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# Analysis of the Volatile Components in Flowers of *Paeonia lactiflora* Pall. and *Paeonia lactiflora* Pall. var. *Trichocarpa*

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## Abstract

*Paeonia lactiflora* Pall. var. *trichocarpa* is a variety of *Paeonia lactiflora* Pall., and is currently the peony herb's principal cultivar group. Here, we study the differences in aromatic components and flowers of different varieties between two groups of cultivars, providing a reference for applying natural fragrance substances of peonies, breeding fragrant flower types, and developing and using improved varieties. Headspace solid-phase microextraction (HS-SPME), gas chromatography-mass spectrometry (GC-MS), peak area normalization for each component relative to content, component library (NIST14/NIST14S) retrieval, and a literature review were used to analyze the volatile compounds in flowers of eight peony varieties, such as "Gaoganhong", and ten comospore peony varieties, such as "Jinshanhong". Results showed that the main volatile compound constituents in flowers of the two groups were terpenes and alcohols. Additionally, the content of eucalyptol, caryophyllene, α-Pinene, citronellol, and 3-Hexen-1-ol, acetate, (Z) was high. Peony cultivars contained linalool, (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene, and 1,4-dimethoxybenzene, while comospore peony varieties contained 1,3,6-octatriene, 3,7-dimethyl-, (Z)-, phenylethyl alcohol, and geraniol. In this study, the differences between the volatile components of flowers of different peony varieties were clarified, laying a foundation for further molecular biology research into the floral fragrance of peonies and the cultivation of new varieties of aromatic peonies. At the same time, it also provides a theoretical basis for the development and application of peony flower by-products.

## Keywords

*Paeonia lactiflora* Pall., *Paeonia lactiflora* Pall. var. *Trichocarpa*, GC-MS, Volatile Components

# 1. Introduction

*Paeonia lactiflora* Pall. is a perennial flowering herb with a characteristic white, bony, serrated leaf margin. *Paeonia lactiflora* Pall. var. *trichocarpa* is a variation of *P. lactiflora* with densely villous carpels, differing from *P. lactiflora*, which has smooth glabrous carpels. It is currently the main peony variety of the two major groups in production [1] [2] [3] [4]. *Paeonia lactiflora* is a recognized traditional flower in China with a long history of cultivation. With large, beautiful flowers, *P. lactiflora* is renowned for its ornamental and medicinal uses. Floral fragrance is one of the most important characteristics of floral plants. The color and shape of peony flowers are rich and varied. The peony that holds fragrance full-bodied is less [1]. Peony flowers contain anthocyanins and natural flavonoids, which are beneficial to the human body. They can nourish the blood, soften the liver, dispel stasis, promote metabolism, improve immunity, delay skin aging, and contain natural antioxidants [5] [6] [7]. It is of great value to study the volatile components of peony flowers in order to select and breed fragrant peony varieties. The development of peony flower by-products in food, medicine, beauty, and health care is equally important. Our findings should help enhance peony flower value and set a direction for future peony breeding research and production.

Studies have shown that the volatile and principal components of peonies at different flowering stages and in different flower organs vary [7] [8] [9]. Comparative analyses of volatile components in flowers of different *P. lactiflora* and *P. lactiflora* var. *trichocarpa* varieties have not been reported. In this study, eight representative varieties of *P. lactiflora* and ten varieties of *P. lactiflora* var. *trichocarpa* were selected for analysis to explore the specificity of volatile components in their flowers and to provide a reference for the breeding and promotion of fragrant peony varieties.

# 2. Materials and Methods

## 2.1. Experimental Details and Treatments

### 2.1.1. Experimental Materials

The experiment was conducted from April to May 2018 at the Peony Resource Garden of the Horticultural Experiment Station (all varieties were three years old after the separation) and the Horticultural Experiment Center, Shandong Agricultural University. The test site was located in Taishan District, Tai'an City, in the central Shandong Province, between 116˚02' and 117˚59' East and between 35˚38' and 36˚28' North, with a temperate semi-humid continental monsoon climate. In this location, light and temperature are synchronized, and the rains coincide with high temperatures. There are four distinct seasons: a dry and windy spring, hot and rainy summer, clear and pleasant autumn, and a cold winter with little snow. The average annual temperature is 13˚C; temperatures are highest in July (an average of 26.4˚C) and lowest in January (an average of 2.6˚C). The average annual precipitation is 697 mm. The test plot had sufficient light with a deep, loose, and fertile sandy loam soil layer that was slightly acidic, moist, and well-drained.

In the *P. lactiflora* subgroup, the leaf margin was bony, fine-toothed, and transparent, and the carpel was smooth and hairless. In the *P. lactiflora* var. t*richocarpa* subgroup, the leaf margin was bony, fine-toothed, transparent, and the carpels were hairy [4] [10] [11].

The tested varieties' floral fragrances were evaluated by olfaction and classified as follows: no fragrance, slight fragrance, light fragrance, strong fragrance, and strong odor.

In this experiment, 18 representative varieties were selected from the two subgroups (**Table 1**).

The "Zifengchaoyang" variety (no aroma) was used as the reference for component analysis.

**Table 1.** Floral type and flowering time of peony varieties.

| Numbering | Variety | Subgroup | Floral type | Flowering period |
|---|---|---|---|---|
| A-1 | "Zifengchaoyang" | *P. lactiflora* | no fragrance | middle |
| A-2 | "Gaoganhong" | *P. lactiflora* | strong fragrance | middle |
| A-3 | "Foguangzhuying" | *P. lactiflora* | strong fragrance | middle |
| A-4 | "Hongxiuqiu" | *P. lactiflora* | light fragrance | middle |
| A-5 | "Jinguangshanshuo" | *P. lactiflora* | light fragrance | late |
| A-6 | "Honglou" | *P. lactiflora* | light fragrance | early |
| A-7 | *Paeonia lactiflora* Pall. | *P. lactiflora* | slight fragrance | early |
| A-8 | "Bingshan" | *P. lactiflora* | light fragrance | late |
| B-1 | "Jinshanhong" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | middle |
| B-2 | "Zifengyu" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | middle |
| B-3 | "Hongfushi" | *P. lactiflora.* var*. trichocarpa* | strong odor | middle |
| B-4 | "Qihualushuang" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | middle |
| B-5 | "Taohuafeixue" | *P. lactiflora.* var*. trichocarpa* | light fragrance | late |
| B-6 | "Dongjingnvlang" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | late |
| B-7 | "Fenyunu" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | early |
| B-8 | "Xuefeng" | *P. lactiflora.* var*. trichocarpa* | light fragrance | middle |
| B-9 | "Yangfeichuyu" | *P. lactiflora.* var*. trichocarpa* | light fragrance | late |
| B-10 | "Fengyuluojinchi" | *P. lactiflora.* var*. trichocarpa* | strong fragrance | late |

Note: According to the flowering period, the selected *Paeonia* spp. were divided into the early flowering type (April 25-May 5), middle flowering type (May 5-May 15), and late flowering type (May 15 onwards). The change in the sequence of early, middle, and late flowering of the species.

Plants with excellent growth and free from diseases and insect pests were selected according to the tested varieties' different flowering periods. Three flowers in bloom from each variety were picked on sunny mornings between 9:00 and 10:00 am. According to the preliminary experiment, selected flowers were picked together with their branches and leaves in an early opening state [12]. The flowers were quickly brought back to the laboratory and placed in water (to ensure that they were completely open at the time of testing) to detect and analyze their volatile components.

#### 2.1.2. Experimental Method

Experimental equipment comprised a GC-MS QP2010 Plus (Shimadzu, Japan); manual SPME sampler (Supelco), and solid-phase microextraction head (model 50/30 m DVB/CAR/PDMS; Supelco).

1) Solid-phase microextraction

Peony flowers in full bloom were cut. If the flowers were too large, half or a quarter of the flower head was cut, ensuring that the integrity of the floral organs was maintained. The cut flowers were placed in a 220 ml headspace sample bottle and sealed with aluminized paper, subsequently heated to 40˚C. Headspace solid-phase microextraction was conducted at an aging temperature of 250˚C for 40 min. The extraction head was placed 1 - 2 cm above the flower and adsorbed for 30 min.

2) GC-MS analysis conditions

Using a GC-MS QP2010 Plus unit, the injection port temperature was set to 250˚C; the starting column temperature was 40˚C, this was maintained for 3 min, and increased to 120˚C at a rate of 6˚C/min. The temperature was further increased to 180˚C at a rate of 10˚C/min and finally to 230˚C at 20˚C/min, where it was maintained for 4 min. Helium (99.999%) was used as the carrier gas; column flow was set to 1.0 mL/min. The ionization mode was set to electron ionization (EI) with an electron energy of 70 eV, ion source temperature of 200˚C, and interface temperature of 230˚C. The scanning quality range was 45 - 450 aMU. Unsplit injection was used.

### 2.2. Data Processing and Analysis

According to the detected total ion flow pattern, the chemical components of volatile substances in the varieties were identified and analyzed using a combination of computer spectral library (NIST14/NIST14S) retrieval and literature review. The relative content of each component was obtained by using the peak area normalization method. The flower aroma was analyzed by HS-SPME-GC-MS [13] [14] [15]. The volatile constituents were classified into terpenes, alcohols, esters, alkanes, ketones, aldehydes, aromatic compounds, and other compounds [16]. The volatile constituents of flowers of a single variety were analyzed according to their relative content [17].

Microsoft Excel software was used to process the data. OriginPro 2016 32Bit software was used for mapping and analysis.

# 3. Results

## 3.1. Analysis of Volatile Components

### 3.1.1. Flowers from *P. lactiflora* Varieties

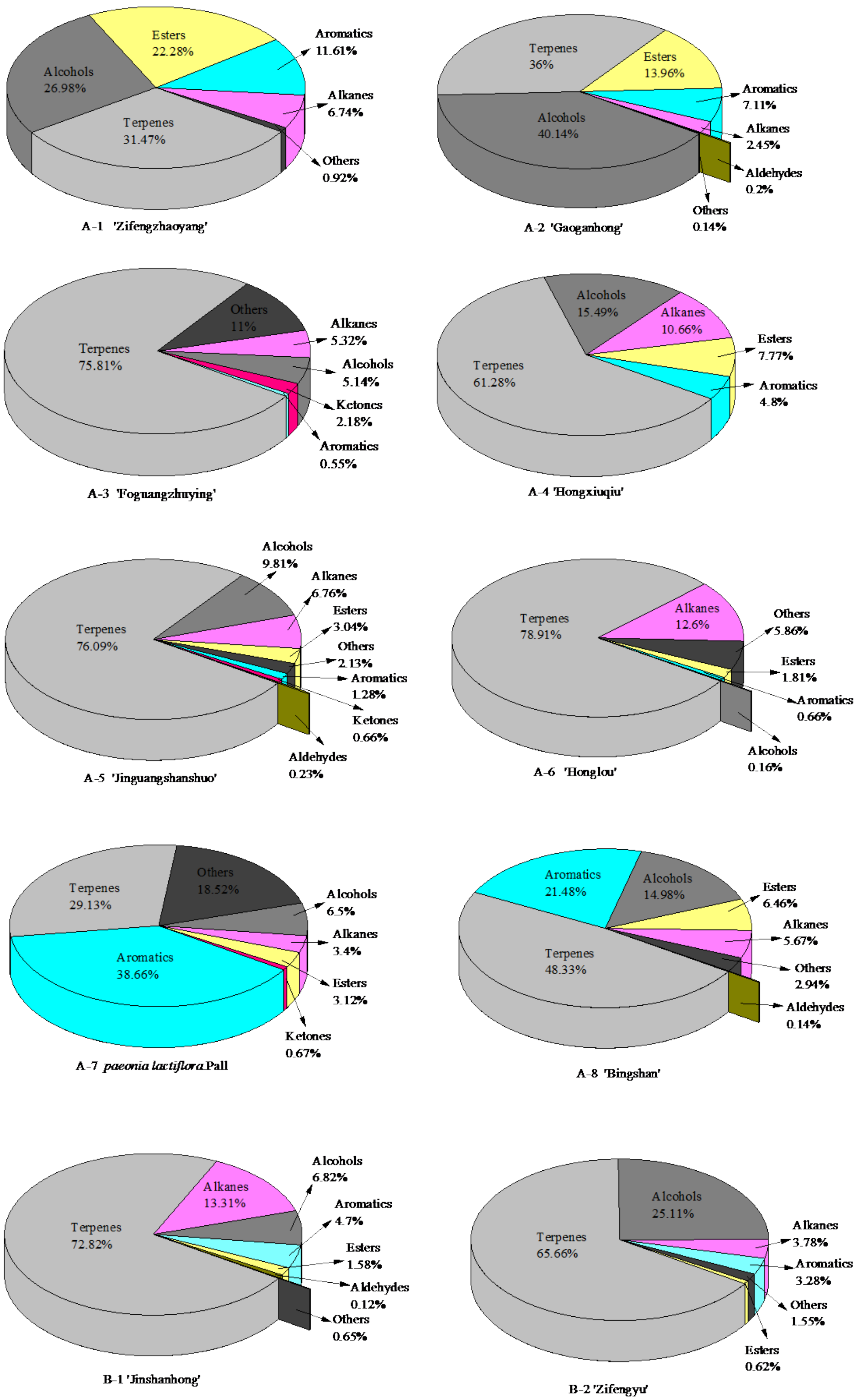
The main volatile components and their relative contents in the flowers of the eight *P. lactiflora* varieties are shown in Figure 1(A-1-8). Eighteen volatile floral components were detected in the "Zifengchaoyang" variety, accounting for 100% of the total composition. The relative content of terpenes was 31.47%; this included eucalyptols (18.09%), caryophyllene (5.27%), (E)-4,8-Dimethyl-1,3,7-nonatriene (3.62%), and α-Pinene (2.51%). Alcohol content reached 26.98% (linalool accounted for 23.42%). Ester relative content was 22.28%, including 3-Hexen-1-ol, acetate, (Z)-(14.11%) and acetic acid, hexyl ester (7.7%). The relative content of aromatic compounds was 11.61% (mainly 1,4-dimethoxybenzene and p-xylene; 9.26% and 1.52%, respectively). Only a small variety of alkanes and other unidentified compounds were found.

Thirty-six volatile substances were detected in the "Gaoganhong" variety, accounting for 100% of the total composition. There were terpenes, esters, alcohols, aromatics, alkanes, aldehydes, and other unidentified compounds. The relative alcohol content was 40.14%, with the most abundant being citronella alcohol (34.99%). There were 15 species of terpenes, accounting for 36% of the total. The main terpenes were 1,3,6-octatriene, 3,7-dimethyl-, (Z)-(10.57%), caryophyllene (9.06%), and eucalyptol (5.58%). The relative ester content was 13.96%; this was mainly 6-octen-1-ol, 3,7-dimethyl-,acetate (8.91%). Aromatics, alkanes, aldehydes, and other compounds accounted for 9.9%.

The "Foguangzhuying" variety had 24 volatile substances, including terpenes, alcohols, aromatics, alkanes, and ketones, accounting for 97.29% of the total components. No esters were detected. The most abundant terpene species, out of the eleven identified (75.81%), were (1R)-2,6,6-trimethylbicyclo[3.1.1] hept-2-ene (27.61%), caryophyllene (13.09%), β-Myrcene (10.87%), D-limonene (8.4%), eucalyptols (4.8%), and 1,3,6-octatriene, 3,7-dimethyl-, (Z)-(4.76%). In addition, phenylethyl alcohol, an aromatic compound, was found in relatively high quantities (4.48%).

The flowers of the "Hongxiuqiu" variety contained 28 volatile substances, including eleven terpenes, three esters, five alcohols, six aromatics, and three alkanes, accounting for 95.19% of the total components. The terpenes were the most abundant at 61.28%, with caryophyllene (27.28%) having the highest content, followed by eucalyptol (21.57%). The relative alcohol content was 15.49%, consisting chiefly of geraniol (6.76%) and 3-hexen-1-ol, (E)-(3.01%). Alkanes accounted for 10.66%, with the largest contribution being made by bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-(8.95%). Esters and aromatics accounted for 12.57% of the total ingredients.

Thirty-six volatile substances were identified in the "Jinguangshanshuo" variety, accounting for 99.9% of the total components. This peony variety's floral components were the most complex in this subgroup, with eight categories:


Esters 22.28%
Aromatics 11.61%
Alcohols 26.98%
Alkanes 6.74%
Terpenes 31.47%
Others 0.92%
A-1 'Zifengzhaoyang'
Terpenes 36%
Esters 13.96%
Aromatics 7.11%
Alcohols 40.14%
Alkanes 2.45%
Aldehydes 0.2%
Others 0.14%
A-2 'Gaoganhong'
Others 11%
Alkanes 5.32%
Terpenes 75.81%
Alcohols 5.14%
Ketones 2.18%
Aromatics 0.55%
A-3 'Foguangzhuying'
Alcohols 15.49%
Alkanes 10.66%
Esters 7.77%
Terpenes 61.28%
Aromatics 4.8%
A-4 'Hongxiuqiu'
Alcohols 9.81%
Alkanes 6.76%
Terpenes 76.09%
Esters 3.04%
Others 2.13%
Aromatics 1.28%
Ketones 0.66%
Aldehydes 0.23%
A-5 'Jinguangshanshuo'
Terpenes 78.91%
Alkanes 12.6%
Others 5.86%
Esters 1.81%
Aromatics 0.66%
Alcohols 0.16%
A-6 'Honglou'
Terpenes 29.13%
Others 18.52%
Alcohols 6.5%
Alkanes 3.4%
Aromatics 38.66%
Esters 3.12%
Ketones 0.67%
A-7 *paeonia lactiflora* Pall
Aromatics 21.48%
Alcohols 14.98%
Esters 6.46%
Alkanes 5.67%
Terpenes 48.33%
Others 2.94%
Aldehydes 0.14%
A-8 'Bingshan'
Alcohols 6.82%
Alkanes 13.31%
Aromatics 4.7%
Terpenes 72.82%
Esters 1.58%
Aldehydes 0.12%
Others 0.65%
B-1 'Jinshanhong'
Alcohols 25.11%
Terpenes 65.66%
Alkanes 3.78%
Aromatics 3.28%
Others 1.55%
Esters 0.62%
B-2 'Zifengyu'

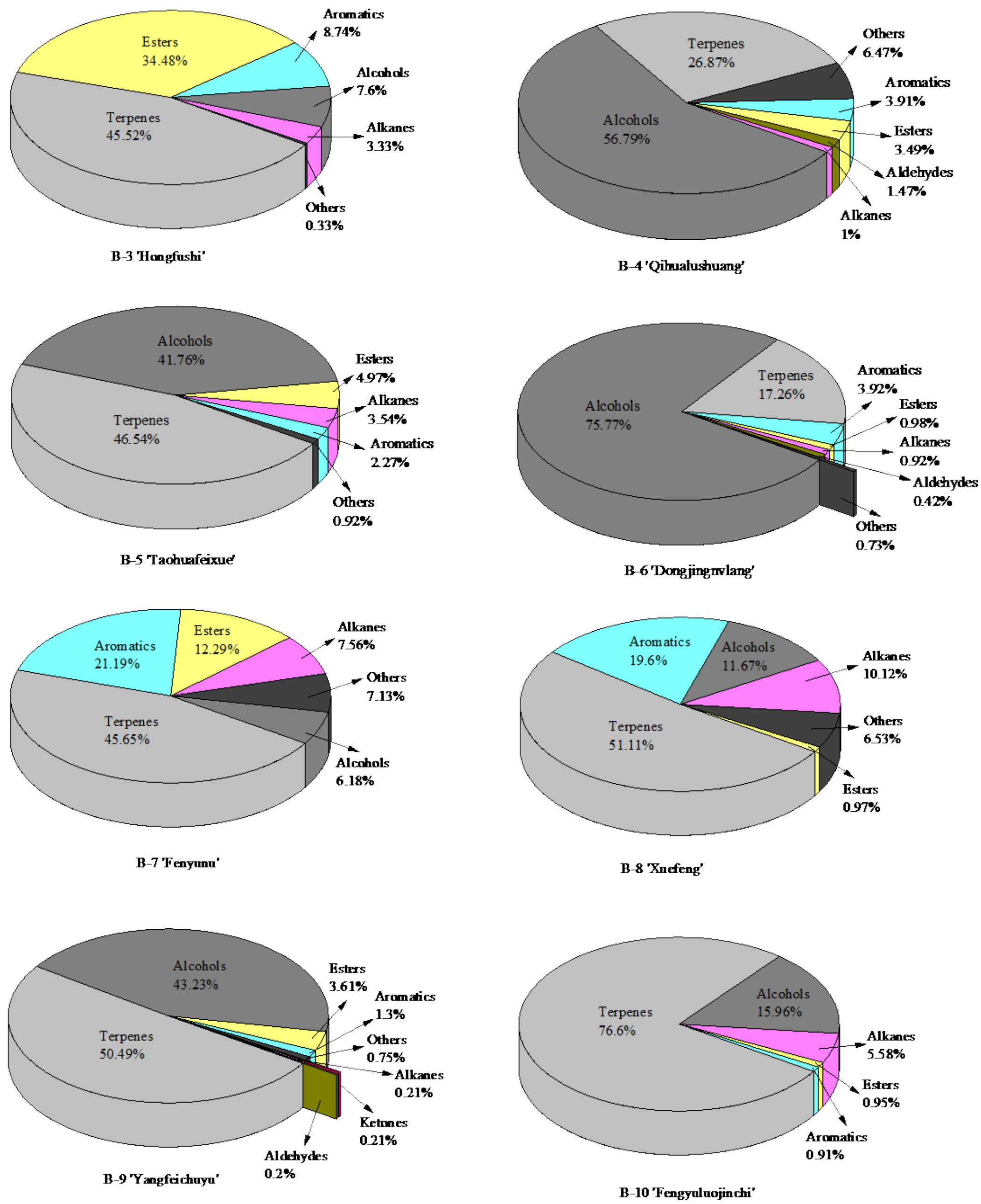



**Figure 1.** Relative contents of flower aroma components. Note: A1-A8 relate to the *P. lactiflora* subgroup, B1-B10 relate to the *P. lactiflora* var. *trichocarpa* subgroup.

thirteen terpenes, six esters, four alcohols, three aromatics, four alkanes, a single aldehyde and ketone, and four unidentified compounds. The terpenes were the largest group at 76.09%. Among the aromatic compounds, eucalyptol (32.73%) had the highest content, followed by α-Pinene (24.72%), caryophyllene (9.39%), phenylethyl alcohol (8.49%), and bicyclo [3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(5.87%).

We identified 22 volatile substances in the "Honglou" variety, making up 95.19% of the total composition. Terpenes, esters, alcohols, aromatics, and alkanes were present; the relative content of terpenes was the highest, accounting for 78.91%. The primary aromatic components were eucalyptol (44.1%), caryophyllene (25.1%), bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(11.07%), and humulene (2.39%).

Twenty-three volatile substances were identified in P. lactiflora: 98.43% of the total constituents, including nine terpenes, a single ester, three alcohols, two aromatics, two alkanes, a single ketone, and five unidentified compounds. The relative contents of terpenes and aromatics were 29.13% and 38.66%, respectively. The main components were 1,4-dimethoxybenzene (36.74%), eucalyptol (12.54%), caryophyllene (6.52%), α-Guaiene (4.38%), (E)-4,8-dimethylnona-1,3,7-triene (3.54%), phenylethyl alcohol (3.4%), and citronellol (2.8%).

The "Bingsham" variety contained 46 volatile substances (accounting for 100% of the components), split among seven categories of compounds, with the exception of ketones. The relative contents of terpenes, aromatics, and alcohols were 48.33%, 21.48%, and 14.98%, respectively. The major components were 1,4-dimethoxybenzene (18.68%), caryophyllene (15.38%), eucalyptol (14.29%), geraniol (9.32%), bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(4.61%), (E)-4,8-dimethylnona-1,3,7-triene (3.421%), and geranyl acetate (3.36%).

According to the data (**Figure 1(A-1-8)**), there are five kinds of volatile substances in the original varieties of peony: terpenes, alcohols, esters, alkanes, and aromatics. The relative contents of aldehydes and ketones in some varieties are minimal. Among the varieties, the components that contributed most to floral aroma were terpenes, esters, and alcohols in the "Zifengchaoyang" variety, terpenes and alcohols in "Gaoganhong", and terpenes and aromatics in "Bingshan" and *P. lactiflora*. Only terpenes contributed in the "Foguangzhuying", "Hongxiuqiu", "Jinguangshanshuo", and "Honglou" varieties. The "Zifengchaoyang" variety was not fragrant, while the flower of *P. lactiflora* was slightly fragrant. The proportion of terpenes in the volatile components of these two species' flowers was lower than that of other varieties (31.47% for "Zifengchaoyang" and 29.13% for *P. lactiflora*). It is speculated that the relative content of terpenes is an important factor affecting this subgroup's aroma intensity. The relative content of aromatic compounds was high in "Bingshan" and *P. lactiflora* and both had a light floral scent. "Foguangzhuying" belonged to the strong fragrances, with fewer aromatic compounds. It is thought that the relative content of aromatics has little effect on aroma intensity. Compared with other varieties, the relative content of alcohols in the volatile components of "Zifengchaoyang" and "Gaoganhong" flowers was higher. The former had no floral scent, while the latter had a strong floral scent; both had a relatively high alcohol content. In addition, no esters were detected in "Foguangzhuying", while the rest of this subgroup contained esters, with the highest ester content observed in the "Zifengchaoyang" and "Gaoganhong" varieties. Hence, it is hypothesized that the relative alcohol and ester content needs to reach a certain threshold in order to affect the aroma intensity.

#### 3.1.2. Flowers from *P. lactiflora* var. *Trichocarpa* Varieties

The volatile compounds and their relative contents identified in the ten varieties of the *P. lactiflora* var. *trichocarpa* subgroup after NIST14 spectrum detection and analysis are shown in **Figure 1(B-1-10)**.

Thirty-three volatile components were detected in the "Jinshanhong" variety of flower, accounting for 99.7% of the total components. There were fourteen terpenes, four esters, five alcohols, two aromatics, five alkanes, one aldehyde, and two unidentified compounds. Among them, the relative content of terpenes was significantly high, reaching 72.82%, and was comprised mainly of caryophyllene (33.04%), eucalyptol (25.16%), and bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl) (3.58%). The main volatile components were bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl) (9.53%), phenylethyl alcohol (5.04%), and 1,4-dimetho-xybenzene (3.14%).

Eighteen volatile compounds were identified in the "Zifengyu" variety, representing all (100%) constituents. The observed volatile compounds were classified into the following six chemical categories: terpenes, esters, alcohols, aromatics, alkanes, and other compounds. The relative content of terpenes was 65.66%, followed by alcohols at 25.11%. The components with relatively high contents were 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-(37.7%), (E)-4,8-Dimethyl-1,3,7-nonatriene (7.34%), 2,4,6-Octatriene, 2,6-dimethyl-, (E,E)-(6.76%), caryophyllene (5.22%), geraniol (12.51%), and citronellol (10.7%).

Sixteen volatile components were distinguished in the "Hongfushi" variety (100% of the total), composed of terpenes, esters, alcohols, aromatics, and alkanes. The relative contents of terpenes and esters were 45.52% and 34.48%, respectively. The main components were caryophyllene (31.32%), 3-Hexen-1-ol, acetate, (Z)-(22.79%), eucalyptol (7.94%), 3-hexen-1-ol, (E)-(7.6%), hexyl chloroformate (5.63%), acetic acid, hexyl ester (5.43%), and humulene (4.05%).

Twenty volatile components were identified in the "Qihualushuang" flower variety, accounting for 98.83% of the total constituents and consisting of terpenes, esters, alcohols, aromatics, alkanes, and aldehydes. The relative contents of alcohols and terpenes were 56.79% and 26.87%, respectively. The main components were geraniol (34.62%), phenylethyl alcohol (20.2%), caryophyllene (18.78%) β-Myrcene (2.6%), and geranyl acetate (2.5%).

Nineteen volatile compounds were detected in the "Taohuafeixue" variety, accounting for 100% of the total components, which included terpenes (46.54%), alcohols (41.76%), esters (4.97%), aromatics (2.27%), and alkanes (3.54%). The substances with relatively high contents were caryophyllene (39.03%), citronellol (29.05%), phenylethyl alcohol (3.69%), humulene (3.53%), and 3-Hexen-1-ol, acetate, (Z)-(2.75%).

Nineteen volatile compounds were identified in the "Dongjingnvlang" variety, accounting for 100% of the total and made up of terpenes, esters, alcohols, aromatics, alkanes, and aldehydes. The relative content of alcohols was the highest (75.77%), followed by terpenes (17.26%). The main components were phenylethyl alcohol (72.02%), caryophyllene (11.62%), 3-Hexen-1-ol, (E)-(3.1%), and 1,4-dimethoxybenzene (2.99%).

The "Fenyunu" variety contained 16 volatile constituents, which accounted for 92.87% of the total, including seven terpenes with a relative content of 45.65%. The relative content of the two types of esters was 12.29%. Two types of alcohol were identified with a total relative content of 6.18%. The relative content of the three aromatic species was 21.19%, and that of the two alkanes was 7.56%. The main components were 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-(29.9%), 1,4-dimethoxybenzene-(17.24%), 3-Hexen-1-ol, acetate, (Z)-(8.44%), eucalyptol (7.58%), bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(5.66%), and 3-Hexen-1-ol, (E)-(4.72%).

Thirty-two volatile components consisting of terpenes, esters, alcohols, aromatics, and alkanes were detected in the "Xuefeng'" variety, accounting for 97.44% of the total. The relative content of terpenes was the highest at 51.11%, followed by aromatics at 19.6%. The main components were caryophyllene (24.72%), eucalyptol (12.43%), geraniol (7.56%), bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(6.79%), and α-Guaiene (5.62%).

Thirty volatile compounds were identified from the "Yangfeichuyu" variety, accounting for 100% of the total. These components were the most complex among the ten varieties in this subgroup. Volatile components in eight categories were distinguished, including ten terpenes, six esters, seven alcohols, three aromatics, one alkane, one aldehyde, one ketone, and one other compound. The relative content of terpenes was the highest at 50.49%, followed by alcohol at 43.23%. The main volatile components were α-Pinene (32.52%), phenylethyl alcohol (23.69%), geraniol (10.7%), β-Myrcene (5.97%), D-Limonene (4.04%), and caryophyllene (3.05%).

Twenty-five volatile components were distinguished in the 'Fengyuluojinchi' variety, accounting for 100% of the total. Fifteen terpenes were observed, with a relative content of 76.6%. There were two kinds of esters (0.95%), three kinds of alcohols (15.96%), two aromatics (0.91%), and three kinds of alkanes (5.58%). The main volatile components were 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-(30.01%), caryophyllene (26.45%), phenylethyl alcohol (8.64%), eucalyptol (8.4%), citronellol (7.05%), and bicyclo [3.1.0]hexane, 4-methylene-1-(1-methylethyl)-(4.3%).

The data indicates that the volatile substances detected in the ten *P. lactiflora* var. *trichocarpa* subgroup varieties were terpenes, alcohols, esters, alkanes, aromatics, aldehydes, and ketones. The main volatile constituents in "Jinshanhong", "Zifengyu", and "Fengyuluojinchi" were terpenes, with considerably high relative contents (72.82%, 65.66%, and 76.6%, respectively); in all three varieties, the floral aroma was strong. The main volatile components of "Hongfushi" were terpenes and esters. The primary volatile components of "Qihualushuang", "Dongjingnvlang", "Taohuafeixue", and "Yangfeichuyu" were alcohols and terpenes, while "Xuefeng" and "Fenyunu" primarily contained terpenes and aromatic compounds.

Relatively high alcohol contents were found in all ten varieties of P. lactiflora var. trichocarpa. Ester compounds and aromatics were also found in all ten, but the relative content was lower in some varieties. The relative content of "Hongfushi" esters was the highest (34.48%), mainly 3-Hexen-1-ol, acetate, (Z)-(22.79%), which may be the main factor contributing to its strong odor.

## 3.2. Analysis of the Main Flower Aroma Substances

### 3.2.1. *Paeonia lactiflora* Subgroup Varieties

Data showed (Figure 2(A-1-8)) that the flowers in the *P. lactiflora* subgroup contained a high amount of the following substances: linalool, eucalyptol, 3-Hexen-1-ol, acetate, (Z)-, citronellol, (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene, caryophyllene, α-Pinene, and 1,4-dimethoxybenzene. The "Zifengchaoyang" variety contained up to 23.42% linalool, which has a light fragrance that is not lasting. The lack of scent in "Zifengchaoyang" may be related to this characteristic. Linalool has antibacterial, antifungal, and antiviral characteristics [18]. "Gaoganhong" contains 34.99% citronellol, which has a sweet rose aroma, endowing it with a unique, intense fragrance. Additionally, citronellol inhibits the spore germination and mycelial growth of mold and has potential applications in food storage and preservation, insect control, and bacteria inhibition [19]. There was a relatively high (1R)-2,6,6-Trimethylbicyclo[3.1.1]hept-2-ene content in "Foguangzhuying" (27.61%), the effects and functions of which have not been reported. The 1,4-dimethoxybenzene content was relatively high in the "Bingshan" variety and the original peony species (18.68% and 36.74%, respectively). This compound has a clove-like odor, and is primarily used for preparation of nuts for eating, tobacco flavor, and deodorant. Eucalyptol and caryophyllene are found in all the eight varieties in this experimental subgroup. Eucalyptol was found in high amounts in "Jinguangshanshuo" and "Honglou", has a camphor-like scent, and has antibacterial, anti-inflammatory, insecticidal, analgesic, and transdermal absorption-promoting properties. Caryophyllene was abundant in "Honglou" and "Hongxiuqiu"; it has a mild fragrance with spicy, woody, citrus, and camphor notes and is often used as a flavoring for cloves, pepper, and herbs. "Jinguangshanshuo" has a relatively high α-Pinene content (24.72%); this compound has a pine aroma and good insecticidal properties [20] [21] [22].

### 3.2.2. *Paeonia lactiflora* var. *Trichocarpa* Varieties

From Figure 2(B-1-8), it can be seen that the substances found in relatively high quantities in the flowers of different *P. lactiflora* var. *trichocarpa* varieties are 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-, caryophyllene, citronellol, phenylethyl alcohol, eucalyptol, 3-Hexen-1-ol, acetate, (Z)-, geraniol, and α-Pinene. Additionally, the substances with relatively high content in each variety differ. The relative content of 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- was only high in "Zifengyu", "Fengyuluojinchi", and "Fenyunu", all of which contained approximately 30%. The relative content of 1,3,6-octatriene, 3,7-dimethyl-, (Z)- in "Yangfeichuyu"

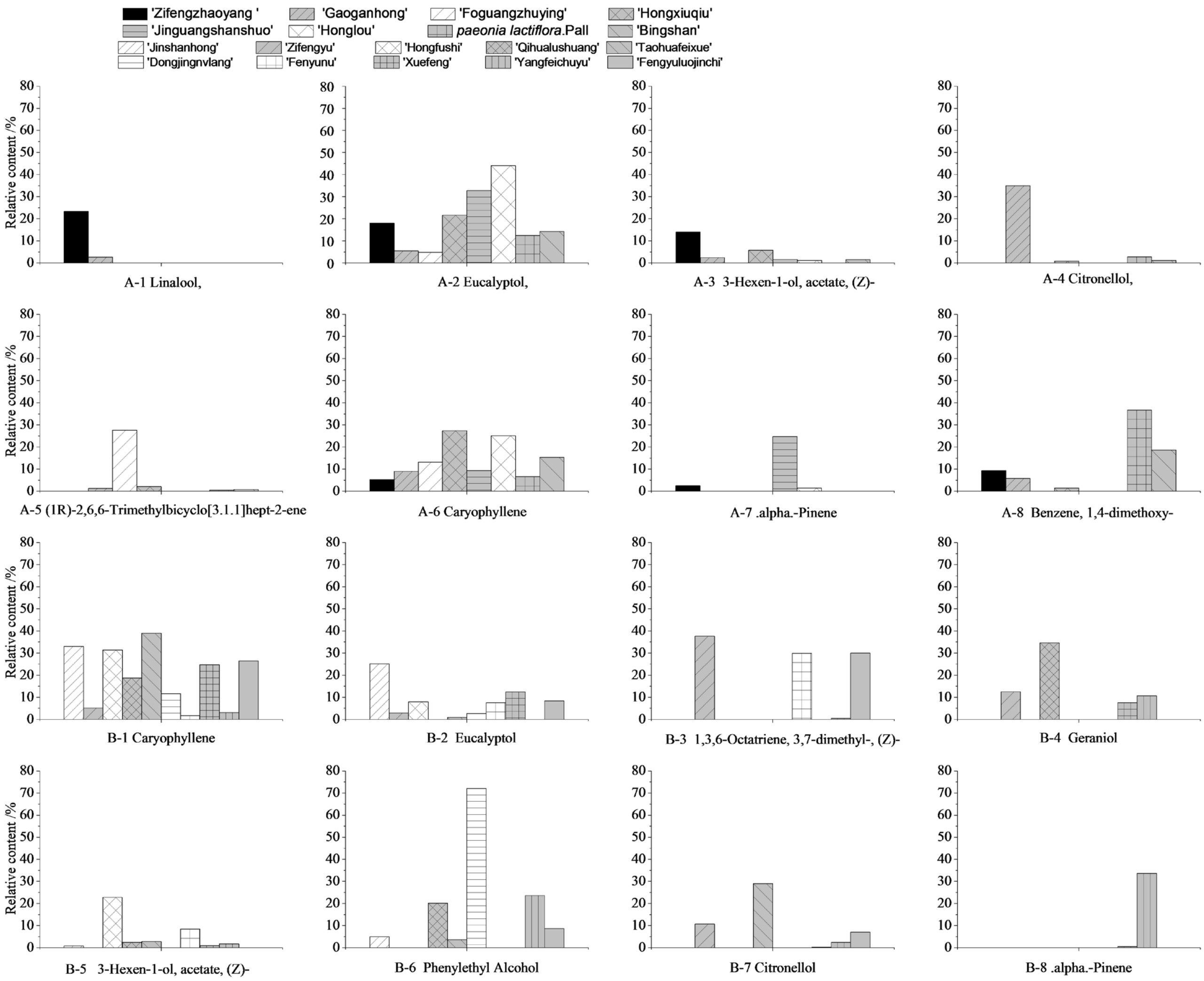



**Figure 2.** Volatile substances with a high relative content in *P. lactiflora* and *P. lactiflora* var. *trichocarpa* flower varieties.

was minimal (0.48%) and its presence was not detected in the other varieties. This compound is fragrant, smelling of vanilla flowers and orange blossom oil, and can be used in various chemical flavor formulas. Seven varieties contained relatively high levels of caryophyllene, but not "Zifengyu", "Fenyunu", and "Yangfeichuyu". Citronellol content was high in "Taohuafeixue" and "Zifengyu", at 29.05% and 10.7%, respectively. The relative content of phenylethyl alcohol in "Dongjingnvlang" was considerably high, reaching 72.02%. This compound has many fragrances, such as rose, violet, and jasmine, which confer the strong floral scent of "Dongjingnvlang". It is widely used as the main fragrance or base fragrance in the production of edible flavors [23] and is the primary raw material in the preparation of rose-flavor edible additives. Geraniol content was highest in "Qihualushuang" (34.62%); moderate geraniol produces a full-bodied aroma of rose and has antitumor, antibacterial, anti-asthma, and insect repellent effects. It has also been used as a treatment for cancer [24]. The primary components of "Qihualushuang" have been used in cancer treatment. Geraniol was also found in the volatile components of flowers of the "Zifengyu", "Xuefeng", and "Yangfeichuyu" varieties (approximately 10%), while it was not detected in other varieties. The relative content of α-Pinene was only high in "Yangfeichuyu", at 32.52%, and the relative content of volatile components in the "Xuefeng" flower was minimal, accounting for only 0.52%. No α-Pinene was detected in the other varieties. The relative content of eucalyptol in "Jinshanhong" was 25.16%. The content of 3-Hexen-1-ol, acetate, (Z) in "Hongfushi" was relatively high, up to 22.79%; this compound has a strong banana flavor [25]. It was speculated that the strong smell of the flowers of this variety may be caused by the odor resulting from the mixture of 3-Hexen-1-ol, acetate, (Z)-, and other volatile substances.

The experimental data showed no positive correlation between the floral aroma of peony and the number of volatile substances. Although "Zifengyu" and "Fenyunu" had intense aromas, only 18 and 16 kinds of volatile substances were detected, respectively. In contrast, "Jinshanhong", which also had a robust aroma, contained 33 kinds of volatile substances contributing to its floral aroma. It is speculated that there are several substances with weak scents or even no smell; this requires further investigation.

## 4. Discussion

The volatile components of ornamental flowers are secondary metabolites and include terpenes, alcohols, aromatic compounds, and fatty acid derivatives. Terpenes and alcohols are widely used in traditional Chinese medicine and health care [18] [26]. The secondary metabolites differ among species and genotypes [7] [26]. Terpenes, alcohols, and linalool primarily produce sweet olive fragrances. Benzoyl acetate, α-pinene, β-pinene, and 3-carene can be mixed in different proportions to produce plum blossom aroma. In *Cymbidium* Sw. spp., fragrant linalool is found only in *Cymbidium faberi.* Terpenes, alcohols, and esters are common components in *Cymbidium* and *Selenipedium* spp. The increase in ester content enhances the strong smell of green cymbidium orchids. Nonaldehyde is a unique component of *Cymbidium* spp., while citronellal is a component of *Selenipedium* spp., component [27] [28] [29]. The main aroma components of cape jasmine are esters [30], while jasmine aroma is derived from esters and ketones [31]. Monoterpenoids and esters produce the characteristic aroma of *Hedychium coronarium* Koenig [32]. The 1,8-eucalyptus oleic acid, eugenol, β-Pinene, cis-auxin, and other compound contents in rose and azalea flowers are relatively high [33] [34] [35]. Terpenes and methyl benzoate are the typical aroma components of the flower varieties of Oriental and Longiflorum hybrids with a strong fragrance. *Magnolia officinalis* flower aroma components are also primarily terpenes [18]-[26]. Terpenes are essential components of floral aroma and are closely related to aroma intensity [36]. The relative content of terpenes in varieties of peony, such as "Honglou", "Jinguangshanshuo", "Foguangzhuying", and "Hongxiuqiu" accounted for over 60% of the total aroma components.

The primary floral constituents of varieties in both experimental subgroups were terpenes and alcohols. The relative content of alcohols in *P. lactiflora* var. *trichocarpa* varieties was between 40% higher than and equal to terpenes. In the current study, 30 volatile components were detected in "Yangfeichuyu" flowers, including α-Pinene and phenylethyl alcohol. Sixteen volatile components were detected in "Fenyunu" flowers, including 1,3,6-Octatriene, 3,7-dimethyl-, (Z)- and 1,4-dimethoxybenzene-. These compound compositions are different from those in the studies of Huang Xue and Song Chaowei [8] [9]. The type and content of floral substances may be related to the sampling method, sampling location, and time. The different environmental conditions of the planting site may also affect types and content of floral volatile compounds [18] [26] [37].

The data indicate that the main floral aroma components of each of the experimental subgroups are the same. However, the relative alcohol content in the *P. lactiflora* var. *trichocarpa* varieties was more significant than in the other original species experimental subgroup. The main components in the fragrance-free "Zifengchaoyang" in the *P. lactiflora* subgroup were terpenes and alcohols, comprising 31.47% and 26.98%, respectively. The relative content of alcohols and terpenes in strong fragrances ("Gaoganhong") was 40.14% and 36.00%, respectively. It is speculated that the relative content of terpene and alcohol may have a threshold that determines aroma intensity.

This experiment showed that the intensity of the flower fragrance and the number of flower aroma volatile substances are not positively correlated. One reason for this may be that in peony flowers in vitro, the physiological and biochemical reactions and stress affects the aroma composition, synthesis mechanism, and normal release due to the lack of some floral components present in living flowers [38]. It is also possible that the intensity of the peony's floral aroma is only related to certain kinds of compounds. This needs further investigation involving more varieties.

Recent studies in the molecular biology of flowers indicate that it is feasible to improve the quality of flowers or breed new aromatic varieties by transgenic methods [39] [40]. In this study, the differences in floral components in different varieties of peony were clarified, laying a foundation for further molecular biology research into the floral fragrances of peony and the cultivation of new aromatic varieties. At the same time, it also provides a theoretical basis for the development and application of peony flower by-products.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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