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Characterization of key volatile compounds in jasmine tea infusion with different amount of flowers

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ABSTRACT

The quality of jasmine tea is related to the volatiles of its infusion. In this study, the volatiles of jasmine tea infusion were extracted under the optimal conditions with a 50/30 μm DVB/CAR/PDMS fiber, tea/water ratio of 1:25 and extraction time of 5 min. A total of 204 volatiles were analyzed by comprehensive two-dimensional gas chromatography-quadrupole time-of-flight mass spectrometry (GC × GC-Q-TOF-MS). Twenty-five compounds were identified as the key volatile compounds by fold change (FC), orthogonal partial least squares discriminant analysis (OPLS-DA), and two-way orthogonal partial least squares analysis (O2PLS). Then optimal amount of flowers (80%–120%) was obtained by the equation describing key volatiles and quality of jasmine tea infusion. And 80% amount of flowers was more appropriate considering the production cost and more pleasant taste. This study laid a foundation for the extraction and research of volatiles of tea infusion and guided the reasonable amount of flowers to produce jasmine tea.

Introduction

China is the largest producer and consumer of tea in the world and has abundant tea products and a long history of tea culture. People also want to enjoy the cheerful feelings of tea-drinking processes (tea art/culture), in addition to the need for thirst quenching and health care (Pan et al., 2022). Usually, tea leaves are brewed, and then the tea is filtered out for people to smell the aroma of tea and taste the taste of tea, which is more in line with Chinese tea drinking habits. The covered porcelain cup, fair mug, and tea cup are the most common utensils for drinking tea in China.

Jasmine green tea (jasmine tea in short) is a typical scented tea that is reprocessed from processed green tea by absorbing the fragrance of jasmine (*J. sambac*), mainly produced in Fujian Province, Guangxi Province, Sichuan Province in China (Wu & Yang, 2021). The finished jasmine tea has been favoured since antiquity because of its characteristic "lovely fresh" flavour (Chen et al., 2016) and various beneficial health effects, such as antioxidant (Tang et al., 2021), amelioration of depression-like symptoms (Zhang et al., 2022), sedative effects on autonomic nerve and mood states (Inoue et al., 2003; Kuroda et al., 2005). Jasmine tea that was exported 6,130 tons in 2020 is one of the main agricultural commodities in international trade, widely sold to Japan, Russia, the United States, and other countries (Ito et al., 2002; Liu, 2021). In addition, with the people's yearning for healthy and quality life and the continuous development of trade globalization, the jasmine tea industry is in great potential for development.

Generally, jasmine tea is manufactured by many steps, including preprocessing the base tea, maintaining the fresh flowers, mixing the flowers with the base tea and scenting, spreading out the mixture for heat dissipation, re-heaping up and scenting, separating the flowers from the tea, heating, and cooling (Chen et al., 2016; Hara et al., 1995). The aroma freshness, concentration, purity, and persistence of jasmine tea gradually intensifies as the number of scenting processes increases, and the last round of scenting process without drying plays a significant role in improving the refreshing aroma (Zhang et al., 2023). Depending on the desired aroma intensity, some steps may be adjusted during the actual production. It is a fact, universally acknowledged, that scenting is the most important process in forming the characteristic flavour of

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jasmine tea (Shen et al., 2017). Further, the quality and amount of jasmine flowers are the most direct factors not only affecting the ability of base tea to absorb the fragrance but also changing the taste compounds and flavor substances of jasmine tea during the scenting process.

Aroma is the main criterion in the sensory evaluation of jasmine tea (Lin et al., 2013) and high-quality jasmine tea has good aroma characteristics that are fresh lovely and rich but not unpleasant. Meanwhile, the volatile metabolites in tea confer a pleasant aroma and are the core components of tea quality (Zhou et al., 2023). Therefore, identifying tea aroma has attracted great interest due to the extremely complex composition of the aromatic compounds (Feng et al., 2019). It is believed that the aroma of jasmine tea is absorbed from the fragrant components of the fresh *J. sambac* flowers by the base tea (Ito et al., 2002) and the different composition and proportion of these volatile compounds influence the concentrations and flavor types of jasmine tea (An et al., 2022). To the best of our knowledge, the aroma identification of various types of jasmine tea leaves has been thoroughly studied by simultaneous distillation extraction (SDE) and headspace solid-phase micro-extraction (HS-SPME) (Chen et al., 2016; Fang et al., 2004; Zhang et al., 2022). However, no systematic study has been carried out on the volatile compounds of jasmine tea infusion without tea leaves, which was in line with Chinese tea-drinking habits and tea-drinking culture (Fig. 1A), and the dynamic changes of the characteristic volatile compounds of jasmine tea infusion in different amount of jasmine flowers are still unclear.

This study aimed to optimize the detection method of volatile components of jasmine tea infusion, which is consistent with Chinese tea-drinking habits, and interpret the characteristic volatile compounds of jasmine tea infusion and their effects on the aroma quality of jasmine tea samples with different amount of flowers. In addition, the influence of the amount of flowers on the characteristic volatile compounds of jasmine tea infusion was explored to provide theoretical guidance for scenting technology of jasmine tea. The jasmine tea samples were scented with different amount of flowers and subjected to sensory evaluation and the acquisition method of jasmine tea infusion was optimized according to the tea-drinking habits of Chinese people. The HS-SPME and comprehensive two-dimensional gas chromatography-quadrupole time-of-flight mass spectrometry (GC × GC-Q-TOF-MS) technology was performed to determine the volatile compounds of jasmine tea infusion. The characteristic volatile compounds of jasmine tea infusion were obtained by principal component analysis (PCA), hierarchical cluster analysis (HCA), fold change (FC) and orthogonal partial least squares discriminant analysis (OPLS-DA), and their relationships with the aroma quality and aromatic characteristics were analyzed by two-way orthogonal partial least squares (O2PLS) analysis. Through the above analysis, the reasonable amount of flowers of jasmine tea can be clarified and the results can guide enterprises to improve jasmine tea processing technology.

Materials and methods

Materials

For studying the volatile compounds of jasmine tea infusion with different amount of flowers, the jasmine tea samples were produced in July 2021 according to the reported method (An et al., 2022) and named S0, S1, S2, S3, S4, S5, S6, S7, S8, S9, S10 (Table A1). All the jasmine tea samples were made from green tea (*Camellia sinensis L.*) that were

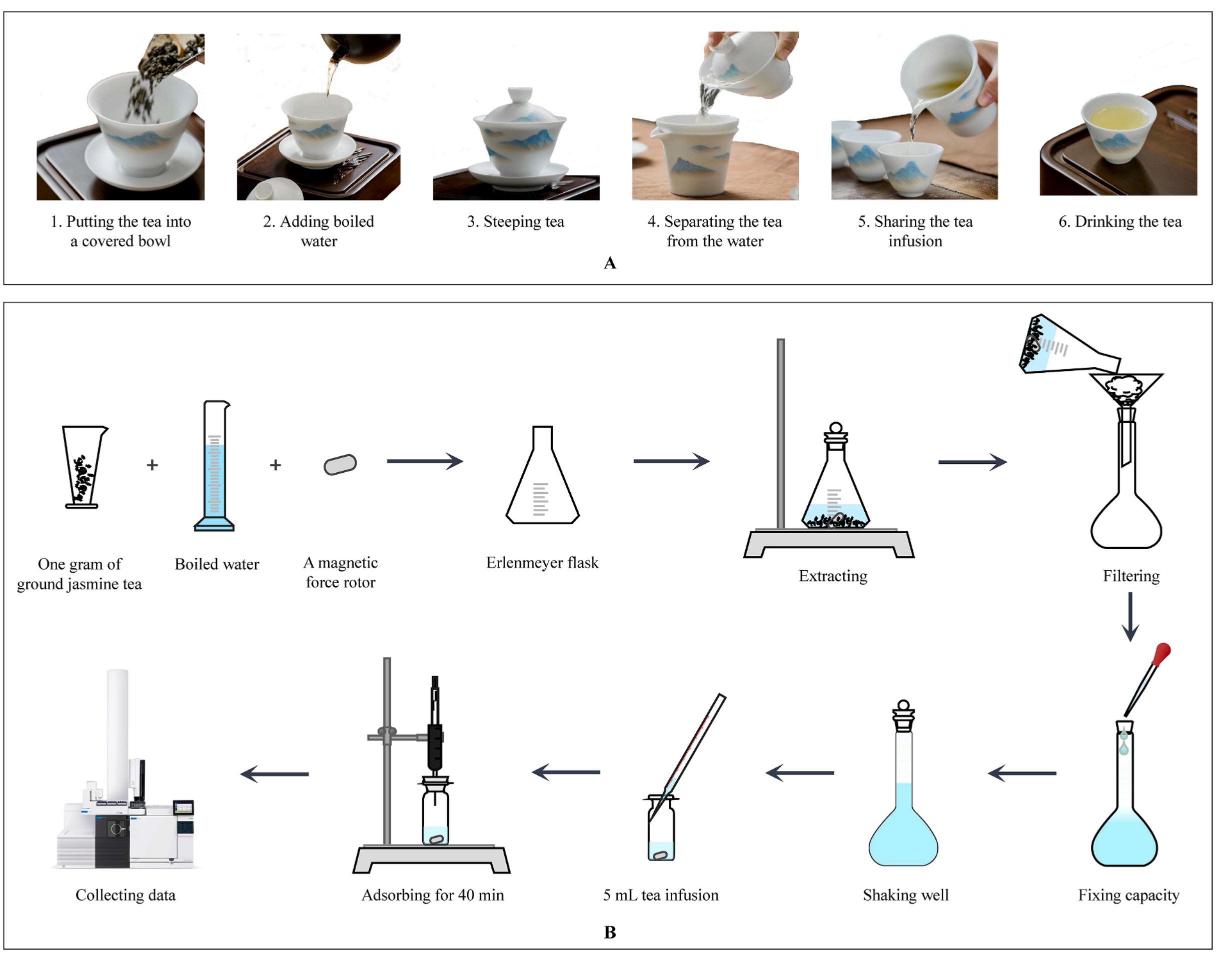

Fig. 1. Chinese tea-drinking habits (A) and volatiles extraction of jasmine tea infusion by HS-SPME (B).

produced by one bud and one leaf in spring in Shimen County, Changde City, Hunan Province, China, and jasmine flowers (*Jasminum sambac*.) that were cultivated in the plantations of Yunbiao Town, Hengzhou City, Guangxi Province, China. The jasmine tea samples were stored at −20 °C after manufacture.

Aroma quality evaluation of jasmine tea infusion

For studying the aroma quality of jasmine tea infusion, the Methodology for Sensory Evaluation of Tea (China National Standard, GB/T 23776–2018) with slight modifications was carried out to evaluate the aroma quality of jasmine tea infusion and a quantitative descriptive analysis method (Du et al., 2021) was performed to evaluate their aroma profiles, including the jasmine fragrance, grassy and dull odour. Seven expert panelists that have more than seven years of work experience on tea sensory evaluation, including three men and four women (aged 25–55 years), were trained by the same tea samples with different amount of flowers prior to scoring aroma attributes. Three g of tea sample was infused with 150 mL boiled filter-water, and the tea soup was poured out after brewing for 5 min, and then seven experts evaluated aroma quality and rated the intensity of the jasmine fragrance, grassy and dull odour in jasmine tea ranging from 0 (absent) to 10 (extremely strong). The average score evaluated by seven experts was used to represent the final aroma quality (comprehensive score) and each aroma attribute strength. The scores of each aroma attribute and comprehensive scores were shown in Table A1. All the jasmine tea samples were subjected to aroma quality evaluation in December 2021.

Analysis of volatile compounds in jasmine tea infusion

Chemicals and reagents

Ethyl decanoate (as internal standard) was provided by Sigma-Aldrich Co. Ltd. (St. Louis, Missouri, USA). A mixture of C7-C25 hydrocarbons (Sigma-Aldrich) was employed to determine linear retention indices (RIs). Ethanol (chromatographic alcohol) and Sodium chloride (analytical grade) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China).

Volatiles extraction of jasmine tea infusion by HS-SPME

All jasmine tea samples were ground using a mixer mill (SMF 2002, Supor, China) for 1.5 min at 50 Hz for volatile compounds analysis of jasmine tea infusion. All the crushed samples are mixed equally and uniformly. This is the Quality Control (QC) sample, which is carried out according to Li Jianxun's method (Li, 2014). The jasmine tea infusion was extracted using the Tea-Determination of water extracts content (China National Standard, GB/T 8305–2013) with slight modifications. The detailed operation was shown in Fig. 1B.

To explore the influence of the concentration of tea infusion on the volatile compounds of jasmine tea infusion, the QC (1.0 g) was accurately weighed (to 0.001 g) and added to a 250 mL erlenmeyer flask with a bottle stopper. Then the magnetic force rotor of 13 × 35 mm (Anpel, Shanghai, China) and boiled water (10, 25, 50, 100, and 200 mL) were added respectively according to the experimental design (Table A2). After 10 min, the tea infusion was filtered to the volume bottle and fixed up to the set volume (10, 25, 50, 100, 200 mL) respectively and named A1, A2, A3, A4 and A5 (Table A2). The 5 mL tea infusion, 50 ppm ethyl decanoate (10 μL) and a magnetic force rotor of 6 × 8 mm (Anpel, Shanghai, China) was added to a 15 mL headspace vial (Agilent, Santa Clara, CA, USA). The headspace vial cap was immediately tightened and placed on a magnetic stirring heating table (EPFO − 984TA7CHSEUA, Talboys, USA) and then stirred at 600 rpm for 10 min at 80 °C. After that, the 65 μm PDMS/DVB (Supelco, Bellefonte, PA, USA) extraction head was pushed out to a position 1 cm from the liquid surface and adsorbed for 40 min at 80 °C at an agitation rate of 600 rpm too. Then the extraction head was inserted into the inlet of the gas chromatograph for data collection and analysis, and thermal analysis was performed at 250 °C for 5 min. Three replicates were performed for each sample.

Factors, affecting jasmine tea infusion, including extraction time (3, 5, 10, 20, and 30 min) and fibers (50/30 μm DVB/CAR/PDMS, 65 μm PDMS/DVB, and 100 μm PDMS) (Table A3), were studied with reference to the above-described methods (Table A2). All the fibers were purchased from Supelco Inc. (Bellefonte, PA, USA). and preconditioned in the injection port of the gas chromatograph according to the manufacturer's instructions prior to the analysis.

Based on the above results of QC samples that extraction conditions effected on the type and content of volatile components, the optimal conditions were applied to extract the volatile components of jasmine tea infusion with different amount of flowers. The optimal conditions were shown in Section 3.2 and all the jasmine tea samples were subjected to volatile compounds detection in December 2021.

GC × GC - Q -TOF -MS conditions

For studying the volatile compounds of jasmine tea infusion, the GC × GC-Q-TOF-MS technology was performed (Yang et al., 2021). An Agilent 8890 GC-7250 Q-TOF-MS (Agilent, Santa Clara, CA, USA) was used for detecting the volatile compounds.

An Agilent HP-5MS ultra-inert capillary column (30 m × 0.25 mm × 0.25 μm) was combined with an Agilent DB-17 ms column (2.89 m × 0.18 mm × 0.18 μm) in the splitless injection mode with a high-purity helium (99.999%) flow at 1.0 mL/min with a split ratio of 20:1. The temperature of the GC injector was maintained at 250 °C. The initial column temperature was set at 40 °C, and after a 1 min hold, increased to 230 °C at the rate of 6 °C/min, then increased to 250 °C at the rate of 20 °C/min and kept for a 1 min final hold. A solid-state modulator SSM1800 (J&X Technologies, Shanghai, China) was placed between the two columns for the heating and cooling stage. The cold zone temperature was set at −51 °C and the modulation period at 4 s. The mass spectrometer conditions were as follows: electron ionization energy of 70 eV, ion source temperature of 200 °C, transmission line temperature of 280 °C, and mass scan range of 45–450 amu.

Qualitative and quantitative analysis of volatile compounds

The volatile compounds of jasmine tea infusion were analyzed qualitatively and quantitatively according to Yin's method (Yin et al., 2022).

The volatiles compounds were qualitatively analyzed by comparing the mass spectrometry data, retention index with the NIST 20 library. The *n*-alkanes (C5-C25) were used to determine the linear RIs for each compound using the following equation.

$$RI = 100 \times n + 100 \times (RT_x - RT_n)/(RT_{n+1}) - RT_n)$$

where RI represents the retention index of the volatile compound, n is the number of atoms of the previous carbon label of the compound, RT_x is the retention time of the compound, RT_n is the retention time of the previous carbon label of the compound, and RT_{n+1} is the retention time of the next carbon label of the compound (Van den Dool et al., 1963).

The concentrations of the volatiles were calculated in μg/L based on the internal standard solution using the following equation.

$$C_i = (C_{is} \times A_i)/A_{is}$$

where, C_i is the mass concentration of any component (μg/L), C_{is} is the mass concentration of the internal standard (μg/L), A_i is the chromatographic peak area of any component, and A_{is} is the chromatographic peak area of the internal standard (Wang et al., 2020).

Statistics

All analyses were repeated thrice. PCA, HCA, OPLS-DA and O2PLS were carried out to cluster samples based on the concentration of all the identified volatile compounds using Simca-p (v 14.1, MKS Umetrics AB, Umeå, Sweden). The scatter plot of differential volatile compound was performed at https://www.omicshare.com/tool. The heatmaps and upset plot were performed using the OmicStudio tools at https://www.omicstudio.cn/tool. And the other plots were drawn using Origin Pro (v2017c, Originlab Corporation, Northampton, MA, USA) and Adobe Photoshop (v 12.0.3, Adobe Systems Incorporated, California, USA).

Results and discussion

The amount of flowers affects the aroma type and aroma quality of jasmine tea infusion

According to Chinese tea-drinking habits, people will smell the aroma of jasmine tea and taste the tea when drinking jasmine tea. And the amount of flowers is the most important factor to form the fresh and heavy aroma of jasmine tea during the scenting processes (Fang et al., 2004). In this study, the aroma quality of jasmine tea infusion samples with different amount of flowers was analyzed in Table A1 and Fig. 2A. The S0 with no jasmine flowers had a higher grassy aroma (9), but no jasmine fragrance (0). With the increase of the amount of flowers, the intensity of the grassy aroma decreased rapidly, and when the amount of flowers increased to 60%, the grassy aroma did not appear in the sample (S3). However, the intensity of jasmine fragrance showed the opposite trend that the intensity of jasmine fragrance of the samples increased with the increase of amount of flowers. It is worth noting that when the amount of flowers was higher than 100%, the intensity of jasmine fragrance increases slowly, which means that the ability of tea to absorb jasmine fragrance may reach its maximum. Some studies believed that the more rounds of the scenting process the tea undergoes, the higher the quality grade (Chen et al., 2016). However, the number and cost of jasmine flowers also increase markedly with an increase in repeated rounds of the scenting process. In addition, the jasmine fragrance in S9 (180%) and S10 (200%) was identified by the panel as a dull and rotten jasmine fragrance, which is different from the fresh and lovely fragrance in other samples. Dull odour, an unpleasant aroma that is unpleasant and detrimental to the aroma quality of jasmine tea, appeared in samples with the amount of flowers more than 80% (S5, S6, S7, S8, S9 and S10). Moreover, as the amount of flowers increased, the score of dull odour showed a rising tendency, S9 (180%) and S10 (200%) had a stronger dull odour, which were 6 and 7, respectively. This trend showed that too much jasmine flower will not only cause a waste of flower resources, but also will give jasmine tea a dull odour, which is not conducive to the fresh and lovely fragrance of jasmine tea.

In fact, the aroma quality of jasmine tea is not determined by the intensity of jasmine fragrance, but is the result of the coordination of various types of aroma attributes and intensity (An et al., 2020). Therefore, this study used the comprehensive score of jasmine tea aroma to characterize the aroma quality of jasmine tea infusion (Table A1). The S0 had the lowest comprehensive score (86) because of no jasmine fragrance, S5 had the highest comprehensive score (97) and good aroma quality. Although S10 was produced with the highest amount of flowers (200%) and its jasmine fragrance intensity was the strongest, it had the lower comprehensive score (89) due to the strong dull odour caused by too much jasmine flower during the scenting process. Moreover, it is worth noting that the factors, including the varieties of jasmine, the origin of jasmine, and the picking period, will affect the aroma quality of jasmine, and further affect the aroma quality of jasmine tea, which is a direction of our future research (Wang et al., 2022).

Optimization of the volatiles extraction of jasmine tea infusion

The extraction and adsorption of volatile compounds are critical steps in the analysis. In order to determine the ideal conditions for the extraction of volatiles by SPME, the effects of the ratio of tea to water, extraction time and the types of fibers for the volatiles of jasmine tea infusion were evaluated. The surface plot of the volatile compounds of the QC sample of jasmine tea infusion analyzed by GC $\times$ GC-Q-TOF-MS

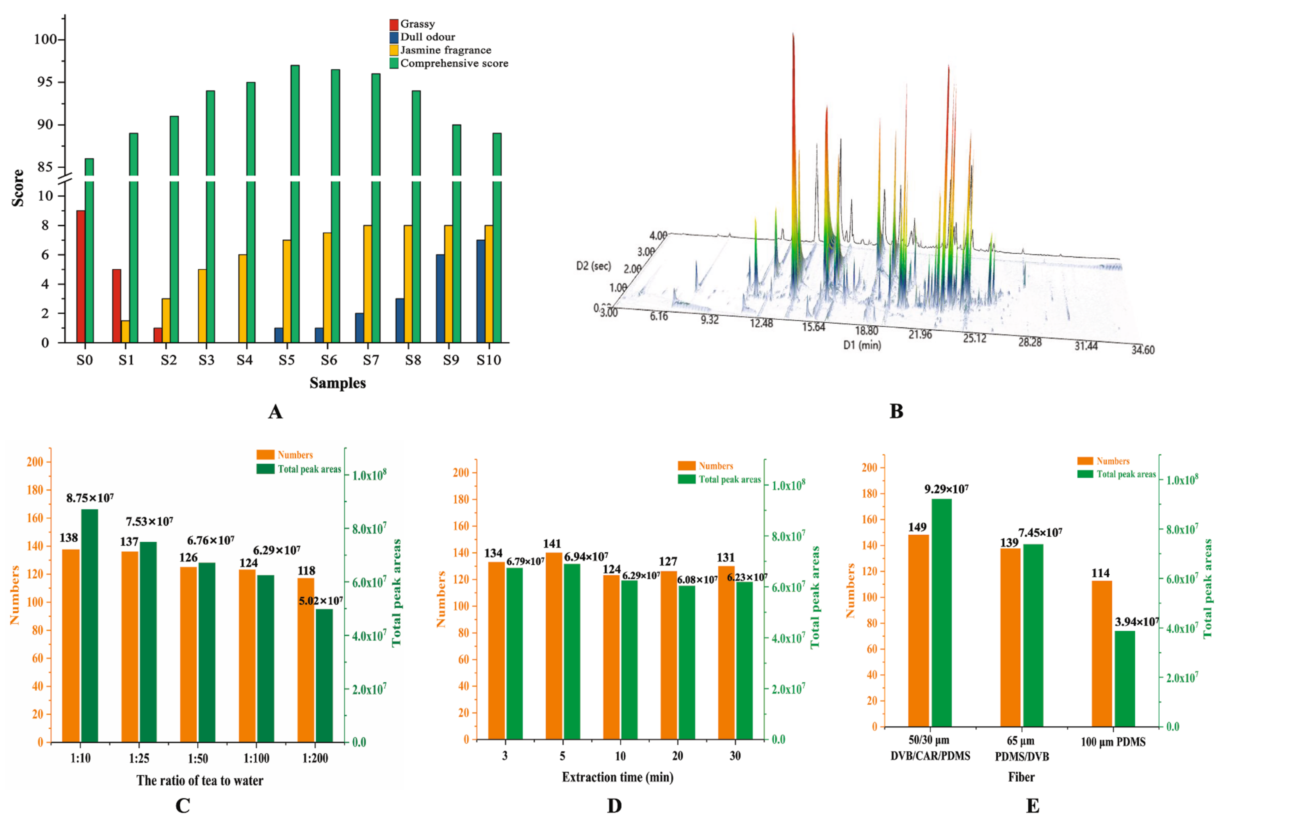

Fig. 2. Sensory evaluation of jasmine tea infusion (A) and surfact plot (B) and effects of different extraction conditions on volatile compounds. (C) The ratio of tea to water. (D) Extraction time. (E) The fibers.

was shown in Fig. 2B. There are many kinds of volatile compounds in jasmine tea infusion, and the contents were quite different. Further, the number and total peak areas of volatile compounds from the different extraction conditions were shown in Table A2, Fig. 2C, D and E.

Firstly, the proportion between tea and boiling water was screened, aimed at facilitating the operation and extracting the maximum amount of volatile compounds from jasmine tea infusion. As shown in Fig. 2C, the total peak areas of extracted volatiles decreased as the proportion between tea and boiling water increased from 1:10 to 1:200. On the other hand, the maximum number of volatile compounds was detected in the tea infusion with the 1:10 (1 3 8) and 1:25 (1 3 7) proportion between tea and boiling water, and the remaining proportions (1:25, 1:50, 1:100 and 1:200) exhibited lower number. Further, in combination with practice, the 1:25 proportion between tea and boiling water is more convenient to operate because of a more suitable volume bottle. Hence, the 1:25 proportion between tea and boiling water was selected.

Next, the extraction time of volatile compounds in the jasmine tea infusion was studied (Fig. 2D). The results proved that the extraction time was not proportional to the extraction efficiency, the number and summation of peak areas of volatile compounds were the greatest after 5 min. And this extraction time corresponded to the time of aroma quality evaluation of jasmine tea in *2.2*, which was much more reasonable.

Finally, three common silica fiber materials (50/30 μm DVB/CAR/PDMS), DVB-PDMS (65 μm PDMS/DVB), and (100 μm PDMS) were tested under the same conditions that the ratio of tea to water was 1:25 and the extraction time was 5 min to optimize the extraction fiber. As shown in Fig. 2E, the number of volatile components extracted by different types of fibers was 149 (50/30 μm DVB/CAR/PDMS), 139 (65 μm PDMS/DVB), and 114 (100 μm PDMS), respectively. Meanwhile, the total peak areas of volatile compounds demonstrated wide variation among the three fibers, with 50/30 μm DVB/CAR/PDMS showing the strongest extraction ability, followed by the 65 μm PDMS/DVB and 100 μm PDMS fibers. Thus, 50/30 μm DVB/CAR/PDMS fiber was chosen as the optimal fiber for SPME.

Consequently, the extraction efficiency to extract volatile compounds in the jasmine tea infusion was the best when using a 50/30 μm DVB/CAR/PDMS fiber, a 1:25 proportion between tea and boiling water, and an extraction time of 5 min.

Comparison of volatile compounds in jasmine tea infusion with different amount of flowers

To illuminate the volatile compounds of jasmine tea infusion, a comprehensive analysis was performed by HS-SPME/GC × GC-Q-TOF-MS. It is well known that GC × GC can provide superior chromatographic peak capacity, selectivity, and lower detection limit for the analysis of small molecules (Winnike et al., 2015), which provided a powerful technique for the analysis of volatile compounds of tea. Also, HS-SPME coupled with GC × GC had been successfully used to analyze volatile compounds of different tea samples (Yang et al., 2021; Zhu et al., 2020). In this study, a total of 204 volatiles were identified and quantified in all jasmine tea infusion samples, which included 36 kinds of alcohols, 24 kinds of aldehydes, 18 kinds of ketones, 49 kinds of esters, 11 kinds of heterocyclics, 3 kinds of phenolics and 63 kinds of hydrocarbons (Table A3). The total content of volatile compounds in jasmine tea infusion was much higher than that in base tea infusion and showed an upward trend as the amount of flowers increased (Fig. 3A). Esters were the most abundant volatile category in jasmine tea infusion followed by alcohols, hydrocarbons and heterocyclics. And most of the volatile compounds were also detected in jasmine tea (An et al., 2022) and jasmine flower (Zhang et al., 2022), which explained that the unique fragrance of the jasmine tea mainly roots in the jasmine flower and they can be dissolved in jasmine tea infusion without tea leaves.

To obtain a preliminary overview of similarities and differences among the jasmine tea infusion with different amount of flowers, PCA and HCA analyses were carried out based on the identified volatile

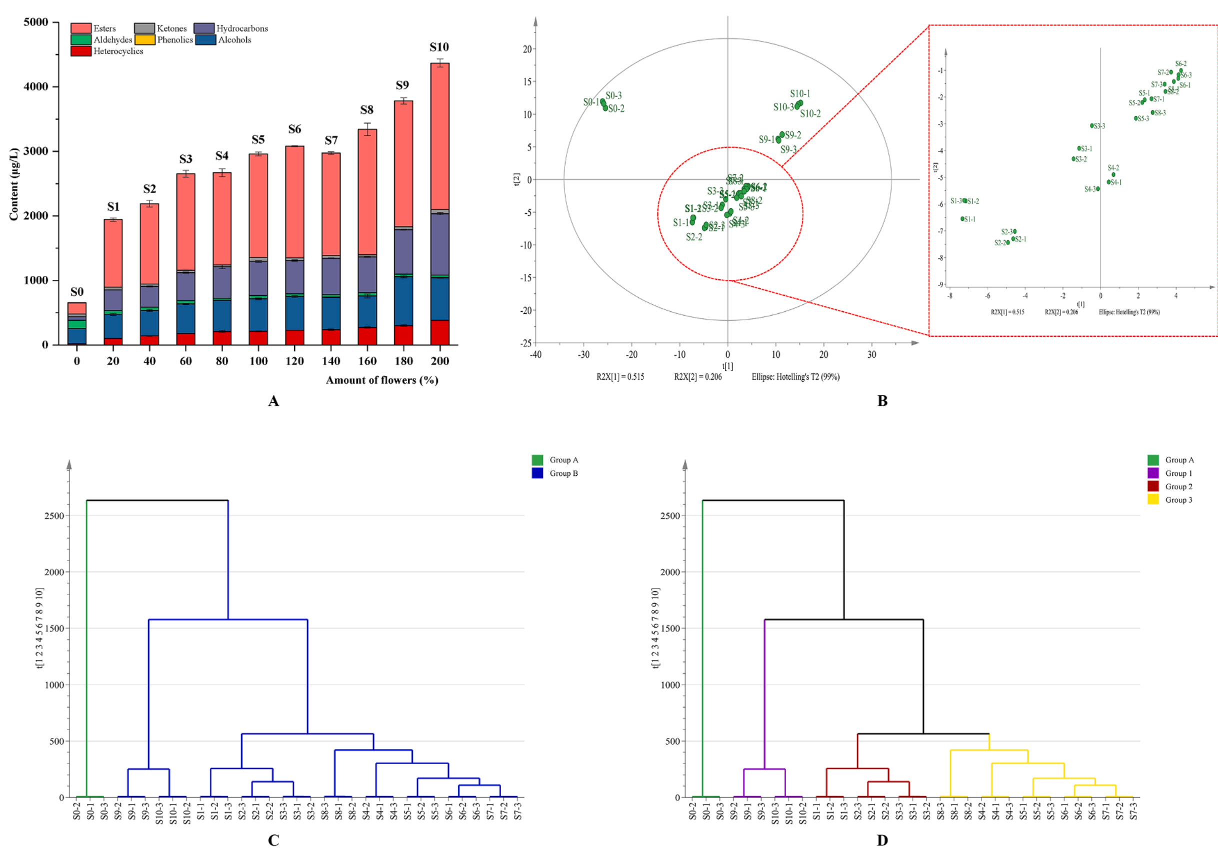

Fig. 3. Total volatile compounds (A) in jasmine tea infusion with different amount of flowers and their PCA analysis (B) and HCA analysis (C, D).

compounds. The PCA analysis results (Fig. 3B) showed that the first two principal components (PC) explained 72.1% of the total variation (PC1 = 51.5%, PC2 = 20.6%). In the direction of PC1 from left to right, the jasmine tea infusion samples were arranged by the amount of flowers increasing from 0% to 200%, which indicated that the volatile compounds changes of jasmine tea infusion were closely related to the amount of flowers. HCA grouped all the samples into two main clusters (Fig. 3C) and four graded branches (Fig. 3D), group A included the base

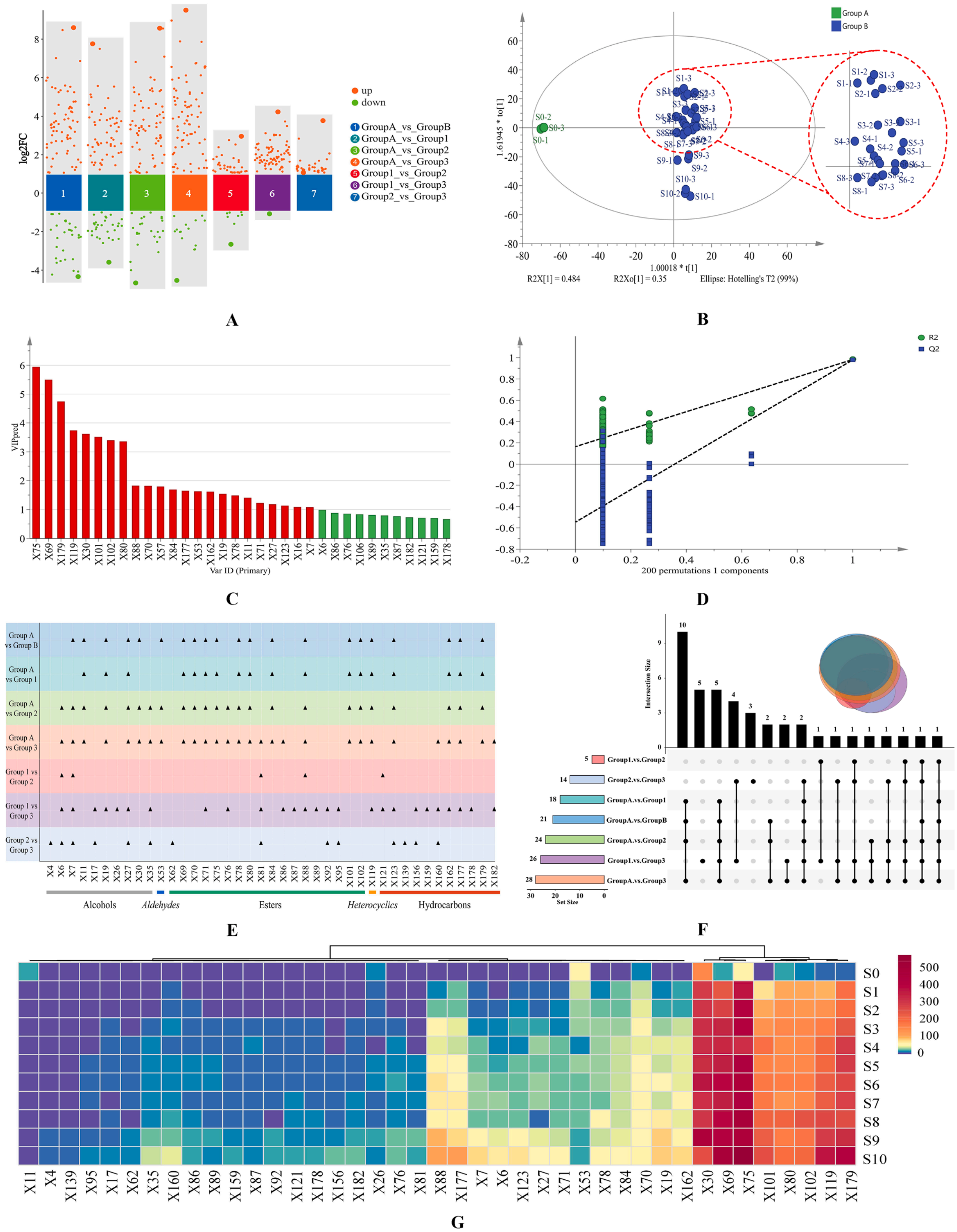

Fig. 4. Analysis of the key volatile compounds of jasmine tea infusion. (A) Scatter plot of differential volatile compounds in different groups based on the log2FC > 1.0 and P < 0.05. (B) OPLS-DA score plot of the jasmine tea in group A and group B. (C) The VIPpred of the volatile compounds. (D) Permutation tests plot. (E) Key volitile compounds in different groups. (F) The upset plot of key volitile compounds in different groups. (G) The content of differential volitile compounds in jasmine tea infusion samples.

tea infusion with 0% amount of flowers and group B consisted of jasmine tea infusion samples with at least 20% amount of flowers, indicating that the volatile compounds of jasmine tea infusion are significantly different from those of the base tea infusion. Furthermore, HCA divided the jasmine tea infusion samples into three groups in more detail, samples with 20–60% amount of flowers (S1, S2, and S3) were included in group 1, samples with 80–160% amount of flowers (S4, S5, S6, S7, and S8) were included in group 2, samples with 180–200% amount of flowers (S9 and S10) were included in group 3, which indicated that the volatile compounds of jasmine tea infusion were affected by the amount of flowers. This result correlated with the sensory review of jasmine tea.

Therefore, it can be speculated that the content and proportion of some volatile compounds in jasmine tea infusion change due to the amount of flowers, which in turn affects the aroma quality of jasmine tea. Moreover, 80% amount of flowers may be the key parameter affecting the jasmine fragrance intensity and comprehensive score in jasmine tea infusion, and 180% amount of flowers may be a key parameter that contributes to the low quality of jasmine tea with a strong dull odor.

The identification of differential volatile compounds in jasmine tea infusion

To determine the differential volatile compounds of jasmine tea infusion, all the volatile compounds of samples in different groups were analyzed by Fold Change (FC) and P-value. Based on the criteria that the values of $\log_2$FC > 1.0 and a P-value < 0.05, differential volatile compounds were identified (Fig. 4A). Obviously, the differences between group A and the other groups were significant, and the number of differential volatile compounds in group A was more abundant, indicating that the volatile compounds between the base tea infusion and the jasmine tea infusion were different significantly. The contents of some volatiles that are present in jasmine flowers (Zhang et al., 2022), such as guaiol (X27), methyl anthranilate (X101), and acetic acid phenylmethyl ester (X75) in group B were higher than that in group A, which indicated that these volatiles were absorbed from jasmine flower by the base tea during the scenting process. However, the contents of some volatile compounds, such as 1-octanol (X11), acenaphthylene (X138), and nonanoic acid, methyl ester (X105) in group B were lower than that in group A, which may be caused to the following reasons. On the one hand, these volatile compounds are easy to volatilize, because the base tea was exposed to the air for a long time and the moisture increased during the scenting process. On the other hand, these volatile compounds in the base tea are masked by the newly absorbed large amount of volatile compounds due to their smaller content. Also, Fig. 4A showed that group 1 vs group 3 had more differential compounds than group 1 vs group 2, and group 2 vs group 3 had fewer differential components than other groups, indicating that the content of volatile compounds in jasmine tea infusion could be changed by the amount of flowers, and there was a significant difference between the volatile components in jasmine tea infusion with the high and low amount of flowers respectively.

However, the method of using FC and P values to determine the differential compounds has certain limitations. Therefore, to further identify the differential volatile compounds in jasmine tea infusion, we constructed the variable importance projection (VIP) charts, in which a VIP greater than 1 indicates an important differential compound (Lenhardt et al., 2015). As shown in Fig. 4B–D and Fig. S, seven OPLS-DA models were established based on PCA and HCA results, including OPLS-DA model 1 (Group A and Group B) (Fig. 4B–D), OPLS-DA model 2 (Group A and Group 1) (Fig. S1–S3), OPLS-DA model 3 (Group A and Group 2) (Fig. S4–S6), OPLS-DA model 4 (Group A and Group 3) (Fig. S7–S9), OPLS-DA model 5 (Group 1 and Group 2) (Fig. S10–S12), OPLS-DA model 6 (Group 1 and Group 3) (Fig. S13–S15), OPLS-DA model 7 (Group 2 and Group 3) (Fig. S16–S18), respectively. Cross-validation analysis showed that the seven OPLS-DA models were reliable. Three conditions need to be fulfilled for identifying the differential volatiles of jasmine tea infusion in different groups: the values of predictive component variable importance in the projection (VIP pred) $>$ 1.0, $\log_2$FC $>$ 1.0 and a P-value $<$ 0.05. Based on the criteria, 41 volatile compounds were identified as differential volatiles, including 10 alcohols, 1 aldehyde, 18 esters, 1 heterocyclic compound and 11 hydrocarbons (Fig. 4E). It can be seen from Fig. 4E and F that the differential compounds of jasmine tea infusion in different groups were not identical, but there were 10 common differential compounds. Meanwhile, group A and group 3 had the most kinds of differential volatiles (28 kinds), while group 1 and group 2 had the least kinds of differential volatiles (5 kinds), which was similar to the rule shown in Fig. 4A, indicating that the volatile compounds of green tea infusion and jasmine tea infusion with different amounts of flowers are quite different, but there was little difference in the volatile components of jasmine tea infusion between samples with 20%−80% amount of flowers (group 1) and samples with 100% −160% amount of flowers (group 2).

More interestingly, the VIP pred of some volatile compounds, such as linalool (X30), acetic acid phenylmethyl ester, and α-farnesene (X179), were greater than 1, but their $\log_2$FC were less than 1 in different groups of jasmine tea infusion samples. This may be due to the higher content of these volatiles in each group. Therefore, in order to comprehensively analyze the differences of jasmine tea infusion, the above 41 volatile compounds were identified as the differential volatile compounds. Most of those differential volatile compounds were also detected in jasmine black tea (Li et al., 2018) and jasmine flower (Zhang et al., 2022), and their content showed an upward trend as the amount of flowers increased (Fig. 4G), indicating that the unique fragrance of the jasmine tea mainly roots in jasmine flowers and played important roles in jasmine tea aroma quality. However, 1-octanol and decanal (X53) that have aromas similar to citrus or lemon (Xiao et al., 2017) were more abundant in green tea infusion (S0) than in jasmine tea infusion (S1-S10), which may be due to their volatile nature or minimal presence in jasmine flowers (Bera et al., 2015; Zhao et al., 2015). In addition, the content of some volatile compounds such as linalool that accounts for 70% of main floral fragrances in nature (Knudsen et al., 2006), acetic acid phenylmethyl ester that conveys the aroma of jasmine and rose (McGinty et al., 2012), α-farnesene that is one of the major volatiles of *J. sambac* (L.) Ait. flowers and presents floral odor (Edris et al., 2008), etc., were much higher in jasmine tea infusion than other volatile compounds, which may have a greater contribution to the aroma quality of jasmine tea infusion.

The key volatile compounds affecting the aroma types and quality of jasmine tea infusion and their association with the amount of flowers

In order to explore the key volatile compounds that affect the aroma types and quality of jasmine tea infusion, a correlation analysis heat map was drawn (Fig. 5A) based on 41 differential volatile compounds and aroma quality evaluation of jasmine tea infusion (Table A1). The results showed that 41 kinds of differential volatile compounds were related to the aroma types of jasmine tea infusion in different degrees and could be further divided into four groups. The two volatile compounds (X11 and X53) of Group Gr were significantly and positively correlated with the grassy odour. The 17 volatile compounds in Group Ja were more significantly correlated with jasmine fragrance and the 17 volatile compounds in Group Du were more significantly correlated with dull odour. The remaining five volatile compounds (X27, X62, X71, X88, and X121) were in Group Ja&Du, which were close to the correlation of jasmine fragrance and dull odour. However, although these volatile compounds were significantly correlated with one or several flavor attributes of jasmine tea infusion, none of them were directly significantly correlated with the comprehensive quality of jasmine tea infusion except 1-octanol, which indicated that the content or proportion of some differential volatile compounds may affect the comprehensive quality of jasmine tea infusion.

Therefore, in order to further identify the key volatile compounds

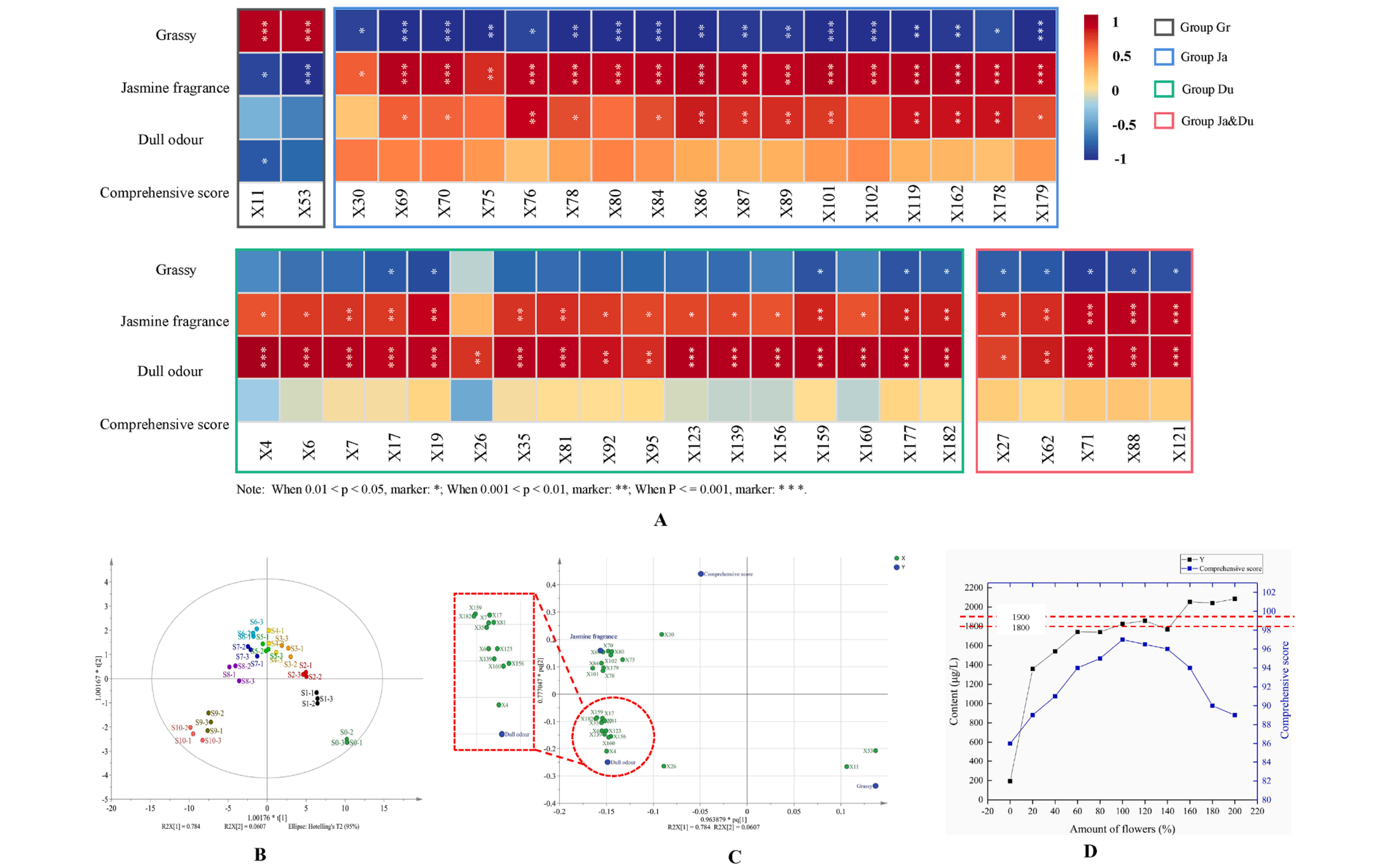

Fig. 5. Identification of key volatile compounds in jasmine tea infusion and their association with the amount of flowers. (A) Heat map of correlation between differential volatile components and aroma of jasmine tea soup. (B) O2PLS score chart. (C) The 25 key volatile compounds obtained from O2PLS analysis. (D) The change plot of Y and comprehensive quality of jasmine tea infusion.

affecting the aroma quality of jasmine tea infusion, the O2PLS analysis was conducted on 41 differential volatile compounds in jasmine tea infusion with different aroma types and aroma quality (Fig. 5B, C). According to the results of loading values, we screened out the top 25 volatile compounds of the square sum of the loading values of the first two dimensions, that is, the key volatile compounds with the greatest correlation with the aroma quality of jasmine tea infusion (Fig. 5C). 1-Octanol (X11) and decanal (X53) were related to the grassy odor and mainly reflected in the sample with 0% amount of flower (S0), which may be the key volatile compounds to form the fragrance of green tea infusion, but were negatively correlated with the comprehensive quality of jasmine tea infusion. Some volatile compounds, such as linalool (X30), acetic acid phenylmethyl ester (X75) and α-farnesene (X179), were the key volatile compounds of jasmine fragrance, which had a positive effect on the comprehensive quality of jasmine tea infusion. This was consistent with the analytical results of Fig. 5A. Other volatile compounds, such as geraniol (X26) with a rose-like aroma (Mączka et al., 2020), aromandendrene (X139) and longifolene (X160) that is a woody aroma compound (Hassan et al., 2020), were the key volatile compounds of dull odour, which were mainly reflected in the samples with the amount of flowers not less than 180% (S9 and S10), and were not conducive to the comprehensive quality of jasmine tea infusion.

To sum up, the total of 25 volatile compounds (X4, X6, X7, X11, X17, X26, X30, X35, X53, X69, X70, X75, X78, X80, X81, X84, X101, X102, X123, X139, X156, X159, X160, X179, and X182) are the key volatile compounds affecting the aroma types and quality of jasmine tea infusion. More interestingly, some of these key volatile compounds were also the key aroma compounds of jasmine tea (An et al., 2022), such as linalool (X30), acetic acid phenylmethyl ester (X75), α-farnesene (X179), indicating that these volatile compounds exist not only in jasmine tea leaves but also can be dissolved in jasmine tea infusion. This is why when drinking jasmine tea according to Chinese tea-drinking habits, the nose can smell the fresh and elegant fragrance, and the tongue can taste the fresh taste of jasmine. At the same time, it is worth further exploring whether these volatile compounds will enter the human body with the tea infusion and play some roles.

On the other hand, the amount of flowers is an important technical index in the scenting process of jasmine tea, during this process, the characteristic scented tea aroma gradually forms and this elegant aroma plays an important part in the appeal of jasmine tea (Ye et al., 2005). To further explore the relationship between the key volatile compounds and the amount of flowers, we drew the change diagrams of 25 key volatile compounds with the amount of flowers (Fig. W). The results showed that X53 and X11 decreased with the increase of the amount of flowers, while most of the other volatile compounds increased with the increase of the amount of flowers, which further indicated that the key volatile compounds of jasmine tea infusion mainly came from the aroma of jasmine flowers absorbed during scenting, but the content of these volatile compounds was not positively correlated with the aroma quality of jasmine tea infusion. Moreover, the correlation between the content of key volatile compounds and the aroma quality of jasmine tea infusion (Y) was studied based on the positive and negative correlation between the 25 key volatile compounds and the comprehensive quality of jasmine tea infusion (Fig. 5D), i.e,

$$Y = X30 + X69 + X70 + X75 + X78 + X80 + X84 + X101 + X102 + X179 - X4 - X6 - X7 - X17 - X26 - X35 - X81 - X123 - X139 - X156 - X159 - X160 - X182 - X53 - X11.$$

Fig. 5D showed that there was no positive correlation between Y and the comprehensive aroma quality of jasmine tea infusion, which meant that there might be more complex synergistic or antagonistic effects among these key volatile compounds (An et al., 2022). However, it is speculated that there may be a suitable Y interval to obtain higher quality jasmine tea infusion, and lower or higher than this interval is not conducive to the quality of jasmine tea infusion. In this study, the reasonable Y interval was about 1800–1900, and the Y values of jasmine tea infusion samples with 80%−120% flower ratio were close to or in this interval, and the aroma quality of these jasmine tea infusion samples was higher. And our research showed that the excessive amount of flowers also made the jasmine tea infusion astringent and unpleasant. Therefore, in order to obtain high-quality jasmine tea and save the production cost, it is feasible to set the amount of flowers to 80%. Moreover, in the actual processing process, the amount of jasmine flowers should be determined in combination with the actual factors, because the base tea, the origin of jasmine flowers, scenting temperature, and even the weather on the day of producing jasmine tea, all have an important impact on the aroma quality of jasmine tea (An et al., 2022; Fang et al., 2004; Yang et al., 2019).

Conclusion

In this study, the extraction conditions of volatile compounds in jasmine tea infusion were optimized according to Chinese tea-drinking habits, and the relationship between volatile compounds and aroma quality of jasmine tea infusion with different amount of flowers were discussed. The results showed that the extraction efficiency to extract volatile compounds was the best when using a 50/30 μm DVB/CAR/PDMS fiber, 1:25 proportion between tea and boiling water, and an extraction time of 5 min. Then all the volatile compounds were determined by GC × GC-Q-TOF-MS, including 204 kinds of compounds classified into seven classes (alcohols, aldehydes, ketones, esters, heterocyclics, phenolics, and hydrocarbons). Combined with FC and OPLS-DA analysis, forty-one volatile compounds were identified as differential volatile compounds that made the aroma of jasmine tea infusion with different amount of flowers different, and most of them showed an upward trend as the amount of flower increased. Further, twenty-five key volatile compounds were identified that were responsible for the formation of aroma types and quality of jasmine tea infusion based on the correlation analysis and the O2PLS analysis. Among them, the two volatiles of grassy aroma and thirteen volatiles of dull odour were negatively correlated with the aroma quality of jasmine tea infusion (Y), while the ten volatiles of jasmine fragrance were positively correlated with Y (Y = X30 + X69 + X70 + X75 + X78 + X80 + X84 + X101 + X102 + X179 - X4 - X6 - X7 - X17 - X26 - X35 - X81 - X123 - X139 - X156 -X159 - X160 - X182 - X53 - X11). Based on this result, we found that 80% −120% of the amount of flowers was beneficial to the aroma quality of jasmine tea infusion. Moreover, in order to save the production cost of jasmine tea and obtain a more pleasant taste of jasmine tea infusion, 80% of the amount of flowers was more appropriate in the actual production process, which can also improve the production efficiency and provide theoretical guidance for the intelligent development of jasmine tea scenting process.

CRediT authorship contribution statement

Huimin An: Writing – original draft, Data curation. **Jiashun Liu:** Data curation. **Yuan Chen:** Formal analysis. **Yiwen Huang:** Trial implementation. **Jinhua Chen:** Data curation, Formal analysis. **Zhonghua Liu:** Conceptualization. **Shi Li:** Funding acquisition, Investigation. **Jianan Huang:** Project administration, Conceptualization.

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

The data that has been used is confidential.

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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.2023.100750.

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