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ARTICLE

Floral Volatiles in Natural Populations of *Paeonia lactiflora*: Key Components and Cultivar Differential Analysis

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ABSTRACT: Floral scent serves as a key criterion for evaluating the ornamental value of flowering plants. Herbaceous peony (*Paeonia lactiflora* Pall.), a traditional Chinese ornamental species, is valued for its vibrant coloration, intricate floral morphology, and positive cultural symbolism. In this study, dynamic headspace adsorption coupled with gas chromatography-mass spectrometry was employed to analyze volatile organic compounds (VOCs) in flowers of 120 herbaceous peony cultivars at the half-opening stage. We detected 86 VOCs, comprising 25 aromatic compounds (79.70%), 21 hydrocarbons (10.51%), 29 terpenoids (8.37%), 7 fatty acid derivatives (1.03%), and 4 heterocyclic compounds (0.38%). The cultivar 'Dr. Alexander Fleming' demonstrated the highest total VOC content, followed by 'Yuezhao Shanhe' and 'Daiyu'. The top five cultivars based on principal component analysis composite scores were 'Shajin Guanding', 'Many Happy Returns', 'Edulis Superba', 'Huicui', and 'Madame de Verneville'. The volatile compositions of these cultivars showed statistically representative characteristics. Aroma activity value analysis revealed 22 key aroma components (e.g., 3-hexen-1-ol, acetate, (Z)-, limonene, (E)-β-ocimene) and 15 modifying components (e.g., methyl hexanoate, α-pinene, benzaldehyde). Domestic cultivars exhibited greater VOC diversity and higher content levels compared to introduced cultivars, with introduced cultivars demonstrating more pronounced compositional variation. Introduced cultivars primarily released nonanal and 1,4-dimethoxybenzene, associated with fruity-sweet notes, whereas domestic cultivars predominantly released 1,4-dimethoxybenzene and phenylethanol, characterized by sweet-floral aromas. Aromatic compounds primarily contributed to the overall aroma, with terpenoids as secondary contributors. This study systematically characterized the floral aroma components of herbaceous peony, providing a theoretical foundation for germplasm resource utilization in cut flower production, essential oil extraction, and aromatherapy applications.

KEYWORDS: Herbaceous peony; floral scent; DHS-GC-MS; PCA; OAV; 1,4-dimethoxybenzene

1 Introduction

Floral scents, released during flowering, comprise complex mixtures of lipophilic, low molecular weight volatile organic compounds (VOCs) with relatively high melting points [1]. These compounds play essential roles in plant survival, reproduction, and ecosystem dynamics [2–4]. Floral scent formation results from the combined effects of diverse floral VOCs. The distinct fragrance profiles originate from variations in VOC composition and concentration, resulting in unique scent characteristics. Based on their biosynthetic pathways, floral VOCs can be classified into three major groups: terpenoids, phenylpropanoids/benzenoids, and fatty acid derivatives [5]. Terpenoids represent the most diverse class of floral VOCs and play crucial roles in aroma formation [6]. These compounds are primarily biosynthesized through the mevalonate (MVA)

and methylerythritol phosphate (MEP) pathways [7,8]. Phenylpropanoids/benzenoids compounds originate from the shikimic acid pathway and are widely present in plant floral fragrances, making them an important component of floral fragrances [9]. As the third major group of floral scent components, fatty acid derivatives are biosynthesized through the lipoxygenase (LOX) pathway. These biologically active compounds often exhibit specific biological functions [10]. This classification of VOCs is not only taxonomically informative but also functionally relevant to our research. Terpenoids and phenylpropanoids/benzenoids compounds are particularly important in essential oil extraction due to their high volatility and aromatic properties, while fatty acid derivatives play a key role in determining the longevity and consumer appeal of cut flowers. By understanding the relative contributions of these VOC classes to fragrance quality, we can better identify cultivars with desirable aromatic profiles for both breeding programs and industrial applications. The commercial and ecological significance of floral VOCs has driven their extensive applications in food, cosmetic, and pharmaceutical industries, consequently accelerating research progress [11].

Recent studies have primarily focused on fragrant ornamental plants, including herbaceous species such as *Lilium* [12], *Lavandula angustifolia* Mill. [13], *Dianthus caryophyllus* L. [14], *Chrysanthemum* × *morifolium* (Ramat.) Kitamura [15], and *Tulipa gesneriana* L. [16], as well as woody plants including *Jasminum sambac* [17], *Paeonia sufruticosa* [18], and *Rosa rugosa* [19]. These investigations have primarily focused on the VOCs of aromatic ornamental plants, the intensity of their fragrance, and how these factors influence consumer preferences. However, herbaceous peony (*Paeonia lactiflora* Pall.), as a significant woody ornamental plant, has not had its unique floral scent characteristics and aromatic components adequately explored in these studies.

Herbaceous peony, a traditional Chinese ornamental species with a long cultivation history [20], is highly valued for its vibrant coloration and intricate floral morphology, possessing significant economic and cultural importance. Previous research on herbaceous peony has primarily focused on flower color [21–23], while floral scent studies remain limited. During breeding processes, floral fragrance traits have often been overlooked, resulting in most cultivars exhibiting faint or negligible aromas, with fragrant varieties being rare. To address market demands, enhancing floral fragrance traits and selecting aromatic cultivars have emerged as crucial objectives in herbaceous peony breeding. Recent years have witnessed progress in herbaceous peony floral fragrance research [24–28]. These investigations have primarily focused on preliminary identification of floral scent components and characterization of fragrance-related biosynthetic genes. However, existing studies are limited in their cultivar coverage. To comprehensively investigate herbaceous peony aroma profiles, this study systematically analyzed scent components across 120 cultivars, including 40 introduced varieties and 80 indigenous Chinese cultivars, significantly expanding the scope of floral scent research in herbaceous peony.

Accurate and effective methodologies are crucial for in-depth investigation of herbaceous peony aroma. Currently, headspace solid-phase microextraction (HS-SPME) and dynamic headspace sampling (DHS) are widely employed for aroma collection, while gas chromatography-mass spectrometry (GC-MS) and automated thermal desorption-gas chromatography/mass spectrometry (ATD-GC/MS) are commonly utilized for aroma analysis in herbaceous peony [29]. HS-SPME operates through adsorption of volatile components onto quartz fiber coatings, followed by thermal desorption at the GC inlet for component collection. DHS directly captures floral components from living plants, offering operational simplicity, minor contamination, and accurate representation of aromatic characteristics while avoiding issues associated with petal damage and aroma alteration. GC-MS enables rapid analysis of flavor compound composition and concentration, offering high efficiency, selectivity, and sensitivity. This technique has become a primary analytical tool in organic matter research. Lo et al. recommend the headspace method as the preferred approach for floral aroma collection, followed by GC-MS analysis for compound identification [30]. Building

upon these methodological advantages in precise volatile component capture and efficient structural analysis, combined with previous research from our team on herbaceous peony floral scent, this study employed DHS coupled with GC-MS to analyze floral scent components across 120 herbaceous peony cultivars. This study analyzed the volatile organic compounds (VOCs) content and composition of different herbaceous peony cultivars, revealing significant differences in aromatic components among cultivars. Using principal component analysis (PCA), we identified superior cultivars with optimal aromatic component ratios. These findings provide breeders with critical insights for selecting high-VOC cultivars as parental material to develop new cultivars with stronger and longer-lasting fragrances, thereby improving breeding efficiency and shortening breeding cycles. Additionally, the results offer direct guidance for the herbaceous peony cut flower and essential oil industries. By selecting cultivars with high VOC content, cut flower producers can enhance product marketability, while essential oil extraction companies can optimize extraction efficiency and improve product quality. Furthermore, this study recommends prioritizing cultivars with higher proportions of aromatic and terpenoid compounds to achieve more desirable fragrance characteristics.

2 Materials and Methods

2.1 Plant Materials

In this study, floral scent samples were collected from 120 herbaceous peony cultivars over two years (2023–2024). The majority (n = 116 cultivars, e.g., 'Alertie', 'Angel Cheeks', and 'Belle Chinoise') were sampled at the Luoyang Academy of Agriculture and Forestry Sciences from mid-April to early May. Additionally, four cultivars ('Dinner Plate', 'Nick Shaylor', 'Sarah Bernhardt', and 'Shirley Temple') were collected in early June 2023 from the Beijing PEONY FARMER Cultivation Base. The Luoyang Academy of Agriculture and Forestry is located in Luoyang City, Henan Province (112°16′–112°37′ E, 34°35′–34°46′ N), while Beijing Peony Farmer Cultivation Base is situated in Yanqing District, Beijing (115°50′–116°29′ E, 40°26′–40°37′ N). Both locations experience a temperate continental monsoon climate, providing suitable environmental conditions for herbaceous peony growth. This climate type provides optimal light, thermal conditions, precipitation, and humidity for herbaceous peony development. However, Luoyang exhibits higher annual temperatures and greater precipitation compared to Yanqing, which experiences lower temperatures, larger diurnal temperature variations, and relatively less precipitation. The 120 herbaceous peony cultivars were systematically numbered from 1 to 120, with cultivars 1–40 representing introduced varieties from Europe and America, and cultivars 41–120 comprising indigenous Chinese varieties. Detailed information, including cultivar names and morphological characteristics at the half-opening stage, is presented in Table 1 and Fig. 1, respectively.

Table 1: Names of 120 herbaceous peony cultivars

120 Herbaceous Peony Cultivars					
'Alertie'	'Gardenia'	'Bingqing'	'Fen Louzi'	'Xiao Louzi'	'Modeng Nvlang'
'Angel Cheeks'	'Jacorma'	'Chunxiao'	'Fen Yinzhen'	'Yingxiong Hua'	'Mozi Cunjin'
'Belle Chinoise'	'Kansas'	'Cibai'	'Fugui Lan'	'Yu Hudie'	'Qihua Chunyu'
'Blush Queen'	'Karl Rosenfield'	'Daiyu'	'Guanyin Zuo'	'Zhongsheng Fen'	'Qingkong Wanli'
'Bowl of Cream'	'Krinkled White'	'Huaju'	'Hong Louzi'	'Zhuangyuan Hong'	'Shajin Guanding'
'Buckeye Belle'	'Lemon Chiffon'	'Huicui'	'Jiguan Zi'	'Zi Lianhua'	'Sishui Liunian'
'Charlie's White'	'Madame de Verneville'	'Lanmeng'	'Jixue Hong'	'Dadi Lushuang'	'Shoufu Yinxu'
'Command Performance'	'Many Happy Returns'	'Lvlian'	'Jinhuan Hong'	'Dieluo Fenchi'	'Shuiying Chunlan'
'Coral Charm'	'Miss America'	'Moyun'	'Jinjiang Hong'	'Donghai Chaoyang'	'Taohua Feixue'
'Coral Sunset'	'Mobsieur Jules Elie'	'Wanbai'	'Lan Chonglou'	'Fenchi Jinyu'	'Taohua Zhengchun'
'Coral Supreme'	'Mother's Choice'	'Wanxia'	'Linglong Yu'	'Foguang Zhuying'	'Taoli Yanzhuang'
'Cytherea'	'Nick Shaylor'	'Xishi'	'Mantang Hong'	'Hongxia Yingri'	'Xuanli Duocai'
'Dinner Plate'	'Old Faithful'	'Xuefeng'	'Qingyun Hong'	'Hongxia Zhenghui'	'Xueshan Pixia'
'Do Tell'	'Paula Fay'	'Yanxiang'	'Ruixia Hong'	'Jinxing Lanman'	'Yinbian Hongge'

(Continued)

Table 1 (continued)

120 Herbaceous Peony Cultivars					
'Dr. Alexander Fleming'	'Peter Brand'	'Zaohong'	'Shanhe Hong'	'Jinxing Shanshuo'	'Yuezhao Shanhe'
'Duchesse de Nemours'	'Pink Hawaiian Coral'	'Bai Yupan'	'Shaonv Qun'	'Kunshan Xiaguang'	'Zilian Wangyue'
'Eden's Perfume'	'Red Charm'	'Chuitou Hong'	'Taohua Fen'	'Lanhai Bibo'	'Zitan Shengyan'
'Edulis Superba'	'Red Sarah Bernhardt'	'Daye Hong'	'Wuxia Yu'	'Lantian Biyu'	'Jinzhen Cihongling'
'Festiva Maxima'	'Sarah Bernhardt'	'Erhao Hong'	'Xiyang Hong'	'Linghua Chenyu'	'Xuri Zhaohongyan'
'Flame'	'Shirley Temple'	'Fen Fushi'	'Xianhe Bai'	'Meigui Piaoxiang'	'Yinie Sandianhong'

Note: According to the order in the table, all herbaceous peony cultivars are numbered 1–120 successively from top to bottom, left to right: Cultivars are ordered by origin (Introduced: 1–40; Domestic: 41–120).

Figure 1: Morphological characteristics of 120 herbaceous peony cultivars at the half-opening stage. The order of the pictures is consistent with that of the 120 herbaceous peony varieties in Table 1

2.2 DHS-GC-MS Analysis

2.2.1 Floral Scent Collection

The floral scent collection method followed the protocol described by Wang et al. [28]. Floral scent samples were collected on clear, windless days to minimize environmental variability that could influence VOC profiles. The tested flowers were placed in an odorless, transparent sampling bag (250 mm × 380 mm, Huadr, Beijing, China) with both ends open. The upper end of the bag was connected to an activated charcoal tube, while the lower end was connected to a glass tube filled with Tenax TA adsorbent. Both ends were sealed with plastic clips, and the setup was connected to an atmospheric sampler (QC-1S model, manufactured by the Beijing Municipal Institute of Labour Protection, Beijing, China) via silicone tubing. The atmospheric sampler drew air from around the flowers, simulating the natural release of floral scent. Simultaneously, the volatile organic compounds (VOCs) emitted by the flowers were directed into the Tenax TA adsorption tube for collection. The air drawn by the sampler was purified through the activated charcoal tube to remove moisture and dust. The flow rate of the atmospheric sampler was set to 400 $mL{\cdot}min^{-1}$, and the collection time was 3 h. After collection, hexane was added to the adsorption tube to elute the adsorbent, yielding the eluate. The eluate samples were stored at −20°C until instrumental analysis.

2.2.2 Floral Scent Detection

Gas Chromatography conditions (Agilent 8890 gas chromatograph, Agilent Technologies, Santa Clara, CA, USA): chromatographic column: HP-5MS flexible quartz capillary column (30 m × 0.25 mm × 0.25 μm); carrier gas: high-purity helium at 0.8 $mL{\cdot}min^{-1}$; injection temperature: 250°C; column flow rate: 1.2 $mL{\cdot}min^{-1}$; column temperature: initiated temperature at 40°C (hold 1 min), followed by five sequential ramps: (1) 6°C·min^{-1} to 100°C, (2) 3°C·min^{-1} to 136°C, (3) 0.5°C·min^{-1} to 138°C, (4) 2°C·min^{-1} to 142°C, and (5) 12°C·min^{-1} to 250°C; injection mode: split injection (9:1 ratio); injection volume: 2 μL.

Mass Spectrometry conditions (Agilent 5977B mass spectrometer, Agilent Technologies, Santa Clara, CA, USA): electron ionization: electron impact (EI) source, 70 eV; interface temperature: 250°C; ion source temperature: 230°C; quadrupole temperature: 150°C; EM voltage: 1247 V; mass range: 29–386 amu.

2.3 Data Analysis

Qualitative Analysis: A 500 $mg{\cdot}L^{-1}$ N-alkane standard solution was diluted 50-fold with n-hexane and analyzed under the aforementioned GC-MS conditions. The retention times for individual n-alkanes were recorded, and the calculated retention indices (RIs) were compared with reference values from the National Institute of Standards and Technology (NIST) library. The RI calculation followed this formula:

$$RI = 100 \times n + 100 \times (t_x - t_n)/(t_{n+1} - t_n)$$

where RI represents the retention index of the target analyte, n denotes the carbon number of the preceding n-alkane, t_x indicates the retention time of the target analyte, t_n corresponds to the retention time of the preceding n-alkane, and t_{n+1} refers to the retention time of the succeeding n-alkane, with t_x falling between t_n and t_{n+1} in the n-alkane series.

Quantitative analysis: A 69.32 $mg{\cdot}L^{-1}$ ethyl decanoate solution in ethyl acetate served as the internal standard. For each 80 μL sample, 0.4 μL of the internal standard solution was added, and the relative content of aroma components was calculated using the following formula:

Relative content of each component ($\mu g \cdot g^{-1}$) = [peak area of each component/peak area of internal standard] × internal standard concentration ($mg \cdot L^{-1}$) × internal standard volume (μL)/sample weight (g) × f (f is the correction factor of each component to the internal standard, $f = 1$).

Principal Component Analysis: The 19 volatile compounds with the highest content were selected as the objects of analysis, and the original data of their relative content were subjected to extreme value standardization. The standardized relative contents of selected floral VOCs were denoted as ZX_1, ZX_2, ZX_3, ..., ZX_{19}, comprising a total of 19 indicators. The coefficients for each indicator were obtained by dividing the corresponding eigenvector data in the principal component loading matrix by the square root of the respective initial eigenvalues. The calculation formula for the principal component composite score is as follows:

$$F_1 = 0.234 * ZX_1 - 0.046 * ZX_2 - 0.039 * ZX_3 - 0.029 * ZX_4 - 0.025 * ZX_5 - 0.043 * ZX_6 - 0.064 * ZX_7 - 0.050 * ZX_8 - 0.067 * ZX_9 - 0.031 * ZX_{10} - 0.077 * ZX_{11} + 0.297 * ZX_{12} + 0.343 * ZX_{13} + 0.361 * ZX_{14} + 0.361 * ZX_{15} + 0.359 * ZX_{16} + 0.351 * ZX_{17} + 0.361 * ZX_{18} + 0.269 * ZX_{19}$$

$$F_2 = 0.055 * ZX_1 + 0.130 * ZX_2 + 0.493 * ZX_3 + 0.421 * ZX_4 + 0.102 * ZX_5 + 0.269 * ZX_6 - 0.050 * ZX_7 + 0.572 * ZX_8 - 0.171 * ZX_9 + 0.297 * ZX_{10} - 0.157 * ZX_{11} + 0.045 * ZX_{12} + 0.000 * ZX_{13} + 0.018 * ZX_{14} + 0.030 * ZX_{15} + 0.021 * ZX_{16} + 0.025 * ZX_{17} + 0.018 * ZX_{18} - 0.015 * ZX_{19}$$

$$F_3 = -0.061 * ZX_1 + 0.196 * ZX_2 - 0.091 * ZX_3 - 0.129 * ZX_4 - 0.157 * ZX_5 + 0.326 * ZX_6 - 0.050 * ZX_7 + 0.112 * ZX_8 + 0.553 * ZX_9 + 0.368 * ZX_{10} + 0.563 * ZX_{11} + 0.105 * ZX_{12} + 0.030 * ZX_{13} + 0.051 * ZX_{14} + 0.053 * ZX_{15} + 0.045 * ZX_{16} + 0.057 * ZX_{17} + 0.054 * ZX_{18} - 0.052 * ZX_{19}$$

$$F_4 = 0.081 * ZX_1 - 0.181 * ZX_2 + 0.326 * ZX_3 + 0.380 * ZX_4 + 0.053 * ZX_5 - 0.429 * ZX_6 - 0.178 * ZX_7 + 0.096 * ZX_8 + 0.385 * ZX_9 - 0.442 * ZX_{10} + 0.359 * ZX_{11} - 0.083 * ZX_{12} + 0.006 * ZX_{13} + 0.016 * ZX_{14} + 0.017 * ZX_{15} + 0.022 * ZX_{16} + 0.022 * ZX_{17} + 0.013 * ZX_{18} + 0.013 * ZX_{19}$$

$$F_5 = 0.225 * ZX_1 + 0.569 * ZX_2 - 0.207 * ZX_3 + 0.019 * ZX_4 + 0.646 * ZX_5 - 0.153 * ZX_6 - 0.358 * ZX_7 - 0.076 * ZX_8 + 0.013 * ZX_9 + 0.056 * ZX_{10} + 0.012 * ZX_{11} - 0.058 * ZX_{12} - 0.029 * ZX_{13} + 0.001 * ZX_{14} - 0.005 * ZX_{15} - 0.019 * ZX_{16} - 0.003 * ZX_{17} - 0.012 * ZX_{18} - 0.026 * ZX_{19}$$

Odor Activity Value Analysis: The formula for calculating the Odor Activity Value is as follows:

$$OAV_i = C_i\left(\mu g \cdot g^{-1}\right)/OT_i\left(mg \cdot kg^{-1}\right)$$

where C_i is the relative content of volatile compound i and OT_i is the odor threshold of volatile compound i in water.

Statistical analyses were performed using Microsoft Excel (version 16.0) and IBM SPSS Statistics (version 21.0).

3 Results

3.1 Major Floral VOCs Composition and Content from Herbaceous Peony Cultivars

A total of 86 floral VOCs were identified, categorized into 29 terpenoids, 25 aromatic compounds, 21 hydrocarbons, 7 fatty acid derivatives, and 4 heterocyclic compounds (Table 2). Aromatic compounds constituted the largest proportion (79.70%), followed by hydrocarbons (10.51%) and terpenoids (8.37%), while fatty acid derivatives (1.03%) and heterocyclic compounds (0.38%) represented the smallest fractions. Comparative analysis of introduced and domestic cultivars revealed that aromatic compounds, hydrocarbons, and terpenoids predominated in both groups. However, introduced cultivars exhibited higher proportions of hydrocarbons relative to terpenoids, whereas the inverse pattern was observed in domestic cultivars.

Table 2: The flora VOCs of 120 herbaceous peony cultivars

Category	RT/min	Compounds	CAS/No.	Chemical formula	Retention index	
					Calculated value	Reference value
Terpenoids	4.058	α-Pinene	80-56-8	$C_{10}H_{16}$	937	937
	4.672	Sabinene	3387-41-5	$C_{10}H_{16}$	976	977
	4.820	6-Methyl-5-hepten-2-one	110-93-0	$C_8H_{14}O$	986	988
	4.915	Myrcene	123-35-3	$C_{10}H_{16}$	992	992
	5.160	3-Hexen-1-ol, acetate, (Z)-	3681-71-8	$C_8H_{14}O_2$	1006	1006
	5.565	p-Cymene	99-87-6	$C_{10}H_{14}$	1027	1028
	5.648	Limonene	138-86-3	$C_{10}H_{16}$	1031	1030
	5.713	Eucalyptol	470-82-6	$C_{10}H_{18}O$	1034	1031
	5.764	(E)-β-Ocimene	13877-91-3	$C_{10}H_{16}$	1037	1037
	5.975	(Z)-β-Ocimene	3338-55-4	$C_{10}H_{16}$	1047	1039
	6.230	γ-Terpinene	99-85-4	$C_{10}H_{16}$	1060	1060
	7.050	Linalool	78-70-6	$C_{10}H_{18}O$	1101	1103
	7.521	β-Thujone	471-15-8	$C_{20}H_{32}O_2$	1119	1121
	8.787	Borneol	507-70-0	$C_{10}H_{18}O$	1169	1168
	9.067	4-Terpineol	562-74-3	$C_{10}H_{18}O$	1180	1179
	9.419	α-Terpineol	98-55-5	$C_{10}H_{18}O$	1193	1190
	9.987	Verbenone	1196-01-6	$C_{10}H_{14}O$	1213	1211
	10.427	Citronellol	106-22-9	$C_{10}H_{20}O$	1227	1228
	10.449	Nerol	106-25-2	$C_{10}H_{18}O$	1228	1231
	11.237	Geraniol	106-24-1	$C_{10}H_{18}O$	1253	1252
	11.779	Citral	5392-40-5	$C_{10}H_{16}O$	1271	1272
	14.536	Citronellyl acetate	150-84-5	$C_{12}H_{22}O_2$	1353	1354
	14.932	Neryl acetate	141-12-8	$C_{12}H_{20}O_2$	1365	1367
	15.247	β-Patchoulene	514-51-2	$C_{15}H_{24}$	1374	1377
	15.611	Geranyl acetate	105-87-3	$C_{12}H_{20}O_2$	1384	1382
	16.936	β-Caryophyllene	87-44-5	$C_{15}H_{24}$	1421	1423
	18.148	α-Caryophyllene	6753-98-6	$C_{15}H_{24}$	1453	1449
	27.127	3,7,11-Trimethyl-6,10-dodecadien-1-ol	51411-24-6	$C_{15}H_{28}O$	1693	1696
	29.539	Phytone	502-69-2	$C_{18}H_{36}O$	1849	1847
Aromatic compounds	3.782	Anisole	100-66-3	C_7H_8O	919	918
	4.341	n-Propylbenzene	103-65-1	C_9H_{12}	955	950
	4.457	Benzaldehyde	100-52-7	C_7H_6O	963	961
	5.472	4-Methylanisole	104-93-8	$C_8H_{10}O$	1022	1021
	5.940	Phenylacetaldehyde	122-78-1	C_8H_8O	1047	1038
	6.395	Acetophenone	98-86-2	C_8H_8O	1068	1067
	6.702	Phenylethyl methyl ether	3558-60-9	$C_9H_{12}O$	1084	1089
	6.921	N,N-Dimethylaniline	121-69-7	$C_8H_{11}N$	1094	–
	7.002	Methyl benzoate	93-58-3	$C_8H_{8O}2$	1099	1092
	7.395	Phenylethanol	60-12-8	$C_8H_{10}O$	1114	1112
	8.166	1,2-Dimethoxybenzene	91-16-7	$C_8H_{10}O_2$	1144	1143
	8.645	1,4-Dimethoxybenzene	150-78-7	$C_8H_{10}O_2$	1163	1165
	9.255	Naphthalene	91-20-3	$C_{10}H_8$	1187	1187
	9.544	Methyl salicylate	119-36-8	$C_8H_8O_3$	1198	1195
	11.355	Phenylethyl acetate	103-45-7	$C_{10}H_{12}O_2$	1257	1255
	12.941	α-Methylcinnamaldehyde	101-39-3	$C_{10}H_{10}O$	1307	1309
	15.176	1,2,4-Trimethoxybenzene	135-77-3	$C_9H_{12}O_3$	1372	1374

(Continued)

Table 2 (continued)

Category	RT/min	Compounds	CAS/No.	Chemical formula	Retention index	
					Calculated value	Reference value
	15.586	Methyl cinnamate	103-26-4	$C_{10}H_{10}O_2$	1384	1380
	16.259	Methyleugenol	93-15-2	$C_{11}H_{14}O_2$	1405	1405
	16.504	1,3,5-Trimethoxybenzene	621-23-8	$C_9H_{12}O_3$	1409	1418
	19.596	Phenethyl isovalerate	140-26-1	$C_{13}H_{18}O_2$	1491	1490
	20.463	2,4-Di-tert-butylphenol	96-76-4	$C_{14}H_{22}O$	1511	1509
	20.533	Butylated hydroxytoluene	128-37-0	$C_{15}H_{24}O$	1513	1512
	28.425	Benzyl benzoate	120-51-4	$C_{14}H_{12}O_2$	1768	1763
	30.881	Dibutyl phthalate	84-74-2	$C_{16}H_{22}O_4$	1969	1960
Hydrocarbon compounds	3.484	Nonane	111-84-2	C_9H_{20}	900	900
	4.555	3-Methylnonane	5911-04-6	$C_{10}H_{22}$	969	970
	5.044	Decane	124-18-5	$C_{10}H_{22}$	1000	1000
	6.195	Decalin	91-17-8	$C_{10}H_{18}$	1058	1055
	6.606	1-Decene	872-05-9	$C_{10}H_{20}$	1079	1088
	7.040	Undecane	1120-21-4	$C_{11}H_{24}$	1100	1100
	7.444	(3E)-4,8-dimethylnona-1,3,7-triene	19945-61-0	C11H18	1116	1116
	9.605	Dodecane	112-40-3	$C_{12}H_{26}$	1200	1200
	12.709	Tridecane	629-50-5	$C_{13}H_{28}$	1301	1300
	16.163	Tetradecane	629-59-4	$C_{14}H_{30}$	1400	1400
	19.941	Pentadecane	629-62-9	$C_{15}H_{32}$	1500	1500
	24.687	Hexadecane	544-76-3	$C_{16}H_{34}$	1601	1600
	26.550	2-Methylhexadecane	1560-92-5	$C_{17}H_{36}$	1671	1671
	27.324	Heptadecane	629-78-7	$C_{17}H_{36}$	1700	1700
	27.438	2,6,10,14-Tetramethylpentadecane	1921-70-6	$C_{19}H_{40}$	1707	1705
	28.523	3-Methylheptadecane	6418-44-6	$C_{18}H_{38}$	1774	1774
	28.843	1-Octadecene	112-88-9	$C_{18}H_{36}$	1794	1794
	28.941	Octadecane	593-45-3	$C_{18}H_{38}$	1800	1800
	30.176	Nonadecane	629-92-5	$C_{19}H_{40}$	1901	1900
	31.205	Eicosane	112-95-8	$C_{20}H_{42}$	2000	2000
	32.113	Heneicosane	629-94-7	$C_{21}H_{44}$	2100	2100
Fatty acid derivatives	3.845	Methyl hexanoate	106-70-7	$C_7H_{14}O_2$	923	925
	4.105	3-Methoxybutyl acetate	4435-53-4	$C_7H_{14}O_3$	940	–
	5.271	Hexyl acetate	142-92-7	$C_8H_{16}O_2$	1012	1011
	7.156	Nonanal	124-19-6	$C_9H_{18}O$	1105	1106
	9.788	Decanal	112-31-2	$C_{10}H_{20}O$	1206	1207
	13.543	Methyl geranate	1189-09-9	$C_{11}H_{18}O_2$	1325	1324
	28.121	2-Octyl-1-dodecanol	5333-42-6	$C_{20}H_{42}O$	1762	–
Heterocyclic compounds	4.240	2-Methylpiperazine	109-07-9	$C_5H_{12}N_2$	949	–
	4.325	1,2;7,8-Dibenzocarbazole	239-64-5	$C_{20}H_{13}N$	954	–
	10.112	4,7-Dimethylbenzofuran	28715-26-6	$C_{10}H_{10}O$	1217	1218
	12.515	Indole	120-72-9	C_8H_7N	1294	1293

Note: "-" indicates that no reference retention index was found for this compound.

The number of floral VOCs detected in each cultivar ranged from 4 to 38 across the studied herbaceous peony cultivars. 'Hongxia Yingri' and 'Sishui Liunian' contained the highest number of floral VOCs, while

'Angel Cheeks' and 'Fenchi Jinyu' contained the fewest. The distribution patterns of floral VOCs also varied substantially: nonane was universally detected across all cultivars, whereas γ-pinene and 4-terpineol were exclusively identified in 'Hongxia Yingri'. Further analysis revealed that domestic cultivars contained 17 unique VOCs, significantly exceeding the four identified in introduced cultivars, highlighting the superior VOC diversity within domestic herbaceous peony germplasm.

3.2 Comparative Analysis of Total VOCs Contents among Herbaceous Peony Cultivars

Significant variation in total floral VOC contents were observed among herbaceous peony cultivars (Fig. 2). 37 cultivars, including 'Coral Supreme', 'Command Performance', and 'Zitan Shengyan', exhibited contents below 10 $\mu g \cdot g^{-1}$. 77 cultivars, such as 'Yingxiong Hua', 'Ruixia Hong', and 'Chuitou Hong', showed contents between 10 and 100 $\mu g \cdot g^{-1}$. Only six cultivars exceeded 100 $\mu g \cdot g^{-1}$: 'Lvlian', 'Kansas', 'Modeng Nvlang', 'Daiyu', 'Yuezhao Shanhe', and 'Dr. Alexander Flaming'. 'Dr. Alexander Flaming' demonstrated the highest VOC content (318.06 ± 74.26 $\mu g \cdot g^{-1}$), followed by 'Yuezhao Shanhe' (291.74 ± 68.59 $\mu g \cdot g^{-1}$) and 'Daiyu' (192.75 ± 15.88 $\mu g \cdot g^{-1}$). The lowest contents were observed in 'Zitan Shengyan' (3.00 ± 0.97 $\mu g \cdot g^{-1}$), 'Command Performance' (2.82 ± 1.79 $\mu g \cdot g^{-1}$), and 'Coral Supreme' (1.95 ± 0.42 $\mu g \cdot g^{-1}$).

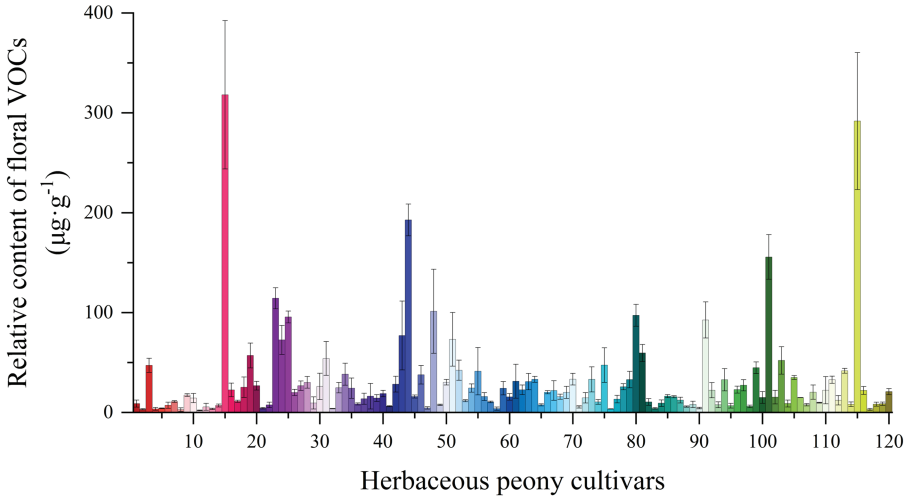

Figure 2: Relative content of floral VOCs in 120 herbaceous peony cultivars

Comparative analysis of introduced and domestic cultivars revealed similar content distributions, with most cultivars below 50 $\mu g \cdot g^{-1}$. 16 cultivars exceeded this threshold, comprising 6 introduced and 10 domestic cultivars. Introduced cultivars exhibited a mean content of 30.53 $\mu g \cdot g^{-1}$ (median: 15.29 $\mu g \cdot g^{-1}$), whereas domestic cultivars demonstrated a mean of 31.24 $\mu g \cdot g^{-1}$ (median: 20.12 $\mu g \cdot g^{-1}$). The elevated mean values relative to medians in both groups suggested right-skewed distributions. While most cultivars displayed relatively low contents, several exhibited exceptionally high values. The higher mean and median values of total VOC contents in domestic cultivars compared to introduced cultivars suggest superior overall floral aroma release capacity in domestic herbaceous peony germplasm. Furthermore, the greater discrepancy between median and mean values in introduced cultivars reflects more substantial inter-cultivar variability.

3.3 Comparative Analysis of Floral VOCs Categories among Herbaceous Peony Cultivars

Aromatic compounds, hydrocarbons and terpenoids were the three types of floral VOCs with the highest content in the aroma of herbaceous peony, so they were further analyzed and compared. Fig. 3

presents the distribution patterns of total VOCs contents and content of each VOCs categories. It can be seen that the fluctuation of the total content and the content of aromatic compounds therein showed a relatively obvious consistency. Similar to total VOC content, most cultivars exhibited low levels across all VOC categories, with only a few demonstrating exceptionally high concentrations.

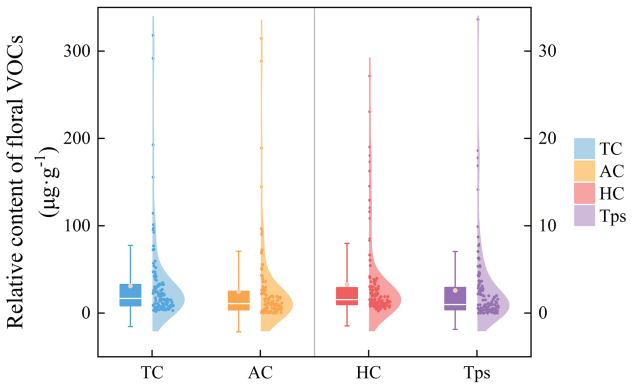

Figure 3: Semi-violin-box plot of various floral fragrance VOCs of 120 herbaceous peony cultivars at the half-opening stage. From left to right, they are total content (TC), aromatic compounds (AC), hydrocarbon compounds (HC), and terpenoids (Tps). The relative contents of TC and AC are relatively high, corresponding to the left *y*-axis; while the relative contents of HC and Tps are relatively low, corresponding to the right *y*-axis. In the semi-violin-box plot, the mean values are presented in small dots inside the box bodies, the centre line represents the median. Violin edges show the 25th and 75th percentiles, and whiskers extend to 1.5× the interquartile range

Aromatic compounds were detected in all cultivars except 'Linglong Yu'. 'Dr. Alexander Fleming' exhibited significantly higher aromatic compound contents (314.61 ± 73.13 $\mu g \cdot g^{-1}$), accounting for 98.92% of its total VOC content. The relative content of aromatic compounds ranged from 0 to 314.61 $\mu g \cdot g^{-1}$, with 85 cultivars showing >50% aromatic compound composition. Notably, phenylethanol and 1,4-dimethoxybenzene were widely distributed with high relative contents. While dibutyl phthalate, though present in 101 cultivars, showed lower contents.

Hydrocarbon compounds were universally present across all cultivars. 'Shajin Guanding' exhibited the highest hydrocarbon emissions (27.15 ± 1.37 $\mu g \cdot g^{-1}$), representing 77.66% of its total VOC content. The relative content of hydrocarbon compounds ranged from 0.25 to 27.15 $\mu g \cdot g^{-1}$, with 18 cultivars showing >50% hydrocarbon composition. Nonane was the most prevalent hydrocarbon, detected in all cultivars.

Comparative analysis of terpenoid content revealed their absence in 6 cultivars, including 'Belle Chinoise', 'Charlie's White', and 'Coral Sunset'. The cultivar 'Qihua Chunyu' exhibited the highest terpenoid emission (33.63 ± 8.47 $\mu g \cdot g^{-1}$), representing 64.62% of its total VOC content. The relative content of terpenoids ranged from 0 to 33.63 $\mu g \cdot g^{-1}$, with only 8 cultivars showing >50% terpenoid composition. Among the detected terpenoids, citronellol and geraniol showed high concents.

Analysis of dominant VOCs revealed distinct compositional patterns across cultivars. Aromatic compounds predominated in 90 cultivars, including 'Belle Chinoise', 'Blush Queen', and 'Bowl of Cream'. Among these, 44 cultivars exhibited 1,4-dimethoxybenzene as their dominant volatile compound, while 42 cultivars showed phenylethanol as the primary volatile component. Methyl cinnamate was dominant in cultivars such as 'Huaju', 'Ruixia Hong', and 'Jinzhen Cihongling', whereas 'Cytherea' uniquely featured dibutyl phthalate. Hydrocarbon dominance was observed in 18 cultivars, including 'Alertie', 'Angel Cheeks', and 'Charlie White'. Nonane served as the dominant volatile compound in 17 cultivars, while 'Many Happy Returns' uniquely

exhibited heptadecane as its primary volatile component. Terpenoid dominance was identified in 11 cultivars, including 'Bingqing', 'Moyun', and 'Xuefeng'. Geraniol served as the dominant volatile compound in 7 cultivars, while citronellol was the primary volatile component in 4 cultivars, including 'Taohua Fen' and 'Qihua Chunyu'. Notably, 'Barker's Beauty' was unique in having decanal, a fatty acid derivative, as its dominant VOC.

3.4 Principal Component Analysis of Major Floral VOCs

Principal component analysis (PCA) is a statistical dimensionality reduction technique that transforms original data into composite variables, facilitating more intuitive interpretation of complex datasets. To accurately identify key volatile components at the half-opening stage of herbaceous peony cultivars, 19 major floral VOCs—including nonane, (Z)-β-ocimene, and phenylethanol—were selected from 86 detected compounds for PCA. Data suitability for PCA was confirmed through SPSS analysis, with a Kaiser-Meyer-Olkin (KMO) value of 0.821 and Bartlett's test of sphericity significance level <0.01. As shown in Table 3, the first five principal components had initial eigenvalues >1, collectively explaining 76.51% of the total variance, effectively representing the primary information of volatile components across 120 cultivars.

Table 3: Eigenvalues of principal components and their variance contributions

Principal component	Eigenvalue	Contribution rate	Cumulative contribution rate
1	7.368	38.781	38.781
2	2.372	12.484	51.265
3	2.004	10.547	61.811
4	1.523	8.018	69.83
5	1.268	6.675	76.505
6	0.974	5.125	81.63
7	0.84	4.423	86.053
8	0.641	3.375	89.428
9	0.455	2.393	91.821
10	0.446	2.348	94.168
11	0.409	2.153	96.321
12	0.274	1.441	97.761
13	0.155	0.818	98.579
14	0.111	0.583	99.162
15	0.092	0.482	99.644
16	0.037	0.197	99.841
17	0.017	0.09	99.931
18	0.009	0.047	99.978
19	0.004	0.022	100

The first principal component (PC1) accounted for 38.78% of the total variance, with significant contributions from nonane, tetradecane, pentadecane, butylated hydroxytoluene, hexadecane, heptadecane, pentadecane, 2,6,10,14-Tetramethylpentadecane, octadecane, and dibutyl phthalate. PC2 explained 12.48% of the variance, primarily influenced by phenylethanol, 1,2-dimethoxybenzene, and phenylethyl acetate. PC3 contributed 10.55% of the variance, mainly driven by α-methylcinnamaldehyde and methyl

cinnamate. PC4 represented 8.02% of the variance, with citronellol and citronellol acetate as the major contributors. Finally, PC5 accounted for 6.68% of the variance, predominantly influenced by (Z)-β-ocimene, 1,4-dimethoxybenzene, and geraniol.

After calculating the principal component scores for the 120 herbaceous peony cultivars, these scores were weighted according to the ratio of the variance contribution rate of each principal component to the cumulative variance contribution rate. The weighted scores were then summed using the weighted sum method to obtain the composite score for each cultivar (Table 4). Based on the composite scores, the top five cultivars with the highest scores were identified: 'Shajin Guanding' (1.427), 'Many Happy Returns' (1.159), 'Edulis Superba' (1.040), 'Huicui' (1.021), and 'Madame de Verneville' (0.899). A comparison of the composite scores between domestic and introduced herbaceous peony cultivars revealed that 7 out of the top 10 cultivars and 21 out of the top 40 cultivars were introduced. Introduced cultivars dominated the higher rankings, with their overall distribution concentrated in the high-score segment, while most domestic cultivars were clustered in the middle- and low-score segments. These results indicate that, based on PCA composite scores, introduced cultivars generally outperform domestic cultivars.

Table 4: The PCA composite scores of floral VOCs in 120 herbaceous peony cultivars

No.	Score	No.	Score	No.	Score	No.	Score	No.	Score	No.	Score
1	0.025	21	0.053	41	0.036	61	0.041	81	0.078	101	0.167
2	0.035	22	0.489	42	0.093	62	−0.010	82	0.035	102	0.053
3	0.044	23	0.079	43	0.096	63	0.061	83	0.060	103	0.213
4	0.626	24	1.040	44	0.092	64	0.005	84	0.028	104	0.084
5	0.032	25	0.095	45	0.162	65	0.026	85	0.024	105	1.427
6	0.239	26	1.159	46	1.021	66	0.063	86	0.150	106	0.130
7	0.153	27	0.590	47	0.016	67	0.108	87	0.055	107	0.076
8	0.120	28	0.680	48	0.075	68	0.064	88	0.046	108	0.072
9	0.899	29	0.030	49	0.039	69	0.043	89	0.019	109	0.102
10	0.150	30	0.236	50	0.001	70	0.036	90	0.058	110	0.067
11	0.756	31	0.029	51	−0.076	71	0.076	91	0.127	111	0.041
12	0.055	32	0.226	52	0.038	72	0.096	92	0.130	112	0.104
13	0.880	33	0.106	53	−0.002	73	0.082	93	0.016	113	0.146
14	0.103	34	0.074	54	0.049	74	0.165	94	0.062	114	0.123
15	0.204	35	0.305	55	0.129	75	0.194	95	0.048	115	0.101
16	0.044	36	0.105	56	0.580	76	0.019	96	0.139	116	0.091
17	0.053	37	0.195	57	0.026	77	0.077	97	0.035	117	0.029
18	0.046	38	0.086	58	0.052	78	0.045	98	0.038	118	0.099
19	0.145	39	0.082	59	0.083	79	0.076	99	0.134	119	0.064
20	0.090	40	0.014	60	0.095	80	0.105	100	0.100	120	0.011

3.5 Odor Classification and OAVs of Floral VOCs

The odor activity value (OAV) combines the relative content of volatile compounds with their odor thresholds, serving as a key indicator to evaluate the contribution of aroma components to the overall aroma profile. Generally, the higher the OAV of a volatile compound, the greater its contribution to the overall aroma. Volatile compounds with an OAV below 0.1 are considered to have a negligible impact on the aroma.

Those with an OAV greater than 1 are regarded as modifying aroma components, while those with an OAV exceeding 10 are identified as key aroma components.

To investigate the contribution of floral VOCs to the overall aroma profile of herbaceous peony and to identify key aroma components, this study conducted a detailed analysis based on OAVs. Among the 86 identified floral VOCs, odor thresholds were obtained from the literature for only 54 VOCs; the remaining VOCs lacked available threshold data. The odor characteristics, odor thresholds, and aroma classifications of these 54 VOCs are summarized in Table 5. Through OAV analysis, 46 floral VOCs with OAVs greater than 0.1 were identified. Among these, 15 floral VOCs, including methyl hexanoate, α-pinene, and benzaldehyde, exhibited OAVs greater than 1 and were classified as modifying aroma components of the herbaceous peony floral scent. Additionally, 22 floral VOCs, such as 3-hexen-1-ol, acetate, (Z)-, limonene, and (E)-β-ocimene, had OAVs exceeding 10 and were identified as key aroma components. Within the key aroma components, 11 floral VOCs, including myrcene, eucalyptol, and (Z)-β-ocimene, showed OAVs greater than 100. These 11 VOCs comprised 6 terpenoids, 3 aromatic compounds, and 2 fatty acid derivatives, with their distribution characteristics illustrated in Fig. 4. Based on odor descriptions and aroma classifications, most floral VOCs exhibited floral, fruity, or woody notes. In the herbaceous peony floral scent, modifying aroma components were primarily sweet or woody, while key aroma components were predominantly floral, fruity, or woody.

Table 5: Descriptions and water threshold values of the major floral VOCs of 120 herbaceous peony cultivars

Compounds	Odor description	ODT/mg·kg^{-1}	Odor type
Nonane	Mild, Hydrocarbon-like	10	Others
Anisole	Sweet, Floral, Herbal, Clove	0.1525	Sweet
Methyl hexanoate	Fruity, Sweet, Fresh, Apple, Grape, Berry	0.07	Fruity
α-Pinene	Fresh, Woody, Herbal, Pine, Resinous	0.12	Woody
2-Methylpiperazine	Amine-like	60	Others
n-Propylbenzene	Aromatic, Benzene-like	0.17712	Others
Benzaldehyde	Sweet, Floral, Almond, Cherry	0.35	Nutty
Sabinene	Woody, Citrus, Herbal, Fresh, Spicy	0.98	Woody
6-Methyl-5-hepten-2-one	Fruity, Green, Banana, Sweet, Fresh	0.068	Fruity
Myrcene	Herbal, Citrus, Woody, Fresh, Balsamic	0.015	Woody
Decane	Mild, Hydrocarbon-like	10	Others
3-Hexen-1-ol, acetate, (Z)-	Green, Grassy, Apple, Fresh, Fruity	0.013	Green
Hexyl acetate	Sweet, Fruity, Pear	0.115	Fruity
4-Methylanisole	Sweet, Floral, Herbal, Clove	0.6	Sweet
p-Cymene	Aromatic, Citrus, Herbal	0.0114	Fruity
Limonene	Citrus, Fresh, Fruity, Orange	0.034	Fruity
Eucalyptol	Cool, Fresh, Herbal, Camphor-like, Green	0.0011	Herbal
(E)-β-Ocimene	Fresh, Floral, Citrus, Woody	0.034	Floral
Phenylacetaldehyde	Sweet, Floral, Hyacinth, Rose, Honey, Green	0.004	Floral
(Z)-β-Ocimene	Fresh, Floral, Fruity, Green, Woody	0.034	Floral
Decalin	Aromatic, Camphor, Turpentine	0.1	Woody
γ-Terpinene	Citrus, Lemon, Sweet, Herbal, Spicy	1	Fruity
Acetophenone	Sweet, Floral, Fruity, Citrus	0.065	Floral

(Continued)

Table 5 (continued)

Compounds	Odor description	ODT/mg·kg^{-1}	Odor type
Methyl benzoate	Sweet, Floral, Ylang-Ylang, Cherry, Tuberose	0.073	Floral
Undecane	Mild, Hydrocarbon-like	10	Others
Linalool	Floral, Sweet, Citrus, Fresh, Woody	0.00022	Floral
Nonanal	Fatty, Citrus, Waxy, Floral, Green	0.0011	Fruity
Phenylethanol	Sweet, Floral, Rose, Honey	0.14	Floral
1,2-Dimethoxybenzene	Sweet, Herbal, Spicy	0.775	Herbal
1,4-Dimethoxybenzene	Sweet, Floral, Lavender, Coumarin	0.0214	Sweet
Borneol	Cool, Camphor, Woody, Minty	0.18	Woody
4-Terpineol	Earthy, Woody, Floral	1.2	Woody
Naphthalene	Strong, Pungent, Coal tar	0.006	Others
α-Terpineol	Floral, Sweet, Lilac, Lily, Citrus	1.2	Floral
Methyl salicylate	Wintergreen, Cool, Sweet, Minty	0.04	Green
Dodecane	Mild, Hydrocarbon-like	10	Others
Decanal	Fatty, Citrus, Waxy, Floral	0.003	Fruity
Citronellol	Floral, Citrus, Rose, Lemongrass	0.01	Floral
Nerol	Sweet, Floral, Citrus	0.3	Floral
Geraniol	Floral, Rose, Citrus	0.0066	Floral
Phenylethyl acetate	Sweet, Floral, Rose, Honey, Fruity	0.24959	Floral
Citral	Citrus, Fresh, Fruity, Lemon	0.04	Fruity
Indole	Floral, Jasmine	0.011	Floral
Tridecane	Mild, Hydrocarbon-like	0.042	Others
Citronellyl acetate	Fruity, Floral, Lemon, Rose	1	Floral
Neryl acetate	Floral, Fruity, Orange Blossom, Rose	2	Floral
Geranyl acetate	Floral, Fruity, Rose, Citrus	0.15	Floral
Tetradecane	Mild, Hydrocarbon-like	1	Others
Methyleugenol	Sweet, Spicy, Clove, Vanilla	0.775	Spicy
β-Caryophyllene	Woody, Spicy, Peppery	0.064	Woody
α-Caryophyllene	Woody, Spicy, Herbal	0.16	Woody
2,4-Di-tert-butylphenol	Phenolic, Medicinal, Smoky.	0.5	Phenolic
Butylated hydroxytoluene	Mild, Waxy, Phenolic	1	Phenolic
Benzyl Benzoate	Sweet, Floral, Balsamic	0.341	Sweet

Note: The odor descriptions and odor type of each volatile compound were obtained from https://www.femaflavor.org/flavor-library, http://www.flavornet.org/flavornet.html, and http://www.thegoodscentscompany.com/ (accessed on 20 March 2025). The odor detection threshold (ODT) is mainly excerpted from Odour Thresholds: Compilations of Odour Threshold Values in Air, Water and Other Media [31]. Compounds without ODT are not listed.

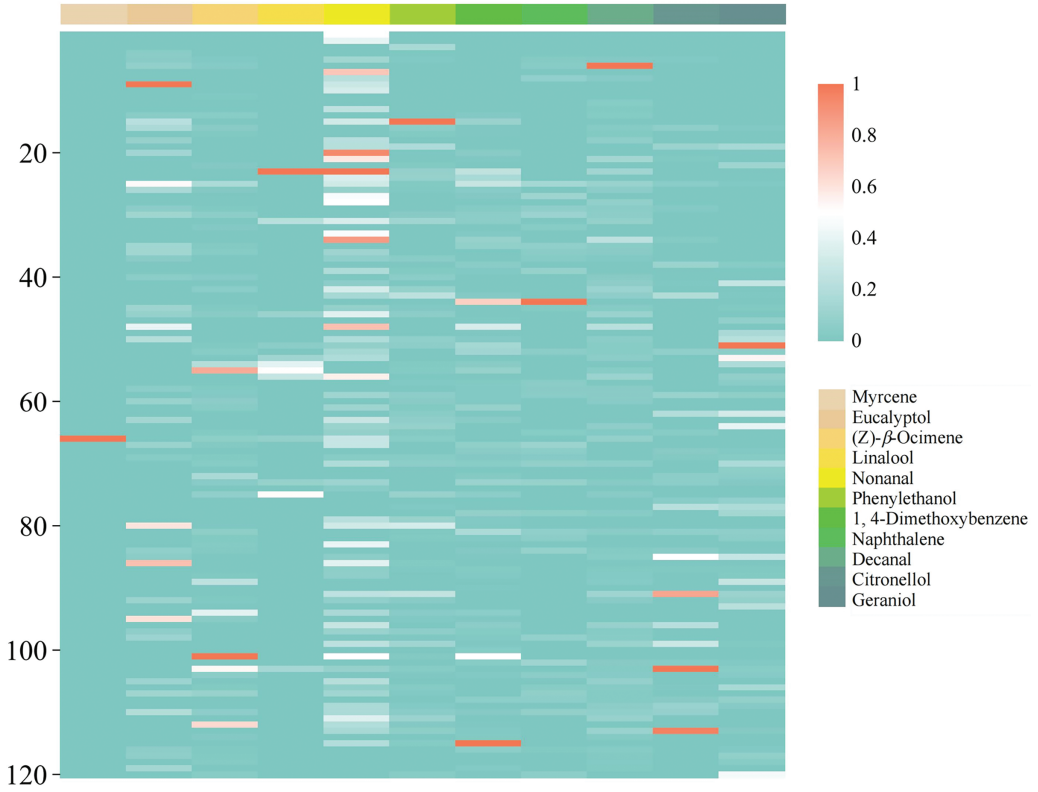

Figure 4: Heatmap visualization of odor activity values for 11 floral VOCs (OAV > 100) across 120 herbaceous peony cultivars. OAV values were normalized per compound to a 0–1 scale using min-max transformation, where 1 represents the maximum OAV value within each compound and 0 indicates the minimum detectable level. Color gradient reflects min-max normalized OAV values per compound. Cultivars are ordered by origin (Introduced: 1–40; Domestic: 41–120)

The sum of OAV values for each cultivar is shown in Fig. 5. The cultivar with the highest total OAV was 'Yuezhao Shanhe', followed by 'Kansas' and 'Daiyu'. The lowest OAVs were observed in 'Coral Supreme', 'Nick Shaylor', and 'Ruixia Hong'. By analyzing the highest-OAV aroma compounds in each herbaceous peony cultivar, aromatic compounds were found to contribute the most to the floral scent of 55 cultivars, including 'Belle Chinoise', 'Coral Supreme', and 'Do Tell'. The highest-OAV aroma compounds in these cultivars were 1,4-dimethoxybenzene, phenylethanol, and naphthalene. terpenoids contributed the most to the floral aroma of 44 cultivars, such as 'Blush Queen', 'Coral Charm', and 'Duchesse de Nemours', with the highest-OAV aroma compounds including geraniol, citronellol, eucalyptol, and linalool. Fatty acid derivatives contributed the most to the floral aroma of 21 cultivars, such as 'Alertie', 'Angel Cheeks', and 'Bowl of Cream', with the highest-OAV aroma compounds being nonanal and decanal. The highest-OAV aroma compounds in introduced cultivars were primarily nonanal and 1,4-dimethoxybenzene, characterized by fruity and sweet aromas, while those in domestic cultivars were mainly 1,4-dimethoxybenzene and phenylethanol, dominated by sweet and floral aromas. Overall, aromatic compounds contributed the most to the overall aroma of herbaceous peony, followed by terpenoids. However, in introduced cultivars, fatty acid derivatives contributed more than terpenoids, which is a key factor in the aroma differences between domestic and introduced cultivars.

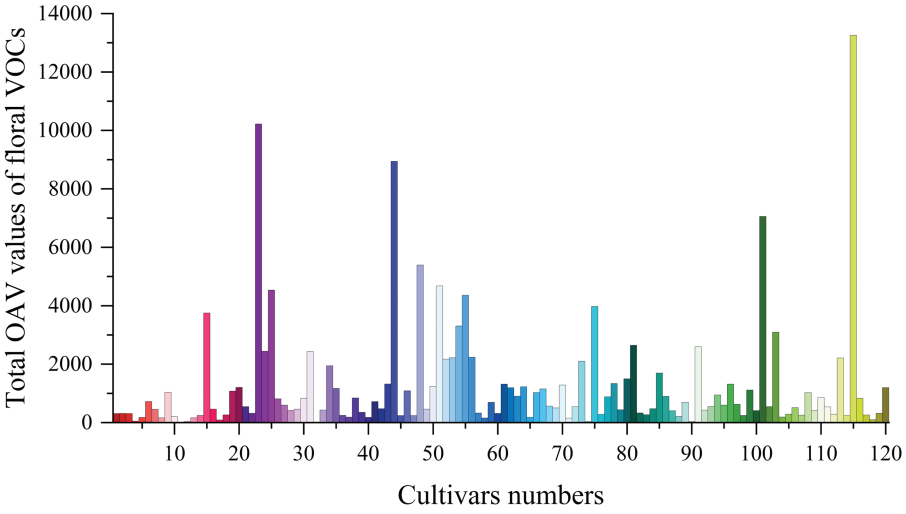

Figure 5: Total OAV values of floral VOCs in 120 herbaceous peony cultivars

4 Discussion

In this study, the volatile components of flowers at the half-opening stage from 120 herbaceous peony cultivars were comprehensively identified and analyzed using multiple analytical methods, aiming to screen aromatic cultivars. The results demonstrated that aromatic compounds, hydrocarbons, and terpenoids constitute the primary components of the floral scent in herbaceous peony. The wide range of VOC diversity observed among herbaceous peony cultivars (from 4 to 38) provides breeders with a valuable resource for selecting parental material with desirable fragrance profiles. By prioritizing cultivars with high VOC diversity, breeders can develop new cultivars with unique fragrances.

Among all the floral scent VOCs, 1,4-dimethoxybenzene exhibited the highest relative content and the largest OAV. Specifically, 1,4-dimethoxybenzene ranked first in relative content among 44 cultivars and was identified as the highest-OAV aroma compound in 47 cultivars. In summary, based on the quantitative analysis and distribution characteristics across cultivars, it can be concluded that 1,4-dimethoxybenzene is the most critical aroma component in the floral scent of herbaceous peony. This conclusion aligns closely with the findings of Wang et al. [28]. 1,4-Dimethoxybenzene is widely present in the floral scents of various plant species and plays a significant role in attracting insect pollinators and facilitating fruit formation. 1,4-Dimethoxybenzene are particularly attractive to common pollinators, such as honeybees [32]. Furthermore, Hoepflinger et al. identified a novel O-methyltransferase, Cp4MP-OMT, which catalyzes the final step in the biosynthesis of 1,4-dimethoxybenzene [33]. This discovery provides critical insights into the biosynthetic pathway of 1,4-dimethoxybenzene.

As a simple and effective data processing method, PCA is widely utilized in the analysis of floral scent composition, as demonstrated in studies by Fu et al., Luo et al., and Zhou et al. [34–36]. In this study, 19 major volatile components, including nonane, (Z)-β-ocimene, and phenylethanol, were selected from the 86 identified floral VOCs for PCA. Five principal components were extracted, which effectively captured the primary information of the volatile components across the 120 herbaceous peony cultivars. The top 5 varieties with the combined scores of principal component analysis were ‘Shajin Guanding’ (1.427), ‘Many Happy Returns’ (1.159), ‘Edulis Superba’ (1.040), ‘Huicui’ (1.021) and ‘Madame de Verneville’ (0.899), and this result can provide parental choices with reference value for aroma breeding of herbaceous peony.

Aroma activity value analysis, a classical method widely applied in food flavor research [37], has gained increasing attention in floral scent studies in recent years [38–40]. Floral scent directly influences the human olfactory system, and the incorporation of odor threshold (ODT) data in the analysis of floral scent components enhances the credibility and persuasiveness of the results. By considering both the relative content of floral VOCs and their corresponding ODTs, it is evident that the contribution of floral VOCs to the overall aroma largely depends on their ODT levels. For instance, in the analysis of herbaceous peony floral scent, hydrocarbons exhibited higher relative content than terpenoids; however, terpenoids played a more critical role in shaping the floral aroma due to the typically higher ODTs of hydrocarbons, which reduced their aroma activity. Furthermore, the odor characterization of herbaceous peony floral VOCs revealed that they primarily exhibited floral, fruity, or woody aromas. The highest-OAV aroma compounds in introduced cultivars were predominantly nonanal and 1,4-dimethoxybenzene, exhibiting fruity and sweet aromas, while those in domestic cultivars were primarily 1,4-dimethoxybenzene and phenylethanol, characterized by sweet and floral aromas. Overall, aromatic compounds contributed the most to the overall aroma profile of herbaceous peony, followed by terpenoids. However, it should be noted that the limited availability of ODT data for many floral VOCs restricted this study to analyzing the aroma activity values of only a subset of floral VOCs. These findings underscore the necessity of further research to expand the ODT database for floral VOCs.

With advancements in science and technology, collection and analytical methods have become increasingly refined, driving rapid progress in floral scent research. Numerous genes associated with floral scent have been identified, establishing floral scent as a prominent research topic in plant sciences [41–43]. The findings of Peng et al. indicate that current floral scent research is progressively deepening, with perspectives expanding from microscopic to macroscopic levels, and research focuses now encompassing biosynthetic pathways, gene regulatory networks, ecological functions, and cultivar selection and breeding [44]. In this study, a systematic investigation of the floral scent composition of herbaceous peony was conducted. Dynamic headspace sampling was employed to collect floral VOCs from 120 herbaceous peony cultivars at the half-opening stage, followed by identification and analysis using GC-MS. The results not only clarified the main components of herbaceous peony floral scent but also analyzed the relative content and aroma activity of each component. These findings provide a solid theoretical foundation and data support for subsequent research on the regulatory mechanisms of herbaceous peony aroma, the identification of key genes controlling scent synthesis, the elucidation of biosynthetic pathways, and the targeted breeding of new aromatic herbaceous peony cultivars. However, it should be noted that this study did not account for environmental factors during VOC collection. Environmental changes can significantly impact plant physiology and VOC emissions [45]. High temperatures, for instance, can accelerate respiration and photosynthesis, altering VOC emission patterns. Increased CO_2 concentrations can also affect stomatal conductance, influencing VOC release. These environmental factors collectively may importantly affect peony floral scent components. The exclusion of these factors may introduce some degree of bias into the results.

5 Conclusion

In this study, we systematically identified and analyzed the floral VOCs from flowers at the half-opening stage of 120 herbaceous peony cultivars using DHS-GC-MS. A total of 86 volatile compounds were identified, primarily categorized into five groups: aromatic compounds, hydrocarbons, terpenoids, fatty acid derivatives, and heterocyclic compounds. Among these, aromatic compounds, hydrocarbons, and terpenoids constituted the majority of the overall aroma profile. The top three cultivars with the highest total VOC release were ‘Dr. Alexander Fleming’, ‘Yuezhao Shanhe’, and ‘Daiyu’, which was significantly higher than that of other cultivars. PCA revealed that the top five cultivars, including ‘Shajin Guanding’, ‘Many Happy

Returns', 'Edulis Superba', 'Huicui', and 'Madame de Verneville', exhibited statistically representative aroma profiles, making them suitable candidates for aroma breeding. Based on OAV analysis, 15 floral VOCs, such as methyl hexanoate, α-pinene and benzaldehyde, were identified as modifying aroma components, while 22 floral VOCs, including 3-hexen-1-ol, acetate, (Z)-, limonene and (E)-β-ocimene, were recognized as key aroma constituents. The highest-OAV aroma compounds in introduced cultivars were primarily nonanal and 1,4-dimethoxybenzene, characterized by fruity and sweet aromas, while those in domestic cultivars were mainly 1,4-dimethoxybenzene and phenylethanol, dominated by sweet and floral aromas. Multivariate statistical analysis (PCA) and OAV evaluation demonstrated that aromatic compounds and terpenoids play a decisive role in shaping the overall aroma of herbaceous peony floral scent. The identification of 1,4-dimethoxybenzene as a key aroma component, characterized by its sweet, clove-like floral scent, underscores its potential as a target for aroma enhancement in herbaceous peony breeding. These findings not only provide a valuable research framework for analyzing the floral scent composition of other herbaceous peony cultivars but also lay the groundwork for the comprehensive development and utilization of herbaceous peony floral aroma in fields such as flavors and fragrances, cut flowers, and floral tea.

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