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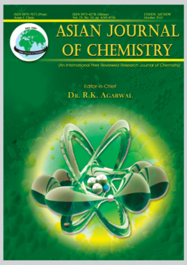

# Comparison of Different Headspace Gas Sampling Methods for the Analysis of Floral Scent from *Lilium* 'Siberia'

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Headspace solid-phase microextraction (HS-SPME) and direct thermal desorption (DTD) coupled to gas chromatography with mass spectrometry (GC/MS) were used to determine the floral scent composition of *Lilium* 'Siberia'. Three types of fiber coating 100 μm PDMS, 2 cm-50/30 μm DVB/CAR/PDMS and 75 μm CAR/PDMS were employed for solid-phase microextraction sampling and Tenax GR was used as adsorbent for direct thermal desorption. The results demonstrated that the major floral scent components were monoterpenoids (73.78-98.52 %) and benzenoids (1.09-25.98 %). Linalool, (*E*)-β-ocimene and methyl benzoate were the most major components. Their total relative amounts were 83.28-97.92 %. In the tests, 26 compounds were separated by DTD-GC/MS, more than the other sampling mediums. As direct thermal desorption provided a more flexible sampling site. We considered that direct thermal desorption with Tenax GR absorbent is more suitable for *Lilium* flower fragrance sampling.

**Key Words: Floral scent, Solid-phase microextraction, Direct thermal desorption, *Lilium* 'Siberia'.**

## INTRODUCTION

In nature, flower colour and scent are two main means to attract pollinators, thereby ensuring plant reproductive success[1,2]. Besides, scent is also an important character of ornamental plants. It's found that aroma influence purchasers' choices higher than flower shape or colour[3]. Many analytical methods have been used to identify the components of floral scent.

Extraction methods, distillative methods and headspace methods are commonly used for isolation of volatiles. Because of losses of flower volatiles in the first two methods, recent research has focused on headspace gas sampling, which provided a more realistic volatile profile than that extraction from the flower tissues[4,5]. These techniques fall into dynamic or static headspace sampling[6]. Solid-phase microextraction (SPME), developed by Pawlisyzyn and coworkers[7], is a very fast, effective and simple method of static headspace sampling. It has a fused-silica fiber coated with different stationary phases for varied samples and was widely used in application of extraction from plants, food, biological and environmental samples[8].

Direct thermal desorption (DTD) is one version of headspace sampling. Volatiles compounds were collected on an adsorbent trap. By heating the trap, volatiles are transferred into a cryofocusing unit to focus the analytes, then transferred to the analytical column and analyzed. Direct thermal desorption has higher desorption efficiency than solvent elution and prevents sample from dilution[5]. Tenax GR were the most widely used solid adsorbent for thermal desorption of air sample[9]. It has high-temperature stability and a higher breakthrough volume for most organic volatiles, yet still has a low affinity for water.

We chose *Lilium* 'Siberia' as the experimental material, which is one of the most typical fragrant ornamental flowers. The main objectives of this study are (1) to characterize the floral scent composition of *Lilium* 'Siberia', (2) to carry out a comparative evaluation of four sampling mediums and (3) to compare these sampling methods and choose the better one for further study.

## EXPERIMENTAL

*Lilium* 'Siberia', a typical oriental hybrid cultivar, has large pure-white flowers with strong fragrance and is one of the most popular cut flowers in China. Flowers of *Lilium* 'Siberia' were purchased from a flower market. Opened flower (1D after anthesis) was sampled on 02:00-04:00 pm (anther removed).

**Solid-phase microextraction (SPME):** Three SPME devices including the 100 μm PDMS, 2 cm-50/30 μm DVB/CAR/PDMS and 75 μm CAR/PDMS were chosen (Supelco, Bellefonte, PA, USA). Before extraction, the fibers were reconditioned by heating in a gas chromatograph injection port at 250 ºC for 10 min (new fibers for 120 min) to prevent contamination. Prior to sampling, a fiber blank was run to confirm no contamination peaks.

For the static headspace analysis, the flower was enclosed in a glass container with purified air for 0.5 h at room temperature. Then the SPME fiber was inserted and exposed to the headspace above the sample for 0.5 h. After adsorption, the SPME fiber was removed from the sample, immediately inserted into the GC injection port and thermally desorbed for 5 min at 250 ºC with a splitless injection mode. Analysis was conducted using a Trace 2000 gas chromatography coupled to a Voyager mass spectrometry. The volatile compounds were separated by a capillary DB-5 column (30 m × 0.25 mm × 0.25 mm) with a 1 mL/min helium flow rate. The GC oven was initially set at 50 ºC for 4 min, then heated at a rate of 10 ºC/min to 270 ºC and maintain at this temperature for 5 min. The mass spectra of volatile compounds were recorded for the m/z of 30-500.

**Direct thermal desorption (DTD):** Before sampling, sorbent tubes (CAMSCO, Houston, TX, USA) containing 200 mg Tenax GR (60/80 mesh) were conditioned by heating them to 270 ºC for 2 h to remove residual components and flowing helium through the tubes at 100 mL/min. Flower was enclosed in another special designed glass container with inlet and outlet ports and swept by continuous 200 mL/min purified air. The volatiles entrained with the exhaust stream were trapped in empty sorbent tubes. After sampling, the sorbent tubes were kept at -20 ºC.

An automatic thermal desorber (ATD, Perkin-Elmer Turbo Matrix 650) was used to desorb the tubes at 300 ºC with a 1.5 mL/min helium flow rate for 10 min. The desorbed volatile compounds were trapped in the cooled trap of Tenax (-25 ºC), then the trap was quickly heating at 40 ºC/s to 300 ºC. Samples desorbed in the ATD were injected in the GC through a capillary transfer line heated to 250 ºC and the sample amount was 4.0 %. Analyses were performed using a Perkin Elmer Clarus 600 gas chromatograph coupled to a Clarus 600T mass spectrometer. A DB-5 fused-silica capillary column (30 m × 0.25 mm × 0.25 mm) was used. The oven temperature programming and other settings were the same as that of SPME experiments.

**Date analysis:** Xcalibur 1.2 was used to for SPME-GC/MS analysis and TurboMass Ver 5.4.2 for DTD-GC/MS analysis. As most of the common compounds of floral scent are easy to identify[10], the volatile compounds were identified by comparing their GC retention times (RI) and mass spectra with NIST 02/08 mass spectral library and retention indexes reported elsewhere (http://www.pherobase.com/). For quantification of volatile compounds emitted, the peak areas of identified compounds were percent normalized and used to calculate relative amount[8,11].

## RESULTS AND DISCUSSION

**Composition of floral scent:** Both SPME and direct thermal desorption extracted the scent substances from the gas around the flower. Most of volatile compounds were identified, except a few too low to identify. Table-1 showed the identified compounds and their relative amounts, which were grouped based on their biosynthetic pathways[4]. The results demonstrated that flower fragrance of *Lilium* 'Siberia' was dominated by only a few compounds.

The mainly components were monoterpenoids [linalool, (*E*)-β-ocimene, (*E,E*)-alloocimene, myrcene], which total relative amounts were 73.78-98.52 %. Some benzenoids/phenylpropanoids, such as methyl benzoate, ethyl benzoate, (*E*)-isoeugenol *etc*., were also identified and total relative amounts were 1.51-26.21 %. Among these compounds, linalool, (*E*)-β-ocimene and methyl benzoate were the most major components (83.28-97.92 %). It is noted that the linalool was the highest amount compound (48.40-77.39 %) in all tests. The odor of *Lilium* 'Siberia' smells sweet, flowery and pleasant, which may result from high levels of linalool and (*E*)-β-ocimene.

The results are similar with previous studies. In *Lilium auratum*, a parent of *Lilium* oriental hybrids, the main floral scent components were monoterpenoids [(*E*)-β-ocimene, linalool] and benzenoids/phenylpropanoids (methyl benzoate, isoeugenol)[12]. While in *Lilium* 'Case Blanca', the major scent compounds also were monoterpenoids [linalool and (*Z*)-ocimene] and benzenoids/phenylpropanoids (benzyl alcohol and isoeugenol)[13]. It's suggested that some common and crucial floral scent components are existed in oriental hybrid lilies.

**Comparison of different sampling mediums:** Floral scent composition varied from different sampling mediums. In the tested four sampling mediums, both 17 compounds were identified from 100 µm PDMS and 2 cm-50/30 µm DVB/CAR/PDMS sampling. While 18, 26 compounds were identified from 75 µm CAR/PDMS and direct thermal desorption sampling, respectively.

Although the major components were similar, their relative amounts were different. We chose the three highest amount common compounds *e.g.*, linalool, (*E*)-β-ocimene, methyl benzoate as comparison factors. Fig. 1 shows the analysis results of the three major comparison factors. The reason for this difference was probably due to the polarity of analytes and mediums.

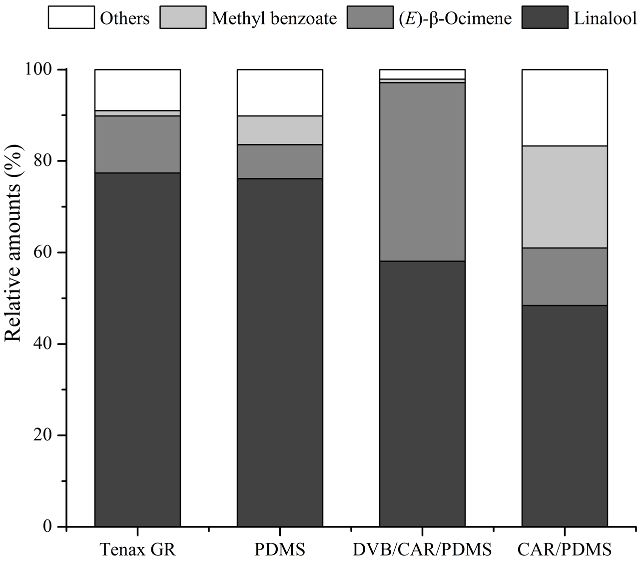

Fig. 1. Comparison of relative amounts of three major scent compounds

TABLE-1
COMPOSITIONS OF FLORAL SCENT EMITTED FROM *Lilium* ‘SIBERIA’ COLLECTED BY DIFFERENT SAMPLING METHODS

| Compounds | Relative amounts (%) | | | | Odour Characteristic* |
|---|---|---|---|---|---|
| | Tenax GR | 100 µm PDMS | 2 cm-50/30 µm DVB/CAR/PDMS | 75 µm CAR/PDMS | |
| **Monoterpenoids** | | | | | |
| α-Pinene | 0.03 | – | – | – | Terpeny, Fruity, Sweet, Green, Woody, Pine, Citrus, Lime, Camphoraceous |
| Myrcene | 1.08 | 0.23 | 0.36 | 2.12 | Metallic, Musty, Geranium, Sweet, Fruity, Ethereal, Soapy, Lemon, Spicy, Woody |
| α-Terpinene | 0.05 | – | – | – | Gasoline-like, Ethereal, Fruity, Lemon |
| *p*-Cymene | 0.03 | – | – | – | Lemon, Fruity, Fuel-like, Sweet, Herbal, Spicy |
| Limonene | 0.16 | – | 0.02 | 0.89 | Licorice, Green, Citrus-like, Ethereal, Fruity |
| (*Z*)-Ocimene | 0.29 | – | 0.11 | 1.37 | Citrus-like, Herbaceous |
| (*E*)-β-Ocimene | 12.50 | 7.45 | 39.05 | 12.61 | Herbaceous, Mild, Citrus, Sweet, Orange, Lemon |
| γ-Terpinene | 0.01 | – | – | 0.05 | Citrus-like, Terpeny, Herbaceous, Fruity, Sweet |
| α-Terpinolene | 0.12 | – | – | 0.08 | Woody, Fruity, Sweet, Piney, Slightly anisic |
| Linalool | 77.39 | 76.13 | 58.10 | 48.40 | Muscat, Sweet, Green, Floral, Lemon, Parsley, Lavender-like, Fruity |
| (*E,Z*)-Alloocimene | 0.18 | – | 0.41 | 3.14 | Fresh, Grassy |
| (*E,E*)-Cosmene | 0.16 | – | 0.15 | – | – |
| (*E,E*)-Alloocimene | 0.04 | 0.14 | 0.27 | 5.10 | Fresh, Grassy |
| α-Terpineol | 0.04 | – | – | – | Peach-like, Anise, Oily, Fruity, Floral, Minty, Toothpaste |
| *cis*-Geraniol | 0.45 | 0.34 | 0.05 | 0.02 | Floral, Rose, Citrus, Marine |
| Geranyl acetate | 0.06 | 0.14 | – | – | Floral, Rose, Fruity, Raspberry |
| **Sesquiterpenoids** | | | | | |
| (*E,E*)-α-Farnesene | 0.11 | 0.28 | 0.03 | – | Woody |
| **Irregular terpenoids** | | | | | |
| 6-Methyl-5-hepen-2-one | 0.14 | – | – | – | Mushroom, Earthy, Vinyl, Rubber, Woody, Blackcurrant, Boiled fruit |
| **Benzenoids** | | | | | |
| Methyl benzoate | 1.10 | 6.29 | 0.77 | 22.27 | Flowery, Honey, Lettuce, Herbal, Watermelon |
| Ethyl benzoate | 0.59 | 0.19 | 0.04 | 3.43 | Chamomile flower, Celery, Fruity, Musty, Tea |
| 2-Methoxy-4-methylphenol | 2.67 | 0.48 | 0.23 | 0.15 | – |
| Methyl salicylate | – | 0.14 | 0.05 | 0.13 | Wine-like, Berry-like, Warm, Sweet, Wintergreen odor |
| **Phenylpropanoids** | | | | | |
| Eugenol | 0.28 | 0.18 | 0.02 | 0.01 | Spicy, Honey, Clove, Balsamic, Herbaceous, Camphoraceous |
| (*Z*)-Isoeugenol | – | 0.12 | 0.01 | – | – |
| (*E*)-Isoeugenol | 2.27 | 7.66 | 0.33 | 0.09 | Flowery |
| **Fatty acid derivatives** | | | | | |
| Sulcatone | 0.14 | – | – | – | Mushroom, Earthy, Vinyl, Rubber, Woody, Blackcurrant, Boiled fruit |
| Nonanal | 0.04 | – | – | – | Gravy, Green, Tallowy, Fruity, Gas, Chlorine, Floral, Waxy, Sweet, Melon, Soapy, Fatty, Lavender, Citrus fruit |
| *n*-Tetradecane | 0.01 | – | – | 0.01 | Mild herbaceous, Sweet, Fusel-like |
| 1-Pentadecene | – | 0.05 | – | – | – |
| *n*-Pentadecane | 0.03 | 0.06 | – | – | Mild green, Fusel-like |
| *n*-Hexadecane | 0.03 | 0.13 | – | 0.01 | Fusel-like, Fruity, Sweet |

*Odor Characteristic were obtained from http://www.pherobase.com/; http://www.flavornet.org/flavornet.html

Generally speaking, PDMS fiber exhibited greater extraction of non-polar compounds than mixed fibers and DVB/CAR/PDMS fiber extracted more polar and middle polar compounds[14]. 75 µm CAR/PDMS fiber was suited for extracting trace level volatiles organic compounds and small analytes (m.w. < 90), but saturates at high level with little displacement[15]. 2 cm-50/30 µm DVB/CAR/PDMS was also recommended for trace compounds analysis[16]. 100 µm PDMS was suggested to be more suitable for floral scent extraction[8,17], because a thick coating was better to retain most floral volatile compounds until desorption[18]. Tenax has a high affinity for lipophilic to medium polarity organic compounds of intermediate molecular weight and low affinity for polar and low molecular weight compounds[14]. As Tenax GR is a composite material of Tenax TA and graphite, the presence of graphite may enhance the conversion or decomposition of certain compounds, such as β-pinene[19].

In this test, Tenax GR extracted and separated the most compounds and compound peaks were well separated and easier to identify. Considering the composition of *Lilium* 'Siberia' floral scent, we recommended that Tenax GR are suited for extract *Lilium* 'Siberia' flower fragrance.

**Comparison of different sampling methods:** Based on the experimental materials, environment and research purpose, a suitable sampling method deserves prudent consideration. Recently, SPME is widely applied and used in many research articles on flower scent published in the last decade[18]. But it also has some limitations. The delicate fiber needs carefully operation. At the same time, samples extracted can be injected only once (not repeated injections). As the samples need to be inserted into GC injection port quickly, it is suit for laboratory scale. However, recently Supelco company provides a portable filed sampler with a CAR/PDMS fiber, which has a sealing mechanism to store samples for later analysis.

Sampling with adsorption tubes is easy and fast[20], while the trapped tubes can be eluted by organic solvent or direct thermal desorption. Internal or external standard compounds are generally added for semi-quantitative. The tubes can be saved for a longer time in a low temperature (to prevent volatile escaped). Outsides the laboratory scale, it is also very convenient when sampling in the field. In limitation, some artifacts would be produced from the trapping medium as a result of thermally instable compounds degradation, besides impossible of repeated sample injection, so the samples are necessary to be controlled.

## Conclusion

By headspace gas sampling of *Lilium* 'Siberia', the main components were monoterpenoids and benzoaids. Linalool, (*E*)-β-ocimene and methyl benzoate were three highest amounts compounds. By evaluating these four sampling mediums and two sampling methods, we recommended Tenax GR as absorbent, and direct thermal desorption is more suitable for lily flower fragrance sampling, for its flexible sampling time and site, as well as higher sensitivity.

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