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HSSPME-GC/MS Study of the Aroma Volatiles of *Allium* species and Chemometric Interpretation for the Aroma Characteristics

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Abstract: *Allium* species are used not only in cookery, but also in many other applications, such as medicinal and nourishing industry. A typical sensorial property of *Allium* species is the strong aroma. Several aroma volatiles contribute to their flavor. In this study, a manual headspace solid phase microextraction (HSSPME) coupled to gas chromatography-mass spectrometry (GC-MS) method was developed to study the aroma volatiles from four kinds of *Allium* species, namely chive, onion, scallion and shallot, with the comparative simultaneous distillation extraction. The GC-MS analysis identified 30 aroma volatiles. The chromatographic data were treated with the chemometric method of principal component analysis (PCA) in order to group different varieties of the aroma characteristics of *Allium* species. Moreover, the continuous investigation during chive growth was conducted to study the relationship between the growth of *Allium* species and the aroma characteristics. It is hoped that the study could provide systematical information of the aroma characteristics of *Allium* species.

Key words: Aroma characteristics, *Allium* species, SPME, PCA, growth

Introduction

Many plants have the typical aroma. The substantial base of the plant aroma is all kinds of aroma volatiles, which could be considered as a kind of terminal metabolites (Feng and Zhao, 2001). *Allium* species are not only important seasoning spices in Chinese food (Chyau and Mau, 2001), but also *Allium* species play an important medicinal role (Zou *et al*., 1999; Lanzotti, 2006). Consumption of *Allium* species can reduce the risk of cancer at specific sites (Teyssier *et al*., 2001). There are many kinds of *Allium* species in China and most of *Allium* species possess the strong aroma, which is an important sensorial property. The typical and stable aroma compositions might be called aroma characteristics, which might be related with the plant metabolism process (Zhang and Li, 2006). The modern chromatographic methodologies such as gas chromatography-mass spectrometry (GC-MS) could describe the plant aroma characteristics effectively via corresponding aroma chromatograms. Although there are many studies focusing on the detection or determination of the aroma volatiles of *Allium* species (Peng *et al*., 1994; Mondy *et al*., 2002; He *et al*., 2004), few systematical works have been underlined for the study of the aroma characteristics of different *Allium* species and the relationship between the growth of *Allium* species and the changing trends of corresponding aroma characteristics.

The earlier studies for the analysis of the aroma volatiles of *Allium* species were usually conducted by the conventional sampling methods such as steam distillation extraction (Peng *et al*., 1994) and simultaneous distillation extraction (He *et al*., 2004). These conventional methods always

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required long extraction time, big solvent amounts and the multiple consequent steps. Moreover, many unstable aroma volatiles would be thermally decomposed and degraded during thermal extraction or distillation (Kimbaris *et al*., 2006). Therefore, these classical sampling methods became unsuitable for the biogenic aroma volatiles. Some advanced sampling methods such as microwave assisted hydro distillation extraction, ultrasound-assisted extraction (Kimbaris *et al*., 2006) and solid phase microextraction (SPME) (Mondy *et al*., 2002) were replacing the conventional methods. Especially, SPME, developed by Pawliszyn and co-workers (Arthur and Pawliszyn, 1990; Pawliszyn, 1995), has been considered as an excellent pre-sampling method, simple and solvent-saving. SPME has been widely used in many fields such as the environmental (Peñalve *et al*., 1999), biological (Mills and Walke, 2000), pharmaceutical (Ulrich, 2000) and the field analyses (Koziel *et al*., 1999). Especially, headspace solid phase microextraction (HSSPME) has been considered as the suitable sampling method for the plant aroma volatiles (Ibáñez *et al*., 1998).

In this study, HSSPME was used to sample aroma volatiles from four kinds of *Allium* species, namely chive, onion, scallion and shallot, followed by GC-MS analysis with the comparison of the traditional simultaneous distillation extraction (SDE) method. An original "chromatographic data processing system" based on Matlab 6.5 was programmed to manage the chromatographic data to specify the corresponding aroma characteristics based on the principal component analysis (PCA). Further, the continuous investigation for the changing trends of the aroma characteristics during chive growth was conducted in order to interpret the relationship between the growth of *Allium* species and the corresponding aroma characteristics.

Materials and Methods

Sample Collection and Preparation

Fresh *Allium* species were purchased from the settled stand of the local vegetable market in Guangzhou. All the samples purchased were selected for uniformity in size and color. Blemished or diseased samples were discarded. The samples were considered fresh as soon as they picked up from the market and analyzed within 24 h. For each measurement, the *Allium* samples were randomly distributed into groups of 250 g. In order to investigate the changing aroma characteristics during chive growth, the chive was seeded during the autumn in 2005. After chive burgeoned (the 13th planting day), the chive samples were continuously collected for the consequent study. The continuous investigation was carrying on until the chive grew mature. During growth, the color of chive samples changed from the shallow yellow to the bottle green.

Before rubbing *Allium* samples were washed with tap water followed by rinsing with deionized water to get rid of the dirt on the surface and dried naturally. Then, 2 g of *Allium* samples from one group were rubbed with 5 mL of NaCl solution (0.05 g mL^{-1}) using a glass mortar. After that, 3 g of sample tissue homogenate were put in a 15 mL glass vial for the following HSSPME procedure.

Headspace Solid Phase Microextraction

Different parameters that affected the HSSPME extraction efficiency were studied and optimized, including the type of SPME fiber coating, extraction time and ionic strength. Replicate measurements were performed for the comparison results during optimization of the extraction method based on peak area. The type of SPME fiber coating was highly influential to the sampling efficiency. Some useful and specific factors should be taken into consideration, such as polarity, matrix, etc. Five common commercial SPME fibers, 100 μm polydimethylsiloxane, 75 μm carboxen-polydimethylsiloxane (CAR-PDMS), 65 μm carbowax-divinylbenzene, 85 μm polyacrylate and 65 μm polydimethylsiloxane-divinylbenzene (Supelco, Inc., PA, USA) were compared. CAR-PDMS fiber coating could sample more species and amounts of aroma volatiles. Therefore, the recommended SPME fiber was 75 μm CAR-PDMS in the study. Secondly, the effect of extraction time was also evaluated. Extraction times of 15, 30, 45, 60 and 90 min were performed to obtain the optimized sampling efficiency. Due to the complexity of aroma composition, the short sampling time (less than 30 min) resulted in incomplete absorption; however, the longer sampling time (45 min) aroused competitive absorption and also caused lower sampling efficiency. Finally, the sampling time of 30 min was preferred in the work. Thirdly, ionic strength was believed to affect the extraction in HSSPME as analytes tended to be in the vapor phase. Increasing ionic strength in the solution could reduce the surface tension and make the analytes volatile to vapor phase easily. However, the overmuch ionic strength would make the analytes dissolved in the matrix solution. The influence of salt addition was studied by adding 0, 0.05, 0.08, 0.20 and 0.35 g mL^{-1} NaCl to the sample tissue homogenate. The addition of 0.05 g mL^{-1} NaCl resulted in the best extraction efficiency.

After the optimization, in the study the HSSPME procedure was carried out in a 75 μm CAR-PDMS fiber coating for 30 min. After extraction, aroma volatiles were thermally disturbed by inserting the fiber into the GC injector set at 250°C in splitless mode for 5 min.

Simultaneous Distillation Extraction

Simultaneous distillation extraction (SDE) was used as a comparative sampling method to HSSPME. The extraction time, solvent and temperature were optimized in the SDE procedure. In the study, 30 g of homogenized *Allium* samples, 150 mL of deionized purified water and two boiling chips were placed in the sample flask. Pentane (30 mL) and two boiling chips were placed in the flask for the solvent. The heating temperatures for the sample and solvent flasks were adjusted to 120 and 45°C, respectively, so boiling in both flasks began at the same time and extraction was carried out for 60 min.

GC-MS Analysis

The Hewlett-Packard (HP) 6890 gas chromatography-HP 5973 mass detector system was used in the study. Chromatographic separation was performed with an HP-VOC (Agilent Scientific, USA) capillary column (60 m length×0.32 mm I.D.×1.8 μm film thickness) with the following instrumental conditions: Ultra-purified helium flow 1 mL min^{-1}; injector temperature 250°C; transfer line temperature 280°C; energy of electron 70 eV; oven temperature from 60 to 115°C at ramp rate of 10°C/min, 115°C for 1 min, from 115 to 125°C at ramp rate of 1°C/min, 125°C for 2 min, from 120 to 160°C at ramp rate of 10°C/min, 160°C for 1 min, from 160 to 172°C at ramp rate of 2°C/min, 172°C for 1 min and from 172 to 260°C at ramp rate of 20°C/min; The parameters of HP 5973 mass detector were: ion mass/charge ratio, 20-550 m/z; scan model.

Data Processing

All the chromatographic data in the study were managed by an original "chromatographic data processing system" based on the Matlab 6.5. The original Total ionization chromatograms (TICs) were smoothed by the chemometric strategies of wavelet transform and polynomial smoothing. The data of the aroma TICs acquired from the GC-MS were exported and transformed to an "m×2" matrix ("m" represented frequencies of MS data-collecting). The first and second column in this "m×2" matrix represented the time of MS data-collecting and corresponding detector's responses, respectively. After normalization, the data of the total chromatograms of all the investigated samples were merged into an "m×n" matrix ("n" represented the numbers of the aroma chromatograms). Finally, PCA analysis was based on this "m×n" matrix.

Results and Discussion

The Composition of the Aroma Characteristics of Allium species

The aroma volatiles were identified by comparing their mass spectra with the National Institute of Standards and Technology (NIST) electronic Mass Spectral Database, available in the equipment.

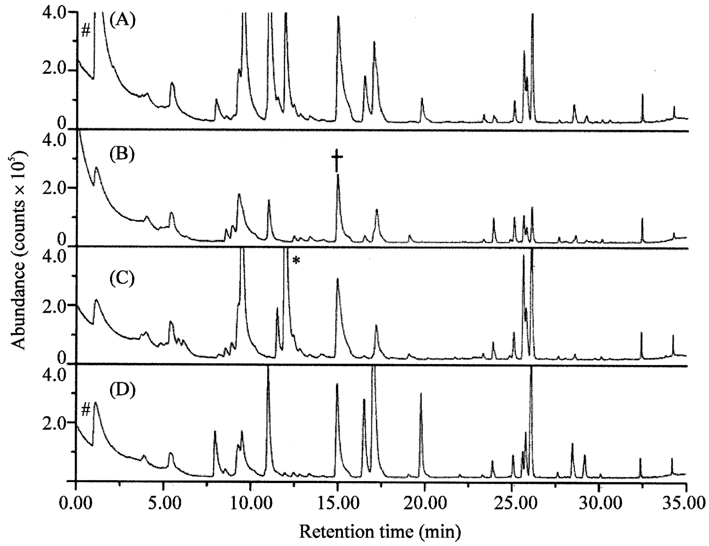

Fig. 1: The aroma chromatograms of chive (A), onion (B), scallion (C) and shallot (D). The marks referred to the aroma volatiles possessing the highest fractions in the corresponding aroma characteristics of *Allium* species ('#' for propanal, '.' for 2,5-dimethyl-thiophene and '*' for [E]-2-hexenal)

Aroma volatiles were considered "identified", when their fit values of mass spectra were at the default value of 85 or above. The aroma chromatograms of different *Allium* Species were illustrated in Fig. 1. Table 1 showed the 30 "identified" aroma volatiles from chive, onion, scallion and shallot, thermally disturbed from the SPME fiber coating, which could be divided into seven groups such as alkene, alkane, alcohol, aldehyde, ketone, organic sulfide and other compounds.

In order to evaluate the reliability of the HSSPME method, the conventional simultaneous distillation extraction (SDE) was performed to sample the aroma volatiles from the *Allium* species as the comparative method. Table 2 showed the qualification result of SDE. SDE could sample 13 aroma volatiles from four kinds of *Allium* species and most of the aroma volatiles could also be sampled by the HSSPME. Because of the high temperature during SDE process, the thermal unstable aroma volatiles were only presented in the HSSPME sampling projects. These thermal unstable compounds such as 1-propanethiol (Jiang, 1996) would change into the other compounds in the SDE process. SDE could sample some typical thermal stable aroma volatiles such as heptane, methyl cyclohexane, octane, diethyl disulfide and ethyl 1-methylethyl disulfide, which were not presented in the HSSPME sampling projects. The results tentatively suggested that HSSPME was a more efficient method for sampling the aroma volatiles of *Allium* species.

Most aroma volatiles identified in the study were the short-chain organic compounds below 10 carbon atoms. Many aroma volatiles sampled by SPME possessed the conjugated structures such as D-limonene, (Z)-3-methyl-1,3-pentadiene, 2-methyl-2-pentenal, [E]-2-hexenal, 2,5-dimethyl thiophene and the like. Aldehydes and organic sulfides consisted of the major aroma characteristics of *Allium* species. According to the previous reports organic sulfides were considered as the characteristic aroma volatiles of *Allium* species, which were also responsible of their characteristic pungent aroma and taste (Lanzotti, 2006). Some organic sulfides identified, such as 2,4-dimethyl-thiophene, dimethyl trisulfide, dipropyl disulfide and so on, have been reported in the previous works (Chu and Hsu, 2001; Teyssier *et al*., 2001). These sulfide compounds of *Allium* species have been considered to potentially benefit people's health (Smith and Yang, 1994; Suschetet *et al*., 1998). Chive possessed more identified

Table 1: Identification of the aroma volatiles of *Allium* species by HSSPME

Aroma volatiles	Retention time (min)	Fit[a]	Fraction of aroma volatiles (%)[b] (n = 7) Chive	Onion	Scallion	Shallot
Alkenes						
(Z)-3-Hexene	3.29	86	---	1.29±0.18	---	---
trans-1,4-Hexadiene	4.82	89	0.25±0.03	1.00±0.08	---	<0.10
(Z)-3-Methyl-1,3-pentadiene	5.14	95	0.14±0.02	0.49±0.05	---	---
3-Ethyl-2-pentene	11.51	90	0.41±0.02	---	1.06±0.08	---
Limonene	21.68	92	<0.10	<0.10	1.10±0.13	---
Alkanes						
Methyl Thirrane	4.01	89	1.18±0.14	5.41±0.63	---	0.23±0.02
Decane	19.06	89	0.75±0.08	0.47±0.02	0.45±0.04	0.65±0.06
Undecane	23.87	93	0.89±0.07	1.15±0.07	1.25±0.07	2.03±0.21
Alcohols						
3-Hexen-1-ol	11.74	86	---	---	<0.10	---
2-Ethyl-1-hexanol	21.32	86	<0.10	---	---	---
5-Methyl-2-(1-methylethyl) -cyclohexanol	28.85	91	<0.10	---	---	---
Aldehydes						
Propanal	1.10	91	22.85±0.79	16.06±0.37	8.85±0.52	12.09±0.94
Hexanal	9.50	92	10.17±0.50	---	12.05±0.69	1.57±0.23
2-Methyl-2-pentenal	10.98	95	13.95±0.98	11.22±1.23	4.46±0.22	9.53±0.86
[E]-2-Hexenal	11.94	95	8.10±0.62	---	39.40±3.26	0.27±0.01
Nonanal	24.81	95	0.77±0.10	0.47±0.03	0.18±0.01	0.15±0.02
Ketones						
3,3-Octanedione	18.67	86	<0.10	---	---	---
(1R)-1,7,7-trimethyl-bicyclo [2.2.1]heptan-2-one	28.20	92	<0.10	0.12±0.01	<0.10	<0.10
2-Undecanone	32.72	86	<0.10	<0.10	---	<0.10
Sulfides						
1-Propanethiol	3.71	91	<0.10	0.13±0.005	1.31±0.08	0.14±0.01
Dimethyl disulfide	7.98	94	1.34±0.11	<0.10	0.10±0.009	4.24±0.50
2,4-Dimethyl-thiophene	13.35	87	0.36±0.02	1.01±0.05	0.58±0.07	0.32±0.02
2,5-Dimethyl-thiophene	14.94	90	12.21±1.11	17.36±0.96	10.23±0.57	11.06±0.72
Methyl propyl disulfide	16.48	87	3.42±0.30	1.05±0.10	1.62±0.16	5.85±0.36
Dimethyl trisulfide	19.74	91	1.31±0.12	0.23±0.02	<0.10	6.37±0.62
Dipropyl disulfide	25.59	94	0.61±0.05	1.84±0.15	4.99±0.50	0.90±0.08
2-Propenyl propyl disulfide	26.05	87	3.49±0.23	4.59±0.34	12.20±1.11	11.27±1.17
Dipropyl trisulfide	34.17	87	0.26±0.02	0.71±0.08	0.44±0.04	0.47±0.03
Miscellaneous						
2-Pentyl furan	19.32	87	<0.10	---	---	---
Naphthalene	30.56	86	<0.10	---	---	---

[a]Fit value was referred to what degree the target spectrum matched the standard spectrum in the NIST library (100 relates to a perfect fit). [b]Fractions of aromatic components (%)= $\frac{\text{Area of the peak of an aromatic component}}{\text{Total area of all aromatic components}} \pm \text{SD}$

The SDS of stable main aroma volatiles (Fraction>0.10%) are calculated in the table

aroma volatiles disturbed from the SPME fiber than the other three kinds of *Allium* species. The major aroma volatiles of *Allium* species were different. Chive and shallot possessed the highest fraction of propanal whereas scallion and onion possessed the highest fractions of [E]-2-hexenal and 2,5-dimethyl-thiophene, respectively. The result tentatively suggested that although organic sulfides were the characteristic aroma components, the aldehydes also contributed to the aroma characteristics of *Allium* species.

When the fractions of aroma volatiles were more than 0.10%, they possessed the stable present frequencies. Therefore, the reproducibility of aroma characteristics could be evaluated from the standard deviation (SD, n = 7). From the SDE listed in Table 2, the corresponding relative standard deviations (RSDs) could be calculated with the satisfying range from 2.3% (propanal of onion) to 14.6% (hexanal of shallot). The fluctuation of the retention time of all the identified peaks was within 0.05 min.

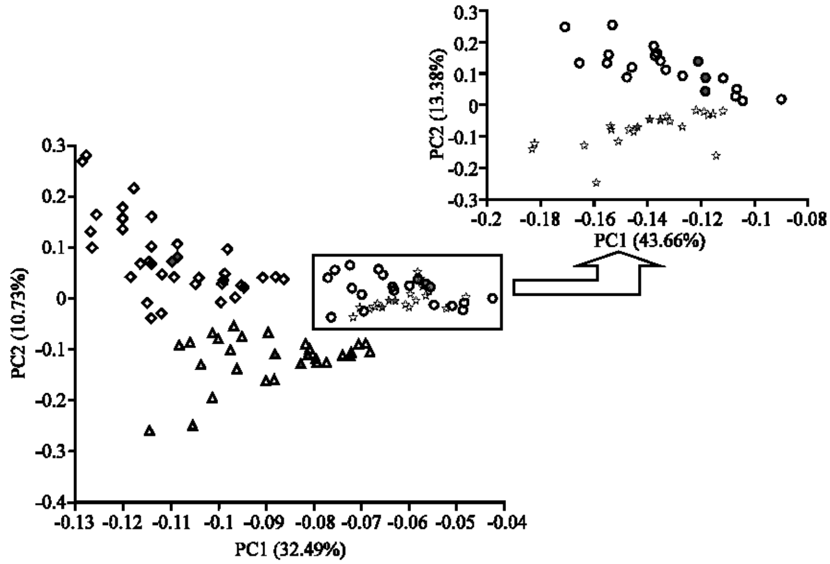

Fig. 2: PCA for the various aroma characteristics of chive ('◇') onion ('☆') scallion ('△') and shallot ('o'). The red marks represented the corresponding unknown samples purchased from the local market

Table 2: Identification of the aroma volatiles of *Allium* species by SDE

Aroma volatiles	Retention time (min)	Fit [a]	Fraction of aroma volatiles (%)[b]			
			Chive	Onion	Scallion	Shallot
Alkanes						
Heptane	11.91	87	5.12	5.70	7.80	4.41
Methyl cyclohexane	13.09	96	1.11	1.16	1.50	---
Octane	15.43	96	4.24	4.64	8.30	3.30
Aldehydes						
2-Methyl-2-pentenal	17.53	93	2.44	4.33	---	---
(E)-2-Hexenal	18.40	97	3.79	---	5.07	---
Sulfides						
Dimethyl disulfide	14.27	87	3.36	---	---	1.47
2,5-Dimethyl-thiophene	20.41	87	1.56	0.67	1.16	---
Diethyl disulfide	20.86	86	0.68	0.56	0.87	0.59
Methyl propyl disulfide	21.15	87	5.15	---	1.76	3.04
Ethyl 1-methylethyl disulfide	22.18	86	0.23	---	0.36	---
Dimethyl trisulfide	22.49	91	4.31	---	0.18	1.40
Dipropyl trisulfide	24.81	87	1.38	---	3.83	1.51
2-Propenyl propyl disulfide	24.97	87	1.09	---	1.47	0.76

[a]Fit value was referred to what degree the target spectrum matched the standard spectrum in the NIST library (100 relates to a perfect fit), [b]Fractions of aromatic components $(\%) = \frac{\text{Area of the peak of an aromatic component}}{\text{Total area of all aromatic components}}$

PCA for the Aroma Characteristics of Allium species

Table 1 suggested that the aroma compositions of four kinds of *Allium* species were various. To interpret the aroma characteristics statistically, the PCA method was established to manage the chromatographic data in the original chromatographic data processing system. Figure 2 showed the different clustering rules of various *Allium* species. Although the clustering rules of onion and shallot were similar to some extent, they could be separated from each other when PCA was conducted to analyze the chromatographic data of only onion and shallot. The different chromatographic data

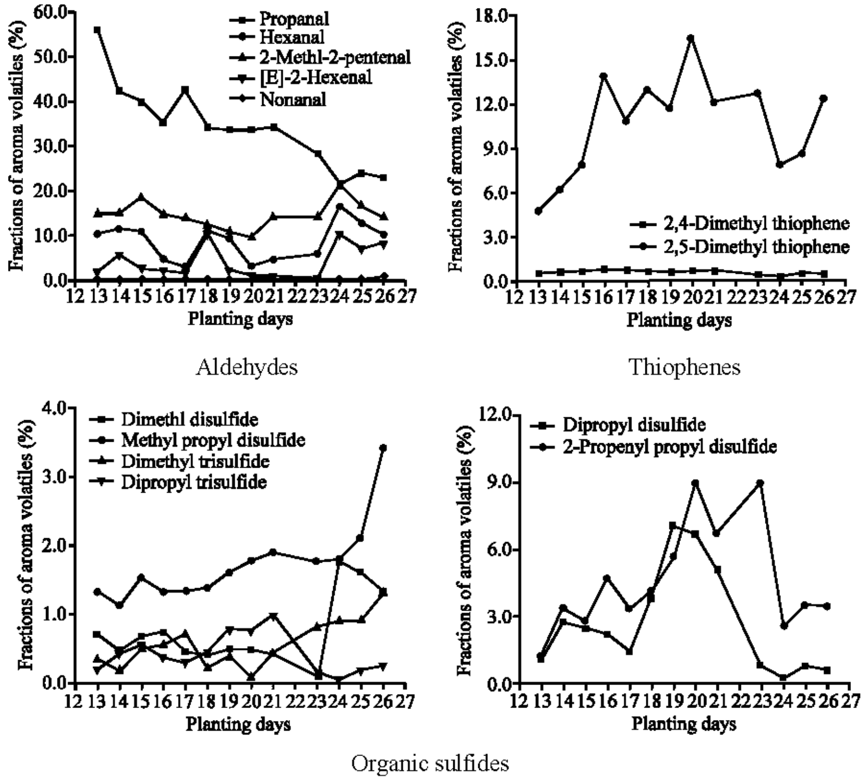

Fig. 3: The changing trends of the typical aroma volatiles during chive growth. The investigation in the 22nd planting day was interrupted due to an instrumental error

segregations in the PCA models tentatively suggested the various aroma characteristics of the different *Allium* species in statistics. After the PCA model was established, the fresh samples were purchased from the local market and analyzed as the above procedure. The chromatographic data of the entire unknown samples could fall into the corresponding PCA segregations. The potential reason for the various aroma characteristics might be related with the different *Allium* specimen. Because the total normalization data in the chromatograms were introduced to the PCA model, it could be considered that the various clustering rules were aroused by the entire aroma characteristics but not individual aroma volatiles.

The Changing Trends of the Aroma Characteristics during Chive Growth

As the most important spices in cuisine, the aroma compositions of *Allium* species have drawn much attention from the analysts. However, there are still no reports focusing on the relationship between the growth of *Allium* species and their aroma characteristics. Among four kinds of *Allium* species in the study, chive possessed the most species of the identified aroma volatiles and was often used in the Chinese cuisine. Therefore, the continuous investigation for the changing trends of the typical aroma volatiles during chive growth was conducted. The results were demonstrated in Fig. 3. For aldehydes, except the propanal the fractions of other aldehydes remained nearly at the same levels during growth. As the typical aromatic components of *Allium* species, most of the sulfides demonstrated the accumulating trends besides dipropyl trisulfide and 2,4-dimethyl thiophene, although the fluctuations were presented. Thus, with the growth of chive the typical aroma became

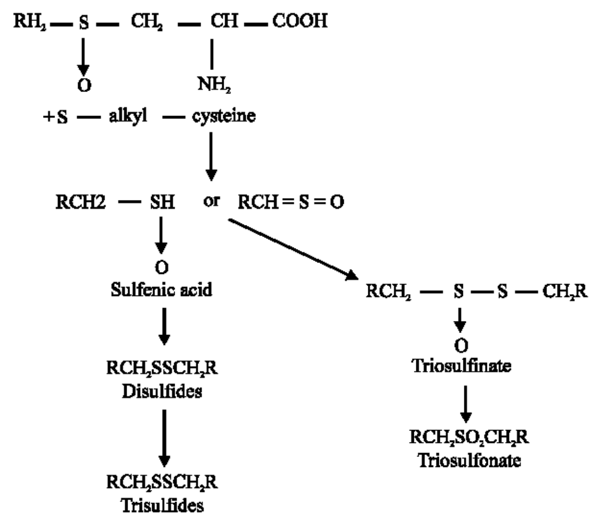

Fig. 4: The tentative pathway of the characteristic organic sulfides in the aroma of *Allium* species during growth (Igarashi, 1994)

thicker. When the tissues were damaged, *Allium* species produce distinct sulfur volatiles through the hydrolysis of flavor precursors such as s-alk(en)yl-L-cysteine sulfoxides (ACSOs) with the corresponding enzyme (Alliinase) catalysis. The tentative pathway for the volatile sulfides in the aroma of *Allium* plants (Igarashi, 1980) was showed in Fig. 4. The potential reason for the accumulating trends of the volatile sulfides might be that the amounts of sulfur precursors increased and the activities of corresponding enzymes improved. It is hoped that the changing trends of aroma characteristics of *Allium* species during growth would provide the helpful clues for the study of metabolism process during *Allium* species growth.

Conclusions

A preliminary method based on HSSPME and chemometric strategy followed by GC-MS detection was developed to study the aroma characteristics of *Allium* species, namely chive, onion, scallion and shallot, followed by GC-MS detection with the comparative sampling method of SDE. In total, 30 aroma volatiles were identified. The aroma characteristics of chive, onion, scallion and shallot were specified by PCA. Different *Allium* species possessed the various aroma characteristics in the PCA model. During chive growth the continuous investigation was conducted to study the changing trends of the typical aroma characteristics and interpret the relationship between the growth of *Allium* species and the aroma characteristics. The study preliminarily clarified the corresponding aroma profiles and characteristics of *Allium* species and might provide helpful clues for the study of metabolism process of *Allium* species during growth.

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