-, methyl ester	C10H10O2	0.61	89
8	Phenol, 4, 4'-(1-methylethylidene) bis-	C8H9NO2	2.03	92
9	Acetic acid,ethyl ester	C4H8O2	2.08	90
10	Benzeneacetic acid, hexyl ester	C13H18O2	3.15	95
11	Benzene, 1, 3-dimethyl-	C8H10	0.84	89
12	Methane,sulfinylbis-	C2H6OS	1.22	91
13	Benzene,1, 2-dimethyl-	C8H10	0.26	91
14	Decane	C10H22	0.65	90
15	Dimethyl sulfide	C2H6S	21.06	94
16	Propionic acid	C3H6O2	0.12	87
17	Chloroform	CHCl3	0.21	89
18	Heptane	C7D16	1.28	92
19	1, 5-Hexadiene, 3, 4-dimethyl-	C8H14	0.44	91
20	Octane, 4-methyl	C9H20	0.31	89
21	Hexanal	C7H16	1.37	93
22	6-Undecylamine	C12H22O2	4.21	91
23	Pyridine-3-carboxamide, oxime, N-(2-trifluoromethylphenyl)	C6H6N2O	1.91	89

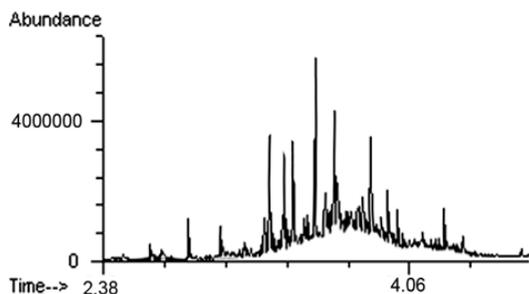


Fig. 2: Total ion current chromatogram of Ruditapes philippinarum flavor compound

Seen from the Table 1, there were 23 kinds of volatile compounds, including 1 kind of amines, 2 kinds of acids, 4 kinds of carbonyl compounds, 2 kinds of sulfur compounds and 14 kinds of others. 6-Undecylamine was also detected in the beef, while there was no trim ethylamine TMA. It was mainly because the sample was frozen with higher fresh and the volatile compound existed some loss. 2 kinds of acids were small molecule acids, which may be produced by the microbial action. Among the sulfur-containing compound, the content of Dimethyl sulfide was up to 21.06%. It may produce the special flavor, which was an important flavor substance in the Ruditapes philippinarum meat.

Moreover, Ruditapes philippinarum meat may be influenced by the environmental pollution. Some of the benzene compounds were transferred from the environmental pollution species to Ruditapes philippinarum (Nguyen *et al.*, 2013).

### CONCLUSION

The research of flavor chemistry starts from Vogel's extraction of benzoic acid from the almond in 1818, while the research of meat quality flavor begins

in the 1950s. The meat quality flavor includes the volatile aroma material and water-soluble non-volatile flavor material and it refers to the meat delicate flavor and aroma. The delicate flavor is composed by the non-volatile flavor active substance, while the aroma is formed by volatile aroma compound. The identified meat flavor compounds mainly include the heterocyclic compounds of alcohols, aldehydes, ketones, ketones, N, O, S and others and part free amino acid and inosinic acid also participate and compose a certain flavor. Twenty three kinds of flavor compounds were monitored successfully from Ruditapes philippinarum in the test and it can provide the reference basis for the detection of aquatic product's flavor compounds with Headspace solid-phase microextraction and Gas Chromatograph-Mass Spectrometer-computer method.

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