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Effect of shaking on the improvement of aroma quality and transformation of volatile metabolites in black tea

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A B S T R A C T

Shaking is an innovative technology employed in black tea processing to enhance flavor. However, the effects of shaking on the evolutionary mechanisms of volatile metabolites (VMs) remain unclear. In this study, we compared the effects of a shaking–withering method with those of traditional withering on the flavor and VMs transformation of black tea. The results showed that black tea treated with shaking exhibited excellent quality with floral and fruity aroma. Based on gas chromatography-tandem mass spectrometry, 128 VMs (eight categories) were detected. Combining variable importance projection with odor activity value analysis, eight key differential VMs were identified. Shaking could promote the oxidative degradation of fatty acids and carotenoids and modulate the biosynthesis of terpenoids to facilitate the formation of floral/fruity VMs (such as (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, *trans*-β-ionone, and decanal). Our findings provide theoretical guidance for the production of high-quality black tea with floral and fruity aromas.

1. Introduction

Black tea has become the most widely consumed type of tea globally because of its distinctive flavor and nutritional benefits that come from its properties, such as antidiabetic, antioxidant, and antimicrobial activities (Bhattacharya, Gachhui, & Sil, 2013; Qu et al., 2020; Zhang, Qi, & Mine, 2019). Aroma is an important contributor to the quality and market value of black tea (Yang et al., 2020; Yang, Baldermann, & Watanabe, 2013), and the quality of the aroma and characteristics vary with the concentration, proportion, and category of volatile metabolites (VMs). Keemun black tea is abundant in geraniol, benzeneacetaldehyde, methyl salicylate, and linalool, which contribute to its floral and honey-like flavor (Su, He, Zhou, Li, & Zhou, 2022), while Dianhong black tea is rich in phenylacetaldehyde, linalool, and geraniol, which provide it with a floral, sweet, and caramel-like aroma (Ma et al., 2022).

The pathways responsible for the formation of VMs can be classified into four categories based on the synthetic pathways of plant endogenous volatiles: fatty acid-derived volatiles, carotenoid-derived volatiles, volatile terpenoids, and amino acid-derived volatiles (Chen et al., 2022; Liu et al., 2023; Yang, Baldermann, & Watanabe, 2013). The VMs content of finished tea is closely linked to the tea variety, cultivation conditions, and processing techniques (Yang, Baldermann, & Watanabe, 2013; Zeng, Watanabe, & Yang, 2019; Zheng, Li, Xiang, & Liang, 2016). Among these factors, processing techniques play a crucial role in promoting terpenoid biosynthesis, glycoside hydrolysis, fatty acid and carotenoid degradation and oxidation, and amino acid polymerization, which directly impact the conversion of VMs and aroma formation in tea (Feng et al., 2019; Guo et al., 2021a; Ho, Zheng, & Li, 2015; Liu et al., 2023; Wang et al., 2019).

The processing of black tea, a fully fermented tea, typically involves withering, rolling, fermentation, and drying. Withering is the initial process that is crucial for the quality of black tea (Hou et al., 2020). During this process, leaves gradually shrink and soften as they lose water, and hydrolytic enzyme activity is enhanced, which promotes the hydrolysis of glycoside aroma precursors, while picking and slight dehydration stress trigger the expression of genes associated with

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Abbreviations: TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combines with the shaking process; VMs, volatile metabolites; IRAE-HS-SPME-GC–MS, infrared-assisted headspace solid-phase microextraction coupled with gas chromatography-tandem mass spectrometry; VIP, variable importance in projection; OAV, odor activity value; PLS-DA, partial least-squares discriminant analysis; MEV, multiple experiment viewer.

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volatile biosynthesis (Hou et al., 2020; Wang et al., 2019; Zeng, Watanabe, & Yang, 2019). Ultimately, this results in changes in both the amount and concentration of VMs, thereby laying the foundation for the formation of specific aromatic qualities. Traditional methods include withering under natural conditions, sun withering, using a withering tank, and continuous machine withering (Huang et al., 2022). To enhance the aroma and flavor of black tea, researchers have developed innovative methods, such as red-light withering (Li et al., 2022), freeze withering (Muthumani & Senthil Kumar, 2007), and dynamic withering (Hou et al., 2020). However, owing to concerns about their effectiveness and cost, these technologies have not yet been widely adopted.

Shaking is critical to form the distinctive aroma of oolong tea (Hu et al., 2018; Ma et al., 2018). Shaking can improve the cell fragmentation rate through leaf flipping, increase the contact between enzymes, such as oxidase and hydrolase with substrates, and promote the oxidation reaction of polyphenols and glycoside hydrolysis (Liu, 2008; Xue et al., 2022; Zeng, Watanabe, & Yang, 2019; Zhang et al., 2019). Meanwhile, stress-induced biochemical reaction occurs in tea leaves under the dual stress of dehydration and damage caused by shaking, triggering the secondary metabolic pathway,inducing upregulation of the coding genes of lipoxygenase (LOX), hydroperoxide lyase (HPL), *trans*-nerolidol synthase (CsNES), etc., and promoting the formation of α-farnesene, methyl jasmonate, indole, *trans*-β-ionone, etc., thereby contributing to the floral and fruit-like aroma of oolong tea (Hu et al., 2018; Ma et al., 2018; Zeng, Watanabe, & Yang, 201; Zhou et al., 2017). In recent years, to meet the demands of consumers and enrich the quality of black tea, researchers have attempted to introduce shaking into the withering process as a new withering process to accelerate volatile and non-volatile biochemical reactions within withered leaves (Lei et al., 2017; Shi et al., 2018; Xue et al., 2022). This shaking–withering process could increase the contents of nerol, benzaldehyde, and *trans*-β-ionone in black tea, and produce a tea with sweet and floral fragrance (Lei et al., 2017). Furthermore, this process could enrich the biosynthesis of non-volatile metabolites, such as amino acids, flavonoids, and linoleic acid (Xue et al., 2022), and produce tea with a sweet mellow taste and floral aroma (Shi et al., 2018). However, most studies on black tea shaking technology were based on the results of sensory evaluation and VMs comparison of the finished black tea product, and it remains unclear how shaking affects the evolution and transformation of VMs throughout the entire process of black tea production.

In this study, we assessed black tea processed using traditional methods combined with the shaking process (SBT) as research objects, and black tea processed using traditional methods (TBT) was used as a control. First, artificial sensory evaluation, infrared-assisted headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (IRAE-HS-SPME-GC–MS) were utilized for analyzed finished tea samples to explore the differences in sensory flavors and VMs between TBT and SBT. Subsequently, partial least-squares discriminant analysis (PLS-DA), multiple experiment viewer (MEV) analysis, odor activity value (OAV) analysis, and significant difference analysis were performed to determine the key differential VMs between TBT and SBT. Finally, the evolutionary laws and transformation mechanisms governing the formation of key differential VMs during black tea processing were further clarified. Our findings have implications for enhancing the flavor quality of black tea and provide theoretical guidance for the targeted and standardized manufacture of high-quality black tea.

2. Materials and methods

2.1. Materials and reagents

Fresh tea shoots composed of one leaf and a bud of the Jinmudan (*Camellia sinensis* L.) cultivar were collected from Shengzhou tea comprehensive experimental base, Tea Research Institute, Chinese Academy of Agricultural Sciences (Zhejiang Province, China), in April 2021.

Purified water was acquired from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). Ethyl decanoate (purity ≥ 98.0 %) was obtained from TCI Chemical Industry Development Co., Ltd. (Shanghai, China). The *n*-alkanes ranging from C7-C40 (purity ≥ 98.0 %) were supplied by J&K Scientific (Beijing, China).

2.2. Preparation of tea samples

Fresh tea leaves were divided into two equal parts and subjected to traditional withering or shaking–withering, followed by rolling, fermentation, and drying. In-process tea samples from each process were collected and immediately frozen in liquid nitrogen before being subjected to freeze-drying using a Scientz-100F freeze dryer (Ningbo Scientz Biotechnology Co., Ltd., Ningbo, China) for subsequent analysis. Finished tea samples were collected and immediately stored in a −80 °C refrigerator for further analysis. The production processes and technological parameters were as follows.

(1) Withering. For the TBT withering process, fresh leaves were spread in an artificial climatic incubator (PRX-450D type, Ningbo Prandt Instrument Co., Ltd., Ningbo, China) at 28–30 °C for 15 h with relative humidity 70–75 % until the moisture reached 60–62 % to produce withered leaves. For the SBT withering process, the fresh leaves were spread in the artificial climatic incubator for 10 h, while maintaining the same temperature and humidity parameters as TBT withering until the moisture content reached 70 %, according to previous studies (Xue et al., 2022); subsequently, the leaves were subjected to a shaking machine (6CWY-60 type, Fujian Jiayou Tea Machinery Intelligent Technology Co. LTD, Quanzhou, China); the leaves were turned over twice for 10 min each, with an interval of 1.0 h between flips. The tea leaves continued to wither until the moisture content reached 60–62 % to produce withered leaves.
(2) Rolling. Withered leaves were rolled in a rolling machine (6CR-35 type, Zhejiang Chunjiang Tea Machinery Co., Ltd., Hangzhou, China) for 75 min, consisting of pressure-free rolling for 25 min, light rolling for 20 min, intermediate pressure for 15 min, heavy pressure for 10 min, and finally light pressure again for 5 min. to produce rolled leaves.
(3) Fermentation. The rolled leaves were placed in the artificial climatic incubator for 3.5 h of fermentation at 28–30 °C and a relative humidity of 90–95 % to yield fermented leaves.
(4) Drying. The fermented leaves were dried in a hot-air drying machine (6CHZ-7B type, Fujian Jiayou Machinery Co., Ltd., Quanzhou, China) at 110 °C for about 15 min until the moisture content reached 20–25 % before being spread out for another 30 min. Subsequently, the samples underwent a second round of drying at 90 °C for an additional 30 min to obtain dried leaves with approximately 6 % moisture.

2.3. Sensory evaluation

The finished tea was assessed by five professional tea tasters following the Methodology of Sensory Evaluation of Tea (GB/T 23776-2018) (Su, He, Zhou, Li, & Zhou, 2022). Specifically, a white plate was used to present 50 g tea sample for appearance evaluation. To obtain tea infusion, 3 g of the tea samples were brewed with 150 mL boiling water for a duration of 5 min. Subsequently, the tasters assessed the appearance, aroma, liquor color, taste, and the infused leaves by combining comments with 100-mark system.

2.4. Analysis of VMs in black tea samples

2.4.1. Extraction and detection of VMs

For extraction, 0.5 g of tea samples were accurately weighed and transferred into a 20 mL headspace bottle. Then, 1 mL of distilled water, heated to boiling point and 10 μL ethyl caprate with a concentration of 20 mg/L were added to the sample, and the cap of the headspace bottle was immediately tightened and the protective cap was pierced using a DVB/CAR/PDMS fiber tip with a manual handle (SPME, 50/30 μm, Supelco, PA, USA). Finally, the vial was exposed to a 100 W infrared device for 15 min, followed by the insertion of the fiber head into the injector port at a temperature of 250 °C for 5 min (Yang et al., 2020).

The volatile fingerprints were analyzed using Agilent Technologies 7890B–7000C, with sample separation employing an HP-5 ms ultra-inert capillary column (30 m × 0.25 mm × 0.25 μm). High-purity helium (99.999 %) was utilized as a carrier gas at a flow rate of 1.0 mL/min, while maintaining a constant GC injector temperature of 250 °C. The initial column temperature was maintained at 50 °C for 5 min, followed by a ramp to 150 °C at a rate of 4 °C/min and held for an additional 2 min. Finally, the temperature was increased to 270 °C at a rate of 10 °C/min and held for another 6 min (Wang et al., 2020).

2.4.2. Qualitative and quantitative analyses of VMs

Qualitative analysis was conducted using the Agilent Mass Hunter Unknowns analysis program and tentatively identified compounds, which were then screened for similarity greater than 80 % using the NIST 11 standard library search. The retention index of each compound was calculated based on the linear formula of *n*-alkanes (C7-C40). Meanwhile, in comparison to the values documented in the NIST database for the same columns the compounds were re-screened, according to the criteria of the retention index difference of less than 30 (https://webbook.nist.gov/chemistry/). Quantitative analysis was conducted to determine the mass concentration of VMs by referencing the ion current response of internal standards with known concentrations.

2.4.3. OAV analysis

The odor activity value (OAV) is defined as the ratio between the concentration of aroma components in a sample and their corresponding odor threshold values (Guo et al., 2021a; Ma et al., 2022). An OAV > 1 indicates that the volatile metabolite contributes significantly to a characteristic aroma (Guo et al., 2021a; Li et al., 2022; Ouyang et al., 2022; Wang et al., 2020).

2.4. Statistical analysis

The experiments were performed in triplicate and the average of the three replicates was reported as the result for each test. SPSS (version 22.0; SPSS Inc., Chicago, IL, USA) was used to analyze the significance of differences in the concentration and content of black tea VMs using different processing technologies by Student's *t*-test ($p < 0.05$). SIMCA-P13 (Umetrics, Umea, Sweden) was utilized to identify the principal components that impact the aroma of black tea processed using different technologies. Multi Experiment Viewer (version 4.9.0, https://mev-tm4-org.caas.cn) was utilized to assess the contributions of VMs under different processing technologies. Origin 8.0 (Origin Lab, USA) was utilized to construct graphs of the dynamics changes of the VMs throughout the processing procedure.

3. Results and discussion

3.1. Effect of shaking treatment on sensory quality of black tea

As shown in Table 1, shaking had a significant impact on the quality of black tea, particularly in terms of enhancing its aroma and taste, while exerting a minor influence on its appearance and leaf infusion. SBT exhibited a flowery and fruity aroma superior to that of TBT, which was characterized by a sweet aroma ($p < 0.05$). In terms of taste quality, SBT exhibited a sweet, brisk, and floral taste, which was considered to be better than that of TBT with sweet, brisk, and grassy taste ($p < 0.05$). Regarding liquor color, SBT demonstrated significantly better quality, with comments noting its orange-red hue and brightness ($p < 0.05$). Furthermore, the total score obtained from sensory evaluation of SBT was higher than that of TBT ($p < 0.05$).

For SBT processing, the dehydration and mechanical damage stress caused by shaking stimulated the expression of aroma-forming genes and promoted the conversion of aromatic substances (Hu et al., 2018; Ma et al., 2018; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008), resulting in a richer and more prominent aroma quality than TBT. In addition, stress leads to a reduction in catechin content, especially ester catechins, and an increase in theaflavin and amino acid content by promoting the enzymatic oxidation of catechins and degradation of proteins, which results in the formation of theaflavins and amino acids (Xue et al., 2022; Zhang et al., 2019), ultimately enhancing the color and taste quality of black tea liquor. In other words, shaking can significantly improve the flavor quality of black tea, which is consistent with the outcomes of prior studies (Lei et al., 2017; Shi et al., 2018; Xue et al., 2022).

3.2. Key differential VMs screening between TBT and SBT

3.2.1. Identification of VMs

In the finished black tea, 128 VMs were detected (Table S1), including 29 esters, 26 alkanes, 25 alcohols, 11 ketones, 11 terpenes, 9 aldehydes, 8 aromatic hydrocarbons, and 9 others. As shown in Table 2, alcohols and aldehydes possessed the highest concentrations of VMs in TBT and SBT, followed by esters, whereas aromatic hydrocarbons exhibited the lowest content. Compared to TBT, SBT exhibited a significant increase in ester and ketone content ($p < 0.05$), whereas the aldehyde, terpene, and aromatic hydrocarbon content significantly decreased ($p < 0.05$).

Compared with the traditional process, shaking increased the content of ketones and esters of SBT, such as ethyl hexanoate with fruity aroma (Conde-Martínez et al., 2013), (Z)-hexanoic acid 3-hexenyl ester with fruity fragrance (Ouyang et al., 2022), and *trans*-β-ionone with

Table 1
Sensory evaluation of TBT and SBT.

Sample	Appearance		Liquor color		Aroma		Taste		Infused leaves		Total score
	Evaluation	Score	Evaluation	Score	Evaluation	Score	Evaluation	Score	Evaluation	Score	
TBT	Tight and heavy, bend, black bloom wih tippy	86.33 ± 0.58	Orange bright	88.17 ± 0.76b	Sweet aroma with slightly floral flavor	86.33 ± 0.58b	Still sweet and brisk, with grass taste	85.67 ± 1.5b	Still red with buds, more green leaf	81.33 ± 1.15	85.82 ± 0.55b
SBT	Tight and heavy, bend, black bloom wih tippy	86.17 ± 0.29	Orange bright	90.00 ± 0.50a	floral and fruity flavor	91.00 ± 1. 00a	Sweet and brisk, with floral flavor	92.83 ± 0.29a	Red bright with buds,with green leaf	83.33 ± 0.58	89.48 ± 0.35a

Notes: TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combined with the shaking process; data are presented as mean ± standard deviation (n = 5). Different lowercase letters in the same column indicate significant differences at $p < 0.05$ level.

Table 2
Volatile types contents of TBT and SBT.

Volatile types		Sample	
		TBT	SBT
Esters	Content (μg/L)	541.35 ± 17.40b	623.26 ± 9.89a
	Proportion	6.80 ± 0.40b	8.66 ± 0.79a
Alcohols	Content (μg/L)	3491.52 ± 163.79	3113.19 ± 339.82
	Proportion	43.79 ± 0.30	42.97 ± 0.47
Aldehydes	Content (μg/L)	3409.90 ± 149.68	2988.90 ± 332.28
	Proportion	42.77 ± 0.08a	41.25 ± 0.55b
Ketones	Content (μg/L)	112.26 ± 3.24b	131.34 ± 8.63a
	Proportion	1.41 ± 0.05b	1.82 ± 0.15a
Terpenes	Content (μg/L)	282.40 ± 18.91a	222.66 ± 12.77b
	Proportion	3.54 ± 0.08a	3.09 ± 0.21b
Aromatic hydrocarbons	Content (μg/L)	16.07 ± 0.77a	12.08 ± 1.42b
	Proportion	0.20 ± 0.00a	0.17 ± 0.00b
Alkanes	Content (μg/L)	46.98 ± 6.29b	56.55 ± 9.15a
	Proportion	0.59 ± 0.06b	0.78 ± 0.06a
Others	Content (μg/L)	72.04 ± 8.94	91.51 ± 17.56
	Proportion	0.90 ± 0.09b	1.26 ± 0.16a
Total content of volatile compounds (μg/L)	Content (μg/L)	7972.52 ± 341.00	7239.49 ± 717.55

Notes: TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combined with the shaking process. Data are presented as mean ± standard deviation (n = 3). Different lowercase letters on the same line indicate significant differences at $p < 0.05$ level.

floral aroma (Ho et al., 2015), and reduced the content of aldehydes, terpenes, and aromatic hydrocarbons of SBT, such as citral, 2,6-dimethyl-2,4,6-octantriene, and o-cymene. Shaking could promote the accumulation of VMs with floral and fruity flavors and change the content and proportion of VMs, thus enhancing the aromatic quality of black tea.

3.2.2. *PLS-DA analysis*

PLS-DA is a supervised pattern discriminant analysis technique that is extensively utilized for evaluating tea quality (Shevchuk, Jayasinghe, & Kuhnert, 2018; Yang et al., 2022). Based on the 128 VMs identified in our black tea samples, differential VMs between TBT and SBT were analyzed. As depicted in Fig. 1A, black tea processed using the different withering methods were clearly distinguished, as indicated by the scoring plots of TBT on the left side and that of SBT on the right side of the chart, respectively. This was consistent with the sensory results (Table 1). The model accurately distinguished the different aromas produced by the different treatments. The independent variable fit index, R^2X (cum), was 0.771; the dependent variable fit index, R^2Y (cum), was 0.995; and the prediction fit index, Q^2 (cum), was 0.945, indicating robust cumulative interpretation and prediction, and that the model is stable and reliable.

The spatial distribution of VMs in the different treatments was further observed in the loading scatter plot of the PLS-DA model (Fig. 1B). For instance, the concentrations of some VMs, such as *trans*-nerolidol, 2,6-dimethyl-2,4,6-octatriene, 1-isocyano-3-methylbenzene, (1S)-(−)-β-pinene, and geraniol, were significantly higher in TBT ($p < 0.05$), whereas concentrations of (Z)-butanoic acid, 3-hexenyl ester, *trans*-β-ionone, 2-propyl-furan, *cis*-3-hexenyl *cis*-3-hexenoate, ethyl hexanoate, cyclobutane, decanal, and (Z)-3-nonen-1-ol were significantly higher in SBT ($p < 0.05$). Additionally, the variable importance in

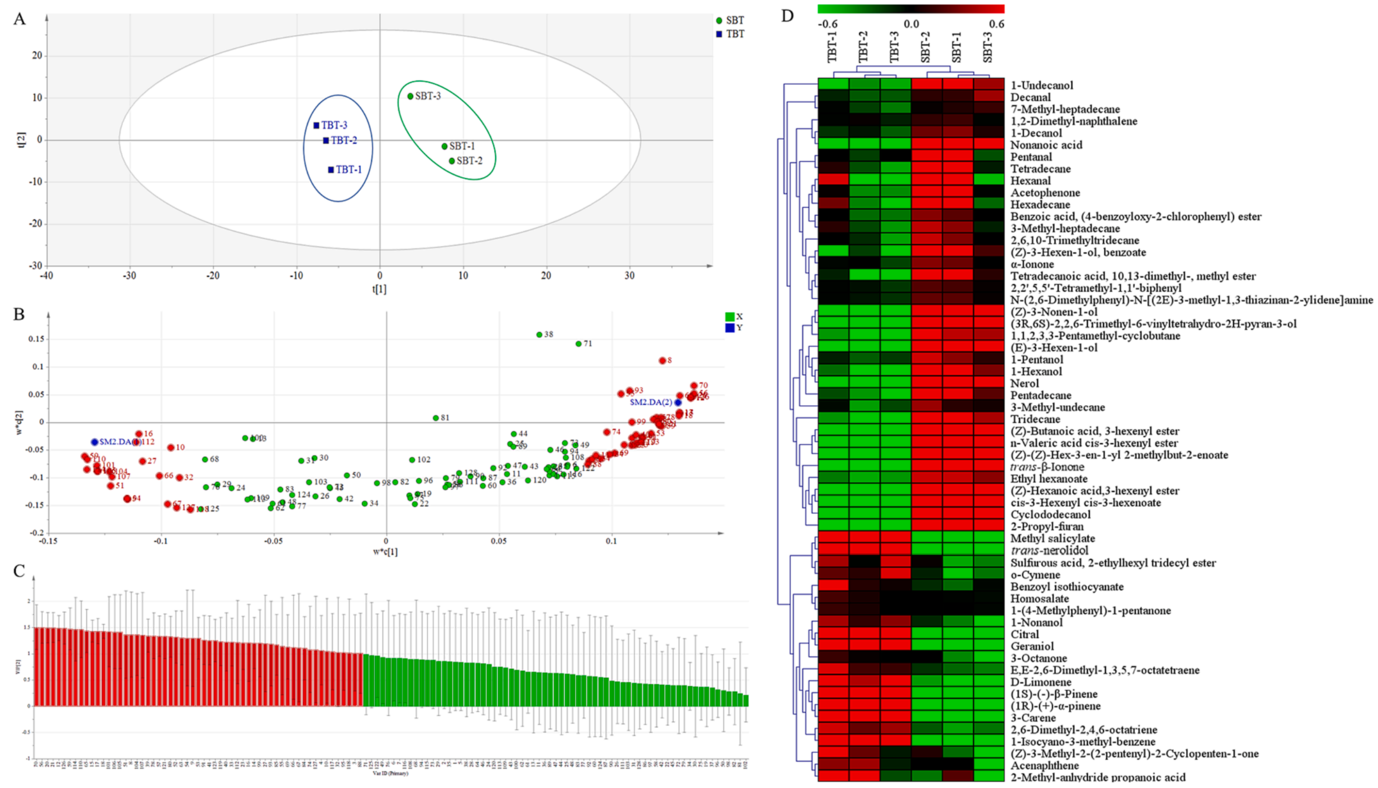

Fig. 1. Partial least-squares discriminant analysis (PLS-DA) results of volatile metabolites (VMs) and heat map of the distribution of differential VMs in black tea processed using different methods. (A) PLS-DA score scatter plot; (B) PLS-DA loading diagram (SM2.DA(1), black tea processed using traditional methods; SM2.DA (2), black tea processed using traditional methods combines with the shaking process); (C) PLS-DA variable importance in projection (VIP) (red indicates VIP > 1.0, green indicates VIP < 1.0); (D) Heat map of the distribution of 59 differential VMs of black tea processed using different methods. TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combines with the shaking process.

projection (VIP) was employed to identify the differential VMs between TBT and SBT (Su, He, Zhou, Li, &Zhou, 2022). Based on VIP > 1, 59 differential VMs, including *trans*-β-ionone, (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, *cis*-3-Hexenyl *cis*-3-hexenoate, *trans*-nerolidol, (Z)-butanoic acid 3-hexenyl ester, 2,6-dimethyl-2,4,6-octatriene, (1S)-(-)-β-pinene, and decanal, were obtained (Fig. 1C). These compounds could serve as markers to distinguish the sweet aroma and the floral, fruity flavor of black tea.

3.2.3. MEV analysis

To visualize the contributions of differential VMs between SBT and TBT, a heat map was generated using the MEV software. As depicted in Fig. 1D, TBT with a sweet flavor was grouped separately from SBT with floral, fruity flavors, consistent with the PLS-DA results. Moreover, these 59 VMs were classified into two categories: the first exhibited a stronger positive correlation with SBT and included decanal, 1-decanol, nonanoic acid, nerol, *trans*-β-ionone, acetophenone, α-ionone, nonanoic acid, and ethyl hexanoate, and these VMs presented floral and fruity flavors; the second type had a sweet and herbal fragrance and included D-limonene, (1S)-(-)-β-pinene, 3-carene, 2,6-dimethyl-2,4,6-octatriene, geraniol, *trans*-nerolidol, citral, and methyl salicylate, and these VMs were present at a higher content in TBT. These results indicated that the varying contents and compositions of the VMs contributed to the distinct aroma types of TBT and SBT.

Combined with the results of PLS-DA and MEV, a significance analysis was conducted on different compounds (Table S2), resulting in the identification of 32 important differential VMs between TBT and SBT, such as decanal, citral, ethyl hexanoate, (Z)-hexanoic acid-3-hexenyl ester, 1-pentanol, 3-nonen-1-ol, geraniol, 1-decanol, *trans*-nerolidol, *trans*-β-ionone, (1S)-(-)-β-pinene, 3-carene, 2,6-dimethyl-2,4,6-octatriene, and D-limonene. Among these, 11 substances were significantly more abundant in TBT ($p < 0.05$), whereas 21 substances exhibited significantly elevated levels in SBT ($p < 0.05$).

3.2.4. OAV analysis

OAV serves as a reliable indicator for objectively assessing the contribution of individual VMs to aroma (Guo et al., 2021a; Li et al., 2022; Ouyang et al., 2022; Wang et al., 2020). The aromatic properties and thresholds of 32 important differential VMs were searched, the OAV and aroma characteristics of 15 VMs were obtained, and eight key differential VMs associated with aroma with OAV > 1 were identified (Table 3). The OAV of *trans*-β-ionone exceeded 500, indicating its strong contribution to the characteristic aroma of black tea (Wang et al., 2020); however, the OAV values of decanal, citral, ethyl hexanoate, (Z)-hexanoic acid 3-hexenyl ester, geraniol, *trans*-nerolidol, and 2,6-dimethyl-2,4,6-octatriene of TBT or SBT were above 1, indicating their important contribution to black tea aroma.

Combined with the outcomes of the sensory evaluation, these results suggest that TBT showed higher OAVs of geraniol, citral, *trans*-nerolidol, and 2,6-dimethyl-2,4,6-octatriene ($p < 0.05$), which are representative VMs with sweet aromas, whereas SBT had higher OAVs of (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, decanal, and *trans*-β-ionone, which represent fruity and floral aromas. These findings suggest that the aromatic characteristics of black tea are significantly affected by the processing method and that shaking can promote the formation and accumulation of VMs associated with floral and fruity aromas.

Table 3
Aroma characteristics and OAV values of key differences in volatile metabolites (VMs) in TBT and SBT.

Compounds	Odor Characteristic^A	OTs (μg/L)^B	TBT(μg/L)	SBT(μg/L)
Decanal	Citrus-like, floral, sweet, orange	0.1	5.66 ± 0.91b	9.90 ± 1.64a
Citral	Lemon sweet, grassy, woody, musty	53	56.23 ± 2.07a	46.85 ± 4.85b
Ethyl hexanoate	Intense aromas of fruity	1.2	0.32 ± 0.06b	1.05 ± 0.08a
(Z)-Hexanoic acid-3-hexenyl ester	Fruity, sweet, rose	16	0.83 ± 0.06b	3.10 ± 0.22a
1-Pentanol	Bitter, almond, synthetic, balsamic	150.3	0.01 ± 0.00	0.01 ± 0.00
1-Hexanol	Grassy	500	0.01 ± 0.00	0.01 ± 0.00
1-Nonanol	Dusty, oily, green, floral	45.5	0.06 ± 0.00	0.04 ± 0.01
Nerol	Sweet, fruity	53	0.22 ± 0.01b	0.32 ± 0.05a
Geraniol	Sweet, fruity, rose	7.5	397.36 ± 14.64a	330.82 ± 34.22b
1-Decanol	Waxy, sweet, floral, fruity	400	0.00 ± 0.00	0.00 ± 0.00
Trans-nerolidol	Floral, citrus, woody	0.25	97.17 ± 3.39a	79.22 ± 0.05b
Trans-β-ionone	Violet-like, floral, raspberry-like	0.007	3215.79 ± 53.2b	3952.84 ± 16.87a
D-Limonene	Fruity, lemon-like	34	0.38 ± 0.02	0.32 ± 0.02
2,6-Dimethyl-2,4,6-octatriene	Herbal, sweet, floral, woody	0.03	98.67 ± 4.37a	73.57 ± 3.38b
Nonanoic acid	Light fat and coconut aromas	3000	0.00 ± 0.00	0.01 ± 0.00

Notes: TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combined with the shaking process. Different lowercase letters on the same line indicate significant differences at $p < 0.05$ level.

A: Determined according to https://www.thegoodscentscompany.com/search3.php?qOdor=20126–76-5&submit.x=9&submit.y=9.

B: OT, odor threshold in water based on the literature (Conde-Martínez et al., 2013; Guo, Ho, Schwab, & Wan, 2021b; Joshi, & Gulati, 2015; Li et al., 2022; Ouyang et al., 2022).

3.3. Effect of shaking on VMs transformation during black tea processing

3.3.1. Changes in VMs types during TBT and SBT processing

The change laws for alcohols, aldehydes, esters, alkenes, ketones, and aromatic hydrocarbons during TBT and SBT processing differed (Fig. 2). For TBT processing, the ester content displayed a trend of initial increase followed by a subsequent decrease, whereas the others exhibited a fluctuating pattern. However, for SBT processing, the aromatic hydrocarbons showed a decreasing trend, whereas the others exhibited a pattern of first increasing and then decreasing, and the peaks of most types occurred during the withering process. Compared with traditional withering, shaking–withering treatment significantly enhanced the content of esters and ketones ($p < 0.05$) while reducing the content of terpenes and aromatic hydrocarbons. The alcohols and aldehydes of SBT in-process products in the fermentation stage were lower than those of TBT; however, there were no significant differences between the finished black teas ($p > 0.05$).

The increase in VMs during withering is mainly attributed to dehydration stress (Wang et al., 2019). The incorporation of shaking into withering process results in continuous damage that alters water, oxygen, and membrane permeability within the leaf cells, which leads to enhanced oxidative degradation of fatty acids and carotenoids (Dudareva, Klempien, Muhlemann, & Kaplan, 2013; Liu, 2008; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008; Wang et al., 2020), thereby stimulating the formation of VMs, such as ethyl hexanoate, (Z)-hexanoic acid 3-hexenyl ester, and *trans*-β-ionone, and enriching the aroma and flavor of black tea. However, the surface structure of tea leaves was damaged by shaking, and the retention of alkenes and aromatic hydrocarbons decreased.

Compared with withered leaves, leaves subjected to rolling experience cell fragmentation leading to VMs volatilization, and polyphenols in the vacuoles entered the cytoplasm to inhibit the enzymatic formation

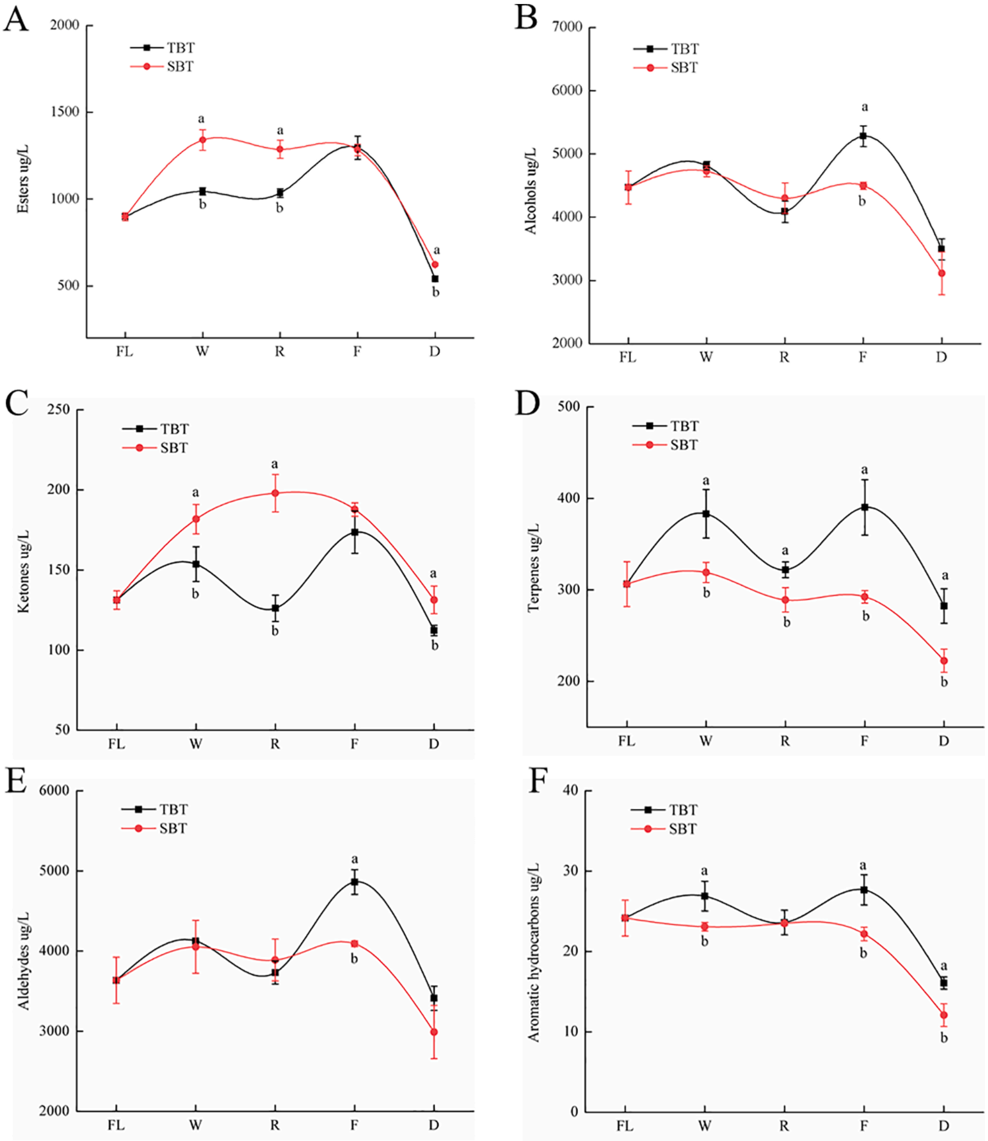

Fig. 2. Changes in main volatile metabolites (VMs) of black tea processed using different methods: (A) esters, (B) alcohols, (C) ketones, (D) terpenes, (E) aldehydes, and (F) aromatic hydrocarbons. TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combines with the shaking process. FL, fresh leaves; W, withered leaves; R, rolled leaves; F, fermented leaves; D, dried leaves. Different lowercase letters in the same process indicate a significant difference at $p < 0.05$ level.

of VMs, thereby decreasing most VMs during the rolling process (Shi, 1987). Fermentation is a pivotal process that transforms a grassy aroma into a sweet floral aroma (Chen et al., 2022). Shaking disturbs the dynamic balance between the production and removal of reactive oxygen species in tea leaves, leading to intensified free radical production, further oxidation of alcohol hydroxyl groups, and transformation of aldehydes during fermentation (Wen, 2013). Thus, the alcohol and aldehyde contents of SBT fermented leaves were lower than those of TBT leaves. The substantial reduction in VMs during drying was presumably due to the increased release of VMs under high-temperature, long-term conditions, which is consistent with prior findings (Li et al., 2022). Consequently, the impact of shaking is not limited to the withered leaves, but also extends throughout the entire processing procedure, ultimately influencing the content and composition of VMs in the finished black tea, thereby affecting its aromatic quality.

3.3.2. Speculation on the effect of shaking on the change and transformation mechanism of eight key differential VMs

The change and transformation mechanisms of eight key differential VMs obtained through PLS-DA, MEV, and OAV analysis during TBT and SBT processing are illustrated in Fig. 3. These eight key VMs could be divided into three groups, fatty acid-derived volatiles ((Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, and decanal), carotenoid-derived volatiles (*trans*-β-ionone), and terpenoid volatiles (geraniol, citral, *trans*-nerolidol, and 2,6-dimethyl-2,4,6-octantriene), according to the transformation and metabolism pathways (Chen et al., 2022; Ho, Zheng, &Li, 2015; Liu et al., 2023; Yang, Baldermann, & Watanabe, 2013; Zeng, Watanabe, & Yang, 2019).

Fatty acids, such as palmitic acid, α-linolenic acid, and oleic acid are important precursors to form black tea aromatic substances via oxidation, cleavage, and isomerization (Dudareva, Klempien, Muhlemann, & Kaplan, 2013; Liu et al., 2023; Schwab, Davidovich-Rikanati, & Lewinsohn, 2008). During TBT and SBT processing, the contents of three fatty acid-derived volatiles first increased and then decreased (Fig. 3A). Dehydration stress induced the enhancement of fatty acid metabolism during the withering process and promoted lipoxygenase-mediated lipid oxidation, resulting in the production of (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, and decanal in considerable quantities (Wang

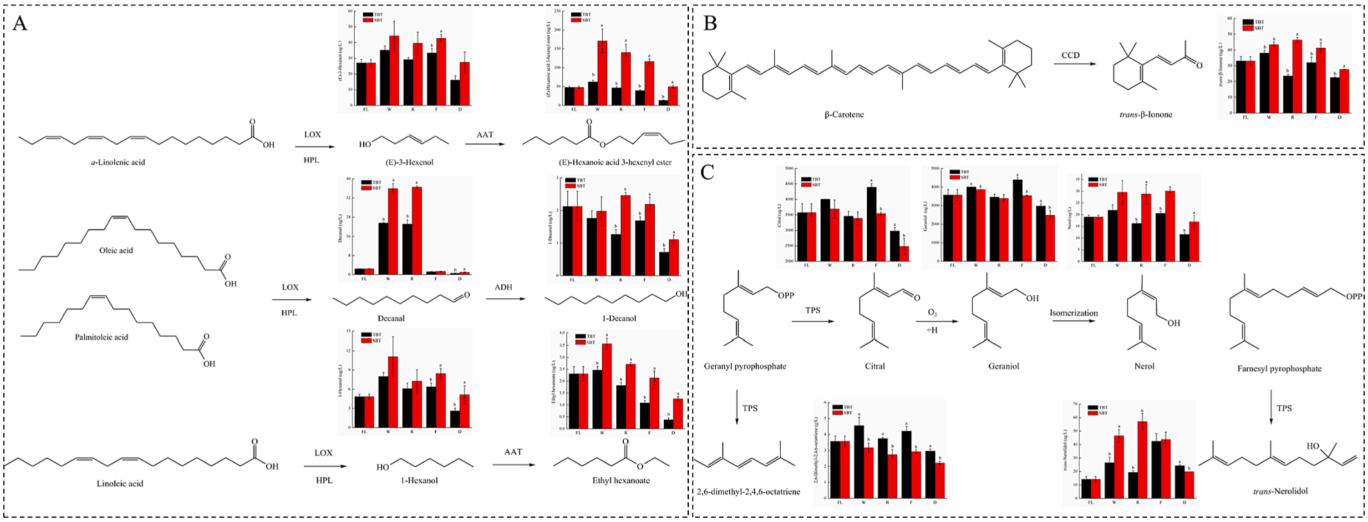

Fig. 3. Evolution characteristics of key differential volatile metabolite-related substances obtained from different sources during TBT and SBT processing: (A) Fatty acid-derived volatiles, (B) Carotenoid-derived volatiles, (C) Volatile terpenoids. TBT, black tea processed using traditional methods; SBT, black tea processed using traditional methods combines with the shaking process. FL, fresh leaves; W, withered leaves; R, rolled leaves; F, fermented leaves; D, dried leaves. LOX, lipoxygenase; HPL, hydroperoxide lyase; AAT, alcohol acyltransferase; ADH, alcohol dehydrogenase; CCD, carotenoid cleavage dioxygenase; TPS, terpene synthase. Different lowercase letters in the same process indicate a significant difference at $p < 0.05$ level.

et al., 2019). However, along with their release and volatilization in subsequent processes, their retention in tea leaves was reduced. Specifically, decanal undergoes a sharp decrease during fermentation, which is further reduced to 1-decanol by alcohol dehydrogenase (ADH) (Ouyang et al., 2022).

The levels of (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, and decanal in SBT and its in-process products were higher than those in TBT and its in-process products (Fig. 3A). This may be because the mechanical damage caused by shaking induced the expression of structural genes in the LOX pathway of unsaturated fatty acids (Hu, Li, et al., 2018), which enhanced the activity of key enzymes and promoted the oxidative degradation of α-linolenic acid, oleic acid/palmitic acid, and linoleic acid to produce (E)-3-hexenol, decanal, and 1-hexanol (Dudareva, Klempien, Muhlemann, & Kaplan, 2013; Hu, Li, et al., 2018; Ho, Zheng, & Li, 2015), and further dehydrogenation synthesis of (Z)-hexanoic acid-3-hexenyl ester and ethyl hexanoate by ADH (Fig. 3A). The rich fatty acid-derived volatiles contributed significantly to the fruity flavor of black tea.

Trans-β-ionone is an important volatile metabolite in black tea with a floral aroma, which is primarily generated through the enzymatic oxidation and auto-oxidation of β-carotene (Ho, Zheng, & Li, 2015; Hou et al., 2020). During TBT processing, *trans*-β-ionone content exhibited an "up-down-up-down" trend, which was consistent with previous research (Liu et al., 2023). However, the *trans*-β-ionone content exhibited an initial increase followed by a subsequent decrease during SBT processing (Fig. 3B). Mechanical damage, resulting from shaking, possibly triggered carotenoid lytic dioxygenases (Wang et al., 2020), and promoted the oxidative degradation of β-carotene (Hou et al., 2020) formation and accumulation of *trans*-β-ionone during the withering and subsequent processing. Consequently, the content of *trans*-β-ionone in SBT and its in-process products was significantly higher than those in TBT and its in-process products ($p < 0.05$), thereby contributing to the formation of floral aroma.

Terpenoid volatiles are predominantly synthesized via the 2-C-Methyl-D-erythritol-4-phosphate pathway and mevalonate pathway in tea plants (Yang, Baldermann, & Watanabe, 2013). During TBT processing, the content of geraniol, citral, *trans*-nerolidol, and 2,6-dimethyl-2,4,6-octantriene exhibited an "up-down-up-down" trend; during SBT processing, geraniol and citral content showed the same trend as that in TBT, 2,6-dimethyl-2,4,6-octantriene showed a decreasing trend, and *trans*-nerolidol exhibited a pattern of first increasing followed by a decrease (Fig. 3C). Furthermore, the content of the four terpenoid-derived volatiles of TBT was higher than that of SBT, whereas the content of nerol, the isomerization product of geraniol, was lower ($p < 0.05$).

The increase in geraniol and *trans*-nerolidol content after withering and fermentation is mainly caused by the hydrolysis of glycoside-bound VMs (Chen et al., 2022) and the upregulation of the expression of related terpene synthesis key rate-limiting enzymes genes (Wang et al., 2019; Zeng, Watanabe, & Yang, 2019). Meanwhile, different withering modes have different effects on the transformation of aroma precursors and the release of VMs (Hou et al., 2020). Shaking accelerated the reduction and isomerization of citral and geraniol to produce nerol because of the high levels of cell disruption in tea leaves (Ouyang et al., 2022), resulting in lower geraniol and citral content in both SBT and its in-process products when compared with TBT and its in-process products (Fig. 3C). Compared to TBT in-process products, the sustained injury stress experienced by tea leaves during SBT processing upregulated the expression of the CsNES gene, thereby promoting the synthesis of farnesyl diphosphate to *trans*-nerolidol (Zhouet al., 2017), whereas the cell damage caused by shaking resulted in high volatility at high temperatures, leading to a decrease in retention in SBT.

The presentation of aroma of black tea is a multifaceted process influenced by various factors, including boiling point, concentration, antagonism, and synergy between VMs. The distinction between traditional withering and shaking–withering in terms of key differential VMs content led to differences in the aromatic characteristics of SBT and TBT.

4. Conclusions

Shaking has a significant effect on the aromatic flavor of black tea and the evolutionary laws and transformation mechanisms that govern the content of VMs. Sensory evaluation revealed that black tea treated with shaking exhibited excellent aromatic quality with products having floral and fruity flavors. Through PLS-DA, MEV, and OAV analysis, eight key differential VMs (geraniol, citral, *trans*-nerolidol, 2,6-dimethyl-2,4,6-octantriene, (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, decanal, and *trans*-β-ionone) were identified as key contributors to the different aromatic flavors of TBT and SBT. Moreover, the evolutionary laws and transformation mechanism during processing were analyzed, and shaking was found to promote the oxidative degradation of fatty acids and carotenoids and modulate the biosynthesis of terpenoids, resulting in higher decanal, (Z)-hexanoic acid-3-hexenyl ester, ethyl hexanoate, and *trans*-β-ionone content (flowery and fruity flavors) in SBT than TBT, and improving the aromatic quality of black tea. This study established a theoretical basis for the directional and standardized production of black tea with floral and fruity flavors. Nevertheless, our results demonstrate the impact of shaking on aroma, flavor, and VMs; however, further exploration is necessary to explore the effects of shaking on taste, color, and non-volatile metabolites of black tea.

CRediT authorship contribution statement

Jinjin Wang: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, Software. **Wen Ouyang:** Conceptualization, Investigation, Data curation. **Xizhe Zhu:** Conceptualization, Resources. **Yongwen Jiang:** Supervision, Investigation, Funding acquisition. **Yaya Yu:** Software, Investigation. **Ming Chen:** Methodology, Data curation. **Haibo Yuan:** Conceptualization, Supervision, Funding acquisition, Project administration. **Jinjie Hua:** Project administration, Conceptualization, Writing – review & editing.

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.2023.101007.

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