

Analytical strategies for aroma VOCs profiling with systematic review of methods and physiological effects

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Abstract: Aroma-related volatile organic compounds (VOCs) may influence the autonomic nervous system (ANS) by stimulating the limbic system and the hypothalamus through the olfactory nerves. Stress, anxiety, and depression may be alleviated, and sedative activity may also be exerted. Since most human studies on aroma inhalation heavily rely on self-reported measures, more valid and objective verification is essential. In addition, comprehensive reviews integrating the chemical diversity, analytical techniques, and physiological effects of aroma VOCs from analytical chemistry perspective remains limited. This systematic review was conducted using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) approach with 51 studies selected from an initial pool of 3,790 records retrieved from multiple electronic databases. Gas chromatography/mass spectrometry (GC/MS) was the predominant analytical method (35 of 38 studies) with headspace solid-phase microextraction (HS-SPME) as the most frequently employed sampling method. Electroencephalogram (EEG), functional near-infrared spectroscopy (fNIRS), blood pressure (BP), and heart rate (HR) were the principal physiological measures used to evaluate stress-relieving and sedative responses. Fragrance inhalation modulated EEG wave activity and induced hemodynamic alterations assessed by fNIRS while reductions in BP and HR suggested parasympathetic nervous system activation. Through this systematic review, it indicates that physiological response may vary depending on aroma type, concentration, exposure duration, and subject characteristics, and such study heterogeneity precluded a formal meta-analysis. Future studies should address to standardize GC/MS profiling conditions, physiological assessment protocols with predefined endpoints, and systematic report of individual differences to strengthen evidence-based applications of aroma VOCs for stress relief and sedation.

Keywords: aroma compounds, volatile profiling, headspace solid-phase microextraction-gas chromatography/mass spectrometry, chemical diversity, stress-relieving and sedative activity

1. Introduction

Odors can act on the neuroendocrine system,

neurotransmitters and neuromodulators, influencing psychological behavior as well as body function.¹

Unlike the other senses, olfactory system is the only

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sensory system that has direct projection to the limbic system² consisting of the hypothalamus which regulates the autonomic nervous system (ANS), the amygdala which is responsible for emotional responses, and the hippocampus which is responsible for memory. Olfactory stimuli pass through the cerebral cortex, hypothalamus, and pituitary gland after reaching the limbic system and are transmitted to the ANS via hormonal pathways.^{3,4} In this context, inhalation of aroma volatile organic compounds (VOCs) can be helpful. Sowndhararajan *et al.* (2015)⁵ investigated the effects of olfactory stimulation using isomeric aroma compounds, (+)-limonene and terpinolene on human electroencephalogram (EEG) activity. These results demonstrate that inhalation of these monoterpenes produced measurable changes in brain electrical activity. Cha (2009)⁶ reported that inhalation of a blended essential oil reduced blood pressure (BP) of hypertension patients. These characteristics of the olfactory system show the possibility that aroma VOCs can act as regulators of stress-relieving and sedative activity. Therefore, continuous related research will have a positive effect on human stress relief and sedation.

In recent decades, research into the inhalation of aroma VOCs has steadily increased. Many studies have reported that olfactory stimulation by fragrance inhalation has various psychological and physiological effects.⁷⁻⁹ Despite this increasing trend, there is a limit to comparing or interpreting results between studies due to differences in sampling methods, analytical methods, and experimental conditions. In particular, fragrance is not composed of a single material but a mixture of several compounds. It is difficult to clearly clarify the correlation between specific fragrance compounds and physiological reactions because qualitative and quantitative information on aroma VOCs is not sufficiently obtained. Therefore, precise compositional analysis of aroma VOCs is essential to clearly understand the physiological effects of fragrance.

Aroma VOCs of flowers or fruits are mainly classified into terpenoids, phenylpropanoids/benzenoids, fatty acid derivatives, and amino acid derivatives.

Among them, terpenoids are composed of more than 40,000 structures derived from isoprene units,¹⁰ and most of them exhibit strong biological activity and fragrance properties.¹¹ Terpenoids are particularly associated with fruity aroma¹²⁻¹⁴ and mainly include isoprene, monoterpenes, sesquiterpenes, and their derivatives.¹¹ The chemical composition of essential oils containing these compounds may vary depending on the harvest seasons and the method of extraction. Guelifet *et al.* (2025) extracted essential oils from wild *R. officinalis* over four seasons using hydro-distillation (HD), microwave-assisted distillation (MD), and steam distillation (SD). The analysis revealed that the percentage composition of the identified compounds varied depending on both the season and the extraction method. Crescente *et al.* (2025)¹⁶ produced essential oils using SD and microwave-assisted hydro-distillation (MAHD). As a result, essential oils extracted by the SD method were rich in hydrocarbons such as limonene, whereas those obtained by the MAHD method contained higher amounts of terpenoids such as carvone. Therefore, the selection of extraction techniques is not just a matter of efficiency but an important factor in determining the quality, stability, and potential applicability of the final product.

Currently, the most commonly used method in aroma VOCs analysis is headspace solid-phase microextraction gas chromatography/mass spectrometry (HS-SPME-GC/MS). This method is suitable for volatile and semi-volatile compound analysis.¹⁷ In addition, the absence of solvents facilitates straightforward sample preparation and automation while coupling with GC through thermal desorption enables simple and rapid sample extraction.¹⁸ However, the adsorbent of SPME fiber can be easily damaged. Standardization and verification are very important in HS-SPME-GC/MS method because analysis conditions can affect limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and reproducibility.

These aroma VOCs act directly on the central nervous system (CNS) which regulates various physiological and neurophysiological changes. Objective indicators such as EEG, functional near-infrared

spectroscopy (fNIRS), BP, and heart rate (HR) are used to evaluate these changes. Cai *et al.* (2025)¹⁹ evaluated the effects of inhalation of *G. jasminoides* on participants by monitoring BP, HR, EEG, and heart rate variability (HRV). Participants first measured BP and HR performed a preliminary evaluation through a questionnaire and then wore a device capable of recording EEG and HRV. After that, the scent of *G. jasminoides* was inhaled and physiological changes were monitored in real time. Kim *et al.* (2018)²⁰ investigated the physiological effects of inhalation of *C. indicum* essential oil on the experimental group by monitoring BP, HR, and EEG. After recording BP and HR during rest, additional measurements were performed with EEG to assess physiological changes before and after essential oil inhalation. Matsubara & Matsui (2025)²⁰ investigated the effects of inhaling various Japanese wood-derived essential oils on the experimental group by monitoring HRV. HRV was measured and a questionnaire was administered during a 2 min essential oil inhalation. Bu *et al.* (2025)²² measured the effects of inhaling oriental lily on the experimental group using BP, HR, HRV, galvanic skin response (GSR), and EEG. The experimental participants underwent these measurements while inhaling the scent of oriental lily for 10 min. Most studies investigate neurophysiological changes using different protocols. Therefore, there are limitations when it comes to comparing results across studies.

Currently, many studies analyze specific aroma VOCs or investigate their effects on animals or humans, but few papers comprehensively review these studies. While surveys are frequently used with humans, these methods are often limited by their inherent subjectivity. Furthermore, many studies rely on only one approach, such as surveys, BP and HR measurements, or chemical composition analyses. Although integrating these three approaches could yield highly informative results, such studies are extremely limited. Studies that focus specifically on an analytical chemistry perspective are rarely available.

Although research on aroma VOCs analysis and inhalation effects has expanded considerably, several important gaps remain. First, there is still a lack of

comprehensive reviews from an analytical chemistry perspective that systematically compare GC/MS methodological parameters such as sampling approaches, SPME fiber selection, column stationary phases, and method validation practices across studies. Second, the physicochemical relationships between specific VOCs structures and their associated physiological responses have not been clearly established. Third, the absence of standardized analytical and physiological measurement protocols makes it difficult to directly compare results across studies or to perform meaningful meta-analyses.

To address these limitations, this systematic review was conducted in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) framework to characterize the chemical diversity of aroma VOCs with particular emphasis on physicochemical properties relevant to GC/MS analysis, to evaluate and compare GC/MS-based pretreatment and analytical conditions including SPME fiber selection, column type, and available validation information, to summarize the stress-relieving and sedative effects of aroma VOCs inhalation in humans based on objective physiological measurements, and to identify key methodological gaps and suggest directions for future standardization. Through this approach, we aim to provide a clear analytical and instrumental foundation for future research on aroma VOCs.

2. Systematic Review using PRISMA

This review adheres to the PRISMA guidelines to ensure a systematic and reproducible analysis. PRISMA is a standardized framework developed to enhance the transparency and comprehensiveness of reporting in systematic reviews and meta-analysis. PRISMA provides a 27-item checklist and a 4-phase flow diagram to guide authors in structuring and reporting their reviews in methodologically rigorous manner.²³ In this review, PRISMA was conducted as follows: identification, screening, eligibility, and inclusion. The literature search was conducted in accordance with the PRISMA guidelines using multiple

electronic databases including Google Scholar, PubMed, and ScienceDirect. These databases were selected to ensure comprehensive and multidisciplinary coverage of studies related to aroma VOCs, analytical methods, and human physiological responses. The use of multiple databases was intended to minimize selection bias and enhance the completeness and reproducibility of the search process.

For identification, the keywords of 'aroma therapy, essential oil, inhalation, olfactory, brain activity, physiological, anxiety, headspace, electroencephalogram' were used. A total of 3,790 records were initially identified. After excluding 240 patents, 3,550 records remained. Among these, 300 studies published before 2005 were further excluded resulting in 3,250 records

for title screening. The titles of the 3,250 records were screened to exclude clearly irrelevant studies such as animal experiments, medical-focused papers, and flavor-related papers, reducing the dataset to 133 were considered potentially relevant. This initial screening step was designed to remove studies that were outside the scope of this review including those unrelated to VOCs, olfactory exposure, analytical methods, or human physiological responses. In eligibility process, the abstracts of the 133 remaining records were further assessed against the predefined inclusion and exclusion criteria. Studies were excluded for the off-topic papers (n=20), animal studies (n=4), biological-only focus (n=1), book chapter (n=1), environmental studies (n=1), food-related studies without olfactory

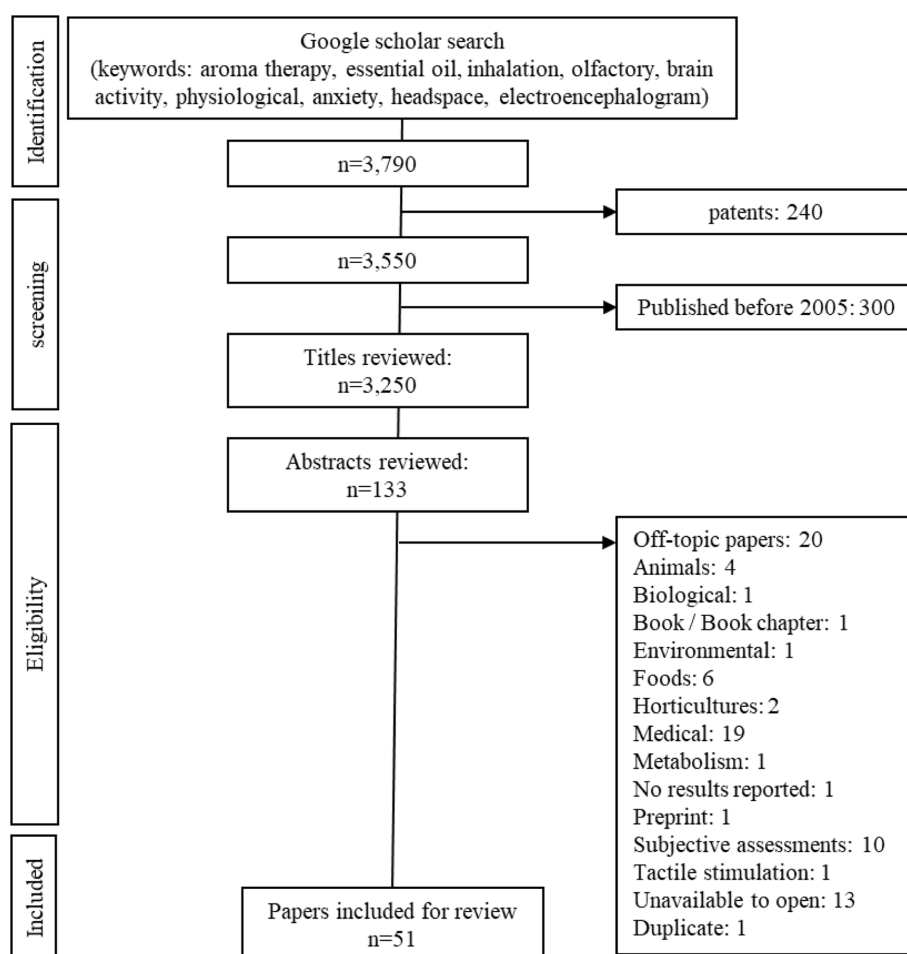


Fig. 1. The flow chart of PRISMA.

relevance (n=6), horticulture studies (n=2), medical studies without analytical relevance (n=19), metabolism-related studies (n=1), studies with no reported results (n=1), preprint format (n=1), subjective assessment-only studies (n=10), studies based on tactile stimulation rather than olfactory exposure (n=1), unavailability of full-text records (n=13), and duplicate studies (n=1). Following this process, a total of 51 studies were selected for detailed analysis (Fig. 1).¹⁹⁻¹³⁸ Methodological quality assessments were independently conducted by two reviewers (C.K. and N.K.). Any disagreements in study inclusion were resolved through discussion until consensus was reached. Among the 51 included studies, 38 studies addressed the analysis of aroma VOCs, 33 studies investigated the physiological effects of aroma VOCs, and 20 studies included both analytical and physiological components.

3. Chemical Diversity of Major Aroma VOCs Detected in the Reviewed Literature

Terpenes constitute a significant portion of aroma VOCs and contribute substantially to their chemical diversity. Terpenes are a group of natural products that are widely found in the plant kingdom, with more than 40,000 terpenoids currently reported.²⁴ Terpenes are lipids composed of repetitions of isoprene units and can be divided into monoterpenes and sesquiterpenes depending on the structure. Monoterpenes are terpenes with two isoprene units, including limonene, α -pinene, and β -pinene. Monoterpenes are widely present in essential oils. Because of their natural and pleasant aroma, they are widely used in the food and fragrance industries and in aromatherapy. They are biologically active with antibacterial, antiseptic, and antioxidant properties, among others.²⁵⁻²⁷ Sesquiterpenes are terpenes with three isoprene units, including nerolidol and denderalasin.²⁸ Among them, nerolidol is a fragrance ingredient used not only in cosmetic products but also in detergents and household cleaners.²⁹

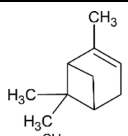
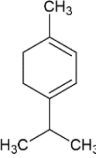
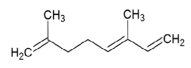
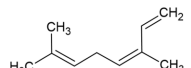
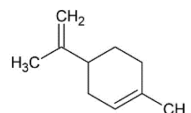
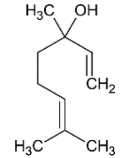
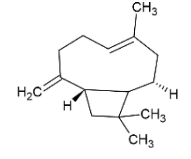
Among the 38 studies analyzing aroma VOCs in this study, pinene was the most frequently reported

aroma VOCs with 25 occurrences, followed by linalool and terpinene with 18 occurrences each, ocimene, limonene and caryophyllene with 17 occurrences each (Table 1). Pinene frequency (n=25) reflects combined reporting of α - and β -pinene isomers, terpinene frequency (n=18) reflects combined reporting of α - and γ -terpinene isomers, and ocimene frequency (n=17) reflects combined reporting of α - and β -ocimene isomers; individual isomer frequencies were not consistently distinguished across reviewed studies. These are all terpenes, which have been reported to exhibit various physiological and pharmacological activities along with fragrance characteristics.

It is noted that pinene (n=25), terpinene (n=18), and ocimene (n=17) each include multiple isomers (α - and β -pinene; α - and γ -terpinene, α - and β -ocimene respectively) which exhibit difference in their physicochemical properties. Consequently, it influences their GC retention behavior and SPME extraction efficiency. For example, β -pinene (boiling point=166.0°C, log P=4.16) and α -pinene (boiling point=155.0°C, log P=4.83) differ in boiling point by approximately 11°C which may lead to differences in retention on nonpolar GC columns. These distinctions highlight the importance of clearly distinguishing between isomers in future studies to improve the accuracy of chemical diversity characterization.

Pinene is a pine flavored monoterpene observed in plants such as neroli, sweet orange, and *C. sativum*.³⁰ Previous studies have shown that they have antibacterial and antifungal effects.³¹ Linalool is a monoterpene with refreshing, floral, and woody scents identified in plants such as ylang-ylang, bergamot, neroli, and clary sage.^{30,32} It is known to inhibit the activity of glutamate receptors that induce excitement of the nervous system and is known to have antibacterial, antifungal, antiviral, and sedative effects. Terpinene is a citrus flavored monoterpene identified in plants such as lemons and *C. sativum*.³⁰ Terpinene is known to have anti-inflammatory properties in animal experiments.³³ Ocimene is a citrus flavored monoterpene identified in plants such as *A. sinensis* and lavender.³⁰ In previous studies, it has been reported that *A. sinensis*

Table 1. Physicochemical properties and detection frequency of the 6 most frequently reported aroma VOCs across 38 GC/MS studies. Note: detection frequency indicates reporting prevalence across reviewed studies and does not directly reflect odor importance, in vivo concentration, or level of human physiological evidence

Chemical type	Name	CAS No.	Molecular weight (g/mol)	Boiling point (°C)	Log P	Structure	Frequency
Monoterpene	α -Pinene	80-56-8	136.23	155.0	4.83 ³⁶		25
	β -Pinene	127-91-3		166.0	4.16 ³⁷		
	α -Terpinene	99-86-5	136.24	174.1	4.25 ³⁷		18
	γ -Terpinene	99-85-4		183.0	4.50 ³⁶		
	α -Ocimene	502-99-8	136.24	170.2	4.30 ³⁸		17
	β -Ocimene	13877-91-3		175.2	4.30 ³⁸		
Oxygenated monoterpene	Limonene	138-86-3	136.24	175.4	4.57 ³⁶		17
	Linalool	78-70-6	154.25	198.5	2.97 ³⁶		18
Sesquiterpene	Caryophyllene	87-44-5	204.36	268.4	6.30 ³⁸		17

essential oil with ocimene as a main ingredient has anti-anxiety activity in animal experiments.³⁴ Caryophyllene is sesquiterpene identified in plants such as basil, cinnamon, pepper, clove, cannabis, lavender, oregano, and rosemary. It has biological effects such as anti-inflammatory and analgesic suppression.³⁵

4. Extraction Methods of Essential Oil

Essential oils, perfumes, and plants are used directly for analysis, but plants are also processed into essential oils through sampling for use. Aroma VOCs are highly volatile and reactive with other compounds in

the atmosphere, can interact with the surfaces of monitoring equipment, and some of those materials may emit VOCs.³⁹ Standardization is crucial because the sampling conditions influence the emission patterns of the aroma VOCs.

Distillation is the most common method for extracting aroma VOCs from heat sensitive plants such as lavender, jasmine, peppermint, and basil. When plant material is placed on a packed bed installed just above the water surface and the water is heated, steam is generated from the heated water. The steam passes over the leaves, disrupting the cellular structure and releasing VOCs.⁴⁰ Distillation has the advantages of low production cost, non-toxicity, and high reproducibility,⁴¹ but it requires a lot of thermal energy for continuous steam generation and carries the risk of burning plant materials.⁴⁰ Kitamoto *et al.* (2024)⁴² extracts essential oil by vaporizing volatile compounds contained in the yuzu peel by generating steam from the bottom of the yuzu peel. Bakhsha *et al.* (2014)⁴³ extracted essential oil from *L. angustifolia*, Gong *et al.* (2024)⁴⁴ extracted leaves from *C. camphora*, and Liu *et al.* (2025)⁴⁵ extracted leaves from *C. lanceolata* using a distillation method.

Cold pressing is a method mainly used to extract aroma VOCs from the peels or seeds⁴⁶ of citrus fruits such as oranges, lemons, limes, bergamots, and grapefruits.⁴⁷ The aroma VOCs can be obtained through centrifugation by pressing seeds or peels at low temperatures. The cold pressing method has the advantages of low production cost and environmental friendliness but the disadvantage of low oil yield as a significant amount of oil remains in the residue after compression.⁴⁸ Kitamoto *et al.* (2024)⁴² extracted the essential oil compounds contained in the flavedo vacuoles of the yuzu peel using cold pressing method.

Solvent extraction is a method of extracting a chemical substance that is easily soluble in an organic solvent such as methanol, ethanol, and ethyl acetate. Solvent extraction has the advantage of being suitable for heat-sensitive plant materials. However, it requires large volumes of solvent and makes it difficult to completely remove the organic solvent from the solute after extraction.^{40,41} Kim *et al.* (2018)²⁰ and

Takemoto *et al.* (2008)²⁰ extracted the essential oils of *C. indicum* and *N. chinensis* root using hexane as a solvent.

Supercritical CO₂ extraction is generally carried out at low temperatures below 60°C and is a method of extracting essential oils without solvents using CO₂.⁵⁰ It can extract essential oils at low temperatures, complementing limitations such as conventional distillation, thermal decomposition of solvent extraction, partial hydrolysis, incomplete extraction, and solvent residue.^{51,52} Cho *et al.* (2013)⁵³ extracted the essential oils of *M. arvensis* using supercritical CO₂ extraction.

5. GC/MS-Based Sampling, Analytical and Data Processing Method for Aroma VOCs Profiling

5.1 Sampling methods

The general problems facing chromatography analysis are the removal of interfering substances and increasing sensitivity. To solve this problem, sample preparation is required. Sample preparation refers to the process of transforming a sample to facilitate analysis when direct analysis using an analytical device is challenging, or when satisfactory results cannot be obtained. Therefore, substances corresponding to the matrix that interfere with the analyte must be removed in the sample preparation process.⁵⁴

In 18 of the 38 studies, HS-SPME was mentioned with the highest frequency as a sampling method. In 8 of the 38 studies, headspace gas from the sample vial was injected into the GC/MS inlet for analysis. In 2 of the 38 studies, VOCs were collected on sampling tube and then thermally desorbed into the GC/MS for analysis. In 1 of the 38 studies, liquid samples were directly injected into the GC/MS inlet for analysis. In 10 of 38 studies, the sampling methods were not specified, while 1 of 38 studies used both HS-SPME and direct injection (headspace gas) methods (Fig. 2-(A)).

5.1.1 Solid-phase microextraction, SPME

HS-SPME method was used in 18 of 38 studies. SPME is a technique that uses a fused silica fiber

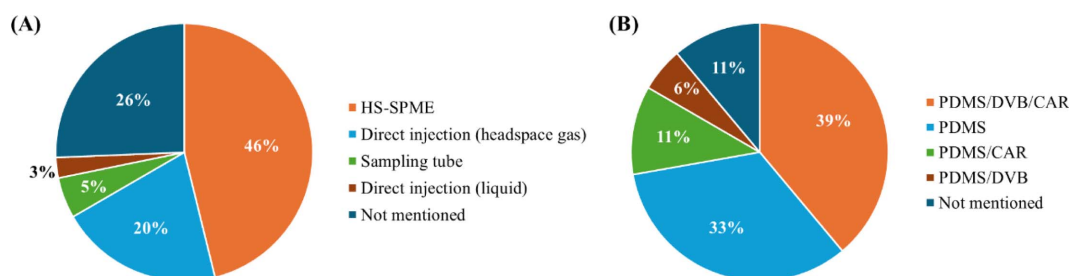


Fig. 2. Analysis results of (A) sampling methods and (B) type of SPME fiber.

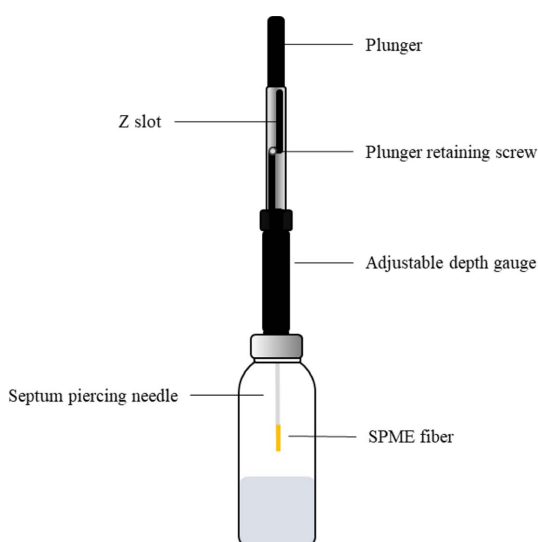


Fig. 3. A scheme of the headspace solid-phase microextraction (HS-SPME).

coated with an adsorbent polymer to adsorb analytes for extraction and concentration (Fig. 3). The extraction technique of SPME consists of two processes. The first process is the distribution of analytes between the sample and the sorbent fiber. The second process is the desorption of analytes concentrated on the sorbent fiber onto analytical instruments such as GC or high-performance liquid chromatography (HPLC). To extract analytes, the sample vial is sealed with a septum or cap. Then, pierce the septum with the SPME fiber and adjust the syringe plunger to immerse the adsorbent fiber directly into the sample or expose it to the headspace. After adsorbing the analytes for a specified period, SPME fiber is adjusted to its original position, withdrawn from the vial, and injected into

the analysis instrument. Expose the SPME fiber in the injection port to desorb the analytes thermally (for GC) or by solvent (for HPLC).⁵⁵ Among them, the HS-SPME method can be used to analyze volatile or semi-volatile compounds from solid samples as well as aqueous solution samples.⁵⁴ HS-SPME has many advantages, including short extraction times, eliminating the need for organic solvents, easy and simple coupling with GC, and high sensitivity.²⁸ However, it has the disadvantages of fiber fragility and limited adsorption capacity.⁵⁶ The adsorbents used in SPME are very diverse. Polydimethylsiloxane (PDMS) and polyacrylate (PA) extract analytes through absorption, and the compound dissolves and diffuses into the adsorbent. Basically, it moves in and out of the adsorbent in the absence of a competition effect. Adsorbents with mixed components such as PDMS/Divinylbenzene (DVB) and PDMS/DVB/Carboxen (CAR) extract analytes through adsorption and physically interact with the analytes. Among them, the most commonly used adsorbent is PDMS/DVB/CAR which contains polar and non-polar properties.⁵⁷ When preparing an analytical sample using SPME, select an adsorbent with similar polarity to the sample. PDMS, PA, CAR, or polyethylene glycol (PEG) are employed in low polar, medium polar, highly polar samples respectively. In this study, PDMS/DVB/CAR was used in 7 of 18 studies, PDMS was used in 6 of 18 studies, PDMS/CAR was used in 2 of 18 studies, PDMS/DVB were used in 1 of 18 studies, and type of SPME fiber was not specified in 2 of 18 studies (Fig. 2-(B)).

5.1.2 Solid-phase extraction, SPE

Solid-phase extraction (SPE) is a method using an

organic solvent and has high recovery and sensitivity. SPE can be applied to some semi solid samples as well as liquid samples. SPE is also suitable for analysis of low and semi volatile compounds. The basic principle is different in that SPE uses the total amount extraction method, while SPME uses the equilibrium extraction method based on the distribution coefficient. SPE was developed as an alternative method to liquid-liquid extraction (LLE).⁵⁸ In the SPE procedure, a disposable cartridge or column filled with an appropriate adsorbent is used as a solid surface and adsorbed until the desired compound reaches equilibrium between the liquid sample and the solid-phase. SPE has the advantage of being able to process sample volume, high sensitivity and recovery rate, shortening analysis time due to reduced procedural steps, easy automation, adaptability to chromatography analysis, and less solvent use compared to LLE.⁵⁸ However, it still requires an organic solvent and has the disadvantage of poor device connection.⁵⁵

5.1.3. In-needle microextraction, INME

Recently, needle-based microextraction has attracted considerable attention. INME is a sample pretreatment technique. In this approach, a nichrome wire is used to coat the inner or outer surface of a needle with an adsorbent for analyte extraction. This method overcomes the inherent fiber fragility associated with SPME. Some studies have directly fabricated adsorbents and coated them onto the needle. It also has the advantage of being able to control particle size, shape, and properties. In several studies, adsorbents are directly synthesized and subsequently coated onto the needle.

This approach not only provides a useful strategy for synthesizing various adsorbents with improved uniformity and purity but also reduces the manufacturing temperature and enhances mixing efficiency in multicomponent systems.⁵⁶ In INME, analytes are first adsorbed onto the inner surface of a needle coated with an adsorbent for extraction. The needle is then introduced into the GC injector, where the analytes are thermally desorbed and subsequently separated on the column. The INME device does not require solvents because extraction and preconcentration are carried out simultaneously resulting in a simple and sustainable process. However, selectivity and extraction efficiency may be highly dependent on the physicochemical properties of the analytes and their interactions with the extraction phase.⁵⁹

5.2 Analytical methods

Instrumental analysis of aroma VOCs by GC/MS is fundamental to the comprehensive volatile profiling and quantitative evaluation of these compounds. Furthermore, it is critical for understanding their physiological relevance to human stress-relieving and sedative responses. Representative analytical instruments include GC/MS, gas chromatography-flame ionization detector (GC-FID), gas chromatography/olfactometry (GC/O), and electric nose (E-nose). Each method is selected based on its measurement principle and intended application. Among the 38 studies analyzing aroma VOCs in this study, 7 studies employed two different analytical instruments, and the most frequently mentioned analytical method is GC/MS (*Fig. 4(A)*).

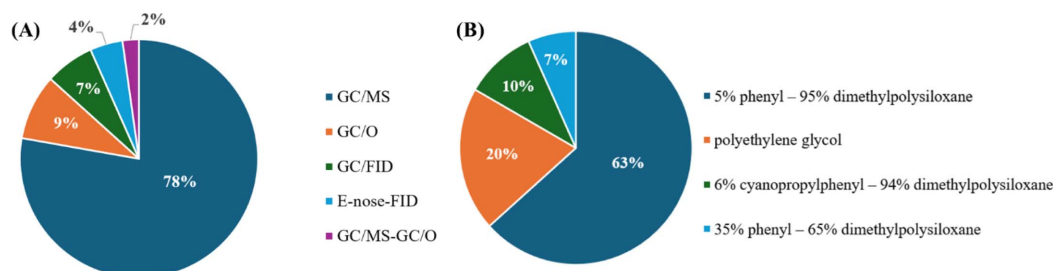


Fig. 4. Analysis results of (A) analytical instruments and (B) column stationary phase.

5.2.1. Gas chromatography/mass spectrometry, GC/MS

GC/MS was the most frequently employed analytical instrument which was mentioned in 35 of 38 studies. In GC/MS, GC separates volatile compounds according to their distribution between the stationary and mobile gas phases. MS subsequently provides structural information by separating ionized species based on their mass to charge ratios. In addition, desorption of analytes within the GC inlet significantly influences chromatographic separation efficiency and quantitative precision. Therefore, optimal conditions must be established through desorption experiments.⁵⁵

Among the reviewed studies, injection port temperatures ranged from 200°C to 280°C, and oven temperature programs were predominantly gradient methods initiating at 40–80°C and ramping to 200–300°C at rates of 2–20°C/min. Helium was universally employed as the carrier gas at flow rates of 1.0–1.7 mL/min. Electron ionization (EI) at 70 eV was the standard ionization mode across most GC/MS studies, whereas one study used 15 eV, and compound identification was achieved by matching mass spectra against the NIST and/or Wiley spectral libraries using a minimum match threshold of 80–90 %.

The stationary phase was explicitly reported in 30 studies. Among these, 19 studies (63 %) used a 5%-diphenyl–95 %-dimethylsiloxane copolymer (e.g., DB-5MS, HP-5MS), a nonpolar phase widely applied for the separation of terpene hydrocarbons and other nonpolar VOCs. 6 studies (20 %) employed PEG (e.g., DB-Wax, TC-WAX), a polar phase that provides complementary selectivity for oxygenated compounds such as linalool and terpinen-4-ol. 3 studies (10 %) used 6 % cyanopropylphenyl–94 % dimethylpolysiloxane (e.g., DB 624MS), and 2 studies (7 %) used 35 % phenyl–65 % dimethylpolysiloxane (e.g., DB-35MS, HP-35MS) (*Fig. 4-(B)*). In the remaining 5 studies, the stationary phase was not specified. The predominance of DB-5-type columns reflects their broad applicability for terpene profiling. However, reliance on a single nonpolar column may lead to co-elution of structurally similar isomers, such as α - and β -pinene or α - and γ -terpinene. The use of dual-column

configurations or heart-cutting GC–GC approaches may improve isomer resolution and could be considered in future standardization efforts (*Fig. 4-(B)*).

However, only a limited number of studies explicitly reported key method validation parameters including the LOD, LOQ, recovery, and precision (%RSD). This lack of consistent validation reporting represents an important gap in the current literature and makes it difficult to directly compare quantitative VOCs data across studies. To improve data reliability and comparability, future studies should follow established analytical validation frameworks such as ICH Q2(R1), AOAC International guidelines, ISO/IEC 17025 standards, and relevant bioanalytical validation frameworks particularly when quantitative interpretation is intended. As a result, even when analyzing the same sample, the interactions among VOCs and their elution order can completely change depending on the selection of polar or nonpolar columns. This can lead to the misidentification of specific compounds or peak overlap phenomena. In addition, differences in separation efficiency caused by column specifications and aging conditions can result in significant variations in VOC profiles among studies. Therefore, the chemical characteristics of the stationary phase must be carefully considered to enable objective comparison of data across studies and efforts toward data standardization using established analytical validation systems are required.

5.2.2. Gas chromatography/flame ionization detector, GC/FID

GC/FID was mentioned in 3 of 38 studies. The FID is widely used in GC and is suitable for the analysis of various organic compounds. During analysis, the column effluent enters a hydrogen–air flame where the analytes undergo combustion and ionization. Then the electrons and ions generated in this process are collected by electrodes, and the resulting current is measured. FID is largely unaffected by variations in carrier gas flow rate and provides high sensitivity. It also offers a wide linear dynamic range and low background noise. However, the sample is destroyed during combustion, and additional gases are required

Table 2. Summary of sampling and analytical methods obtained from 38 references

Sample		Analytical method			Reference
Type	Sampling methods	SPME fiber ^{a)}	Instrument	Number of VOCs ^{b)}	
Plant (4)	Sampling tube	-	GC/MS	39	66
	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	15	19
	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	17	67
	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	16	68
Leaf (2)	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	40	69
	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	28	70
Root (1)	HS-SPME	PDMS/DVB (65)	GC/MS	17	71
Flower (7)	HS-SPME	DVB/CAR/PDMS	GC/MS	16	72
	HS-SPME	-	GC/MS	114	73
	HS-SPME	-	GC/MS	12	22
	headspace	-	GC/MS	11	74
	headspace	-	GC/MS	97	75
	-	-	GC/MS	-	76
	HS-SPME	CAR/PDMS (75)	GC/MS	15	77
Essential oil (23)	headspace	-	GC/MS	45	42
	headspace	-	GC/MS	18	78
	headspace	-	GC/FID	6	
	headspace	-	GC/FID	-	79
	headspace	-	E-nose/FID	34	80
	HS-SPME	CAR/PDMS (75)	GC/MS-GC/O	27	
	headspace	-	GC/MS	28	81
	HS-SPME	PDMS (100)	GC/MS	47	82
			GC/O	4	
	HS-SPME	PDMS (100)	GC/MS	19	83
			GC/O	18	
	HS-SPME	PDMS (100)	GC/MS	7	84
			GC/O	7	
	HS-SPME	PDMS (100)	GC/MS	17	43
			GC/O	4	
	-	-	GC/MS	28	43
	HS-SPME	PDMS (100)	GC/MS	-	49
			GC/FID	-	
	Sampling tube	-	GC/MS	26	21
	HS-SPME	PDMS (100)	GC/MS	42	20
	headspace	-	E-nose/FID	35	86
	-	-	GC/MS	32	53
	-	-	GC/MS	16	45
	HS-SPME	DVB/CAR/PDMS (50/30)	GC/MS	70	87
	-	-	GC/MS	9	44
	-	-	GC/MS	11	88
	-	-	GC/MS	-	89, 90, 91, 92
Perfume (1)	Direct Injection	-	GC/MS	33	93

a) SPME: solid-phase microextraction (PDMS: polydimethylsiloxane; CAR: carboxen; DVB: divinylbenzene); b) VOCs: volatile organic compounds; -: not available

for operation (Table 2).

5.2.3. Gas chromatography/olfactometry, GC/O

GC/O was mentioned in 4 of 38 studies. GC/O refers to a technique in which volatile compounds eluting from the GC column are detected and evaluated by a human assessor. In this approach, the human evaluator replaces conventional detectors such as MS or FID. The assessor smells the column effluent through a specially designed sniffing port to detect the presence of odor active compounds and rates their intensity using a defined scale. GC/O was initially described as a screening method to determine whether compounds identified in a sample exhibit olfactory activity. While its applications have expanded considerably and are now being commonly used to assign relative importance of odor active compounds⁶⁰ (Table 2).

5.2.4. Electronic nose, E-nose

E-nose was reported in 2 of the 38 studies. It consists of gas sensors and a pattern recognition system and is designed to mimic the human olfactory system.⁶¹ It utilizes an array of sensors that interact with analytes on sensitive material surfaces and converts the resulting specific responses into digital signals for odor discrimination.⁶² E-nose offers advantages such as cost effectiveness, portability, flexibility, and time efficiency compared with conventional GC detection methods coupled with MS or FID. Therefore, it enables rapid and accurate characterization of VOCs patterns. While the E-nose cannot match GC/MS in terms of compound-level identification or quantitative precision, it serves as a complementary screening tool for rapid odor classification particularly in field or quality-control settings where full GC/MS analysis may be impractical⁶³⁻⁶⁵ (Table 2).

5.3. Multivariate analysis methods

Multivariate analysis methods were used to analyze various aroma VOCs and to test the statistical significance between groups or to identify patterns. Multivariate analysis methods include principal component analysis (PCA), analysis of variance (ANOVA), and hierarchical clustering (HC). Among

the 38 studies analyzing aroma VOCs in this study, PCA is the most frequently mentioned analytical method.

In addition to the univariate and classical multivariate methods identified in the reviewed studies, more advanced chemometric approaches including partial least squares discriminant analysis (PLS-DA),⁹⁵ orthogonal PLS-DA (OPLS-DA),⁹⁷ and machine learning-based methods such as random forest,^{94,96} and support vector machine (SVM)⁹⁷ have been increasingly adopted in VOCs profiling research in adjacent fields such as food authenticity and clinical breathomics. The absence of such methods in the reviewed aroma VOCs literature suggests a methodological lag that future studies should address, particularly for discrimination among structurally similar terpene isomers and for building predictive models linking VOCs profiles to physiological outcomes.

5.3.1. Principal component analysis, PCA

PCA is the most widely used multivariate statistical technique and is utilized across most scientific disciplines. It is a multivariate technique that analyzes data tables where observations are described by multiple intercorrelated quantitative dependent variables. This technique extracts important information from the table, represents it as a set of new orthogonal variables called principal components, and displays similar patterns of observations and variables as points on a map.⁹⁸ Since it is desirable to reduce the dimensionality when analyzing large-scale multivariate data, PCA is employed for this purpose.⁹⁹

PCA was mentioned in 3 of 38 studies. It was used to visually distinguish chemical differences and cluster patterns between aroma VOCs by dimensional reduction of VOCs composition data. Wang *et al.* (2024)⁶⁹ applied PCA to analyze the compositional VOCs structures of eight bamboo species. It was confirmed that there were significant differences in leaf VOCs among species and distinct clusters representing representation. Adrar *et al.* (2025)⁷⁵ utilized PCA on fragrance mixtures emitted from ten rose cultivars. This confirmed that the aroma VOCs

composition patterns were statistically different among rose varieties, and not all roses have similar fragrance combinations. Alqathama *et al.* (2024)⁸⁷ visually identified chemical differences between samples through the PCA of aroma VOCs for 8 species of *T. foenum-graecum* seeds oil.

5.3.2. Hierarchical clustering, HC

In HC, each cluster is subdivided into smaller clusters to form a tree-like data structure or dendrogram. The resulting dendrogram can be arranged according to predefined criteria and displayed alongside gene expression heatmaps for visualization.¹⁰⁰ HC has the advantage of not requiring a priori specification of the number of clusters and enables visualization of the general similarity structure of the data at multiple resolution levels through the dendrogram.¹⁰¹

HC was mentioned in 2 of 38 studies. It was used to group and distinguish independent or unusual species based on the similarity of VOCs composition. Wang *et al.* (2024)⁶⁹ conducted cluster analysis on the volatile compounds of eight bamboo leaf species to explore differences and similarities in their aroma profiles. As a result, the 8 bamboo species were classified into 2 distinct categories. Alqathama *et al.* (2024)⁸⁷ classified eight species of *T. foenum-graecum* seeds oil into two major macro clusters, confirming that one species exhibits distinct and independent characteristics.

5.3.3 Analysis of variance, ANOVA

ANOVA is examined from the user's perspective and focuses on understanding model construction and the fundamental assumptions of the method.¹⁰² ANOVA is classified into one-way and two-way ANOVA. One-way ANOVA is used when there is one independent variable and one dependent variable and the most widely used method for providing clear and accurate explanations of research findings.¹⁰³ F-test is used to determine the statistical significance of differences between group and correlation coefficient R^2 can measure how well the model describes the data.^{104,105}

ANOVA was mentioned in 1 of 38 studies. Wang

et al. (2024)⁶⁹ compared the VOCs and individual components of eight bamboo leaf species through one-way ANOVA to see if they contribute significantly to the overall aroma profile.

6. Stress-Relieving and Sedative Effects of Aroma VOCs on the Human Body

Aroma VOC molecules enter the nose and stimulate the olfactory system. When odor molecules reach the cilia of the nasal mucosa, they stimulate the limbic system and hypothalamus via the olfactory nerve. This stimulation activates neurotransmitters in the CNS inducing emotional states such as relaxation, arousal, calming, and excitement.¹⁰⁶ During this process, inhaled fragrances reach the limbic system including the hypothalamus, amygdala, and hippocampus. The hypothalamus regulates hormonal secretion and instinctive behaviors, the amygdala mediates emotional responses, and the hippocampus is involved in object recognition and memory. The stimuli are then transmitted to the hypothalamus, which modulates the ANS and endocrine activity to suppress the activation of sympathetic nervous system. This review focused only on quantitative physiological indicators obtained by measuring instruments for more objective evaluation excluding subjective assessments such as surveys or behavioral indicators such as task performance ability. As a result, studies measuring EEG, fNIRS, BP, HR, skin temperature, and pupil light reflection were reviewed.

6.1. Relaxation and inducing nervous stability of the CNS

The aroma VOCs entering the nose stimulate the limbic system and the hypothalamus through olfactory nerves. This stimulation activates the central neurotransmitter, which can lead to emotional states such as relaxation and sedation. The effects of nervous stability of the CNS and inducing relaxation were measured with instruments such as EEG and fNIRS.

EEG was mentioned in 28 of 33 studies (Table 3). EEG responses were summarized based on observed

Table 3. Summary of the literature on EEG changes associated with fragrance inhalation

Olfactory stimulus	Number of participants	Participant age	Exposure concentration (%)	Exposure duration (min)	EEG response pattern	Reference
<i>G. jasminoides</i>	60	20 ~ 26	-	10	α : $\uparrow$ β : $\downarrow$	19
<i>C. indicum</i> essential oil	10	-	-	3	α : $\uparrow$ β : $\downarrow$ γ : $\downarrow$ θ : $\uparrow$	20
Lavender, rosemary, jasmine, clary sage oil					α : $\uparrow$ β : $\uparrow$	
Rose, geranium, peppermint oil	23	31.13 $\pm$ 11.42	-	1.5	α : NS β : $\uparrow$	118
Eucalyptus, chamomile, thyme oil					α : $\uparrow$ β : NS	
d-Borneol essential oil	24	21.92 $\pm$ 2.47	-	10	α : NS β : $\uparrow$ δ : NS θ : NS	
d-Camphor essential oil	24	21.29 $\pm$ 1.46	-	10	α : $\uparrow$ β : NS δ : NS θ : NS	119
Methyl eugenol essential oil	24	22.71 $\pm$ 2.97	-	10	α : NS β : NS δ : $\uparrow$ θ : $\uparrow$	
Methyl chavicol essential oil	24	23.17 $\pm$ 3.60	-	10	α : $\uparrow$ β : NS δ : NS θ : NS	
Basil essential oil	12	20s	1	3	α : NS β : NS γ : NS	80
Oriental lily (Siberia)	31	-	-	5	α : $\downarrow$ β : $\uparrow$	74
<i>M. spicata</i>	60	-	-	10	α : $\uparrow$ β : $\downarrow$	70
Oriental lily (Oriental hybrids)	60	21.38 $\pm$ 1.89	-	10	α : $\uparrow$ β : $\downarrow$	22
Lavender, bergamot essential oil	50	20 ~ 65	0.5	5	α : $\downarrow$ θ : $\uparrow$	107
Lavender essential oil	20	23.25 $\pm$ 4.52	-	7	α : $\uparrow$ β : NS θ : $\uparrow$	89
Jasmine oil	20	22.70 $\pm$ 4.27	-	7	β : $\uparrow$	91
<i>M. arvensis</i> essential oil	20	20 ~ 30	-	-	α : $\uparrow$ γ : $\downarrow$	53

Table 3. Continued

Olfactory stimulus	Number of participants	Participant age	Exposure concentration (%)	Exposure duration (min)	EEG response pattern	Reference
White musk aromatic oil	10	23.2 ± 2.3	-	10	α: NS β: ↓ γ: ↑ θ: NS	89
Citronella oil	20	18 ~ 29 21.40 ± 2.76	-	20	α: ↑ β: ↑	92
<i>C. faberi</i>	25	24.5 ± 2.23	-	3	α: ↑ β: ↑ γ: NS θ: NS	76
<i>Rosa hybrida</i> 'Hera'	19	54.3 ± 2.98	-	3	α: NS β: ↑ γ: NS θ: NS	
<i>C. lanceolata</i> essential oil	40	18 ~ 26	3	5	α: ↑ β: ↓ θ: ↑	45
<i>Z. jujuba</i> seeds essential oil	20	20 ~ 30	-	-	α: ↑ γ: ↑ θ: ↑	120
<i>C. citratus</i> essential oil	30	32.4 ± 2.50	-	20	δ: ↑ θ: ↑	121
<i>P. menziesii</i> , <i>L. angustifolia</i> essential oil	15	51.2 ± 12.4	2.5	15	α: ↑ β: ↓ γ: ↓	122
Lemon, rosemary essential oil	32	21.44 ± 2.05	-	30	α: NS β: NS	123
Pine oil	31	-	-	1	α: ↑ θ: ↑	124
Cypress oil			-	1	α: NS θ: NS	
Chamomile oil			-	1	α: NS θ: NS	
Lavender oil			-	1	α: NS θ: NS	
β-Caryophyllene	48	18 ~ 40	-	20	α: ↓ β: ↓ δ: ↑ θ: ↑	125
Linalool				20	α: ↑ β: ↑ δ: ↑ θ: ↑	
Citral				20	α: ↓ β: NS δ: ↑ θ: ↑	

Table 3. Continued

Olfactory stimulus	Number of participants	Participant age	Exposure concentration (%)	Exposure duration (min)	EEG response pattern	Reference
<i>C. camphora</i>	43	18 ~ 25	1	5	α : NS β : ↓ γ : ↓ δ : NS θ : ↑	44
<i>M. azedarach</i> flower	100	24.4 ± 6.79	-	20	α : NS β : NS γ : NS δ : NS θ : NS	77
d-Camphor	24	21.29 ± 1.46	-	8	α : ↑	126
Copaiba oil	22	18 ~ 40	10%	20	α : NS β : ↓	88
Lemongrass essential oil	30	32.4 ± 2.50	-	20	α : ↑ β : ↑	127

–: not measured, NS: not significant

directional changes and only waves showing significant variation were reported to improve clarity and interpretability. EEG records and amplifies electrical activity from the scalp by reflecting the activity of neurons in the cerebral cortex. It offers a non-invasive and relatively objective approach that enables continuous monitoring.¹⁰⁷ Each EEG range is responsible for different physiological mechanisms.¹⁰⁸ The lowest frequency, delta (δ) wave (0.5–4 Hz)¹⁰⁹ is involved in physical recovery and cognitive functions such as maintaining synaptic homeostasis and clearing toxic proteins. When it is reduced, it causes sleep disorders and decreased work efficiency.¹¹⁰ Theta (θ) wave (4–7 Hz) is observed in the early stages of sleep and are associated with activity in the frontal lobe and hippocampus, which is involved in memory enhancement and generation of new ideas.²⁰ Alpha (α) wave (8–12 Hz) activates the parasympathetic nervous system in meditation or rest and is observed in peaceful states such as meditation and rest.^{111,112} Beta (β) wave (16–31 Hz) is associated with improved cognitive performance and improved academic achievement, but excessive activation may be a major cause of anxiety in subjects.^{20,113} The highest

frequency, gamma (γ) wave (36–90 Hz) is activated during complex learning or information processing and decreases during sedation due to reduced BP and HR.^{20,114}

fNIRS was mentioned in 1 of 33 studies. fNIRS measures changes in brain activity based on the optical properties of near-infrared light neurovascular coupling system, which is the change in brain blood flow and oxygen supply according to neuroelectric activity. When neurons in a specific brain region are activated, oxygen is consumed during neural metabolism, oxygenated hemoglobin (HbO) in the blood surrounding this region is depleted, and deoxygenated hemoglobin (HbR) in this area is increased. Reversal of concentration indicates decreased brain activity. The fNIRS has the advantages of fewer restrictions due to its high resistance to motion artifacts and allowing for versatile research.^{115,116} Ohata *et al.* (2022)¹¹⁷ investigated the effects of *C. iyo* and *C. junos* essential oils inhalation on brain activity in 32 subjects using fNIRS. After *C. iyo* inhalation, the HbO concentration was significantly reduced throughout the prefrontal cortex which indicates a cerebral sedation effect. After *C. junos* inhalation, the HbO concentration was significantly

increased throughout the frontal lobe which suggests that it could affect task performance and improve working memory.

Because exposure conditions varied widely across studies, a formal meta-analysis was not feasible. Concentrations ranged from undiluted essential oils to diluted preparations (0.1–10 %) and inhalation times ranged from 2 to 30 min. These differences should be considered when interpreting the reported physiological responses.

6.2. Relaxation and homeostasis recovery of ANS

The ANS maintains the stability of the cardiovascular system by regulating the heart and blood vessels through the sympathetic and parasympathetic nerves.¹²⁸ Exercise, excitement or stress activates the sympathetic nervous system leading to adrenaline secretion, muscle tension, and increases in BP and HR.¹²⁹ Conversely, when at rest, the parasympathetic nervous system is more dominantly activated.¹³⁰ These effects were evaluated by measuring BP, HR, GSR, finger temperature (FT), and pupillary light reflex measurements.

Measurements of systolic blood pressure (SBP), diastolic blood pressure (DBP), HR and HRV after aroma VOCs inhalation have been reported in 25 of 33 studies (Table 4). Their reduction may conclude that aroma VOCs inhalation helps the body reach a state of relaxation and sedation due to an increase in parasympathetic nervous system activity.^{131,132} However some studies showed different results, which suggest that response patterns can vary depending on various factors such as fragrance type, concentration, exposure time, and subject characteristics.

GSR was mentioned in 4 of 33 studies and FT was mentioned in 3 of 33 studies. They can measure phenomena occurring in the sweat glands of the fingers and palms, which are sensitive to the sympathetic nervous system. Sweating increases due to sympathetic nerve activation under stressful situation which results in elevated GSR^{133,134} and decreased FT due to vasoconstriction.^{74,117,125,135} Conversely blood vessels dilate under calm state which results in elevated FT and decreased GSR. Cai *et al.* (2025),¹⁹ Zhang *et al.*

(2024),⁷⁰ Bu *et al.* (2025)²² measured a significant decrease in GSR after inhalation of *G. jasminoides*, *M. spicata*, and oriental lily (Oriental hybrids). Ohata *et al.* (2022)¹¹⁷ measured a significant increase in FT upon inhalation of *C. iyo* and *C. junos* essential oils. Meanwhile Pan *et al.* (2011)⁷⁴ observed a significant increase in GSR and FT after inhalation of oriental lily (Siberia). In most experiments, a decrease in GSR and an increase in FT were observed. This suggests that inhalation of aroma VOCs may induce a calming effect. However, the differing results observed across studies likely reflect substantial heterogeneity in study design including differences in odor source and concentration, exposure duration (ranging from 2 to 30 min across reviewed studies), subject characteristics such as age and aroma preference, and the specific physiological endpoint and measurement timing. These sources of variability precluded a formal meta-analysis in the present review, and the observed response patterns should therefore be interpreted as indicative rather than conclusive.

Pupillary light reflex measurement was mentioned in 1 of 33 studies. It is a suitable parameter for evaluating changes in ANS activity caused by odor stimuli.¹³⁶ The miosis rate increases when the sphincter muscle is regulated by the parasympathetic nervous system or the dilator muscle is regulated by the sympathetic nervous system. This means that the fragrance affects the ANS that dominates the parasympathetic nervous system.¹³⁷ Ohata *et al.* (2022)¹¹⁷ measured a significant increase in the pupillary light reflex after inhalation of *C. iyo* and *C. junos* essential oils. This suggests that the aroma VOCs of the two essential oils influenced ANS, particularly the parasympathetic branch, promoting physiological stability.

7. Research Limitations

In the GC/MS-based volatile profiling of aroma compounds, one important limitation is that physiological outcomes related to stress relief or sedation may vary even for the same compound depending on individual aroma preferences or previous memories associated

Table 4. Summary of the literature on ANS changes associated with fragrance inhalation

Olfactory stimulus	Number of participants	Participant age	SBP ^{a)}	DBP ^{b)}	HR ^{c)}	HRV ^{d)}	Reference
Yuzu synthetic fragrances	34	22.1 ± 1.4	-	-	↓	-	42
<i>G. jasminoides</i>	60	20 ~ 26	↓	↓	↓	NS	19
<i>C. indicum</i> essential oil	10	-	↓	↓	↓	-	20
Lavender, rosemary, jasmine, clary sage, rose, geranium, peppermint, eucalyptus, chamomile, thyme oil	23	31.13 ± 11.42	↓	-	-	-	118
d-Borneol	24	21.92 ± 2.47	↑	NS	↑	-	119
d-Camphor	24	21.29 ± 1.46	↓	NS	↓	-	
Methyl eugenol	24	22.71 ± 2.97	↓	NS	↓	-	
Methyl chavicol	24	23.17 ± 3.60	↓	NS	↓	-	
Basil essential oil	12	20s	↓	↓	↓	-	80
Oriental lily (Siberia)	31	-	↓	NS	NS	-	74
Oriental lily (Oriental hybrids)	60	21.38 ± 1.89	↓	↓	↓	↓	22
<i>Ch. Obtuse</i> , <i>C. japonica</i> , <i>Th. dolabrata</i> , <i>C. glaucophylla</i> , <i>C. camphora</i> , <i>S. album</i> , <i>C. atlantica</i> , <i>S. verticillata</i>	30	-	-	-	-	↑	21
<i>C. zizanioides</i> root	18	20 ~ 28	-	-	-	↑	71
<i>C. iyo</i> , <i>C. junos</i> essential oils	32	20 ~ 24	-	-	-	-	117
<i>M. spicata</i>	60	-	↓	↓	↓	↑	70
<i>C. aurantium</i> oil, rose water	23	23 ~ 61	-	-	NS	↑	138
Jasmine oil	20	22.70 ± 4.27	↓	↓	↓	-	89
White musk aromatic oil	10	23.2 ± 2.3	-	-	-	↑	89
Citronella oil	20	21.40 ± 2.76	↓	NS	↓	-	92
<i>C. lanceolata</i> essential oil	40	18 ~ 26	↓	↓	↓	↑	45
<i>A. truncatum</i> leaf	120	19.5 ± 1.2	NS	NS	NS	-	68
<i>C. deodara</i> leaf	15	51.2 ± 12.4	↓	↓	↓	-	122
<i>P. menziesii</i> , <i>L. angustifolia</i> essential oil	15	51.2 ± 12.4	-	-	↓	↑	
Pine oil	31	-	-	-	-	↑	124
Cypress oil			-	-	-	NS	
Chamomile oil			-	-	-	NS	
Lavender oil			-	-	-	NS	
β-Caryophyllene	48	18 ~ 40	NS	NS	↓	-	125
Linalool			↓	NS	↓	-	
Citral			NS	NS	↓	-	
<i>C. camphora</i>			NS	↓	NS	-	
<i>M. azedarach</i> flower	100	24.4 ± 6.79	NS	NS	NS	-	77
d-Camphor	24	21.29 ± 1.46	↓	↓	↓	-	126
Copaiba oil	22	18 ~ 40	NS	NS	↓	-	88

a) SBP: Systolic blood pressure; b) DBP: Diastolic blood pressure; c) HR: Heart rate; d) HRV: Heart rate variability; NS: Not significant; -: not measured

with fragrance. This variability makes it difficult to directly compare findings across studies. In addition, it is challenging to establish robust relationship between identified VOCs and their reported physiological effects related to structure and activity. Furthermore,

inconsistency in GC/MS profiling conditions, data processing workflows, and physiological assessment methods reduce inter-study comparability.

To address these limitations, future research should pursue standardization along three key areas. First,

GC/MS profiling conditions could be more consistently defined through adoption of consensus protocols specifying SPME fiber type, extraction temperature (recommended: 40–60°C for monoterpenes), extraction time (30–60 min), desorption temperature (220–250°C), and column type (dual-column approach for isomer resolution). Second, physiological measurement would benefit from clearer reporting guidelines including the number of participants, age range, inhalation concentration (expressed as headspace VOCs concentration in $\mu\text{g}/\text{m}^3$ where possible), and exposure duration and baseline correction procedures. Third, the integration of advanced data analysis approaches such as machine learning would substantially enhance the discriminative and predictive capabilities of future volatile profiling studies. It would include convolutional neural networks (CNNs) applied to EEG spectral data and random forest models for VOCs-physiological response mapping which represents a promising direction for identifying compound associated with specific biomarkers of stress relief and sedation. Recent studies in related fields such as breathomics-based disease biomarker discovery have shown that AI-assisted GC/MS data interpretation is feasible which suggests similar potential in aroma VOCs research. These standardization efforts, combined with data-sharing initiatives and open-access VOCs databases are expected to accelerate the translation of analytical chemistry findings into aroma therapeutic applications based on evidence.

8. Conclusion

This review was conducted based on the PRISMA approach and systematically summarizes the chemical diversity of aroma VOCs, GC/MS-based analytical methods, and their stress-relieving and sedative effects in humans. Aroma VOCs are classified into various groups according to their chemical structures and functions, reflecting their chemical diversity, and each group has the potential to elicit olfactory as well as stress-relieving and sedative responses. Comprehensive volatile profiling of aroma compounds has mainly been performed using GC/MS, with HS-

SPME recognized as a commonly applied pretreatment technique. SPME represents a solvent free sample preparation method. Its applicability has been demonstrated through coupling with analytical instruments such as GC and HPLC for the analysis of organic compounds, and its use is expected to expand further. To investigate stress-relieving and sedative effects in humans, studies measuring EEG, fNIRS, and BP or HR were examined. EEG enables the assessment of α , β , γ , δ , and θ waves and has been used to determine whether inhalation of fragrances induces relaxation. Brain activity changes can be evaluated through fNIRS, and fragrance stimulation has been shown to induce hemodynamic alterations in the brain. Measurements of BP and HR have reported reductions in SBP, DBP and HR following essential oil inhalation, suggesting that aroma exposure promotes stress-relieving and sedative physiological states. Some studies have reported inconsistent findings, indicating that responses may vary depending on aroma type, concentration, exposure duration, and participant characteristics. The heterogeneity in analytical platforms, sampling conditions, exposure protocols, and physiological endpoints across the reviewed studies precluded a formal meta-analysis; this represents a key limitation of the current evidence base. Future studies should implement standardized methodology that addresses: (1) controlled and reproducible GC/MS profiling conditions including SPME fiber selection, extraction temperature/time, and GC column specifications; (2) consistent physiological measurement protocols with pre-defined endpoints and baseline controls; and (3) systematic documentation of individual differences such as scent preference and prior experience. Addressing these priorities will enable more rigorous cross-study comparisons and support the practical translation of aroma VOCs research into evidence-based stress relief and sedation applications.

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