HS-SPME-GC-MS Analysis and Principal Component Analysis of Volatile Compounds in Rhododendron fortunei at Different Flowering Stages Postprint

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Abstract

To investigate the volatile components of Rhododendron fortunei, this study employed headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) to conduct qualitative and quantitative analysis of volatile components at different flowering stages, and analyzed the characteristic volatile components through principal component analysis (PCA). The results showed that: 4 -myrcene, -ocimene, copaene, isoledene, eucalyptol, ylangene, (+)-epibicyclosesquiphellandrene, (3R-trans)-4-vinyl-4-methyl

Full Text

Analysis of Volatile Compounds in Rhododendron fortunei at Different Flowering Stages Using HS-SPME-GC-MS and Principal Component Analysis

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Abstract

This study investigated the volatile components of *Rhododendron fortunei* using headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry (HS-SPME-GC-MS) to qualitatively and quantitatively analyze volatile compounds across different flowering stages, with principal component analysis (PCA) employed to identify characteristic aroma components. The results revealed that a total of 50 volatile compounds were detected across four

flowering stages, classified into six categories: phenylpropanoids/benzenoids, terpenoids, alcohols, aldehydes, hydrocarbons, and others. PCA of 29 major volatile substances extracted two principal components with a cumulative variance contribution rate of 88.55%. The analysis identified -myrcene, -ocimene, copaene, isoledene, eucalyptol, ylangene, (+)-epi-bicyclosesquiphellandrene, and (3R-trans)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene as highly positively correlated with the first principal component. The second principal component contributed 31.45% of the variance, with eugenol showing the strongest influence and a high negative correlation. These substances constitute the key aroma components of *R. fortunei*. Notably, among the nine highly correlated substances, seven were terpenoids. In conclusion, terpenoids represent the primary characteristic aroma constituents of *R. fortunei*.

Keywords: *Rhododendron fortunei*, different flowering periods, volatile components, principal component analysis (PCA)

Introduction

Floral scent constitutes an important component of plant volatile compounds (Hu et al., 2017) and comprises a mixture of low-molecular-weight, readily volatile compounds (Inna et al., 2002). For ornamental flowers, fragrance serves as a crucial quality indicator, often described as the "soul of flowers" (Chen et al., 2001), and holds significant commercial value.

Rhododendron is a world-renowned ornamental flower and one of China' s three famous floral species, widely used in landscape greening and celebrated as the "king of woody flowers" (Chen et al., 2003). However, only a few varieties possess fragrance, and these resources have received limited attention with sparse research reports. *Rhododendron fortunei* primarily grows on mountain ridges or under forest canopies at elevations of 600–2000 m (Yang et al., 2018) and represents a precious endemic species in China (He, 2017). Distributed across Shaanxi, Hubei, Hunan, Zhejiang, Jiangxi, Fujian, Guangxi, Guizhou, and Yunnan provinces, this species features large leaves, brightly colored and fragrant flowers, and strong resistance, making it suitable for artificial cultivation and granting it high ornamental value (Wang et al., 2015).

Previous research on rhododendron aroma has primarily focused on volatile oil extraction, analysis, identification, and activity evaluation, with limited investigation into volatile component analysis. Therefore, this study employed HS-SPME-GC-MS combined with PCA to conduct qualitative and quantitative analysis of volatile components in *R. fortunei* at different flowering stages, aiming to elucidate its aroma composition and provide a reference for future development and utilization to enhance its economic value.

Materials and Methods

1.1 Experimental Materials

Rhododendron fortunei samples were collected from Siming Mountain National Forest Park in Ningbo. Based on petal expansion degree, flower development was divided into four stages: bud stage (petals unopened), half-opening stage (petals partially opened), full-opening stage (petals fully opened), and wilting stage (petals beginning to wither).

1.2 Instruments

The experimental instruments included a Mettler Toledo electronic balance (Switzerland), HP7890B GC system/5977A MSD GC-MS (Agilent Technologies, USA), manual solid-phase microextraction device with 65 m PDMS/DVB fiber (Supelco, USA), and a heating magnetic stirrer (IKA, Germany).

1.3.1 SPME Sample Preparation

The type of SPME fiber and extraction conditions significantly influence the adsorption and analysis of aroma components. In this study, SPME extraction conditions were optimized to ensure optimal adsorption of petal aroma components (Fan et al., 2017). A 65 m PDMS/DVB SPME fiber was used to adsorb aroma components from petals at four flowering stages. For each stage, petals were cut into small pieces and 5 g samples were rapidly weighed and placed in 8 mL headspace vials. After sealing, a 65 m PDMS/DVB SPME fiber injector was inserted, and headspace extraction was performed at 25 °C for 1 h with magnetic stirring at 500 r · min ^{1}. Following extraction, the fiber was quickly inserted into the GC injector for 5 min desorption. Before each injection, the fiber was conditioned at 250 °C for 10 min. Each sample underwent three biological replicates.

1.3.2 GC-MS Analysis Conditions

An Agilent 7890B GC system/5977A MSD GC-MS was used with reference to the method of Natalia Dudareva et al. (2003), with slight modifications. Chromatographic conditions: Agilent 19091S-433 column (30 m × 250 m × 0.25 m); initial oven temperature 50 °C; injector temperature 250 °C; carrier gas (helium, 99.999%) flow rate 1.0 mL · min ^{1}. The temperature program began at 40 °C for 2 min, increased to 160 °C at 3 °C · min ^{1}, then to 200 °C at 10 °C · min ^{1}, and finally to 300 °C at 20 °C · min ^{1} with a 3 min hold, using splitless injection.

MS conditions: electron impact ionization (EI) source; ion source temperature 230 °C; interface temperature 250 °C; electron energy 70 eV; quadrupole temperature 150 °C; EMV tune 1149; solvent delay 2.6 min; scan range 15–500 amu.

1.3.3 Data Processing

For qualitative analysis, NIST and WILEY standard mass spectral libraries were searched and verified using a chemical workstation data processing system, combined with manual spectral interpretation using relevant literature (Cong, 1987). Only identification results with match values greater than 80 (maximum 100) were accepted. For quantitative analysis, the relative content of each chemical component was determined using peak area normalization.

1.3.4 Aroma Value Calculation

Components with aroma values > 1 were considered characteristic aroma contributors, with higher values indicating greater contribution to sample aroma (Qin et al., 2017).

Results and Analysis

GC-MS total ion current chromatograms of *R. fortunei* petals at the bud, half-opening, full-opening, and wilting stages are shown in [Figure 1: see original paper], with qualitative and quantitative results presented in .

Note: A. Bud stage; B. Half-opening stage; C. Full-opening stage; D. Wilting stage.

[Figure 1: see original paper] Total ion current chromatograms of volatile compounds in *Rhododendron fortunei* during different flowering stages

Volatile compounds and relative contents in *Rhododendron fortunei* during different flowering stages

2.1 Principal Component Analysis of Major Volatile Components in *R. fortunei*

As shown in , 50 compounds were detected, with five substances—1-methyl-3-(1-methylethylidene)-cyclohexane (39), 1-methylene-4-(1-methylethenyl)-cyclohexane (40), 1,7,7-trimethyl-tricyclo[2.2.1.0(2,6)]heptane (41), (E,E)-1,5-dimethyl-8-(1-methylethylidene)-1,5-cyclodecadiene (46), and (R)-1-methyl-5-(1-methylethenyl)-cyclohexene (47), along with 2-hexenal (33)—detected only in the bud stage. 2-methoxy-6-(2-propenyl)-phenol (4) and -pinene (5) were detected only in the full-opening stage. Six compounds—6-methyl-5-hepten-2-ol (27), 2,6-dimethyl-1,7-octadiene-3,6-diol (28), (3R,6S)-2,2,6-trimethyl-6-vinyltetrahydro-2H-pyran-3-ol (29), (R)-3,7-dimethyl-6-octen-1-ol (30), 3,3,6-trimethyl-1,5-heptadien-4-ol (31), and (1R)-2,6,6-trimethylbicyclo[3.1.1]hept-2-ene (48)—were detected only in the wilting stage. (E)-2-nonenal (36) appeared only in bud and half-opening stages, while 6,6-dimethyl-bicyclo[3.1.1]hept-2-ene-2-methanol (23), (1S)-6,6-dimethyl-2-methylene-bicyclo[3.1.1]heptane (38), 4-methylene-2,8,8-trimethyl-2-vinyl-bicyclo[5.2.0]nonane (42), and hexanal (32)

were undetectable in half-opening and full-opening stages. Due to high variability, these substances were excluded from PCA (Zhao et al., 2015). Therefore, 29 major volatile components present throughout flowering were selected for PCA using SPSS 21.0 software to more objectively reflect characteristic aroma constituents.

As shown in and [Figure 2: see original paper], three principal components had eigenvalues > 1. The first principal component contributed 57.09% of variance, the second contributed 31.45%, and the cumulative contribution of the first two components reached 88.55%. Thus, the first two principal components adequately represented the composition of major volatile components, reflecting most original variable information and achieving dimensionality reduction. Consequently, the first two principal components were extracted for characteristic volatile component analysis.

Eigenvalues of principal components and their variance contributions

[Figure 2: see original paper] Scree plot of principal component

Principal component loading matrix

Loading values reflect correlation coefficients between variables and principal components, with +/- signs indicating positive/negative correlations (Xiao et al., 2017). Larger absolute values indicate stronger relationships, reflecting the degree of variable loading on a given component. According to and [Figure 3: see original paper], eucalyptol (T8) contributed most to the first principal component with a loading value of 1. Other influential substances included -myrcene (T1), -ocimene (T2), copaene (T4), isoledene (T5), ylangene (T10), and (+)-epi-bicyclosesquiphellandrene (T13), along with (3R-trans)-4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-cyclohexene (F4), primarily representing terpenoids with loading values of 0.97, 0.97, 0.99, 0.98, 0.94, 0.92, and -0.91, respectively. PC1 showed high positive correlation with seven terpenoids including -myrcene and -ocimene, and high negative correlation with F4.

For the second principal component, eugenol (P2) had the greatest influence with a loading value of -0.96, showing high negative correlation. Its intense clove scent represents a typical aroma component in aromatic plants (An, 2016). -bourbonene (T11) also contributed substantially with a loading value of -0.93. The most positively influential aroma components were (E,Z)-2,6-nonadienal (AL2) and 1-octen-3-ol (A1), both with loading values of 0.88.

[Figure 3: see original paper] Biplot of principal component analysis (PCA)

2.2 Dynamic Changes of Terpenoids Highly Positively Correlated with the First Principal Component Across Flowering Stages

Terpenoid compounds exhibit complex structures and diverse varieties (Muhlemann et al., 2014), representing the largest class of plant secondary metabolites. Most possess intense sweet, floral, and woody aromas, consti-

tuting important components of floral scent (Hu et al., 2016). Eucalyptol, -myrcene, and -ocimene are monoterpenes, while copaene, isoledene, (+)-epi-bicyclosesquiphellandrene, and ylangene are sesquiterpenes. As shown in [Figure 4: see original paper], only eucalyptol was detectable across all four stages, reaching its maximum relative content (4.11%) at the half-opening stage. Content decreased by 0.65% from half-opening to full-opening stage and by another 0.65% from full-opening to wilting stage, showing a low-high-low pattern. The remaining six components were undetectable in the bud stage and showed gradually decreasing relative contents.

As indicated in , eucalyptol, -myrcene, and -ocimene had relatively low aroma thresholds with aroma values > 1 at all flowering stages, confirming them as characteristic aroma components of *R. fortunei*. Only eucalyptol was present in the bud stage with an aroma value of 84.17 mg $\cdot$ kg ^{1}. At the half-opening stage, all three substances reached peak aroma values: eucalyptol was four times higher than in the bud stage, -myrcene was 2.4 times higher than its minimum (wilting stage), and -ocimene was 2.6 times higher than its minimum (wilting stage). From half-opening to full-opening stage, aroma values declined, with -myrcene decreasing fastest (40.2% reduction), followed by -ocimene (38.8% reduction), and eucalyptol slowest (15.8% reduction). During senescence, aroma values continued declining to 234.17 mg $\cdot$ kg ^{1} for eucalyptol, 34.62 mg $\cdot$ kg ^{1} for -myrcene, and 5.59 mg $\cdot$ kg ^{1} for -ocimene.

[Figure 4: see original paper] Change of relative content of terpenoids with highly positive correlation of the first principal component across different flowering stages

Aroma components and aroma values of terpenoids with highly positive correlation of the first principal component across different flowering stages

Conclusion and Discussion

Plant developmental stages regulate the release of floral compounds, whose types, quantities, and contents gradually increase during flower development, peaking at pollination before declining significantly during senescence, sometimes even altering compound types. Across the four flowering stages, 29, 29, 31, and 33 aroma compounds were detected respectively, showing minimal numerical variation but notable differences in relative content among compound categories. Four changing patterns were observed: phenylpropanoids/benzenoids and terpenoids showed low-high-low trends; alcohols exhibited complex low-high-low-high patterns; aldehydes showed continuous decline; and hydrocarbons displayed high-low-high patterns.

Characteristic aroma results from interactions among multiple volatile compounds, with each component' s contribution determined by its aroma value (Zhao et al., 2010). Eucalyptol, with its camphor scent, was a relatively abundant compound in *R. fortunei* with low aroma threshold (0.012 mg $\cdot$ kg ^{1}) and high aroma value, making it an important aroma constituent. -myrcene, with

floral scent, was undetectable in the bud stage but showed aroma values of 82.31, 49.23, and 34.62 mg • kg ^{1} across the three flowering stages, playing an important role in aroma composition. -ocimene, with neroli oil scent, is a common floral component found in citrus flowers (Li et al., 2018), lily (Zhang et al., 2018), and snapdragon (Natalia Dudareva et al., 2003). In this study, -ocimene relative content remained below 0.5% across stages with a higher aroma threshold (0.034 mg • kg ^{1}), yielding aroma values of 5.59–14.41 mg • kg ^{1} and representing an important aroma component. These compounds constitute essential aroma constituents of *R. fortunei*.

Wang (2012) extracted volatile oils from *Rhododendron qinghaiense* using steam distillation and identified 62 compounds by GC-MS, primarily terpenoids, hydrocarbons, and phenylpropanoids/benzenoids, with terpenoids accounting for 46.41% of relative content. Yang et al. (2015) collected volatile components from *Rhododendron championae* using HS-SPME and identified 17 compounds, with terpenoids (64.09%) most abundant, followed by alkenes (29.65%) and aromatics (6.25%). Wu et al. (2015) analyzed volatile components from *Rhododendron parvifolium* using solvent-free microwave extraction and GC-MS, finding terpenoid relative content as high as 59.02%. These studies demonstrate that terpenoids dominate rhododendron floral scent. Using HS-SPME-GC-MS, this study identified 50 components in *R. fortunei* across six categories: phenylpropanoids/benzenoids, terpenoids, alcohols, aldehydes, hydrocarbons, and others. PCA of 29 major volatile components showed the first principal component explained 57.10% of variance, representing basic aroma composition. Among the eight highly loaded aroma substances, seven were terpenoids showing high positive correlation, confirming terpenoids as the primary characteristic aroma components of *R. fortunei*, consistent with previous reports. Eugenol had the greatest influence on the second principal component, contributing significantly to aroma modulation.

Many terpenoids in plant floral volatiles are major essential oil components beneficial to human health, with substantial commercial value (Yue and Fan, 2011). Increasing attention from industry has led to growing research on applications in food, health products, medicine, and cosmetics (Li, 2012). This study elucidates changes in aroma compounds across *R. fortunei* flowering stages, providing fundamental data and theoretical references for development in fragrance, daily chemicals, food, and health products. However, the release mechanisms of these aroma substances in *R. fortunei* remain unclear and warrant further investigation.

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Note: Figure translations are in progress. See original paper for figures.