



## Sensory-directed flavor analysis reveals the improvement in aroma quality of summer green tea by osmanthus scenting

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### ABSTRACT

Flower scenting is an effective way to enhance the aroma of green tea (GT), including those osmanthus scented green tea (OSGT). However, the mechanism of aroma enhancement by scenting is still unclear. Here, the volatiles of GT, OSGT, and osmanthus were detected by GC-MS. The total volatile content of OSGT was significantly increased compared to GT, with the flowery and coconut aromas enhanced. Furthermore, 17 of 139 volatiles were responsible for the enhancement by GC-olfactometry and their absolute odor activity values (OAVs). Aroma recombination, omission and addition experiments showed that dihydro- $\beta$ -ionone, (*E*)- $\beta$ -ionone, (*E*, *E*)-2,4-heptadienal, geraniol, linalool,  $\alpha$ -ionone, and  $\gamma$ -decalactone were the key aroma volatiles with flowery or coconut aromas. Additionally, the dynamics of the key volatiles (OAVs >1) from different scenting durations were analyzed, proving that the optimal duration was 6–12 h. This study provides new insight into the mechanism of aroma formation during OSGT production.

### 1. Introduction

Green tea is a popular drink worldwide with potential health benefits. According to the season of production, green tea can be divided into spring, summer and autumn varieties. The flavor quality of green tea harvested in spring is better than that harvested in autumn, while the flavor of green tea harvested in summer is the worst. In the summer, tea plants are exposed to high temperatures and strong light, resulting in the accumulation of caffeine and tea polyphenols, compounds related to carbon metabolism. As a result of these conditions, the content of amino acids, compounds related to nitrogen metabolism, decrease; at the same time, metabolic pathways associated with volatile accumulation are inhibited. Seasonal differences result in a much less coordinated taste and aroma of summer green tea compared to spring tea (Guo, Ho, Schwab, & Wan, 2021; Huang et al., 2023; Ji et al., 2018; Shao et al., 2022). This phenomenon causes an increasing amount of summer tea resources to be abandoned, resulting in the waste of tea resources. Therefore, improving the flavor quality of summer green tea is a basic research topic in the tea industry.

Aroma is an important factor in evaluating the flavor of green tea. The charming, flower-like aroma properties are considered the hallmark

of high-grade green tea. To further improve green tea's floral-like properties, a number of flowers have been used to scent the tea, including jasmine, osmanthus, and roses. Among these flowers, osmanthus is loved by consumers because of its rich aroma, leading to osmanthus scented green tea (OSGT), which has become popular among consumers. For OSGT, raw green tea (GT) is used as a *chapi*, absorbing the fragrance of osmanthus flowers so that it retains the original tea flavor but is also endowed with the fragrance of osmanthus flowers (An et al., 2022). Generally, osmanthus can be divided into three types: thunbergii, latifolius and aurantiacus. The color of thunbergii is orange–yellow, and the contents of  $\alpha$ -ionone,  $\beta$ -ionone and  $\gamma$ -decalactone in thunbergii are higher, with a sweet and rich fragrance. The latifolius is yellowish-white or yellowish, and the content of (*E*)- $\beta$ -ocimene in latifolius is higher, with a clear and mild fragrance. The aurantiacus is orange–red, and it lacks compounds such as  $\alpha$ -ionone and  $\beta$ -ionone; the aroma of this type is inferior, and although it has a general osmanthus aroma, the sweet aroma is not as strong as that of thunbergii, and the fresh aroma is not as strong as that of latifolius (Cai et al., 2014; Sheng et al., 2020). During scenting, the GT absorbs volatiles in osmanthus flowers by capillary coagulation due to their loose porous structure. This process promotes the accumulation of a large

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number of floral-like volatiles, giving the GT an obvious osmanthus fragrance. Previous evidence shows that the main components of OSGT include palmitic acid, linolenic acid, (*Z*)-pyranoid linalool oxide, dehydrodihydro-3-epoxy-violyl alcohol, and methyl hexadecanoate (Guo et al., 2021). In fact, the aroma of OSGT is affected by many volatiles, and the different components and proportions of these volatiles affect the aroma intensity and persistence of OSGT. However, some unsolved problems continue to hinder the development of OSGT, that is, which volatiles are absorbed from flowers in large quantities during the scenting process and which volatiles are important contributors to the formation of the charming aroma of OSGT.

Therefore, the aim of this study was to (a) explore the differential volatiles in GT, OSGT, and osmanthus by GC-MS; (b) identify and verify the key aroma volatiles that contribute to OSGT by sensory-directed flavor analysis; and (c) explore the accumulation pattern of these key aroma volatiles during scenting. The results of the study provide a theoretical basis for improving the osmanthus scenting process and the quality of OSGT, thus expanding the resource utilization of summer green tea.

## 2. Materials and methods

### 2.1. Sample preparation and collection

Lu'an Guapian green teas were harvested in July 2022 from a tea plantation located in Jinzhai County, Anhui Province. The finished green teas were immediately sent to cold storage at a temperature of 0 °C until scenting. Fresh osmanthus was picked in September 2022 from osmanthus trees in Jinzhai County, Anhui Province. After picking, the fresh osmanthus flowers were sorted to remove impurities such as stalks and leaves. The subsequent processing of the green tea made from Osmanthus flowers is shown in Fig. 1. Specifically, green teas and fresh osmanthus flowers were mixed by weight in a 2:1 ratio, and the scenting process lasted for 12 h. This process was carried out at room temperature. Upon completion of the scenting, the mixture of osmanthus and green teas was separated, from which the osmanthus was removed, leaving pure green teas. The obtained green teas were dried at a

temperature of 80 °C to obtain OSGT with a moisture content of 6%. Fresh osmanthus, GT, and OSGT were collected as shown in Fig. 1, and tea samples from the scenting process, which lasted for 3, 6, 9, and 12 h, were also collected. These samples were taken in triplicate, sealed in sample bags and subsequently transported to the laboratory. All samples were freeze-dried prior to the experiment and subsequently analyzed for volatiles.

### 2.2. Chemicals and materials

Linalool, (*E*)- $\beta$ -ionone, geraniol, (*E,E*)-2,4-heptadienal, dihydroactinolide, myrcene, (*Z*)-jasmone, heptanal, octanal, decanal, 2,5-dimethylpyrazine, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol,  $\gamma$ -decalactone,  $\delta$ -decalactone, 1-octen-3-ol, (*Z*)-4-heptenal, methyl salicylate, nonanal, phenylacetaldehyde, methyl salicylate, indole, 3-methyl-1-butanol, and (*Z*)-linalool oxide (pyranoid) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China); dihydro- $\beta$ -ionone, linalool oxide II,  $\alpha$ -ionone, linalool oxide I, hexanal, 3-ethyl-2,5-dimethylpyrazine, and 2,3-diethyl-5-methylpyrazine were obtained from Shanghai Aladdin Bio-Chem Technology Co., (Shanghai, China). All reference odorants had a purity >95% for GC. Dichloro-methane (CH<sub>2</sub>Cl<sub>2</sub>) was obtained from Tedia Corporation (Fairfield, OH, USA). Anhydrous ethanol (CH<sub>3</sub>CH<sub>2</sub>OH) was obtained from Damao Chemical Reagent Factory (Tianjin, China). Sodium chloride (NaCl) and anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Distilled water was obtained from Wahaha Group Company (Hangzhou, China). Ethyl capanoate was obtained from Yuanye Biotechnology Co., Ltd. (Shanghai, China). N-Alkanes (C<sub>6</sub>–C<sub>40</sub>) were obtained from Sigma–Aldrich (Shanghai, China). A solid-phase microextraction (SPME) fiber manual sampling holder and divinylbenzene/carboxen/polydimethylsiloxane (50/30  $\mu$ m) microextraction fiber were obtained from Supelco (Bellefonte, PA, USA). Solvent-assisted flavor evaporation (SAFE) was obtained from Xinweier Glass Co., Ltd. (Chongqing, China). Among them, dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) was used after distillation.

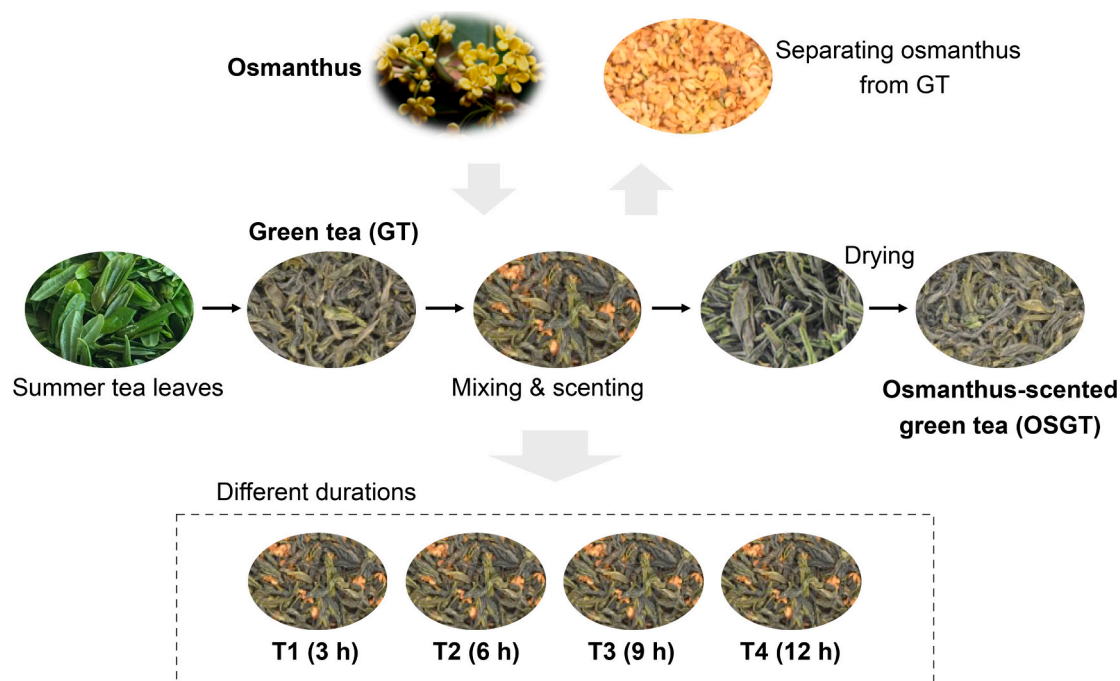


Fig. 1. The manufacturing process of osmanthus-scented green tea. Seven samples including GT, OSGT, osmanthus, T1, T2, T3, and T4 were obtained. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

### 2.3. Extraction of volatile compounds

The volatile components of the three samples were extracted using headspace solid-phase microextraction (HS-SPME) and solvent-assisted flavor evaporation (SAFE). The extraction method of SAFE better retains the original flavor of the samples and can extract the high molecular weight compounds with low volatility in the samples. SPME is easy, efficient and quick, and is suitable for extracting low molecular weight and high volatile compounds. Therefore, we combined the two extraction methods to obtain a more comprehensive aroma profile (Huang et al., 2022).

#### 2.3.1. Preparation of internal standard solution and tea infusion

For SPME, ethyl decanoate (12.50 mg) was first dissolved in anhydrous ethanol (10 mL), after which 80  $\mu$ L of the solution was dissolved in pure water (10 mL). For SAFE, ethyl decanoate (21.95 mg) was first dissolved in anhydrous ethanol (10 mL), after which 2278  $\mu$ L of the solution was dissolved in pure water (10 mL).

Tea samples (3.0 g) were accurately weighed in a conical flask, 150 mL of pure boiling water was added, and the mixture was brewed for 4 min. The tea broth was filtered through 400 mesh gauze, put into an ice-water bath, and cooled quickly to room temperature. The sample volatiles were extracted by three methods.

#### 2.3.2. Extraction of volatiles through headspace SPME

A pipette was used to measure 10 mL of tea broth in a headspace bottle with a rotor, and the SPME internal standard (4  $\mu$ L) was added and mixed thoroughly with NaCl (3.0 g) to promote the precipitation of aroma volatiles. The headspace flask was equilibrated in a constant-temperature water bath at 40 °C for 15 min and adsorbed under this water bath condition for 40 min (Huang et al., 2022; Zhang et al., 2023). Osmanthus volatiles were extracted in the same way as described above, but the ratio of osmanthus to water was changed. Specifically, 1 g of osmanthus was used and brewed with 100 mL of boiling water to simulate a 2:1 ratio by weight of green tea and osmanthus in scenting.

#### 2.3.3. Extraction of volatiles through SAFE

Internal standard (6  $\mu$ L) was added to 150 mL of tea broth and mixed thoroughly. The mixture was passed through the SAFE device under vacuum at  $10^{-3}$  Pa at an extraction temperature of 40 °C and a condensation temperature of -80 °C (liquid nitrogen). The collected SAFE fractions were thawed under tap water buffer, completely thawed, poured into a split funnel, extracted three separate times with distilled dichloromethane (30 mL), and finally dried with anhydrous sodium sulfate (until quicksand-like anhydrous sodium sulfate appeared). Finally, the extract was nitrogen-blown to 100  $\mu$ L at 25 °C in a water bath (Huang et al., 2022).

### 2.4. Detection and identification of volatile compounds

The GC-MS analysis of volatiles was performed using an Agilent system consisting of a gas chromatograph (Model 7890B) and a mass spectrometer (Model 5975B, Santa Clara, CA, USA). During detection, the aroma volatiles were separated on an HP-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu$ m, J & W, Folsom, CA, USA). Pure helium (purity >99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. The inlet temperature and injection method were 250 °C. The nonsplit mode was used. For SPME, the following procedure was employed for the GC: temperature of 40 °C for 5 min, then ramped from 40 to 180 °C at a rate of 4 °C/min. Then, the temperature was raised from 180 to 280 °C at a rate of 15 °C/min (held at 280 °C for 5 min). For SAFE, 2  $\mu$ L of distillate was injected into the GC injection port. The temperature was maintained at 40 °C for 5 min and then ramped from 40 °C to 100 °C at a rate of 5 °C/min. Then, the temperature was raised from 100 °C to 200 °C at a rate of 3 °C/min and finally to 280 °C at 20 °C/min (held at 280 °C for 5 min). For SBSE, the thermal desorption

procedure followed a previous study (Ma et al., 2021; Ma et al., 2023). The temperature was maintained at 40 °C for 5 min and then ramped from 40 °C to 100 °C at a rate of 3 °C/min. Then, the temperature was raised from 100 °C to 130 °C at a rate of 2 °C/min and finally to 250 °C at 10 °C/min (held at 250 °C for 5 min). The mass selective detector was operated in positron ionization mode with a mass scan range from  $m/z$  30 to 350 at 70 eV. The linear retention index was determined by injecting n-alkanes C6–C40 using the same running procedure.

### 2.5. Identification of aroma volatiles

#### 2.5.1. Identification and quantification of volatiles

All volatile compounds were first identified by comparison with mass spectra from the NIST17 library. The retention times (calculated using n-alkanes C6–C40) were used to calculate retention indices, which were compared with those measured under the same conditions in the NIST17 library, and volatile compounds with retention indices within  $\pm 20$  were retained. Finally, the concentrations of volatile compounds were relatively quantified by the ratio of the peak area of the compound to the peak area of ethyl arachidonate (Liu et al., 2023). Additionally, chemical standards with OAVs >1 were employed to achieve absolute quantification.

#### 2.5.2. GC-O analysis

The aroma volatiles were extracted by SBSE, an experimental method that was slightly modified from previous studies (Ma et al., 2021; Ma et al., 2023). Ten milliliters of tea broth mixed thoroughly with NaCl (3.0 g), equilibrated in a constant-temperature water bath at 40 °C for 15 min, and adsorbed for 90 min were used. GC-O analyses were performed using the equipped sniffing port (ODP 3, Gerstel, Germany), and the extracted volatiles were fed into the MS (250 °C) and sniffing port (230 °C) in a 1:1 ratio. The injection procedure was consistent with SBSE in 2. 4.

The GC-O analysis was conducted by a group of experienced evaluators (3 males and 2 females) who had been trained for up to 2 months, as described in Zhang et al. (2023). Then, GC-O analysis combined with the detection frequency method was used to analyze the aroma properties and odor intensity of the aroma volatiles (Ma et al., 2021; Wang et al., 2020). The average of the five evaluators represents the intensity of the aroma volatiles.

#### 2.5.3. OAV calculation

OAV is widely used to assess the contribution of individual flavor volatiles to the overall aroma profile of food and tea samples. Individual volatiles with an OAV value >1 are generally considered to be aroma-active compounds and therefore contribute significantly to the overall aroma of the sample (Wang et al., 2020). The OAV is calculated as the ratio of the detected concentration of each volatile to its odor threshold (OT), which is the odor threshold at which a volatile can be smelled and identified in water. The OT values for the different volatiles were taken from some early reports (Liao et al., 2020; Zhai, Zhang, Granvogl, Ho, & Wan, 2022; Zhu et al., 2015; Zhu et al., 2018).

### 2.6. Recombination, omission, and addition experiments

#### 2.6.1. Quantitative descriptive analysis (QDA)

Ethical permission, to conduct human sensory panel research, is not customary for our institution. The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research, such as no coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge, and ability to withdraw from the study at any time. Attached document is a statement signed by the participants.

QDA was used to analyze the characteristics of the tea broths and to assess the differences between the broths. A total of 12 trained assessors

participated in this experiment (7 females and 5 males). The evaluated tea products and chemical standards were safe for consumption. Representative tea samples (3.0 g) with a tea-water ratio of 1:50 were placed in an evaluation cup, filled with boiling water, and covered with a lid for 4 min. Twenty-five milliliters of tea broth was transferred to a 50 mL brown sniffing bottle, and the evaluator described the aroma attributes of the tea broth and screened out the aroma descriptors that appeared in the top 6 frequencies. Were flowery, roasted, cooked soybean-like, coconut, green, and chestnut-like. The corresponding standards and foods for the 6 aroma attributes were flowery ((*E*- $\beta$ -ionone), roasted (3-ethyl-2,5-dimethylpyrazine), coconut ( $\gamma$ -decalactone), green (hexanal), cooked soybean-like (cooked soybean), and chestnut-like (cooked chestnut) (Zhang et al., 2023). Evaluators used a 4-point scale (0–1 for weak odors; 1–2 for moderate; 2–3 for strong; 3–4 for very strong).

### 2.6.2. Recombination experiments

Recombination experiments were performed on the aroma volatiles with OAV > 1 that we screened in GT (16) and OSGT (18) to validate the qualitative and quantitative results of the aroma volatiles. The aroma specimens used were dissolved in anhydrous ethanol and then dissolved in 25 mL of deionized water at the concentrations detected in the tea broths (Zhang et al., 2023). The concentration of ethanol was below OT

(990,000  $\mu\text{g/L}$ ). The recombinant samples were evaluated according to the same evaluation method of QDA.

### 2.6.3. Omission experiments

Flowery and coconut aroma were the two aroma attributes that differed most between GT and OSGT, and aroma recombination tests were conducted to verify the importance of single aroma volatiles in these two categories for the overall OSGT tea samples. The flowery and coconut aroma volatiles with OAVs > 1 were missing in the recombinant OSGT samples. A total of 11 aroma volatiles were deleted separately. Twelve trained evaluators participated in this experiment (7 females and 5 males) and triangulated samples missing one aroma volatile (three randomly coded vials, two OSGT recombinant sample vials, and one vial missing one aroma volatile) (Wang et al., 2024).

### 2.6.4. Addition experiments

Seven key aroma volatiles screened by the omission experiments were added to *Re*-GT according to the concentration difference between GT and OSGT. Twelve trained evaluators participated in this experiment (7 females and 5 males), who evaluated and scored the key aroma volatiles of the samples to which a single key aroma volatile had been added. Samples were evaluated and scored to calculate the contribution of a single key aroma volatile to the aroma.

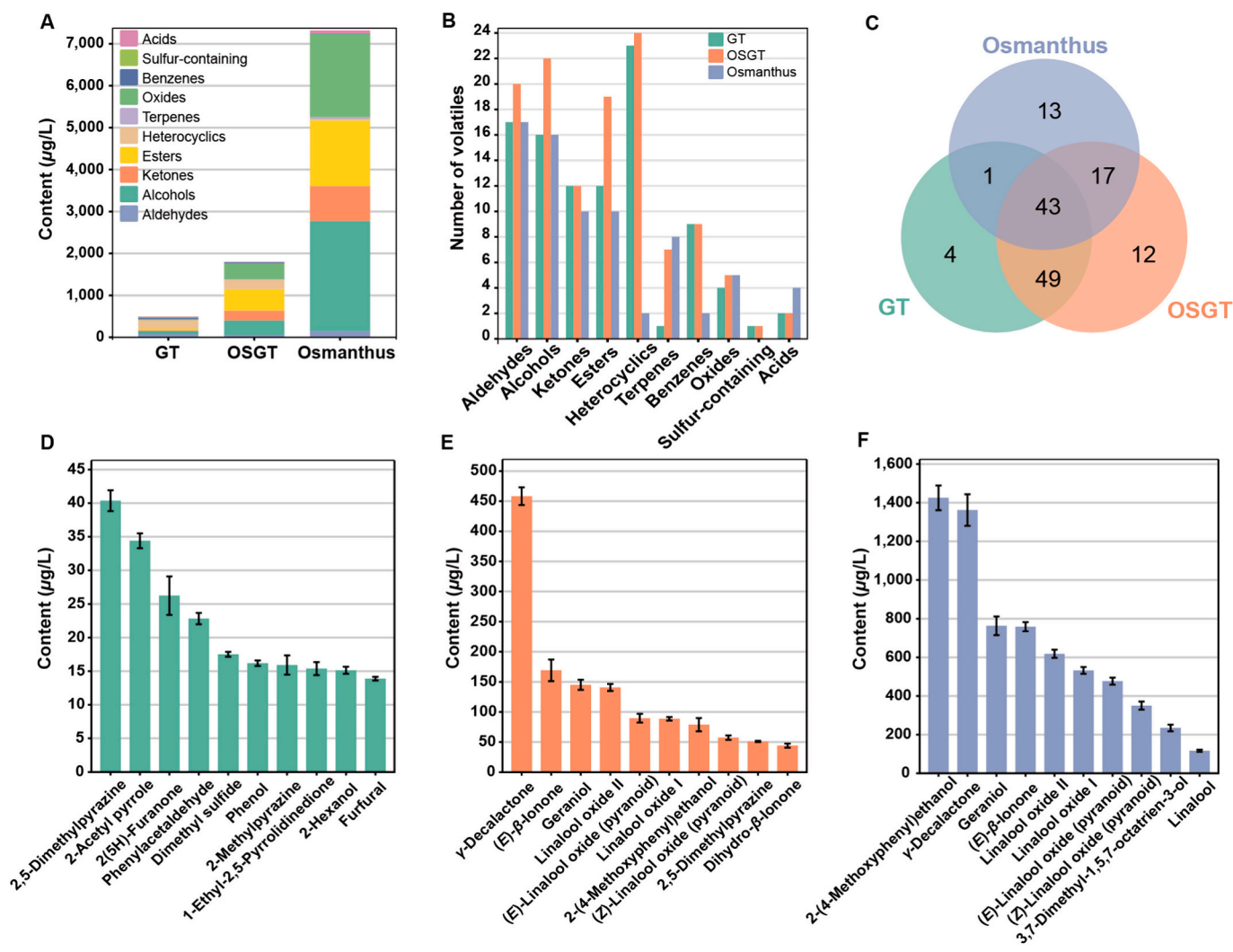


Fig. 2. (A) Total volatile compounds detected by HS-SPME-GC-MS and SAFE-GC-MS in GT, OSGT, and osmanthus. (B) Categories and numbers of volatiles detected in GT, OSGT, and osmanthus. (C) Venn diagram of volatiles detected in GT, OSGT, and osmanthus. Bar graphs of the top 10 volatiles in contents detected by HS-SPME-GC-MS and SAFE-GC-MS in (D) GT, (E) OSGT, and (F) osmanthus.



2.7. Statistical analysis

All experiments and samples analyzed were replicated at least three times, and the levels of volatile compounds detected are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed using SPSS software (IBM, Armonk, NY, USA), with *p* values <0.05 considered significant. Principal component analysis (PCA) for volatile characteristics was performed using SIMCA software (Umea, Sweden). Data were plotted by Origin (OriginLab Co., USA) and GraphPad Prism 8 software (Shen et al., 2023).

3. Results and discussion

3.1. Volatile profiles of GT, OSGT, and osmanthus

A total of 139 volatile compounds were detected by HS-SPME and SAFE (Fig. S1, Table S1). Among them, 97 were contained in GT, 121 in OSGT, and 74 in Osmanthus. The total volatile content of OSGT (1800.33 µg/L) was much higher than that of GT (495.28 µg/L) (Fig. 2A). These volatiles can be divided into 10 classes, including aldehydes, alcohols, ketones, esters, heterocyclics, terpenes, benzenes, oxides, sulfides, and acids. After scenting, the alcohols, ketones, esters and oxides were increased in OSGT, which were the main volatiles in osmanthus (Fig. 2B). Generally, alcohols and ketones are described as

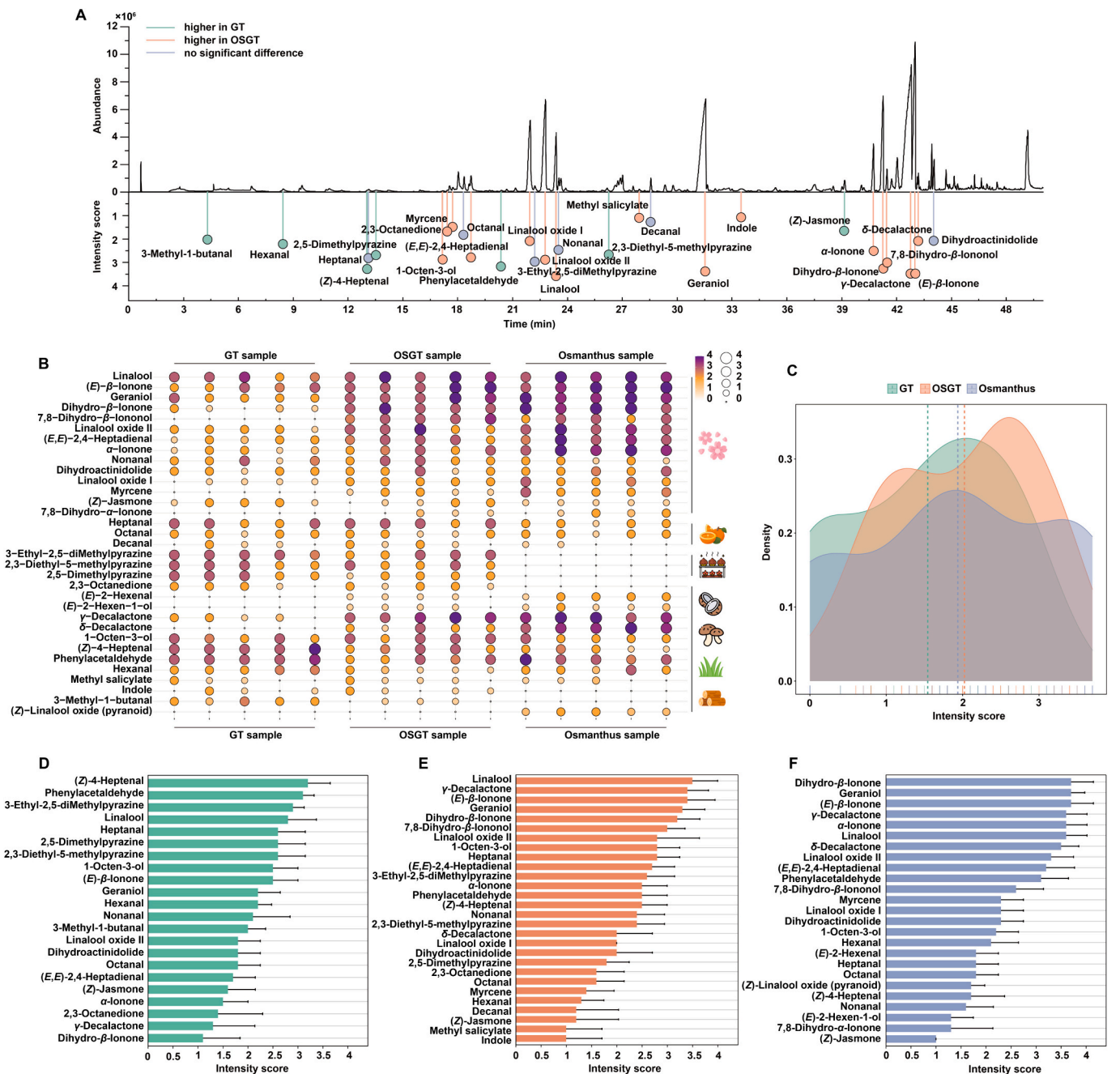


Fig. 3. (A) Total ion flow diagram of aroma volatiles was detected by SBSE-GC-O/MS in OSGT. (B) Score bubble diagram of aroma volatiles was detected by SBSE-GC-O/MS in GT, OSGT, and osmanthus. (C) Density plot of aroma intensity (AI) in GT, OSGT, and osmanthus. The volatiles with aroma intensity (AI) over 1 in (D) GT, (E) OSGT, and (F) osmanthus.

having a flowery aroma, aldehydes possess citrus and green flavors, esters provide sweetness and coconut aroma, and pyrazines are associated with baking and caramel aromas (Meng et al., 2024; Zhai et al., 2022). Therefore, osmanthus is an ideal flower for enhancing the aroma of tea, which is conducive to improving the aroma quality of GT.

A total of 92 volatiles were found simultaneously in GT and OSGT, and 4 and 12 were detected separately in GT and OSGT, respectively (Fig. 2C). Forty-three of the 74 volatile compounds in osmanthus were detected in both GT and OSGT, 17 volatiles were common only to osmanthus and OSGT, and 13 were present only in osmanthus. The top 10 volatiles in GT (Fig. 2D), OSGT (Fig. 2E), and Osmanthus (Fig. 2F) are listed. These dominant volatiles showed considerable variation among the three samples. Compared with GT, volatiles in OSGT are significantly increased in relative content, which is attributed to adsorption from osmanthus flowers. For example, the relative content of  $\gamma$ -decalactone, the most abundant volatile in OSGT, changed significantly from  $2.44 \pm 0.11$  to  $458.27 \pm 14.67$   $\mu\text{g}/\text{kg}$ . (*E*)- $\beta$ -ionone, dihydro- $\beta$ -ionone, geraniol, and linalool are the main contributing compounds in osmanthus black tea, and they are also characteristic volatiles of osmanthus flowers. The black tea absorbed the fragrance of osmanthus, giving it a rich floral aroma (Meng et al., 2024). The significantly increased content of these volatiles may give OSGT its unique flowery flavor.

### 3.2. Identification and quantitation of aroma volatiles

GC-O was conducted to select the aroma volatiles from the volatile profiles. During GC-O, individual aroma volatiles are resolved under different temperature conditions and thus perceived by the assessor (Barba, Beno, Guichard and Thomas-Danguin, 2018). Under these conditions, interactions between aroma volatiles can be avoided, thus ensuring the authenticity of the aroma volatiles, and critical aroma volatiles are not missed. Accurate quantitative analysis using GC-O/MS and external standards ensures the accuracy of the original results.

A total of 32 aroma volatiles were detected in GT and OSGT (Fig. 3A). Among them, 14 aroma volatiles in OSGT were significantly higher than those in GT, and 5 in OSGT were significantly lower than those in GT. These aroma volatiles were described as flowery, citrus, roasted, coconut, and others (Fig. 3B). (*Z*)-Linalool oxide was only perceived in osmanthus, and 7,8-dihydro- $\beta$ -iononol, myrcene, 7,8-dihydro- $\alpha$ -ionone, (*E*)-2-hexenal, (*E*)-2-hexen-1-ol, and  $\delta$ -decalactone were both recognized in OSGT and in osmanthus (Fig. 3B). 7,8-dihydro- $\beta$ -iononol, myrcene, and 7,8-dihydro- $\alpha$ -ionone were floral aroma volatiles derived from osmanthus. The intensity of these aroma volatiles was increased in OSGT through adsorption, enhancing the floral intensity of OSGT.  $\delta$ -decalactone, which has a coconut attribute, was another aroma volatiles that distinguishes GT from OSGT, enhancing the depth of OSGT's aroma profile (Porcelli & Steinhaus, 2022). Fig. 3C further shows the aroma intensity of these three samples, which is based on the aroma intensity (AI) of the 33 aroma volatiles. OSGT had a higher aroma intensity (2.02) than osmanthus (1.94) and GT (1.53), indicating aroma enhancement through the scenting process. The volatiles with AI over 1 in GT (Fig. 3D), OSGT (Fig. 3E), and osmanthus (Fig. 3F) were sorted. (*Z*)-4-heptenal (AI = 3.2; fish oil-like) and phenylacetaldehyde (AI = 3.1; honey-like) were the main aroma volatiles affecting the quality of GT. (*Z*)-4-heptenal not only possesses the property of fish oil but also the aroma property of boiled beans, which is the main contributor to the aroma of beans (Xiao et al., 2022). Linalool (AI = 3.5; flowery, citrus-like),  $\gamma$ -decalactone (AI = 3.4; coconut-like, sweet), (*E*)- $\beta$ -ionone (AI = 3.4; flowery, violet-like), geraniol (AI = 3.3; rose-like, citrus-like), dihydro- $\beta$ -ionone (AI = 3.2; flowery, osmanthus-like), and 7,8-dihydro- $\beta$ -iononol (AI = 3.0; flowery) were strongly aromatic in OSGT. Dihydro- $\beta$ -ionone (AI = 3.5; flowery, osmanthus-like), geraniol (AI = 3.7; rose-like, citrus-like), (*E*)- $\beta$ -ionone (AI = 3.7; flowery, violet-like),  $\gamma$ -decalactone (AI = 3.6; coconut-like, sweet),  $\alpha$ -ionone (AI = 3.6; flowery, violet-like), linalool (AI = 3.6; flowery, citrus-like),  $\delta$ -decalactone (AI = 3.5; coconut-like, citrus-like), linalool oxide II (AI = 3.3; flowery,

woody), (*E,E*)-2,4-heptadienal (AI = 3.2; flowery), and phenylacetaldehyde (AI = 3.1; honey-like) were strongly aromatic in osmanthus. These differences in the aroma intensity of the active volatiles may explain why the three samples differ greatly in their overall aroma.

The 30 volatiles of 32 aroma volatiles were further absolutely quantified by using standard compounds (Table S2), except for the unavailable standards of 7,8-dihydro- $\beta$ -iononol, 7,8-dihydro- $\alpha$ -ionone and 2,3-Octanedione. Thus, 7,8-dihydro- $\beta$ -iononol, 7,8-dihydro- $\alpha$ -ionone and 2,3-Octanedione were expressed as relative contents. For the 14 aroma volatiles described as flowery aroma, all significantly increased ( $p < 0.05$ ) from GT to OSGT, except for nonanal (Fig. 4A). Interestingly, 7,8-dihydro- $\beta$ -iononol, 7,8-dihydro- $\alpha$ -ionone, and myrcene were not found in GT, but all reached high levels in OSGT. The introduction of these active volatiles may have a considerable effect on the flavor of OSGT and promote the formation of an osmanthus-like flavor. The contents of other flowery aroma volatiles were also significantly increased ( $p < 0.05$ ) after scenting, including linalool (1.85–18.18  $\mu\text{g}/\text{L}$ ), (*E*)- $\beta$ -ionone (0.74–52.79  $\mu\text{g}/\text{L}$ ), geraniol (4.95–107.69  $\mu\text{g}/\text{L}$ ), dihydro- $\beta$ -ionone (0.33–29.61  $\mu\text{g}/\text{L}$ ), linalool oxide II (6.17–87.21  $\mu\text{g}/\text{L}$ ), (*E,E*)-2,4-heptadienal (1.29–8.61  $\mu\text{g}/\text{L}$ ),  $\alpha$ -ionone (0.24–8.04  $\mu\text{g}/\text{L}$ ), dihydroactinidolide (6.08–10.89  $\mu\text{g}/\text{L}$ ), linalool oxide I (1.07–24.54  $\mu\text{g}/\text{L}$ ), and (*Z*)-jasmone (3.74–5.44  $\mu\text{g}/\text{L}$ ). Notably, there were 2 coconut-like volatiles with significant increases ( $p < 0.05$ ), including  $\delta$ -decalactone (0.80–3.65  $\mu\text{g}/\text{L}$ ) and  $\gamma$ -decalactone (2.43–301.78  $\mu\text{g}/\text{L}$ ). The unique aromas of these two volatiles may contribute to the enhancement of OSGT's sweet and flowery aromas. A previous study confirmed that the addition of  $\gamma$ -decalactone significantly enhances the sweetness perception of sucrose solutions (Barba et al., 2018).

### 3.3. Screening of aroma volatiles responsible for aroma enhancement

The volatiles with higher content do not indicate a larger contribution. OAV is used to assess and compare the contribution of volatiles. Nineteen of 33 volatiles were selected with OAVs  $> 1$  in GT and OSGT (Table S2). Among them, 10 volatiles were described as having a flowery aroma (Fig. 5A), and the other 9 had other aroma attributes (Fig. 5B). The OAVs of dihydro- $\beta$ -ionone, geraniol,  $\alpha$ -ionone, dihydroactinidolide, (*E*)- $\beta$ -ionone, (*E, E*)-2,4-heptadienal, linalool, (*Z*)-jasmone, myrcene,  $\gamma$ -decalactone and  $\delta$ -decalactone were significantly increased ( $p < 0.05$ ) from GT to OSGT. Dihydro- $\beta$ -ionone is naturally found in strawberry, apricot and osmanthus and has a typical woody and osmanthus aroma. Dihydro- $\beta$ -ionone is also known as the king of Osmanthus and is the main aroma compound in Osmanthus oil with a mellow, sweet and fresh cedar aroma. Because of its unique flavor, it is widely used in the food and beverage industry (Zhang et al., 2023, Xu et al., 2019). The dihydro- $\beta$ -ionone had a lower threshold value of 0.001  $\mu\text{g}/\text{L}$ , and both GT and OSGT had larger OAV values, with the OAV of OSGT being 90.63 times higher than that of GT (Fig. 5C), followed by (*E*)- $\beta$ -ionone (71.34),  $\alpha$ -ionone (33.97), geraniol (21.74), linalool (9.85), and (*E, E*)-2,4-heptadienal (6.67). Geraniol, (*E*)- $\beta$ -ionone, and linalool were the major compounds affecting the overall aroma of Keemun black tea (Xu et al., 2023). Interestingly, the coconut volatiles  $\gamma$ -decalactone and  $\delta$ -decalactone yielded significant increases ( $p < 0.05$ ) from GT to OSGT (Fig. 5B), with increases of 124.19 and 4.55, respectively (Fig. 5C).  $\gamma$ -Decalactone and  $\delta$ -decalactone have the aroma attributes of coconut milk and sweetness, but they have rarely been reported in tea.  $\gamma$ -Decalactone has a high content in osmanthus, which may be conducive to the formation of the characteristic flavor of OSGT (Cai et al., 2014, Meng et al., 2024). A total of 14 volatiles related to flowery and coconut aroma were thus selected, with the total content showing that osmanthus (1792.26  $\mu\text{g}/\text{L}$ )  $>$  OSGT (564.34  $\mu\text{g}/\text{L}$ )  $>$  GT (33.47  $\mu\text{g}/\text{L}$ ) (Fig. 6A). These volatiles are the key to distinguishing the flavor of OSGT from GT, especially in flowery and coconut aromas (Fig. 5A).

Teas typically contain hundreds of volatiles, but not all volatiles play a dominant role in the aromatic qualities of tea (Hu et al., 2022).  $\gamma$ -Decalactone, geraniol, (*E*)- $\beta$ -ionone, linalool, and  $\delta$ -decalactone were

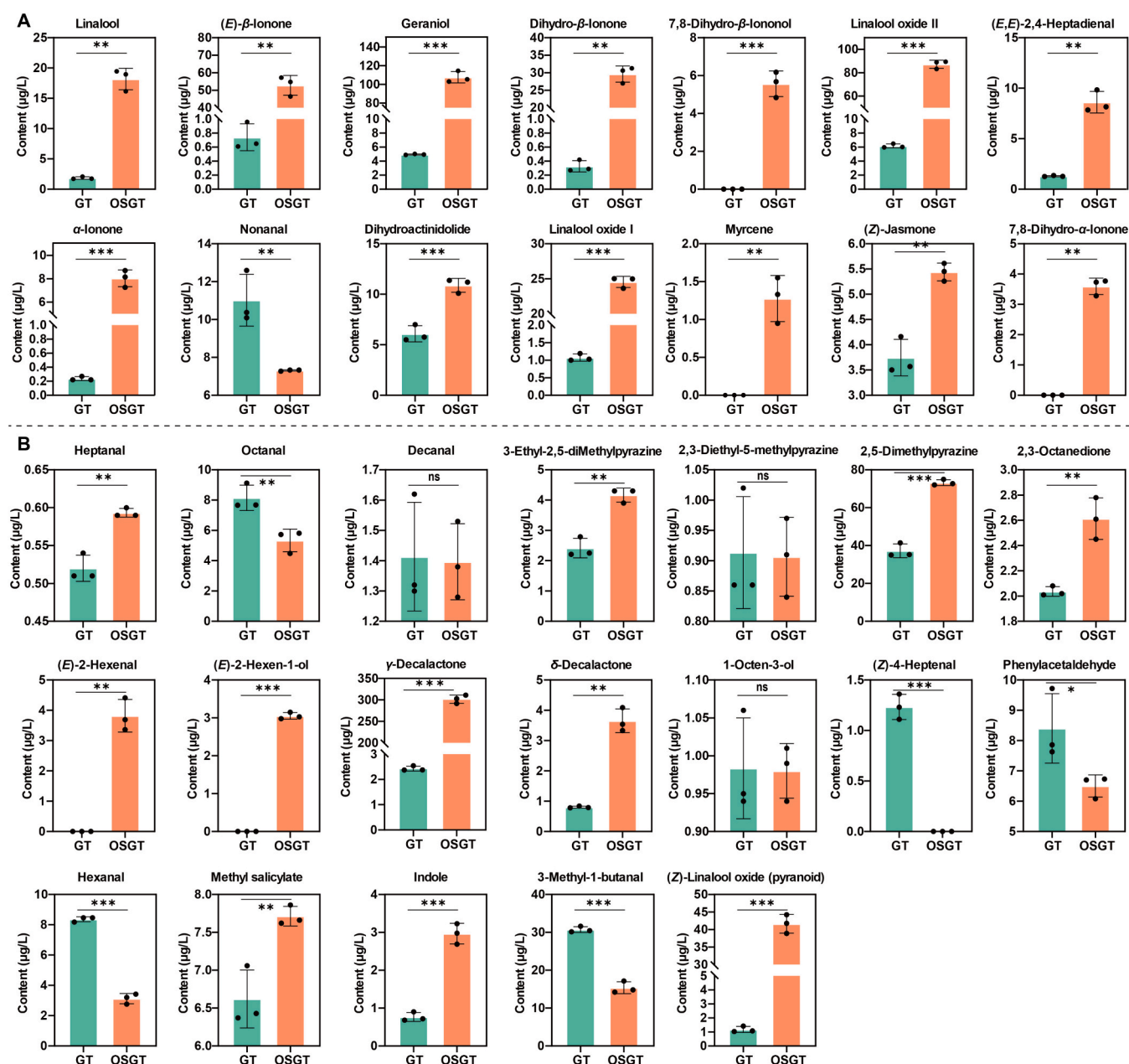


Fig. 4. Box plots of absolute content of identified aroma volatiles in GT, and OSGT, and box plots of relative content of 7,8-dihydro- $\beta$ -ionone, 7,8-dihydro- $\alpha$ -ionone, and 2,3-octanedione.

ranked in the top 5 (Fig. 6A), which were all absorbed from osmanthus. Dihydro- $\beta$ -ionone, (E)- $\beta$ -ionone,  $\gamma$ -decalactone, (E, E)-2,4-heptadienal, and geraniol were ranked in the top 5 for OAV (Fig. 6B). (E)- $\beta$ -ionone, linalool, dihydro- $\beta$ -ionone and 2-ethyl-3,5-dimethylpyrazine are the characteristic volatiles of Wuyi rock tea and are responsible for the formation of the 'rock flavor' of Wuyi rock tea (Guo, Ho, Schwab, & Wan, 2021). The high OAV of dihydro- $\beta$ -ionone may also play a key role in the formation of the special flavor of OSGT.

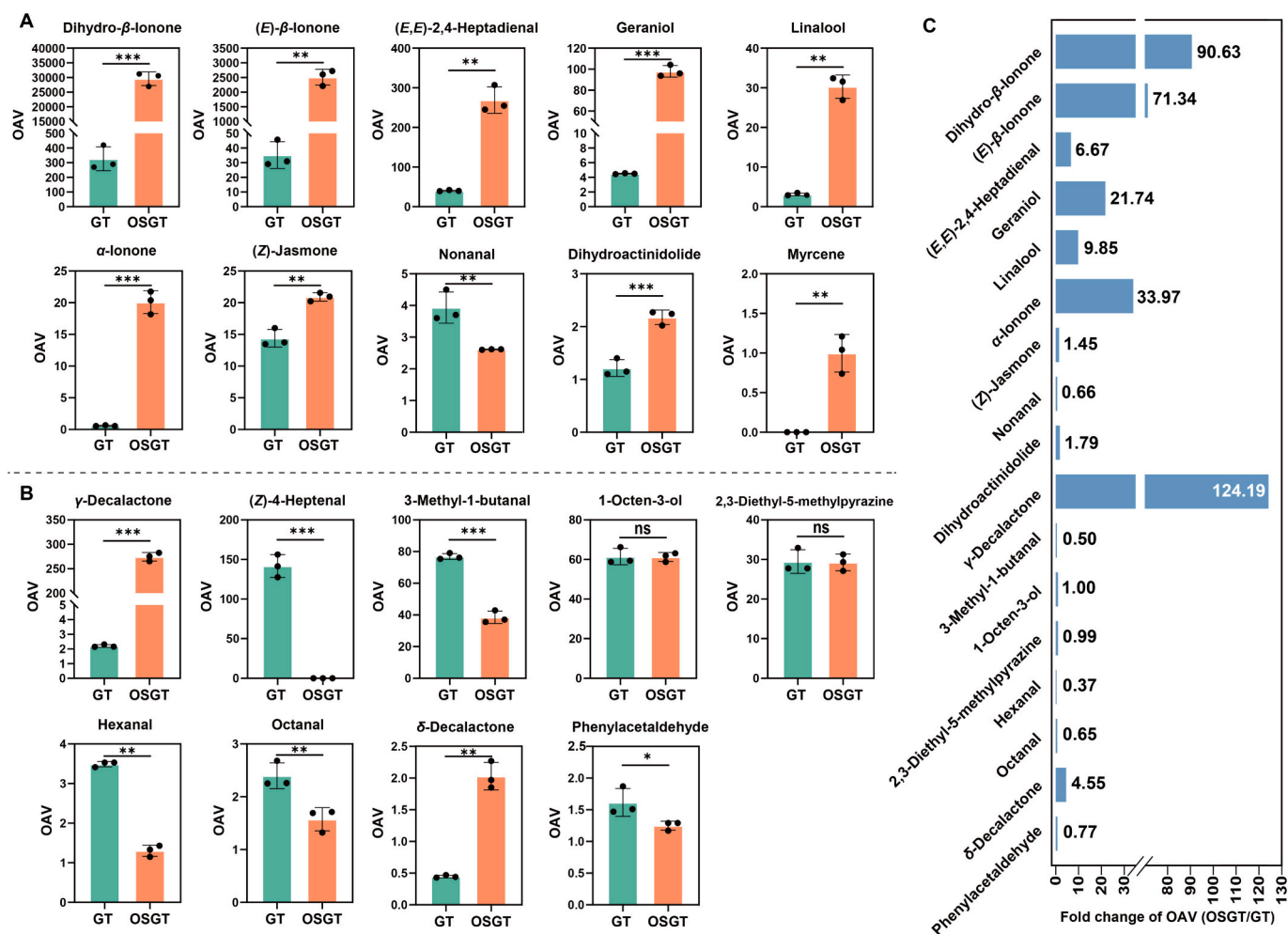
### 3.4. Verification of key aroma volatiles

The trained evaluation panelists gave characteristic attributes that matched the samples by sensory evaluation, and six descriptors were summarized and filtered based on the frequency of occurrence: flowery, roasted, cooked soybean-like, coconut, green, and cooked chestnut-like (Fig. S2A). The contributions of the above volatiles to the aroma were

further verified by aroma recombination, omission, and addition experiments.

**Aroma recombination and omission experiments.** In total, 16 and 18 responsible aroma volatiles were selected in GT and OSGT, respectively. Therefore, these 16 and 18 responsible aroma volatiles were added to deionized water as recombination samples at their true concentrations in the tea samples. Twelve trained panelists evaluated the sensory differences between the recombination (Resample) samples and the original samples. The similarity between the recombination and GT reached 82.75% (3.31/4), proving the successful recombination of all the responsible aroma volatiles in GT (Fig. S2B). The roasted score in the original GT sample was 2.03, while the score in the aroma recombination sample was only 1.63. Among the added responsible aroma volatiles, only 2,3-diethyl-5-methylpyrazine has the aroma attribute of roasted, and the reason for the higher score of roasted in GT may be that there may be an enhancement effect among the other roasted aroma





**Fig. 5.** (A) Box plots of flowery volatiles with OAVs >1 in GT, and OSGT. (B) Box plots of volatiles with OAVs >1 for other aroma attributes in GT, and OSGT. (C) Histogram of OAV ratios for GT and OSGT.

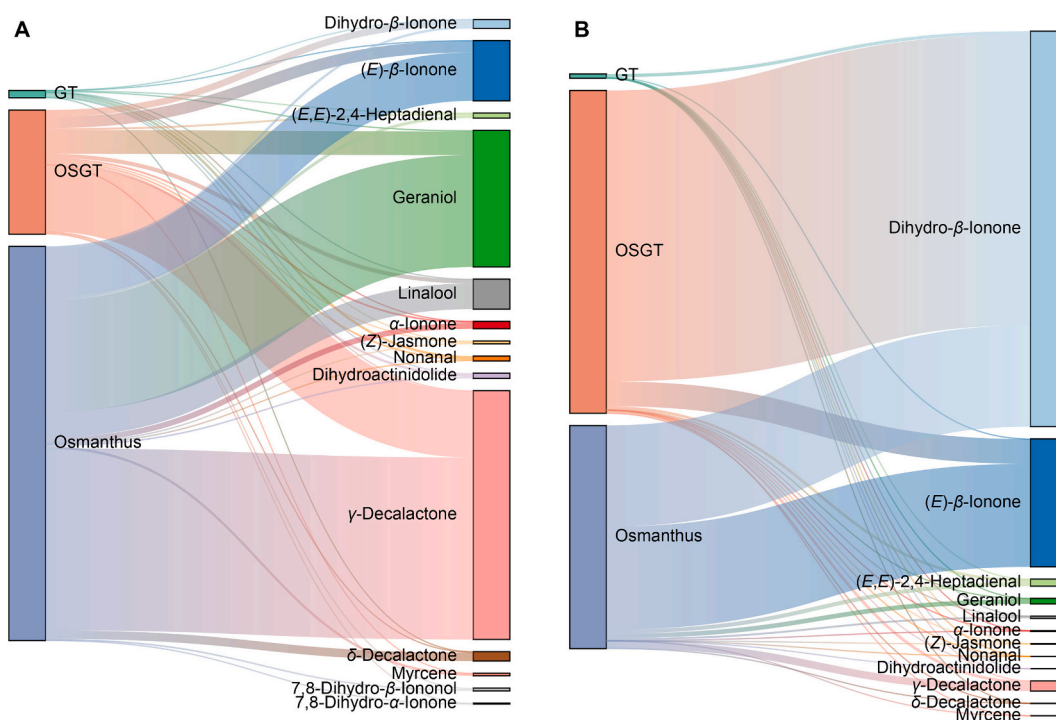
volatiles. There may be an enhancing effect. The similarity between the recombination OSGT and OSGT reached 88.54% (3.54/4), demonstrating the successful recombination of all responsible aroma volatiles in OSGT (Fig. S2C). For the single aroma attribute, the recombination of OSGT and OSGT for roasted (1.14/1.62, 70.47%), green (0.32/0.45, 70.37%), cooked chestnut-like (0.83/1.09, 76.45%), flowery (3.47/3.35, 103.58%), and coconut (2.49/2.37, 105.06%) were successfully recombined.

Flowery and coconut are the key aroma volatile attributes that distinguish GT from OSGT. omission experiments were designed (Table S3). Among the 11 aroma omissions, 7 key aroma volatiles were accurately identified; they were dihydro-β-ionone ( $p < 0.001$ ), (*E*)-β-ionone ( $p < 0.001$ ), (*E, E*)-2,4-heptadienal ( $p < 0.01$ ), geraniol ( $p < 0.001$ ), linalool ( $p < 0.001$ ), α-ionone ( $p < 0.05$ ), and γ-decalactone ( $p < 0.001$ ). The aroma omission experiments effectively demonstrated that dihydro-β-ionone, (*E*)-β-ionone, (*E, E*)-2,4-heptadienal, geraniol, linalool, and α-ionone are the key aroma volatiles for the flowery aroma attributes of OSGT. Geraniol, with flowery and rose odors, is the main aroma volatile in black tea, and (*E*)-β-ionone, with a violet aroma, is an important aroma volatile that constitutes the special flavor of Pu-erh tea (Lv et al., 2012; Xiao et al., 2017). It has been shown that geraniol and (*E*)-β-ionone had sweetness similarity perceptions. The odor-taste interaction enhanced the sweetness of black tea infusion under the condition of consistency between taste and smell (Yu, Liu, Zhang, Luo, & Zeng, 2021). γ-Decalactone is the key aroma volatile for the coconut milk aroma attribute in OSGT and is responsible for the difference in

coconut attributes between GT and OSGT.

**Aroma addition experiments.** The aroma addition experiments were employed to further quantify the contribution of single key aroma volatiles. The six key aroma volatiles selected with flowery aroma attributes were added to each of the six groups, and they all contributed to the enhancement of the flowery aroma attributes of Re-GT (Table S4); among them, the two groups with the addition of dihydro-β-ionone and linalool achieved significant enhancement ( $p < 0.05$ ). The addition of dihydro-β-ionone increased the Re-GT score from 1.94 to 3.10, and the addition of Re-GT + α-ionone increased the Re-GT score from 1.94 to 2.74. The addition of the other four groups had corresponding aroma enhancements with (*E*)-β-ionone of 26.72%, (*E, E*)-2,4-heptadienal of 20.45%, geraniol of 22.85%, and α-ionone of 13.96%. γ-Decalactone is the coconut aroma of interest for the volatiles, and the score of coconut aroma increased from 1.32 to 2.69 in the group with the addition of γ-decalactone only, an increase of 103.91%. It is noteworthy that in the group to which only γ-decalactone was added, his score was higher than that of Re-OSGT (2.49), suggesting that γ-decalactone is the major contributor to coconut aroma. The addition of all the key aroma volatiles from the flowery and fruity aroma categories to Re-GT resulted in a score of 3.37 for the flowery aroma and 2.41 for the coconut aroma, which increased by 73.54% for the flowery aroma and 82.45% for the coconut aroma. The group that added all the key aroma volatiles was compared with Re-OSGT (flowery: 3.47) (coconut aroma: 2.49), and there was not much difference between them. The aroma addition experiments also corroborated the accuracy of the aroma omission experiments, thus





**Fig. 6.** (A) Sankey diagrams on the absolute content of flowery aroma volatiles and coconut aroma volatiles in GT and OSGT, Sankey diagrams on the relative content of 7,8-dihydro- $\beta$ -iononol and 7,8-dihydro- $\alpha$ -ionone. (B) Sankey diagrams on the OAVs of flowery aroma volatiles and coconut aroma volatiles in GT and OSG.

demonstrating that dihydro- $\beta$ -ionone, (*E*)- $\beta$ -ionone, (*E, E*)-2,4-heptadienal, geraniol, linalool, and  $\alpha$ -ionone were the main causes of the differences in the flowery aroma of the two samples, and  $\gamma$ -decalactone was the main cause of the coconut aroma between GT and OSGT.

### 3.5. Dynamics of aroma volatiles of OSGT during scenting

Fig. S3A shows the total content of aroma volatiles for samples scented for 0 h, 3 h, 6 h, 9 h, and 12 h. The total volatile content in OSGT increased continuously with increasing aroma addition time and reached the maximum value after 12 h. In addition, a principal component analysis was performed to characterize the volatiles in the samples (Blasco et al., 2015; Granato, Santos, Escher, Ferreira, & Maggio, 2018). As shown in Fig. S3B, the tea samples with different scenting durations showed a clear distinction. The tea samples with 0 h and 3 h of scenting were in the positive semiaxis of PC1, and the tea samples with 6 h, 9 h and 12 h of scenting were in the negative semiaxis of PC1. This is consistent with the results of Fig. S3A.

The absorption of aroma volatiles is a dynamic process. During scenting, the content of key aroma volatiles of tea samples at 3 h increased rapidly (Fig. S4) and tended to stabilize from 6 to 12 h, and the overall content of aroma reached a maximum at 12 h (Fig. S4). Regarding the dynamic changes in the aroma volatiles with flowery aroma, the content increased significantly ( $p < 0.05$ ) at 6 h, and the content of aroma volatiles reached the maximum value at 12 h. In the comprehensive analysis, it was speculated that there may be a critical point for a better scenting time from 6 to 12 h. Overall, this study is the first to characterize the key aroma volatiles in OSGT, mainly adsorbed from osmanthus. Through sensory-assisted flavor analysis, we identified key volatiles responsible for the floral and sweet aromas, which all showed enrichment patterns during the scenting process. Nonetheless, this is a preliminary study. On the one hand, the aroma profile of green tea (also known as *Chapi* in Chinese) is influenced by various factors, such as cultivation measures and processing techniques (Wang et al., 2024). Therefore, in-depth exploration of the adsorption patterns of characteristic volatiles in osmanthus by different green teas is a basic

topic for the future. On the other hand, how green tea adsorbs the characteristic volatiles from osmanthus, and the deeper mechanisms explaining this specific adsorption phenomenon are also worth being further explored.

## 4. Conclusion

The aim of this study was to evaluate the improvement in the aroma quality of summer green tea with osmanthus scenting. Our results showed that the total volatile content of OSGT was significantly increased compared to that of GT, and the flowery and coconut aromas were enhanced as determined by a sensory panel. Furthermore, 17 of 139 volatiles were responsible for the enhancement of flowery and coconut aromas by GC-olfactometry and their OAVs based on absolute quantitation, including dihydro- $\beta$ -ionone, (*E*)- $\beta$ -ionone, (*E, E*)-2,4-heptadienal, geraniol, linalool,  $\alpha$ -ionone, (*Z*)-jasmone, nonanal, dihydroactinidolide, myrcene,  $\gamma$ -decalactone, (*Z*)-4-heptenal, 3-methyl-1-butanal, hexanal, octanal,  $\delta$ -decalactone, and phenylacetaldehyde. Quantitative descriptive analyses, aroma recombination, omission and addition experiments showed that dihydro- $\beta$ -ionone, (*E*)- $\beta$ -ionone, (*E, E*)-2,4-heptadienal, geraniol, linalool,  $\alpha$ -ionone, and  $\gamma$ -decalactone were the key aroma volatiles with flowery or coconut aromas. Additionally, the dynamics of the key aroma volatiles (OAVs >1) from different scenting durations were analyzed, proving that the optimal duration was 6–12 h. This study, therefore, provides new insight into the mechanism of aroma formation during green tea scenting using osmanthus.

## Ethical statement

The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research, such as no coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge, and ability to withdraw from the study at any time. Attached is a statement signed by the participants.

## CRedit authorship contribution statement

**Yujie Wang:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Guojian Deng:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Data curation. **Lunfang Huang:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Jingming Ning:** Writing – review & editing, Writing – original draft, Validation, Supervision, Funding acquisition.

## Declaration of Competing Interest

The 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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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2024.101571>.

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