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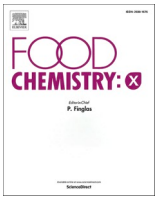

Effects of isolated scenting on the taste quality of broken green tea based on metabolomics

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(−)-Gallocatechin (PubChem CID: 9882981)
Isoschaftoside (PubChem CID: 3084995)
Epigallocatechin gallate (PubChem CID: 65064)
Butein (PubChem CID: 5281222)
L-(−)-Malic acid (PubChem CID: 222656)
(−)-Catechin (PubChem CID: 73160)
trans-3-Indoleacrylic acid (PubChem CID: 5375048)
Palmitic Acid (PubChem CID: 985).

ABSTRACT

Liquid chromatography-mass spectrometry (LC-MS) combined with multivariate analysis were used to characterize the nonvolatile compounds of broken green tea and explore the effect of isolated scenting on metabolic profile and taste quality of broken green tea in this research. A total of 236 nonvolatile compounds were identified and 13 compounds were believed to be the key characteristic taste compounds of scented broken green tea. Meanwhile, the optimal isolated scenting time of broken green tea was determined to be 10 h based on the sensory evaluation and PLS results. The contents and types of flavonoids, organic acids and catechins lead to the difference of taste quality at different scenting times. Overall, these findings provided a theoretical basis for scenting to improve the taste of broken green tea, and provide a new idea for improving the taste of broken green tea.

1. Introduction

Green tea is a non-fermented tea made from fresh leaves with high tenderness. Broken green tea adds the step of kneading and cutting on the traditional production process of green tea, which makes the cell broken rate high and the content of water extract high. Broken green tea is generally made from tea mixed with old and tender tea leaves harvested by low-end machines, which is commonly utilized as a foundational ingredient in both domestic and international tea bags, as well as scented tea products (Ye et al., 2022). Broken green tea tends to have a bitter taste and a relatively flat aroma due to its high content of catechins and old tea leaves. Hence, improving the flavor quality of broken tea has always been a primary focus of research in the tea area (Ye et al., 2021).

Scenting is one of the most important steps in the processing of scented tea, which can be mainly divided into traditional scenting, humidification of continuous scenting and isolated scenting. Among these processes, isolated scenting is the most convenient. It uses a plastic net to separate jasmine and tea for scenting, reducing the process of screening flowers, thereby reducing labor and shortening the production cycle. Some studies have shown that scenting makes the aroma of tea more harmonious and richer, and reduces the bitterness of tea, which is of great significance for the improvement of tea quality (Liu et al., 2023; Chen et al., 2023). During the scenting process, the changes of non-

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volatile components such as tea polyphenols and ester catechins were considered to be related to the bitterness of tea, the increase of volatile components such as α-farnesene and linalool was considered to be an important factor in the aroma quality of jasmine tea (Ye et al., 2022, Zhao et al., 2023). However, the changes in the flavor quality of broken green tea are related to the overall changes of various compounds during the scenting process. In previous studies, Chinese scholars have extensively studied the relationship between the changes of volatile components and tea quality during scenting, but the contribution of the changes of non-volatile components to taste has not been thoroughly studied. Therefore, a comprehensive understanding of the changes of taste compounds in the scenting process of broken green tea is crucial for improving the quality of broken green tea and understanding the mechanism of scenting on taste improvement.

Scenting time is one of the factors affecting the quality of scented tea and it will change with the temperature, amounts of jasmine and the variety of tea (An et al., 2022). If the scenting time is excessive, the tea aroma is dull and taste is tainted; If the scenting time is not enough, the jasmine aroma of tea is light and taste is astringent. Therefore, determining the optimal scenting time is extremely important for improving the quality of broken green tea. In previous studies, the optimal scenting time of jasmine tea was studied from the perspective of aroma, the influence and contribution of different scenting times to non-volatile content remains largely unknown.

Metabolomics can quantitatively and qualitatively analyze hundreds of different metabolites detected, with high sensitivity and wide applicability, making it a popular tool for detecting food chemical components (Wei et al., 2023), plant active ingredients (Lei et al., 2023), pesticide residues (Selim Mohamed et al., 2023) and more. Currently, research on the correlation between flavor quality characteristics and chemical components based on metabolomics has become one of the hotspots in tea research.

In the present study, ultra-high performance liquid chromatography coupled with mass spectrometry (UPLC-Orbitrap/MS) and quantitative descriptive analysis (QDA) were combined to systematically study the key characteristic nonvolatile compounds of scented broken green tea and dynamic changes in the metabolite profile and taste characteristics from samples of base tea (B), scenting for 4 h (I1), 8 h (I2), 10 h (I3), 12 h (I4), and 16 h (I5). Furthermore, the chemical transform pathways of key compounds during the scenting process and the optimal time for scenting of broken green tea were also explored. The material basis of the difference of broken green tea quality caused by different scenting time was studied, which provided a theoretical basis for improving the quality of broken green tea.

2. Materials and methods

2.1. Tea samples and chemicals

The broken green tea was provided by Hunan Shimen Xiefeng Famous Tea Co., Ltd and jasmine flowers were picked from Heng County, Nanning city, China. The jasmine tea samples were produced by two scenting processes by our cooperation with China Tea (Hunan) Co., Ltd. (Changsha, China) in July 2022. Briefly, using plastic mesh to separate tea and flowers, lay a layer of tea leaves and then a layer of flowers for 0 (B), 4 (I1), 8 (I2), 10 (I3), 12 (I4) and 16 h (I5) at a pile temperature of 50 °C. When the scenting time reached, the flowers were separated from the tea and then the scented tea were dried to a moisture content of 6–7 % at about 40 °C. The samples were packaged in sterile polyethylene bags, transported to the laboratory then stored at −80 °C until required. The isolated scenting method was shown in Fig. 1A. The LC-MS grade methanol and acetonitrile were purchased from Merck

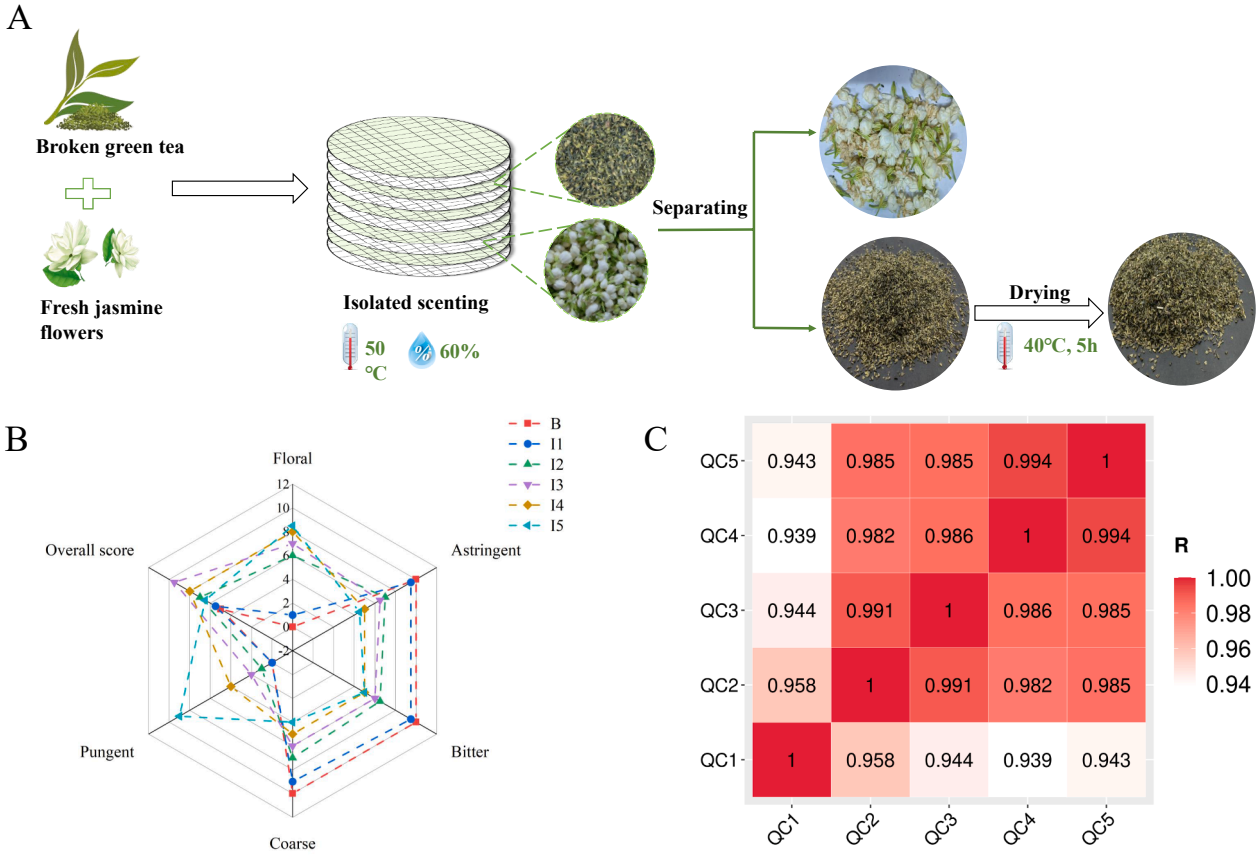


Fig. 1. The processing flow of isolated scenting of broken green tea. (B) Radar plot of sensory taste attributes profile of broken green tea. (B) Pearson correlation coefficient between QC samples.

(Darmstadt, Germany), and formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was obtained from a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Sensory evaluation of broken green tea

In order to initially assess the taste quality of various samples, a team composed of five professional reviewers (3 females and 2 males, aged 25–45) who have obtained the National Senior Tea Evaluator Qualification Certificate carried out sensory evaluation and quantitative descriptive analysis (QDA) on the broken green tea, which were approved by Institutional Review Board Committee of Hunan Agricultural University (#TSF-780-2020). Tea infusion was made following the guidelines outlined in the Methodology for Sensory Evaluation of Tea (China National Standard, GB/T 23776-2018). Briefly, three grams of samples were brewed with 150 mL boiling water for 3 min for the first time and for 5 min the second time in a specialized evaluation tea cup. The tea infusion was then poured immediately into a tea bowl for the panel's evaluation. Then the strength of taste attribute in tea infusion, including floral, pungent, bitterness, coarse, astringency and overall score, were scored respectively from 0 (none) to 10 (extremely concentrated). Among them, the "floral" and "pungent" commonly considered as aroma attributes from sensory science viewpoints referred to the taste that can be felt in the mouth when we drink jasmine tea, which were identified as pleasant jasmine fragrance feeling and unpleasant stuff feeling respectively. "coarse" was a rough, dull, and woody taste. "bitterness" was a taste induced by compounds such as caffeine. "astringency" was a sensation of convergence on the tongue. The overall score represented the degree of coordination among the taste attributes of tea. The higher the score, the better the taste coordination, and the higher the overall taste quality of tea. In the end, the intensity of each taste attribute in the samples was represented by the average score of 5 individuals. Each sample was assessed three times. According to the prescribed jasmine tea evaluation procedure outlined in the "Tea Sensory Evaluation Methods" (GB/T 23776-2018), the experimental scheme involving sensory evaluation is in line with Chinese national law and does not need ethical approval.

2.3. Non-volatile compounds identification methods

2.3.1. Non-volatile compounds extraction

The method for extracting jasmine broken tea solution was adjusted according to the GB/T8313-2008 (China National Standard). 0.5 g (to 0.0001) ground sample was added to a conical flask containing 25 mL of methanol solution with a concentration of 70 %. Subsequently, the sample was subjected to ultrasound at room temperature for 30 min and shaken every 10 min. Then, the supernatant was poured into a centrifuge tube and centrifuged at 12,000 rpm for 10 min at 10 °C. Finally, the extracts were filtrated through 0.22 μm nylon membrane filtration and moved to liquid phase vial for further detection. Each tea sample was set with 3 parallel replicates. QC samples were quality control samples, which were made by mixing the extraction liquid of the same volume of all samples in the centrifugal tube evenly. During the UPLC-Orbitrap/MS analysis, one QC sample is inserted every 10 samples to monitor the stability of the instrument and the reliability of the data.

2.3.2. LC-MS condition

The compound measurements were carried out using an ultrahigh performance liquid chromatography system (UPLC Infinity 1290, Agilent Technologies, Santa Clara, CA, USA) coupled to an Orbitrap mass spectrometer (Agilent Technologies). The UPLC conditions were as follows: the mobile phase was A phase with 0.1 % water formic acid and B phase with 0.1 % acetonitrile formic acid; the B-phase gradients were 0.0–1.6 min, 0 %–5%; 1.6–18.0 min, 5 %–18 %; 18.0–38.4 min, 18 %–95 %; 38.4–42 min, 0 %; the temperature of the column was 35 °C and the volume of injection was 2 μL. The MS conditions were as follows: Ion source type: H-ESI; Spray voltage: positive ion (V) = 3500, negative ion (V) = 2500; Ion transfer tube temperature (°C): 325; Spray temperature (°C): 300; Sheath gas (Arb) = 35; Auxiliary gas (Arb) = 10; Purge gas (Arb) = 1; Orbitrap resolution: 120000; Scanning range (m/z): 100–1500; Scan cycle (Sec) = 3; RF lens (%): 70.

2.3.3. Qualitative and quantitative analysis of nonvolatile compounds

The Compound Discoverer software was used to analyze the raw data. Initially, the molecular formula was predicted based on information such as retention time, mass charge ratio, molecular weight, and adduct ions. This predicted formula was then compared with information from the mz Cloud database, including fragment ions and collision energy for each compound. The compounds that had CV values below 30 % in the QC samples were chosen as the final qualitative results. Integrate the detected chromatographic peaks in the sample, where the peak area of each characteristic peak represents the relative quantitative value of a compound. Normalize the quantitative results using the total peak area, and finally obtain the quantitative results of metabolites.

2.4. Data analysis methods

Principal component analysis (PCA), hierarchical cluster analysis (HCA), and orthogonal partial least-squares discriminant analysis (OPLS-DA) were performed using Simca-p (v 14.1, MKS Umetrics AB, Umeå, Sweden). Radar plot and line chart were obtained by Origin Pro (v2022c, Originlab Corporation, Northampton, MA, USA). Other plots were drawn using Adobe Photoshop (v 12.0.3, Adobe Systems Incorporated, California, USA).

3. Results and discussion

3.1. Sensory evaluation

Fig. 1B showed the taste scores of each sample. The results indicated that when the base tea (B) was scented, samples with different scenting times exhibited different levels of enhanced floral taste and reduced astringent, bitter, and coarse tastes. The pungent taste increased with the prolongation of the scenting time. When the scenting time reached 16 h (I5), the pungent taste is the strongest (9). The overall score of broken green tea demonstrated a pattern of initially rising and then falling, suggesting that the taste quality of scented broken green tea was not determined by a single taste attribute, but by the coordinated presentation of various taste attributes.

The scenting of jasmine tea involves the tea absorbing the floral fragrance, and this can be categorized into physical and chemical absorption. Simultaneously, we believe that the volatile compounds adsorbed by tea during the scenting process will dissolve in the tea soup, thereby affecting the taste (Chen et al., 2023). Besides, the overall perception of flavor is a synchronous sensory perception, including taste, aroma, sensation and trigeminal sensation (Zhu et al., 2021). In the process of taste perception, smell and taste play an important role, which can not only affect the taste of food alone, but also enhance people's perception of taste through the interaction of cross-sensory perception (Tian et al., 2023). Therefore, aroma can also influence taste quality through this interaction mechanism. The fragrance release of jasmine fresh flowers during the scenting process is a slow process, there was little difference in the taste characteristics after 4 h of scenting (I1), possibly because the brief scenting period led to a limited release of fragrance and heat dissipation from the flowers, resulting in a weaker floral taste (1). In the early stage of scenting, the fragrance of flowers was clear and pleasant, and the floral taste of scented broken green tea gradually increased. However, in the later stage of scenting, the jasmine gradually lost its activity and emitted a pungent taste due to the hydrothermal effect, resulting in a stronger unpleasant pungent taste of the scented broken green tea. The decrease in astringency, bitterness, and coarseness may be due to changes in the chemical composition of broken

green tea caused by temperature during the scenting process, which required further research.

3.2. The content changes of nonvolatile compounds in isolated scenting process

Correlation analysis was performed on the metabolites detected in five QC samples, and the results showed that the instrument had high repeatability and stability during the detection and analysis process, ensuring the reliability of the data (Fig. 1C). 4780 ion features were detected during the 42 min of UPLC-Orbitrap/MS analysis, and 236 metabolites were finally identified according to the tea metabolomics databases. These compounds were composed of 13 catechins and their derivatives (37.89 %), 39 flavonoids (16.96 %), 19 terpenoids (0.43 %), 44 organic acids (7.80 %), 7 alkaloids (24.12 %), 15 carbohydrates (0.90 %), 11 fatty acids (0.79 %), 28 amino acids and derivatives (2.01 %), 14 heterocyclic compounds (4.25 %), 7 phenols (2.73 %) and 39 other compounds (2.12 %) (Fig. 2A). All these compounds existed in each sample, indicating that the scenting process and time will not affect the types of nonvolatile compounds, but will affect the content. Among them, catechins and their derivatives, flavonoids and flavone glycosides, and alkaloids, which are generally considered to be as major contributors to the bitterness and astringency of tea (Zhang et al., 2020), were the main nonvolatile compounds in the broken tea sample in this study, which may be the reason why this entire batch of tea samples had strong bitterness and astringency. Following the scenting process, there was a notable decrease in the overall concentration of nonvolatile compounds (Fig. 2B). Except for carbohydrates, the different types of nonvolatile metabolites were significantly fluctuated during scenting process (Fig. 2C-G).

3.3. Effect of scenting on taste compounds of broken green tea

In order to initially determine the variations between the samples, principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed on the peak areas of each sample obtained by UPLC-Orbitrap/MS, with a fitting parameter R^2X of 0.484 (Fig. 3A and B). Even though the explanatory power of the top 3 principal

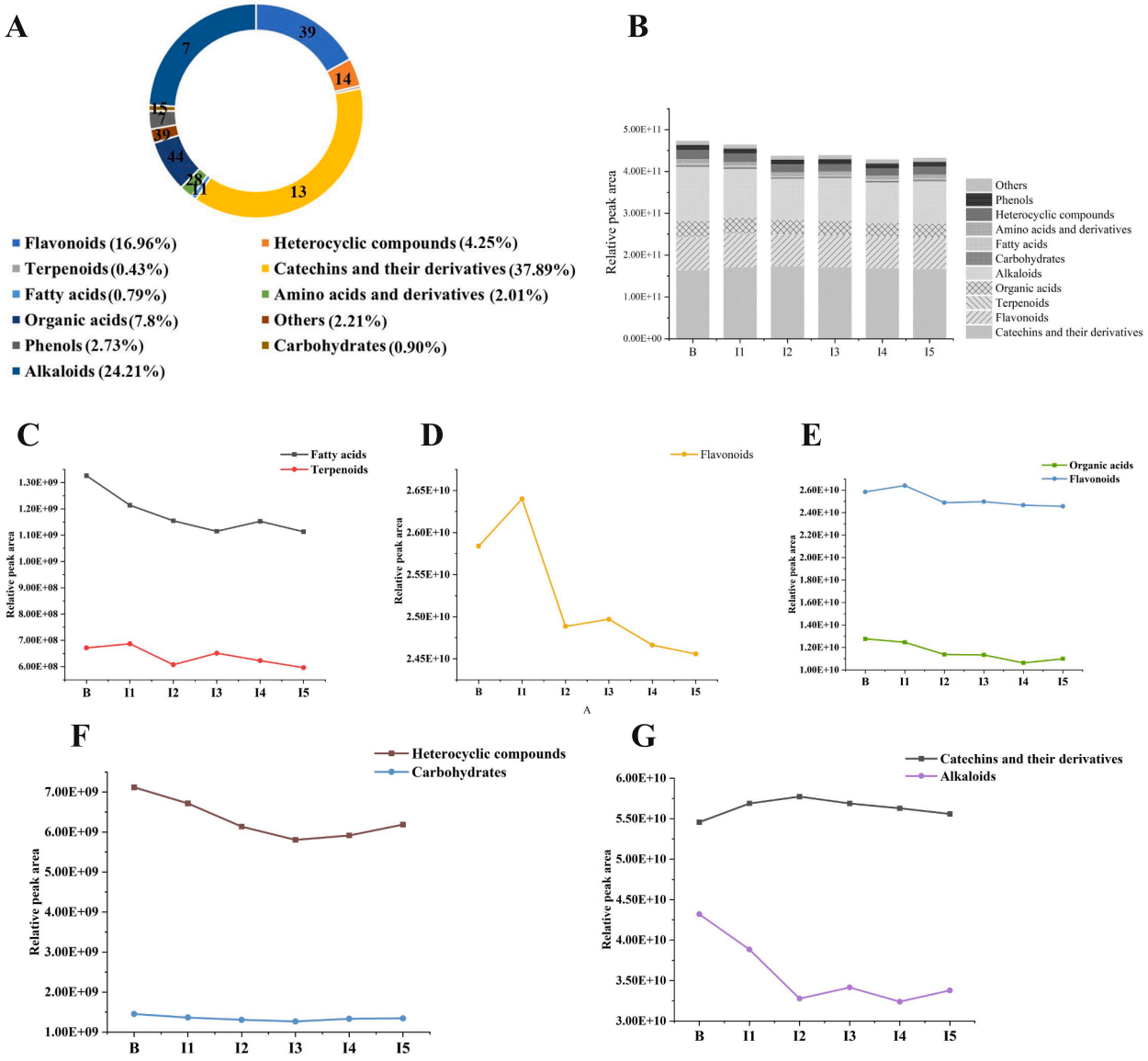


Fig. 2. The overall metabolic profile of broken green tea during scenting process. (A) The classification of the nonvolatile metabolites. (B) The trend of total content of nonvolatile compounds in broken green tea with scenting time. (D-H) Trends of the metabolites change different categories in different scenting time.

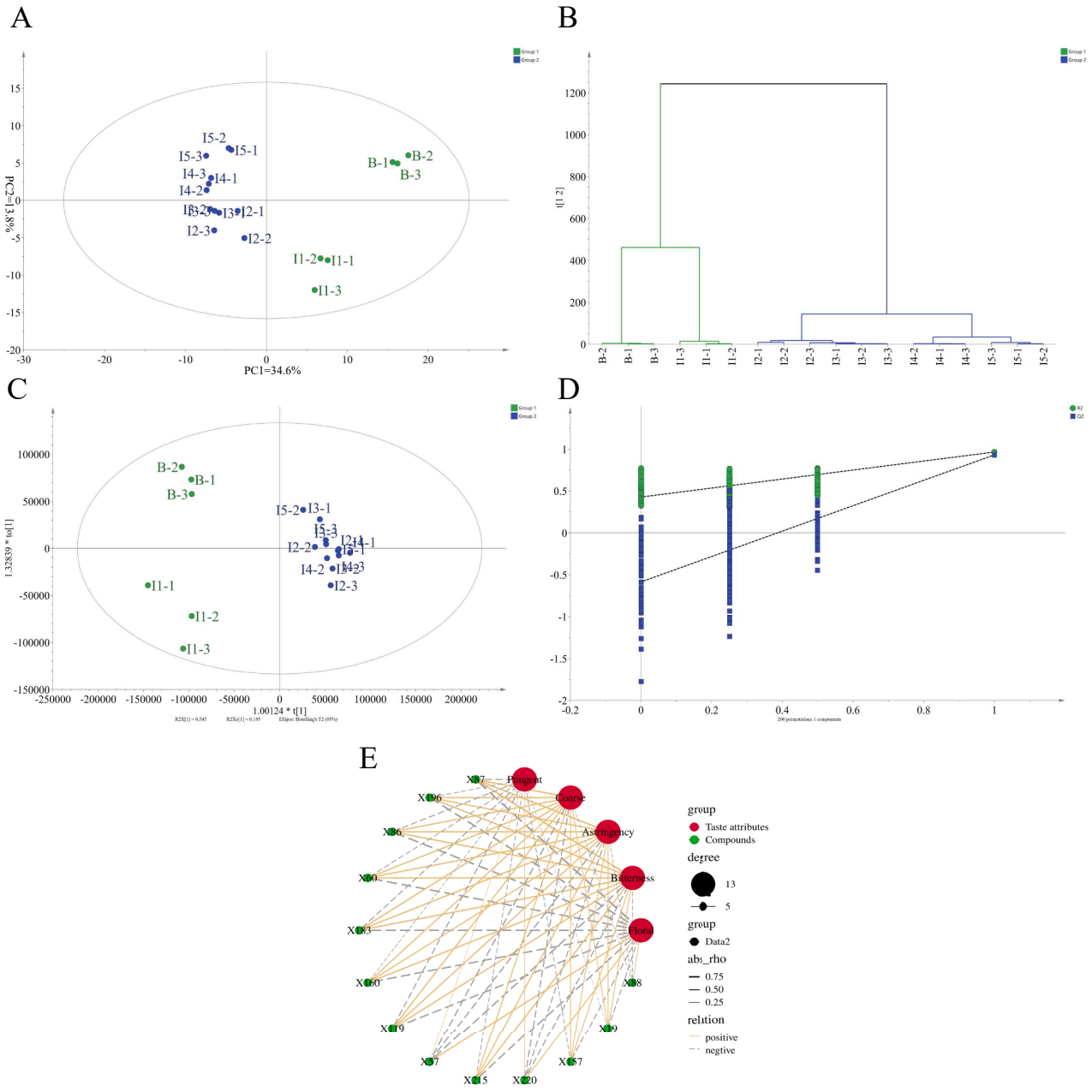


Fig. 3. The differential metabolites of the base tea and scented tea. (A) The PCA analysis. (B) The OPLS-DA model. R2X = 0.739, R2Y = 0.965, Q2 = 0.931. (C) A cluster analysis of the tea samples. (D) Hypothesis testing of the OPLS-DA model. (E) The correlation network diagram between taste attributes and key differential compounds.

components was not high (PC1 = 34.6 %, PC2 = 13.8 %, PC3 = 11.54 %), the combined explanatory power of the top 5 principal components was only approximately 70 %, with significant differences between Group 1 (B and I1) and Group 2 (I2-I5). It is worth noting that base tea (B) and isolated scenting tea (I1) were grouped together, which indicated the I1 that had been isolated scenting for 4 h had not undergone significantly altered in its metabolites, and cannot achieve the effect of scenting. Therefore, the isolated scenting time should not be less than 4 h in the actual production process of jasmine tea. In the direction of PC2, from bottom to top, the group 2 were arranged according to the increase in scenting time from 8 h to 16 h, suggesting a close correlation between PC2 and scenting process induced changes in nonvolatile compounds in scented broken green tea. The HCA tree structure results aligned with the findings of the PCA, and the Group 2 are further divided into two subcategories: I2 and I3; *I4* and I5, indicating that the scenting time also plays a crucial role in the chemical changes.

Based on these results, OPLS-DA analysis (orthogonal partial least squares discriminant analysis) was performed to explore the differences of metabolites in jasmine tea caused by scenting for the data Group 1 vs Group 2 (Fig. 3C). The cross-validation analysis, which included 200 permutation tests, showed that reliability of the OPLS-DA models, with R2 intercepts of 0.428 and Q2 intercepts of −0.585 (Fig. 3D). VIP > 1 and P < 0.05 were used as screening criteria for variables with important contributions to taste of scented broken green tea. According to these criteria, 13 compounds, which composed of 5 catechins and their derivatives, 1 alkaloid, 3 organic acids, 2 phenols, 1 fatty acid, and 1 flavonoid (Table 1), were identified as key differential compounds for the comparisons Groups 1 vs Group 2. In summary, these 13 differential

Table 1
Key characteristic taste compounds (VIP > 1.0) of scented broken green tea.

Name	ID	CAS	VIP	P value	Formula	Mass error (ppm)	*m/z*	Rt (min)	Adduct
(Alkaloids)									
Caffeine	X60	58-08-2	10.93	0.006	C8 H10 N4 O2	−3.63	195.087	8.957	[M+H]+1
(Catechins and their derivatives)									
(−)-Gallocatechin	X196	3371-27-5	3.08	0	C15 H14 O7	−0.32	305.0667	5.481	[M−H]−1
Epigallocatechin 3-O- (3-O-methylgallate)	X87	83104-87-4	2.24	0	C23 H20 O11	0.03	471.0935	15.779	[M−H]−1
Epigallocatechin gallate	X88	989-51-5	1.95	0.001	C22 H18 O11	−0.38	459.0908	11.92	[M+H]+1
(−)-Catechin	X220	18829-70-4	1.39	0.007	C15 H14 O6	−0.1	289.0718	8.394	[M−H]−1
Epiafzelechin 3-O-gallate	X86	108907-43-3	1.02	0	C22 H18 O9	0.88	425.0883	20.106	[M−H]−1
(Organic acids)									
L-(−)-Malic acid	X119	97-67-6	1.39	0.002	C4 H6 O5	0.93	133.0144	1.009	[M−H]−1
Pheophorbide A	X160	15664-29-6	3.56	0	C35 H36 N4 O5	−3.19	593.2738	34.139	[M+H]+1
trans-3-Indoleacrylic acid	X183	29953-71-7	1.08	0	C11 H9 N O2	−2.6	188.0701	6.255	[M+H]+1
(Fatty acid)									
Palmitic Acid	X157	57-10-3	1.08	0.034	C16 H32 O2	−3.6	274.2733	27.188	[M+H]+1
(flavonoids)									
Isoschaftoside	X215	52012-29-0	2.85	0	C26 H28 O14	−0.67	563.1409	15.417	[M−H]−1
(Phenols)									
Butein	X57	487-52-5	1.56	0	C15 H12 O5	−2.36	273.0751	16.589	[M+H]+1
2,2′-Methylenebis(4-methyl-6-*tert*-butylphenol)	X19	119-47-1	1.54	0.045	C23 H32 O2	−0.27	339.2329	32.507	[M−H]−1

metabolites can be used to differentiate unscented broken green tea from scented broken green tea, and the changes of these compounds during scenting may be the reason for the improvement of the taste of broken green tea. Fig. 3E showed the correlation between these 13 characteristic taste compounds and the five taste attributes. The results showed that the 13 compounds were correlated with the taste attributes to varying degrees, indicating that these compounds were closely related to the taste characteristics of scented broken green tea. It is worth mentioning that the correlation coefficient only represents the relationship between the QDA of sensory properties and the concentration of the compounds.

3.4. Contribution of key differential compounds to the taste of broken green tea and their changes during scenting process

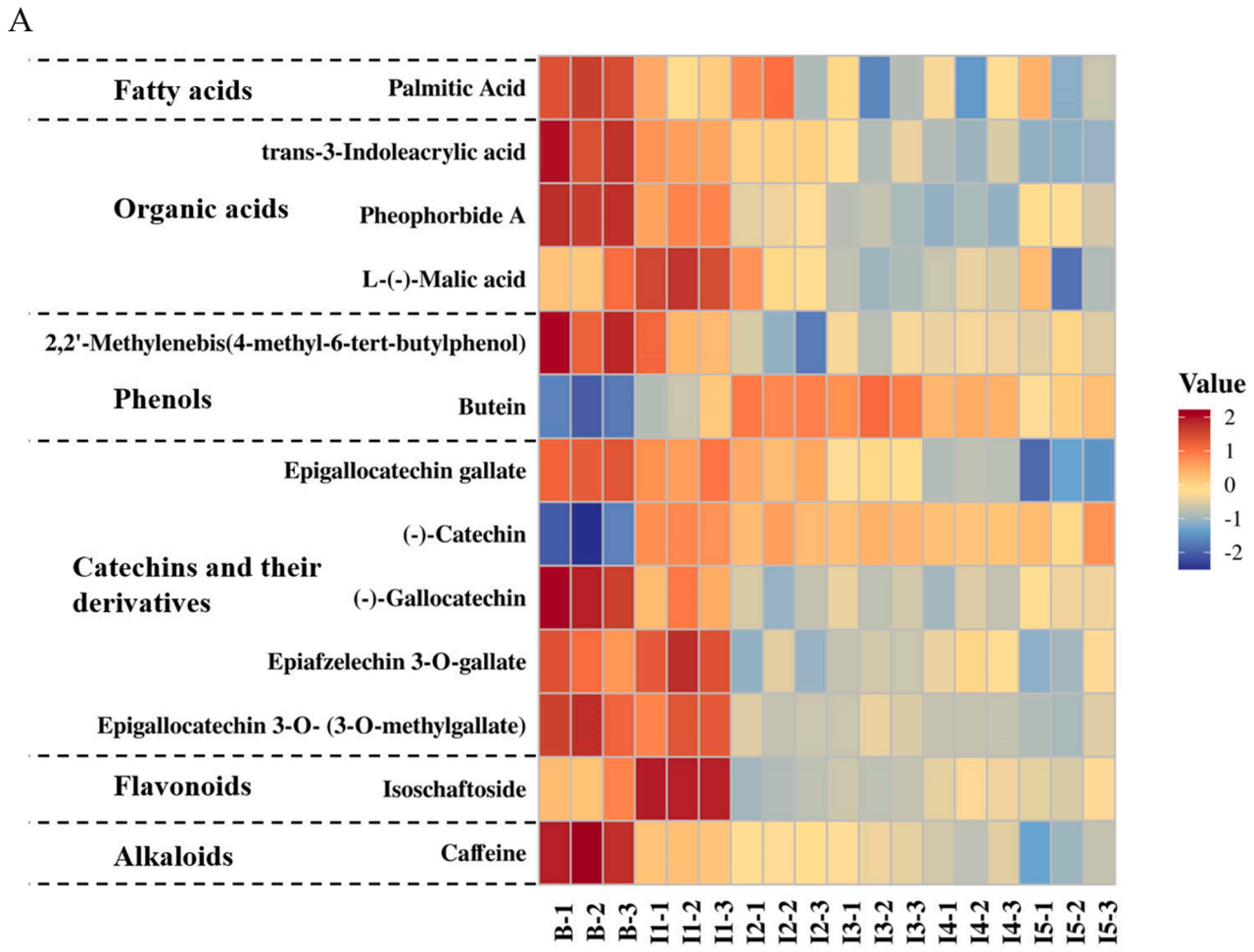
A heatmap was plotted to visualize the variations of the key differential metabolites among samples (Fig. 4A). As can be seen in Fig. 4A, the contents of these compounds in the Group 1 clearly differed from those in Group 2. The changes in the metabolites also showed that most of the contents of differential metabolites decreased significantly after scenting. The content of compounds of I1 was approximately comparable to that of base tea, as indicated by the PCA results, and this similarity may also explain the slight difference in taste between the two.

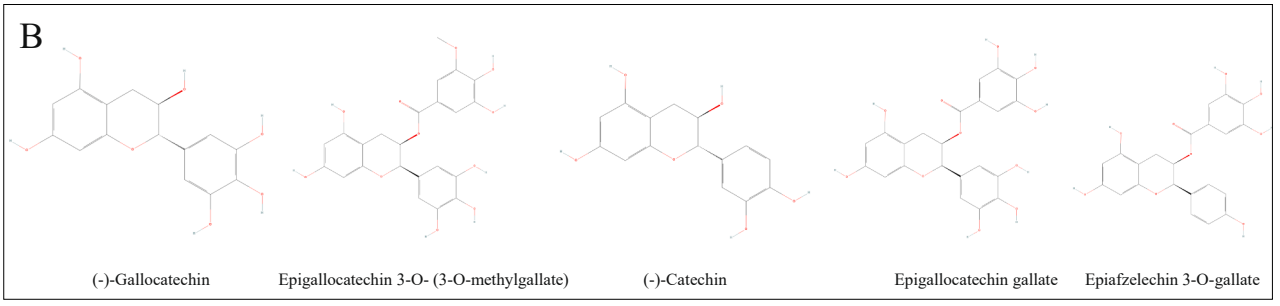
Catechins and their derivatives. Catechins, the primary component of tea polyphenols, are recognized as a significant factor in creating the astringent and bitter tastes of tea. Catechin has been extensively researched for its diverse pharmacological benefits, including its antioxidant, anti-tumor, and antibacterial properties (Chen et al., 2018). In this study, the astringent compounds of broken green tea, including Epigallocatechin gallate (X88), Epigallocatechin 3-O-(3-O-methylgallate) (X87), Epiafzelechin 3-O-gallate (X86), and (−)-Gallocatechin (X196), were significantly reduced after scenting, leading to decreased astringency in the scented tea soup. There was a significant positive correlation between (−)-Catechin (X220) content and floral taste (Fig. 4D). The significant increase in (−)-Catechin content after scenting may be one of the reasons for the increase in floral intensity of scented broken green tea.

Most researches indicate that certain catechins undergo isomerization at the C-2 position of flavane-3-ol when tea is subjected to heat treatment (Wu et al., 2020). For instance, phenotypic catechins like EGCG, ECG, EGC, and EC are transformed into non-phenotypic catechins such as GCG, CG, GC, and C, and, etc. Furthermore, the C-3 ester bonds of ester type catechins are unstable and easily hydrolyzed to non-ester type catechins and gallic acid at relatively high temperatures and humidity (Peng & Shahidi, 2023), which maybe the reason for the decrease of Epigallocatechin 3-O-(3-O-methylgallate) (X87), Epigallocatechin gallate (X88) Epiafzelechin 3-O-gallate (X86), and (−)-Gallocatechin (X196) and increase of (−)-Catechin (X220) (Fig. 4B). However, although the content of these compounds had decreased compared to the base tea (B), there is a clear upward trend during the scenting process. Therefore, we speculate that catechins are also accompanied by a series of polymerization and condensation reactions during the scenting process. At present, some researchers also speculate that ester type catechins are obtained by esterification of non-ester type catechins with gallic acid. For example, Niemezt and Gross et al. believed that 1-O-galloyl-β-Glucose is an effective acyl donor and acceptor for tannin synthesis(Niemetz & Gross, 2005, Gross, 2008); Liu et al. confirmed on this basis that the acylation process of catechin gallate is similar to the synthesis pathway of hydrolyzed tannins, that is, 1-O-galloyl-β-Glucose is a direct acyl donor for the synthesis of ester type catechins, and EC and EGC are present in uridine diphosphate glucose galloyl-1-O-β-D-glucosyltransferase (UDG-galloyl-1-O)-β-D-glucose transfer (UGGT) and epicatechin 1-O-galloyl-β-D-Galloyl transferase (EC-1-O-galloyl)-β-Synthesis of ester type catechins under the action of D-gallic acyltransferase (ECGT) (Liu et al., 2012). In summary, we believe that a series of reactions such as oxidation, isomerization, or hydrolysis occur among the components of catechins during the scenting process, resulting in a decrease in the bitterness and astringency of the tea soup.

Alkaloids. Caffeine is methylxanthine alkaloids, which is the main purine alkaloids and important bitter taste substances in tea, making significant contributions to the flavor and quality of tea (Hollman & Arts, 2000). Besides, caffeine can combine with theaflavin to form a complex, which has been identified as an umami-enhancing compound in tea (Liu et al., 2018). In this research, the only alkaloids that showed notable variations was caffeine, which decreased significantly after scenting. This decrease may be attributed to the creation of hydrogen bonds between the nitrogen atoms in the caffeine molecule and the hydroxyl hydrogen in the catechin molecule, leading to a reduction in caffeine content. The reduction of caffeine after scenting made the tea soup taste more mellow, thus improving the taste of broken green tea.

Organic acids and fatty acids. Organic acids in tea offer health advantages, including antioxidant effects, support for digestion and absorption, speeding up gastrointestinal movement, and balancing intestinal microbiota (Chen et al., 2023). The content of organic acids in tea is about 3 % of the total dry matter of tea, playing a crucial role in regulating the taste of tea soup (Liu et al., 2013). In the present study, the levels of *trans*-3-Indoleacrylic acid (X183), and Palmitic Acid (X157) and Pheophorbide A (X160) decreased over the scenting process, whereas the content of L-(−)-Malic acid (X119) showed a phenomenon of initially increasing and then decreasing during the scenting process.





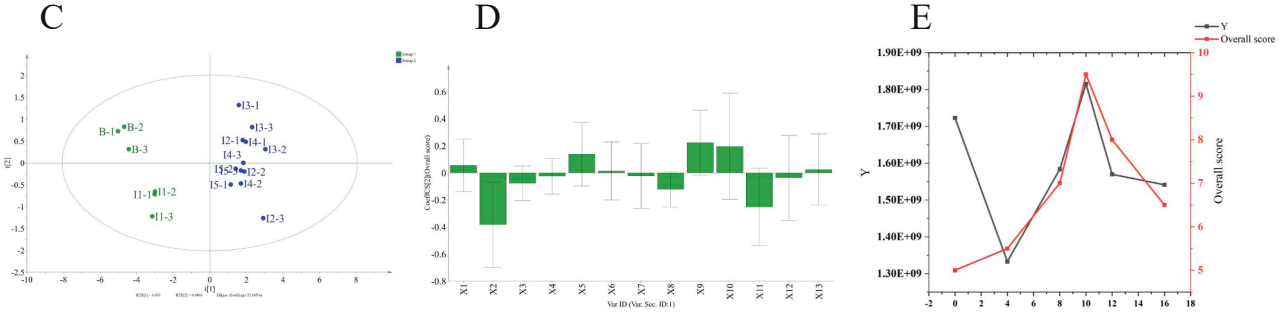


Fig. 4. (A) Heatmap analysis of critical metabolites in base tea and scented tea. (B) Structures of five different catechins. (C) PLS score chart. (D) The regression coefficients plot. (E) The change plot of Y and overall quality of broken green tea.

These organic acids were positively correlated with the bitterness, astringency and coarseness of broken green tea, and the reduction of their content may help to improve the flavor of broken green tea. The transformation mechanisms involved in these compounds need to be further studied.

Flavonoids and phenols. Flavonoid glycosides are significant compounds found in tea, known for their strong antioxidant properties. They also contribute to the bitter taste of tea, with a low threshold for taste and the ability to enhance the bitterness of caffeine (Guo et al., 2020). In this study, Isoschaftoside (X215) and 2,2′-Methylenebis(4-methyl-6-*tert*-butylphenol) (X19) showed an overall decreasing trend after scenting, and Butein (X57) showed an overall upward trend. Isoschaftoside and 2,2′-Methylenebis(4-methyl-6-*tert*-butylphenol) showed a significant positive correlation with bitterness, astringency and coarseness, Butein showed a significant negative correlation with bitterness, astringency and coarseness. Therefore, the change of these compounds makes the overall taste of the broken green tea more harmonious. Isoschaftoside is a biglycoside compound. During the heat treatment of flavonoids, the triglycoside is gradually degraded into biglycoside, monoglycoside and aglycogen, which may be the reason why Isoschaftoside showed a trend of first increasing and then decreasing during the process of scenting.

In the scenting process of broken green tea, there is a dynamic balance between jasmine fragrance release and water loss and tea fragrance absorption and moisture absorption. The aroma released by jasmine was absorbed by the broken green tea and dissolved in the tea soup during brewing, thus forming the characteristics of floral flavor and making broken green tea taste richer. At the same time, the enzymatic reaction of jasmine flowers and the migration of water released heat, making the temperature of the environment and tea pile to rise. The rise of temperature will make catechins, flavonoids, and other compounds isomerization, oxidation or hydrolysis of a series of chemical reactions mentioned above, so that the bitter taste of the broken green tea will be reduced, and the taste will be improved.

3.5. The determination of the optimal time for isolated scenting of broken green tea

In order to further determine the relationship between key nonvolatile compounds and the taste quality of scented broken green tea, the Partial least-squares regression analysis (PLS) was conducted on the overall scores and 13 differential nonvolatile compounds in scented broken green tea. The clustering results of PLS were consistent with the PCA results (Fig. 4C). The coefficients indicated the strength of the relationship between the taste quality (Y) and the systematic part of each of the key nonvolatile factors (X), and the regression model was obtained using the coefficients plot (Fig. 4D), i.e.

$$Y = 0.06X1 - 0.38X2 - 0.08X3 - 0.02X4 + 0.14X5 + 0.01X6 - 0.02X7 - 0.12X8 + 0.22X9 + 0.19X10 - 0.25X11 - 0.04X12 + 0.03X13$$

Fig. 4E showed that there was positive correlation between Y and the overall taste quality of scented broken green tea, which meant that the equation obtained from PLS analysis is reliable. Additionally, the Fig. 4E indicated that when the scenting time was 10 h, the Y score and overall score of scented broken green tea were the highest. Therefore, in order to make the broken green tea after scenting obtain the highest quality and get the maximum improvement effect, it is feasible to set the scenting duration to 10 h.

3.6. Analysis of differential metabolites during scenting of broken green tea

In order to fully understand the metabolites during scenting, we performed differential analysis of samples collected throughout scenting (Fig. S1a-k). A total of 38 compounds were identified during the scenting process of broken green tea, which could be used to distinguish broken green tea with different scenting time (Fig. 5F). The screened compounds and their information are listed in Table S1. When comparing group B and I1 (Fig. 5A), the number of different compounds was the least, which further explained the similar sensory results. A total of 17 differential metabolites were identified in the comparison of the I1 and I2 groups, of which 6 were up-regulated and 11 were down-regulated (Fig. 5B). Significantly reduced levels of compounds, including Isoschaftoside (X215), (−)-Gallocatechin (X196), Epigallocatechin 3-O- (3-O-methylgallate) (X87), Theobromine (X180), and Epiafzelechin 3-O-gallate (X86), are mostly thought to be associated with strong bitterness and astringency. These results showed that the composition of broken green tea compounds changed greatly and the taste was improved when scenting time reached 8 h. When comparing the I2 and I3 groups (Fig. 5C), 13 differential metabolites were identified, of which 6 were up-regulated and 7 were down-regulated. The difference in the number of different compounds in this group may be due to the little difference in scenting time. In the comparison of I3 and *I*4 groups (Fig. 5D), a total of 20 differential compounds were identified, of which 11 were significantly up-regulated. These upregulated compounds may be responsible for the decreased of broken green tea quality at 10 h to 12 h scenting. In the comparison between I4 and I5 groups (Fig. 5D), only 11 differential metabolites were identified.

In general, the relative levels of convergent metabolites such as catechins and flavonoids decreased significantly with scenting process. Over time, these metabolites may play a key role in reducing astringency in broken green tea. The changes of these compounds may be related to the temperature changes during scenting. The jasmine flower has not lost its physiological function after picking, and the bud will continue to breathe for a certain time, thus generating heat. At 4 h scenting, the temperature required for chemical reaction was not reached because the scenting time was too short, so the taste changed little. When scenting time reached 8 h, the temperature gradually increased, which made the compounds undergo a series of reactions, and the taste of the broken green tea became mellow. At scenting time of 10 h–12 h, jasmine flowers opened completely, had the strongest respiration and the most changes of compounds. Then the jasmine began to lose its vitality, gradually shriveling and yellowing, producing an unpleasant odor that reduces the quality of the broken green tea.

4. Conclusion

In this study, UPLC-Orbitrap/MS analysis method was used to systematically analyze the non-volatile metabolites during the scenting process of broken green tea. The results showed that the difference of metabolites was obvious before and after scenting, and the change of contents of 13 compounds such as Caffeine and Epigallocatechin gallate may be the reason for the improvement of taste of broken green tea. A series of chemical reactions such as hydrolysis and isomerization of metabolites in the scenting process make the taste of samples with different scenting time significantly different. Finally, the scenting time of 10 h was considered to be the best scenting time for broken green tea.

In conclusion, this study provided a theoretical basis for scenting to improve the taste quality of broken tea from the perspective of sensory evaluation and metabolites, and provides a new idea for improving the quality of broken green tea. To the best of our knowledge, this research was the first to explore the optimal time for isolated scenting from the perspective of metabolites, which offered technical guidance for enterprises to efficiently produce high-quality jasmine tea. Additionally, this study was the first time to systematically analyze the non-volatile components in the whole scenting process, which is helpful to understand the formation mechanism of taste of jasmine tea. At the same time, we believe that temperature plays an important role in the process of metabolite transformation, so further studies will discuss the mechanism of the influence of scenting temperature on specific compounds.

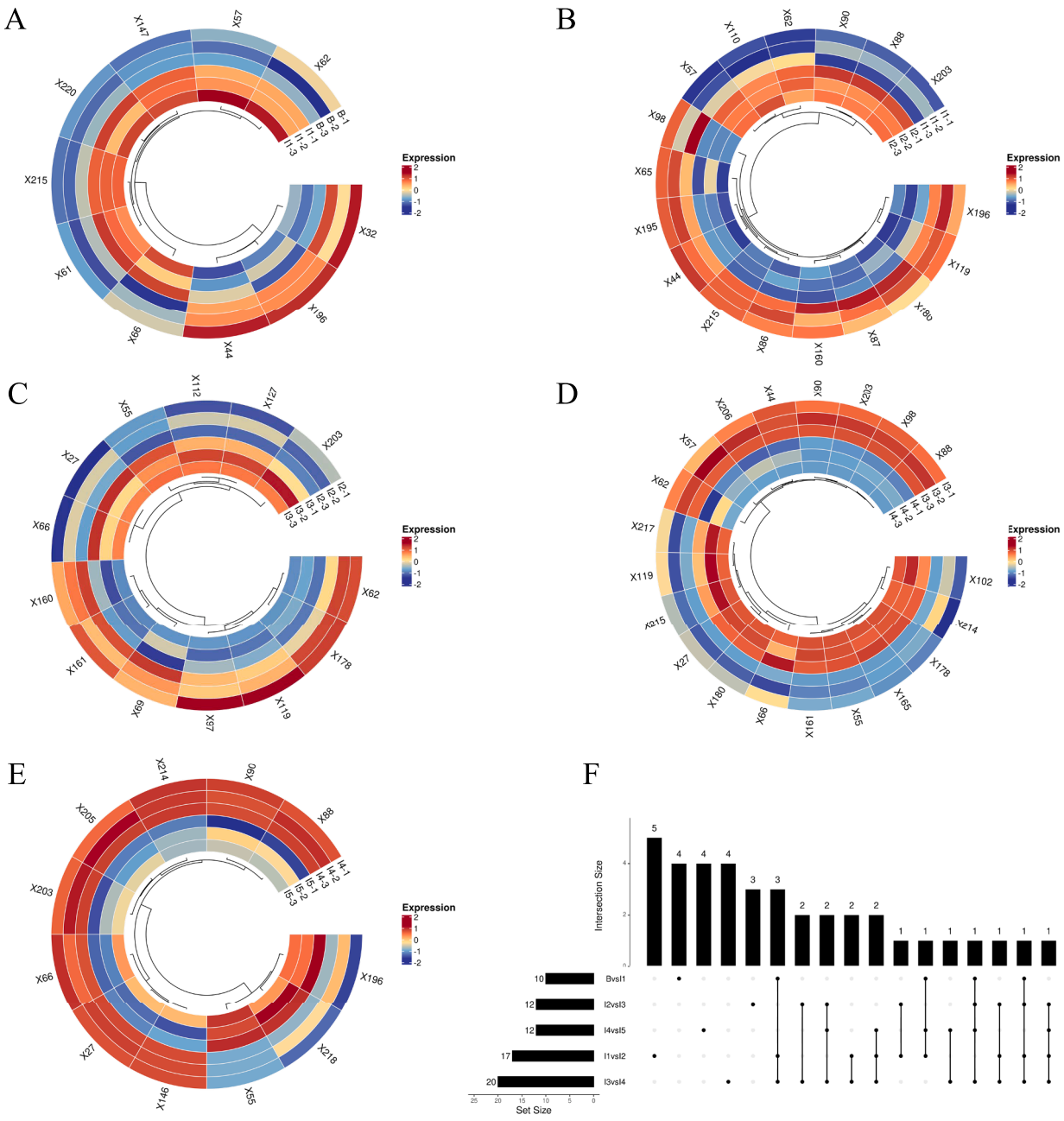


Fig. 5. Analysis of non-volatile differential metabolites during the scenting of broken green tea. (A–E) Ring heat maps; (F) The upset plot of key differential non-volatile compounds in different groups.

CRediT authorship contribution statement

Yuan Chen: Writing – original draft. **Yiwen Huang:** Methodology. **Huimin An:** Investigation. **Jiashun Liu:** Formal analysis. **Youcang Jiang:** Formal analysis. **Jiaqi Ying:** Investigation. **Shi Li:** Investigation. **Zhonghua Liu:** Project administration, Conceptualization. **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

Data will be made available on request.

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Institutional Review Board Statement

According to the prescribed jasmine tea evaluation procedure outlined in the "Tea Sensory Evaluation Methods" (GB/T 23776-2018), the experimental scheme involving sensory evaluation is in line with Chinese national law and does not need ethical approval. In the course of the implementation of this study, no human body, animal violation of law, morality, or "Declaration of Helsinki" was involved. All participants have given written consent.

Appendix A. Supplementary data

Supplementary data (Supplemental Fig. S1. Analysis of differential metabolites during scenting of broken green tea. (A–E) The OPLS-DA model. (F–J) Hypothesis testing of the OPLS-DA model. Supplemental Table S1. Key differential non-volatile compounds of broken green tea during the scenting process.) to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101454.

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