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Research Paper

Comprehensive assessment of fragrance profiles in peony flowers through HS-GC-IMS and metabolic pathway analysis

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ABSTRACT

This study addresses the identification and comparison of volatile organic compounds (VOCs) in different peony varieties to elucidate their distinct aroma profiles and the common VOCs that constitute the unique fragrance of peony flowers, laying a foundation for breeding high-quality peony varieties and developing related products. Headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) combined with principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) was employed to analyze and differentiate the VOCs of eight peony varieties: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN. The study identified 87 VOCs, revealing significant differences in VOC compositions among the samples. Notably, BAITIANE displayed distinct differences in both color and VOC composition compared to the other seven varieties. It exhibited uniquely high levels of butanoic acid-M, butanoic acid-D, hexanal-D, ethanol-D, and ethyl acetate. Twelve key differential VOCs were identified as having significant impacts: ethanol-D, hexanal-D, (e)-2-hexenal-D, (e)-2-hexenal-M, hexanal-M, butanoic acid-M, acetic acid-D, acetic acid-M, butanoic acid-D, ethyl acetate, thiophene, and dimethyl sulfide. KEGG pathway analysis highlighted the roles of xenobiotics biodegradation, terpenoid metabolism, and amino acid metabolism in VOC biosynthesis. The findings are significant for the breeding of fragrant peony varieties and the development of floral-based products. The findings provide theoretical support for breeding new horticultural flower varieties and developing commercial peony products, enhancing the understanding of floral scent chemistry, and offering valuable information for the perfumery, floral food processing, and related industries.

1. Introduction

Peony (*Paeonia* × *suffruticosa*) is a unique and esteemed woody flowering plant native to China, renowned for its large, vibrant blooms and elegant appearance. Often referred to as the “king of flowers”, the peony is highly valued for its ornamental beauty. Globally celebrated as “national grace and divine fragrance,” peonies are prized for their color, form, and fragrance, which significantly enhance their appeal to consumers and play a crucial role in the breeding of ornamental plants. The volatile compounds (VOCs) responsible for a flower’s scent not only facilitate plant communication with the environment, aiding in breeding, pollination, and defense against pests and diseases (Zhu et al., 2023), but also hold potential applications in food, pharmaceuticals, a7nd cosmetics (Mostafa et al., 2022). Peony petals are rich in vitamins, amino acids, sugars, minerals, and flavonoids, making them valuable both nutritionally and medicinally.

Floral scents are primarily derived from VOCs synthesized within plant organs and released into the air. Studies have shown that the VOCs of peonies mainly include terpenes, aromatics, ketones, and alcohols, with smaller quantities of esters, aldehydes, and ethers (Cherri-Martin et al., 2007). The variety and concentration of these compounds differ significantly across peony species and developmental stages,

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contributing to the unique fragrance of each variety. Identifying these VOCs is essential for evaluating plant diversity, improving breeds, and developing new varieties. The collection and analysis of floral VOCs are critical in characterizing the scent properties of plants.

Recent research on VOCs in the petals of ornamental plants such as jasmine (Bera et al., 2017), petunia (Oyama-Okubo et al., 2018), lily (Du et al., 2019), Tillandsia (Lo et al., 2021), Hedychium (Abbas et al., 2021), and rhododendron (Yang et al., 2023) has made significant progress. However, studies on peonies remain relatively limited. Advances in extraction and analytical techniques for floral VOCs now allow for more precise analysis of the components and emission levels of peony VOCs, providing valuable insights for breeding and utilization.

Different extraction methods can yield varying VOCs profiles. The primary identification techniques include sensory evaluation, quality indicator detection, and characteristic component analysis, with chromatographic techniques being widely used. Gas chromatography (GC), liquid chromatography (LC), GC/LC-mass spectrometry (GC/LC-MS), ultraviolet (UV) spectroscopy, near-infrared (NIR) spectroscopy, and thin-layer chromatography (TLC) are among the methods employed (Flaig et al., 2020; Medina et al., 2020; Yao et al., 2021). Headspace gas chromatography-ion mobility spectrometry (HS-GC-IMS) is a novel analytical technique that distinguishes VOCs based on differences in gas retention time and ion mobility (Xiao et al., 2022). Combining the high sensitivity of GC with the uniqueness of ion mobility spectrometry, HS-GC-IMS allows for rapid, simple, and direct detection of VOCs without complex sample preparation, preserving the original scent profile and yielding reliable results (Castell et al., 2023; Wang et al., 2020). This technique has been applied in various fields, including food testing, drug monitoring, hazardous chemical analysis, breath analysis, and biological analysis (Gu et al., 2020; Li et al., 2021a).

In this study, HS-GC-IMS technology combined with principal component analysis (PCA) was used to identify and compare the VOCs of different peony petal varieties. The aim was to determine the key VOCs released by eight peony varieties and to establish a VOCs fingerprint for each, facilitating the evaluation and selection of unique peony germplasm resources. This research provides a theoretical basis for the breeding of fragrant peony varieties and the development and utilization of aromatic floral resources.

2. Materials and methods

2.1. Materials

Eight varieties of peony samples (TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN) were utilized in this experiment (Fig. 1). The samples were collected at Peony Garden, Caojiaping, Lintao County, Dingxi City, Gansu Province, China (35°21′12.08″ N, 103°49′42.52″ E). Six flowers in full bloom, with similar maturity, were collected for each variety at 9:00 a.m., individually placed in preservation bags, and sealed. Within 2 h of collection, the samples were transferred to *a* − 80 °C freezer and stored overnight. The following day, the flowers from each variety were cut into small pieces using scissors and placed on a freeze-drying tray. The samples were freeze-dried at −50 °C and 10 Pa pressure for 48 h. After freeze-drying, the samples were ground using a grinder and passed through a 100-mesh sieve. The sieved samples were collected and used for subsequent HS-GC-IMS analysis.

2.2. Sample preparation

HS–SPME was used to extract the VOCs from the peony samples (Shen et al., 2023). Specifically, 0.5 g of each variety of peony flower was accurately weighed and placed into a 20 mL headspace-glass sampling vial (Shandong Haineng Scientific Instrument Co., Ltd., Shandong, China). The samples were incubated at 60 °C with a shaking speed of 500 r/min for 20 min. After incubation, 500 μL of the headspace gas phase was automatically injected into the injector under splitless injection mode using a syringe heated to 85 °C. Each sample was measured in triplicate.

2.3. Chromatographic conditions

VOCs were analyzed using a HS-GC-IMS flavor analyzer (FlavourSpec®) from Gesellschaft für Analytische Sensorysteme mbH (G.A.S., Dortmund, Germany) and a CTC-PAL 3 static headspace automatic sampling device (CTC Analytics AG, Switzerland). The VOCs were separated using GC with a MXT5 column (30 *m* × 0.53 mm × 1.0 μm, Restek Company, Bellefonte, PA, USA). Nitrogen (99.999 % purity) was used as the carrier gas with the following programmed flow: 2 mL/min for 2 min, then linearly increased to 10.00 mL/min and subsequently to 100.00 mL/min within 10 min, maintained for 40 min. The total

Fig. 1. The eight varieties of peony samples used in this study.

chromatography running time was 60 min, and the injection port temperature was 80 °C. The experiment was conducted under ambient pressure.

2.4. IMS conditions

A tritium source was used with a flow rate of 75 mL/min. The electric field power was 500 V/cm, and the drift gas purity was 99.999 %. The length of the migration tube was 53 mm, with an electric field strength of 500 V/cm, and the temperature of the migration tube was 45 °C. High-purity nitrogen (99.999 % purity) was used as the drift gas.

2.5. Data analysis

A mixture of six n-alkanes (analytical grade, Aladdin, Inc.): 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone, and 2-nonanone were used as external references to calculate the retention index (RI) of VOCs under the same chromatographic conditions as the samples. The VOCs in the samples were characterized using GAS software (G.A.S., Dortmund, Germany, version 2.0.0), the built-in NIST retention index database (NIST 2020, National Institute of Standards and Technology, Gaithersburg, MD, USA), and the IMS drift time database. To compare VOCs between samples, VOCal data processing software plug-ins, such as Reporter (Version 11.x), Gallery Plot (Version 1.1.0.2), and Dynamic PCA (version 0.0.3), were used to generate three-dimensional (3D) spectra, two-dimensional (2D) spectra, difference spectra, fingerprints, and PCA maps. PLS-DA VIPs were calculated using SIMCA software (version 14.0) (Umea, Sweden).

The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a comprehensive integrated database that combines information from genomics, chemistry, and system functions across multiple dimensions (Kanehisa et al., 2007). It categorizes data into four main areas, further subdivided into 17 specialized sub-databases, encompassing extensive knowledge from the molecular level to higher-order biological system functions (Kanehisa et al., 2007). In this study, we uploaded the names of all identified compounds from the GC-IMS results to the online Majorbio Cloud Platform (www.majorbio.com) of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) (Ren et al., 2022). Using the cloud tools available on this platform, we conducted an in-depth KEGG enrichment analysis to reveal the contributions of VOCs in different peony samples to their aroma profiles

3. Results and discussion

3.1. HS-GC-IMS analysis of VOCs in peony samples

HS-GC-IMS was employed to analyze the VOCs in the petals of eight different peony varieties (Leng et al., 2021; Liu et al., 2023; Sun et al., 2023). The Fig. 2a presents a three-dimensional spectral topographical visualization. Here, the X-axis represents the ion drift time for identification, the Y-axis represents the gas chromatography retention time, and the Z-axis represents the peak height for quantification (Li et al., 2021b). In HS-GC-IMS, GC separation was performed based on the interaction strength between the complex VOCs in the sample, followed by separation in the stationary phase of the chromatographic column. The eluted components at different retention times were softly ionized by the ion source in a gaseous form to form molecular ion groups. Due to differences in mass, collision cross-section, charge, and periodic ion pulses, the ions entered the linear drift electric field successively under ambient air pressure and achieved different migration rates for secondary separation (Yang et al., 2021). Various kinds of compounds were effectively distinguished.

The 3D topographic map was relatively rough, making it challenging to observe the data clearly. Therefore, we normalized the ion migration time and reactive ion peak (RIP) position. The top-view plot (Fig. 2b) of the HS-GC-IMS spectrum was then obtained. After data normalization, the red vertical line at 1.0 represents the RIP peak. The ordinate represents the gas chromatography retention time. The dots on either side of the RIP represent the VOCs. The color denotes the substance concentration; white indicates a lower concentration, and red indicates a

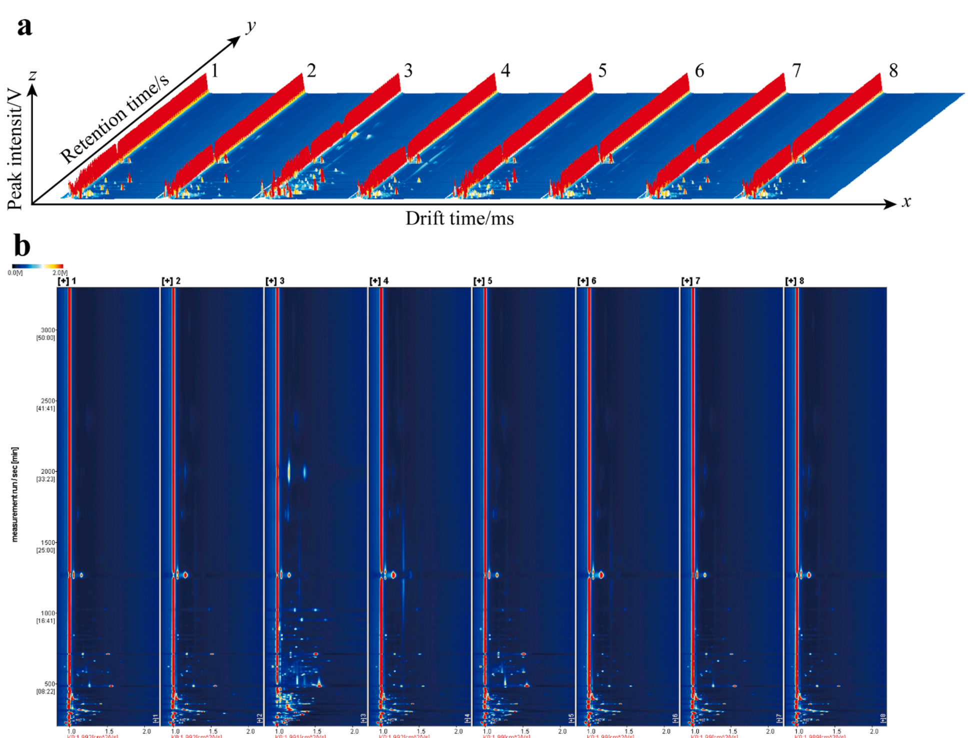

Fig. 2. Volatile Organic Compounds identified by HS-GC-IMS in the eight varieties of peony samples: (a) 3D topographic plot; (b) 2D topographic plots. 1–8 represent the eight varieties of peony samples: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN.

higher concentration. Generally, the darker the color, the greater the concentration. Most signals appeared within a retention time of 1500 s and a drift time of 1.0–1.5 ms. The colors represent the signal intensity of individual substances, with red indicating high intensity and blue indicating low intensity. Darker colors correspond to greater intensity. The VOCs detected from the peony petals showed distinct signal intensities, suggesting variations in the VOCs among the samples.

3.2. Detected VOCs in peony samples by HS-GC–IMS

The peak intensities of all VOCs in the eight peony samples are listed in Table S1. A total of 87 VOCs were detected and identified, corresponding to 109 peaks. These compounds include 23 aldehydes, 17 alcohols, 11 ketones, 8 esters, 4 acids, 6 terpenes, 9 aromatic compounds, and 9 others (with "M" indicating monomers and "D" indicating dimers) (Fig. 3; Fig. S1). The Fig. 3a shows that acid compounds have the highest proportion in the eight peony samples, particularly in BAITIANE

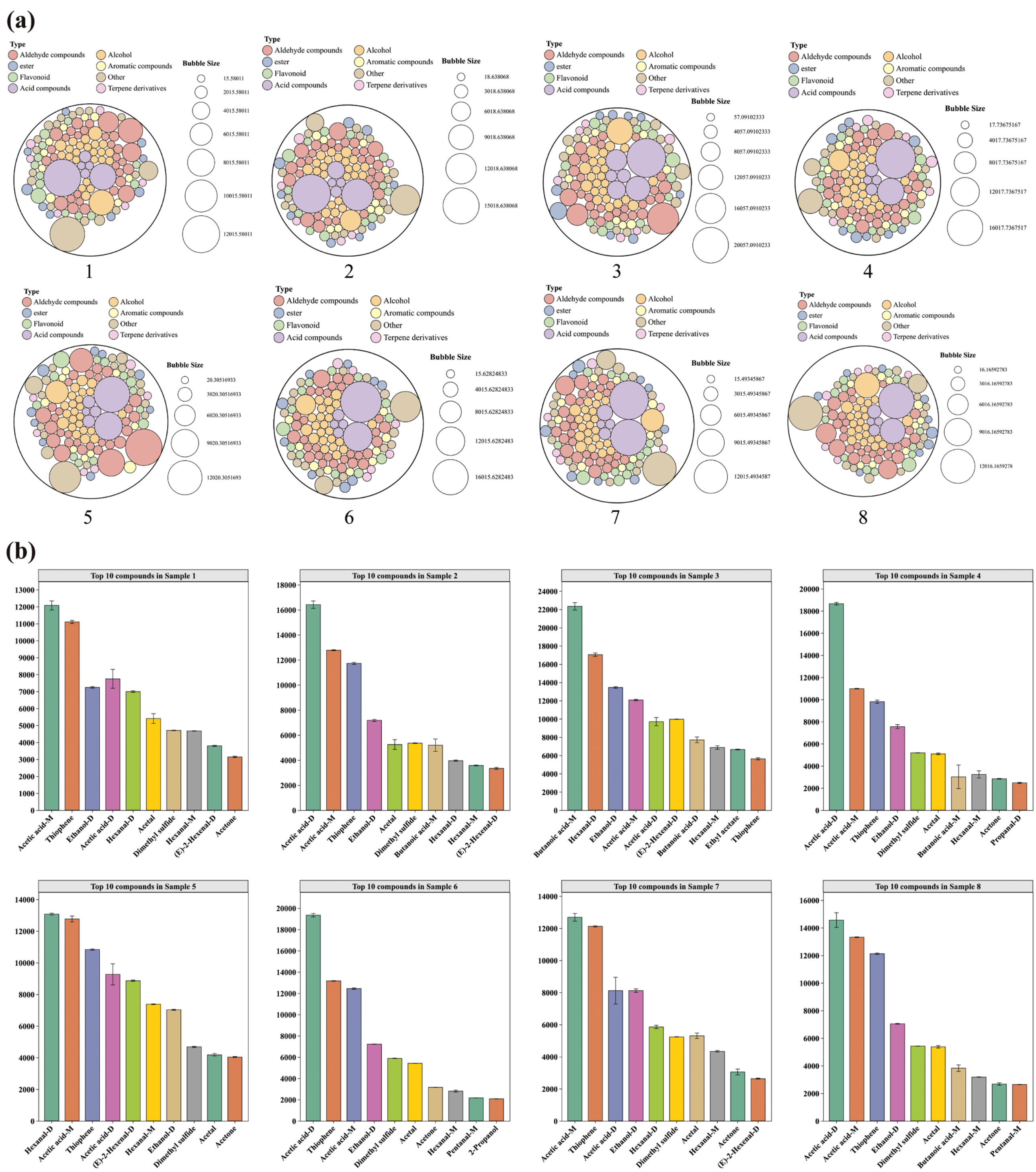

Fig. 3. Circle packing plots of the volatile profiles (a) and bar graphs of the top 10 VOCs by concentration or peak intensity (b) detected by HS-GC-IMS in the eight varieties of peony samples. 1–8 represent the eight varieties of peony samples: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN.

(sample 3) and DAFUGUI (sample 5), which also show a high proportion of aldehydes.

The Fig. 3b presents the top 10 VOCs and their peak intensities in the eight peony samples, detailing VOC distribution across these samples. Five VOCs were common among the samples: acetic acid-D and acetic acid-M (pungent, sour aroma similar to vinegar (Söllner and Schieberle, 2009)), thiophene (sulfurous, earthy odor reminiscent of mushrooms or truffles (Yu et al., 2012)), ethanol-D (alcoholic, slightly sweet aroma similar to spirits or ripe fruit (Steinhaus and Schieberle, 2007)), hexanal-M (grassy, green odor reminiscent of freshly cut grass or green leaves (Steinhaus et al., 2009)). Focusing on specific samples, Fig. 3b highlights unique VOCs: TAOXI (sample 1), DAFUGUI (sample 5), and HONGXIUQIU (sample 7) share identical top 10 VOCs but in different amounts. BAITIANE (sample 3) contains butanoic acid-D (strong, rancid, cheesy odor akin to aged cheeses (Qian and Reineccius, 2003)) and ethyl acetate (fruity and sweet aroma often associated with pears, bananas, or pineapples (Xiao et al., 2014)); YAOHUANG (sample 4) contains propanal-D (light fruity scent reminiscent of green apples (El'odé et al., 1966)); MANAOPAN (sample 6) has 2-propanol (sharp, alcoholic scent contributing a clean, somewhat medicinal aroma (Bai et al., 2019)). In contrast, ZIHELIAN (sample 2) and MANAOPAN (sample 8) do not exhibit any unique top 10 VOCs. The HS-GC-IMS analysis successfully identified a range of VOCs in peony samples, each contributing unique aromatic properties. This detailed understanding of VOCs enhances the potential applications of peony samples in various fragrance and flavor industries.

3.3. Comparison of the fingerprints of VOCs

The pleasant aromas of various peony samples are directly attributed to their VOC profiles, which were characterized and qualitatively analyzed using IMS retention indices and drift times compared with a control. A fingerprint was created based on 116 signals to visualize the differences in VOCs among the eight peony varieties (Fig. 4). Each row in Fig. 4 represents a sample, and each column represents a compound. Dark colors represent high-intensity compounds, while black indicates very low or non-detectable concentrations.

TAOXI (Sample 1) contained relatively high amounts of 2-propanol. ZIHELIAN (Sample 2) exhibited higher levels of nonanal, which has a strong sweet orange aroma. Major compounds in BAITIANE (Sample 3) included butanoic acid-M, 2-methylpropanoic acid, propanoic acid, phenylacetaldehyde, benzaldehyde, (e)−2-octenal, (e)−2-heptenal, (e)−2-hexenal-D, 2-hexenal-D, 2-methyl-2-pentena-D, (e)−2-pentenal-D, 3-methyl-2-butenal, (e)−2-butenal, 2-propenal, 2,4-hexadienal, octanal, heptanal-D, hexanal-D, and propanal-D. YAOHUANG (Sample 4) had high contents of 2-methyl-1-propanol-M, linalool, butyl acetate, methyl acetate-M, methyl acetate-D, and 3-carene. DAFUGUI (Sample 5) contained higher levels of methyl 3-methylbutanoate, pentanal-M, 3-methylbutanal, butanal, 2-methylpropanal, and 2-ethylfuran. In DAOJIN (Sample 6), acetic acid-D, 4-methyl-3-penten-2-one, 1-penten-3-one, thiophene, acetals, and dimethyl sulfide were predominant. HONGXIUQIU (Sample 7) had higher levels of 2-ethyl-1-hexanol, while MANAOPAN (Sample 8) exhibited high amounts of γ-butyrolactone and acetaldehyde.

The identified VOCs significantly contribute to the fragrance profile of peony petals. For instance, nonanal in ZIHELIAN (Sample 2) imparts a strong sweet orange note (Zhang et al., 2021). Key floral scent constituents, phenylacetaldehyde and benzaldehyde, were found in BAITIANE (Sample 3) (Li et al., 2012). Butyric and propanoic acids in BAITIANE (Sample 3) add to its complex aroma profile (Feng et al., 2016). Linalool and ethyl butyrate in YAOHUANG (Sample 4) contribute floral and fruity smells, respectively (Yan et al., 2018; Zhang et al., 2023). The high levels of methyl 3-methylbutanoate in DAFUGUI (Sample 5) highlight its unique composition.

These VOCs profiles underscore the distinct fragrance characteristics of each peony variety and their potential applications in perfumery, food flavoring, and aromatherapy (Wang et al., 2021). The findings align with previous research emphasizing the importance of terpenes and aromatic compounds in floral scents (Li et al., 2012).

3.4. Candidate differential VOCs in peony samples

To investigate the compositional differences in VOCs among eight peonies, we employed HS-GC-IMS combined with PCA. PCA is a technique used to simplify complex data by transforming numerous interconnected original variables into a few orthogonal principal components. This orthogonal transformation converts a set of potentially correlated variables into linearly uncorrelated variables, known as principal components (Sebzalli and Wang, 2001). PCA is particularly effective in emphasizing differences in VOCs based on signal intensity. In the PCA plot, samples that are closer together indicate smaller differences, while those farther apart signify greater differences. As shown in Fig. 5a, the cumulative variance contribution rate of the first and second principal components reaches 92.69 %, demonstrating that PCA effectively captures and reflects the overall characteristics of the samples. In the PCA plot, samples D and F (labeled A-H for samples 1–8, respectively) exhibit high similarity, as do samples B and H. Conversely, samples A, E, and G show closer chemical composition. Sample C, however, displays significant differences in chemical composition compared to the other samples. Furthermore, samples B, D, F, and H are

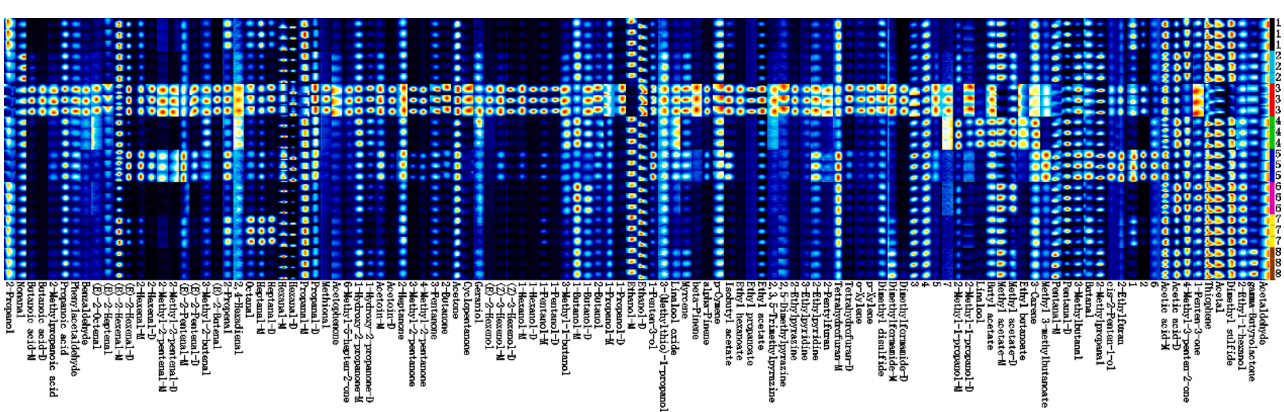

Fig. 4. Fingerprint comparison of volatile organic compounds in the eight varieties of peony samples determined by HS-GC-IMS. Note: The darker the spot, the higher the quantity of VOCs. Each row represents all the signal peaks selected in a sample, and each column represents the signal peak of the same VOCs across different treatments. The HS-GC-IMS analysis was performed in triplicate. 1–8 represent the eight varieties of peony samples: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN.

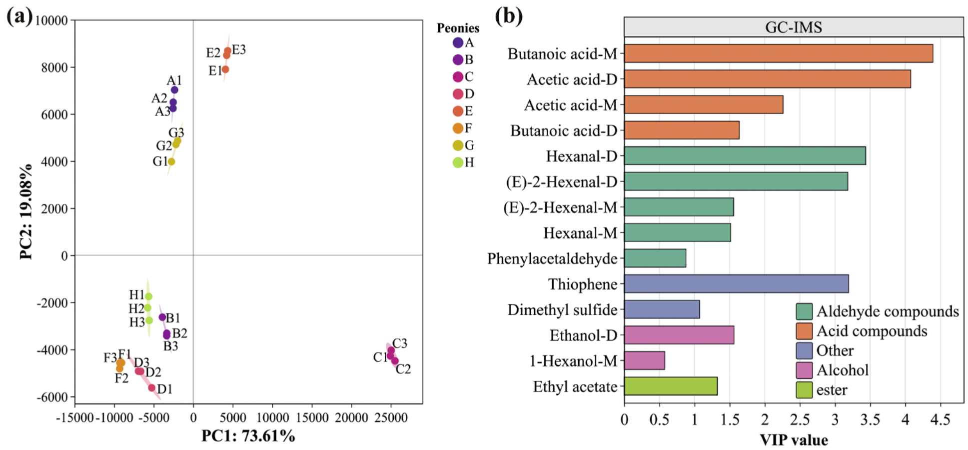

Fig. 5. The PCA score plots of the volatile profiles (a) and the VIP values of the PLS-DA models for the candidate differential VOCs (b) in the eight varieties of peony samples determined by HS-GC-IMS. A-H in (a) represent the eight varieties of peony samples: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN.

concentrated in the lower left region of the PCA plot, while samples A, E, and G are in the upper left region, and sample C is positioned alone in the lower right corner. This indicates that the differences in the VOCs of the eight peony samples are most evident in the first principal component (PC1), which contributes 73.61 %.

To thoroughly evaluate the contribution of each variable in the classification process, we employed the Partial Least Squares Discriminant Analysis (PLS-DA) model and calculated the Variable Importance in Projection (VIP). According to the literature (Liland, 2011; Oliveira et al., 2022), variables with VIP values greater than 1 are considered to significantly contribute to the model. After rigorous testing, our constructed PLS-DA model demonstrates excellent performance without issues of overfitting or underfitting. Based on the VIP assessment results, we identified a total of 12 VOCs as potential differential VOCs. These compounds include one alcohol (ethanol-D), four aldehydes (hexanal-D, (e)−2-hexenal-D, (e)−2-hexenal-M, hexanal-M), four acids (butanoic acid-M, acetic acid-D, acetic acid-M, butanoic acid-D), one ester (ethyl acetate), and two other VOCs (thiophene; dimethyl sulfide). Among these, acetic acid-D, acetic acid-M, ethanol-D, hexanal-D, and thiophene are included in the common top ten VOCs of the eight peony samples (Fig. 3b). This suggests that they are the main components of the characteristic aroma of peonies and form the basis of their distinction from other floral fragrances. The consistent presence and significant levels of these VOCs indicate their crucial role in defining the unique scent profile of peonies. While these compounds are prevalent across different peony varieties, the specific proportions and presence of additional VOCs contribute to the variations in fragrance among peony cultivars. Therefore, the unique composition and concentration of these VOCs in peony flowers not only distinguish them from other floral scents but also create subtle differences among peony cultivars. This diversity is crucial for the development of specialized fragrances in the perfumery industry and enhances the horticultural value of different peony samples.

The Fig. S2 shows the content of 14 potential differential VOCs in the eight peony samples. The box plots reveal that in BAITIANE (Sample 3), the VOCs contents of butanoic acid-M, butanoic acid-D, hexanal-D, ethanol-D, and ethyl acetate are significantly higher than in the other seven samples. However, the thiophene and dimethyl sulfide content in BAITIANE (Sample 3) is much lower. In DAOJIN (Sample 6), the levels of thiophene and dimethyl sulfide are significantly higher, and (e)−2-hexenal-D and (e)−2-hexenal-M are significantly lower compared to the other samples. These findings are consistent with the conclusions obtained from the fingerprint analysis (Fig. 4). These differential VOCs likely play key roles in forming different floral scents. For example, (e)−2-hexenal has a spicy vegetable and fruity aroma (Chen et al., 2019; Feng et al., 2022). Alcohols and acids can react to form esters, which often have floral and fruity aromas (Liu et al., 2004). In BAITIANE (Sample 3), the significantly higher levels of butanoic acid and ethanol may react to produce esters with floral and fruity scents, contributing to its unique aroma (Mukdsi et al., 2009). Overall, there is a clear difference between the VOCs of BAITIANE (Sample 3) and the other seven samples. Interestingly, BAITIANE (Sample 3) has white flowers, which contrasts with the bright colors of the other samples. There appears to be a strong correlation between flower colors and VOCs. Research indicates that the production of floral scents and colors can be linked by shared biosynthetic pathways. For example, in a study on Iranian narcissus, different flower colors and their associated scents are produced through distinct metabolic processes, with specific VOCs correlating with specific colors (Haghshenas et al., 2024). Additionally, a study on *Iris lutescens* demonstrates that anthocyanins, responsible for flower coloration, also affect the emission of certain VOCs, linking visual and olfactory traits (Wang et al., 2013). This suggests that the interplay between color and scent in flowers results from complex biochemical pathways that co-regulate these traits to attract specific pollinators and adapt to their environment.

3.5. KEGG analysis of VOCs in peony samples

The KEGG pathway analysis provides insights into the metabolic pathways and biological functions associated with the VOCs identified in peony flowers. By mapping the detected VOCs to KEGG pathways, we gain a better understanding of the biosynthetic routes and potential regulatory mechanisms involved in producing these compounds. The KEGG analysis reveals several significant pathways in the biosynthesis and metabolism of the identified VOCs, including xenobiotics biodegradation and metabolism, metabolism of terpenoids and polyketides, amino acid metabolism, energy metabolism, and carbohydrate metabolism.

Out of the 87 detected VOCs, 29 were enriched in these pathways (Fig. 6). Xenobiotics biodegradation and metabolism was the most represented pathway, with eight VOCs (benzaldehyde, phenylacetaldehyde, acetophenone, acetaldehyde, p-cymene, p-xylene, propanoic acid, and o-xylene) associated with it, suggesting that the detoxification and degradation of foreign compounds play a significant role in peony flowers' ability to manage and modulate various VOCs, contributing to their unique aroma profiles (Sandermann, 1992). Four VOCs (benzaldehyde, phenylacetaldehyde, acetophenone, and acetaldehyde) were linked to the metabolism of terpenoids and polyketides, highlighting the importance of terpenoids in providing characteristic floral and fruity aromas that enhance the overall fragrance of peonies (Chen et al., 2011). Amino acid metabolism was associated with two

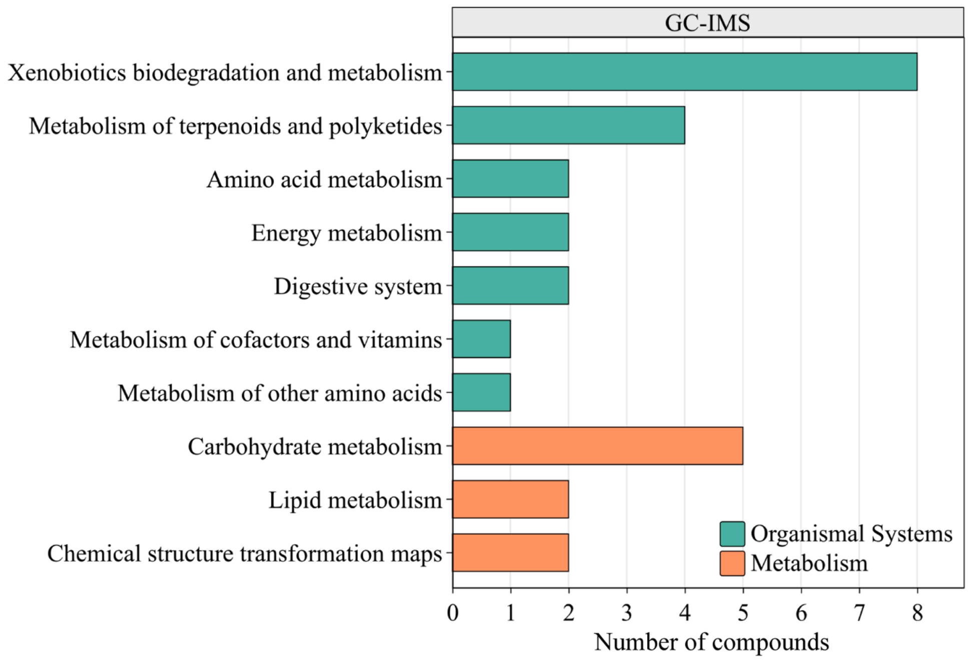

Fig. 6. KEGG pathway of VOCs in the eight peony samples determined by HS-GC-IMS. 1–8 represent the eight varieties of peony samples: TAOXI, ZIHELIAN, BAITIANE, YAOHUANG, DAFUGUI, DAOJIN, HONGXIUQIU, and MANAOPAN.

VOCs (phenylacetaldehyde and acetaldehyde), underscoring its role in producing aroma compounds, as amino acids are precursors for various aromatic compounds, including those contributing to floral scents (Li et al., 2021c). Energy metabolism, linked to two VOCs (phenylacetaldehyde and acetaldehyde), emphasizes the importance of energy production and utilization in synthesizing and maintaining VOCs in peony flowers (Aharoni et al., 2006). Carbohydrate metabolism, associated with five VOCs (acetone, acetaldehyde, butanal, propanoic acid, and 2-propanol), plays a vital role in providing necessary substrates for the biosynthesis of aromatic compounds, leading to the formation of various alcohols and acids essential for ester formation (Dudareva et al., 2005). Lipid metabolism, linked to two VOCs (acetone and acetaldehyde), is integral in forming fatty acid derivatives and esters, contributing to the floral and fruity aromas of peonies (Aharoni et al., 2003). Additional pathways included the metabolism of cofactors and vitamins (propanoic acid), metabolism of other amino acids (acetaldehyde), digestive system functions (propanoic acid and 2-methylpropanoic acid), and chemical structure transformation maps (myrcene and geraniol), each contributing one or two VOCs, further illustrating the complexity and diversity of metabolic processes involved in VOC biosynthesis.

The integration of HS-GC-IMS and KEGG analysis not only identifies the VOCs but also elucidates the biochemical pathways responsible for their synthesis. For instance, in BAITIANE (Sample 3), the higher levels of butanoic acid and ethanol suggest active fatty acid and alcohol metabolism, leading to the formation of esters that impart floral and fruity scents (Mukdsi et al., 2009). This biochemical insight helps explain the unique aroma of BAITIANE compared to other peony varieties.

4. Conclusions

The fragrance of plant flowers is produced by VOCs released from floral organs, primarily petals. Variations in these compounds result in different floral scents. Floral scents are composed of various compounds, classified into terpenes, phenylpropanoids, fatty acids, and amino acids. Fatty acids and amino acids are significant VOCs found in both floral and fruit scents. The primary VOCs associated with peony fragrance are citronellol, geraniol, and linalool.

This study investigated the VOCs in eight peony samples using GC-IMS for comprehensive VOCs profiling. This method, which does not require sample pretreatment and uses dual qualitative techniques of gas-phase retention time and ion mobility time, is simple, fast, and accurate. Non-targeted analysis using HS-GC-IMS fingerprints, combined with PCA and PLS-DA, allowed for the visual identification of differences in VOCs among the samples. A total of 87 VOCs and 109 peaks were detected, including 23 aldehydes, 17 alcohols, 11 ketones, 8 esters, 4 acids, 6 terpenes, 9 aromatic compounds, and 9 other compounds. Statistical analysis revealed that butanoic acid-M, acetic acid-D, hexanal-D, thiophene, (e)−2-hexenal-D, acetic acid-M, butanoic acid-D, ethanol-D, (e)−2-hexenal-M, hexanal-M, ethyl acetate, and dimethyl sulfide were the most significant VOCs in the eight peony samples (Fig. 7). Among them, the concentration or peak intensity of acetic acid-D, acetic acid-M, ethanol-D, hexanal-D, and thiophene were the highest (Fig. 7). Aldehydes, alcohols, and ketones were the main contributing VOCs, with aldehydes contributing the most. Many aldehyde compounds have fruity scents, which may explain the different floral aromas of peonies. These various VOCs contribute to the different floral aromas. Seven peaks were not identified, indicating the database is still incomplete, and further detection using other methods is needed. Applying HS-GC-IMS to analyze VOCs in peony petals helps evaluate and screen peony germplasm with unique aromas. This study provides a theoretical basis for future research in breeding fragrant peony varieties and developing aromatic flower resources.

In conclusion, the integration of HS-GC-IMS with KEGG analysis offers a powerful tool for dissecting the complex biochemical networks that define the unique aromas of peony flowers. This approach enhances our understanding of the metabolic pathways involved in fragrance production and provides valuable information for practical applications in breeding, fragrance manufacturing, and ecological studies.

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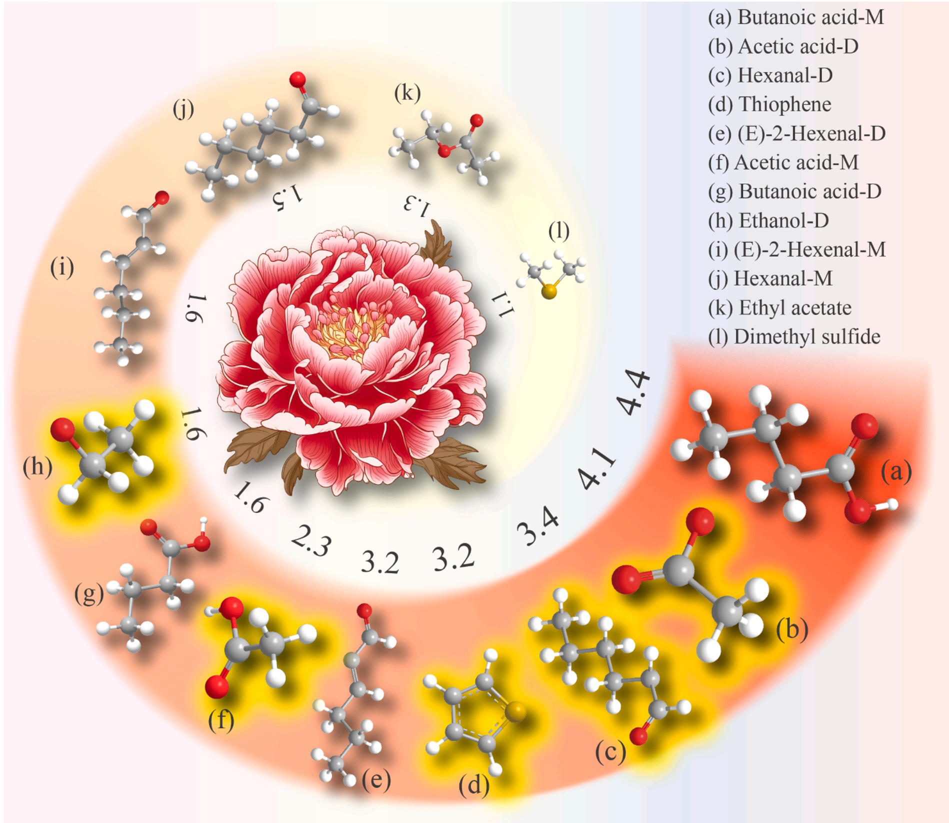

Fig. 7. Potential Differential VOCs with the Largest Contribution Among All VOCs in Eight Peony Samples. (a)-(l) represent butanoic acid-M, acetic acid-D, hexanal-D, thiophene, (e)−2-hexenal-D, acetic acid-M, butanoic acid-D, ethanol-D, (e)−2-hexenal-M, hexanal-M, ethyl acetate, and dimethyl sulfide, respectively. The numbers on each VOC indicate their Variable Importance in Projection values. VOCs with a yellow highlighted background (**b, c, d, f**, and h) are the top ten VOCs common to all eight peony samples.

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CRediT authorship contribution statement

Yanhua Pan: Supervision, Resources, Project administration, Methodology, Investigation, Conceptualization. **Yini Ma:** Writing – original draft, Visualization, Data curation. **Jiadong He:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Lu Zhang:** Writing – review & editing. **Rong Huang:** Writing – review & editing. **Ling Tang:** Writing – review & editing. **Jiaxuan Ren:** Writing – review & editing. **Weicheng Wang:** Writing – review & editing. **Muriel Quinet:** Writing – review & editing. **Yu Meng:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition.

Declaration of competing interest

All authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2024.113646.

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