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Comparative volatiles profiling in milk-flavored white tea and traditional white tea Shoumei via HS-SPME-GC-TOFMS and OAV analyses

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A B S T R A C T

White tea is a mildly fermented tea processed with withering and drying. Milk-flavored white tea has a unique milk flavor compared to the traditional white tea. Little is known about the aromas that make white tea taste milky. Here we conducted the volatile profiling via headspace solid-phase microextraction (HS-SPME)-gas chromatography-time-of-flight mass spectrometry (GC-TOFMS) and chemometrics to explore the key volatiles making milk-flavored white tea taste milky. Sixty-seven volatiles were identified, with 7 volatiles (OAV > 1 and VIP > 1) were characterized as the typical aromas. Green and light fruity scent volatiles, such as methyl salicylate, benzyl alcohol, and phenylethyl alcohol, were richer in TFs than MFs. Strong fruity and cheese aromas, such as dihydro-5-pentyl-2(3H)-furanone, 2-pentyl-furan, (E)-6,10-dimethyl-5,9-undecadien-2-one, and hexanal, were more abundant in MFs than TFs. Dihydro-5-pentyl-2(3H)-furanone, recognized as coconut and creamy aroma, should be the essential volatile for milky flavor. Also, (E)-6,10-dimethyl-5,9-undecadien-2-one and 2-pentyl-furan may contribute to the milk scent formation.

1. Introduction

Tea (*Camellia sinensis*) is nutritional and healthy, and is one of the most popular beverages in the world. White tea belongs to the six major tea types in China and is mainly in Fuding city and Zhenghe county, Fujian Province, China. Beiyuan Tribute Tea, high-quality white tea, has enjoyed a reputation since ancient times. Traditional white tea can be divided into silver needles, Bai Mudan and Shoumei based on the tea leaves' ages. White tea become more and more popular due to its additional health functions, such as effects of hypolipidemic and hypoglycemic, free radical scavenging, and antioxidants (N. Li et al., 2022; Ning, Ding, Song, Zhang, Luo, & Wan, 2016; Sanlier, Atik, & Atik, 2018). White tea is fresh, mellow, and palatable due to its mild fermentation. The processing of white tea consists of withering and drying. Withering is critical for white tea quality since it influences the formulation of white tea's unique fragrance and flavor. After prolonged withering, the activity of amylase and glutamate decarboxylase in tea leaves rose, resulting in a significant increase in theaflavin, γ-aminobutyric acid, and soluble sugar content (C. Zhou et al., 2022). Meanwhile, both enzymatic and non-enzymatic oxidations stimulated the synthesis of tea fragrance (Y. Zheng et al., 2022). It has been demonstrated that tea scents were mostly produced from carotenoids, lipids, and glycosides or through the Maillard reaction (Ho, Zheng, & Li, 2015). Aroma compounds were generated depending on the withering process rather than the tea leaves themselves.

In recent years, white tea has been gain increasing attention. Feng et al. found that geraniol and linalool were the main contributors to making white tea floral and sweet (Feng, Li, Li, Yin, Wan, & Yang, 2022). White tea tastes different from region to region. C. Ma et al. (C. Ma et al., 2022) found that Yunnan white tea was more astringent than Fuding white tea. Tea aroma always determines the white tea quality. The withering environment affects the synthesis of aromas and flavors. Several studies have been conducted on the effects of light (Mu, Li, Tang, Liu, & Wang, 2021), time (Dai et al., 2017; Wang et al., 2019; B. Zhou et al., 2022), temperature (Shao, Zhang, Lv, & Shen, 2021; Yu et al., 2020) on the quality of white tea. Compared to green tea, the prolonged

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withering of white tea could facilitate the accumulation of flavonoids, flavanone, amino acids, and sugars (C. Zhou et al., 2022). As reported by Mu et al. (Mu et al., 2021), tea polyphenols were highest when withered under green light, but lowest when withered under blue light. Red-light can boost the activity of glycosidase in the later withering to improve the aroma quality of tea (Li, He, et al., 2022). Also, red-light withering could make a sweet, fresh, and mellow taste (Fu et al., 2015; Hao et al., 2016). The withering temperature influenced not only the dehydration rate but also the sensory quality and biochemical compositions in white tea. The withering process was shortened at 25 °C while remaining the same sensory quality as natural withering.

Milk-flavored white tea is a newly patented white tea from Zhenghe county, and is well-known for its rich floral and fruity smells with an obvious milk scent. When compared to Shoumei, a traditional white tea, milk-flavored white tea is made from mature shoots that have been withered at high temperatures and humidity. Few studies have been conducted on the aroma of milk-flavored white tea, it is critical to explore the volatile components of milk-flavored white tea. TOF-MS (time-of-flight mass spectrometry) is suited for applications such as rapid GC separation, resulting in peak time compression with sufficient sensitivity and speed. Importantly, the fast spectral generation rate and the nonbias spectra allow for the analytical resolution of chromatographically unseparated compounds (Song, Gardner, Holland, & Beaudry, 1997). Gas chromatog-raphy-time-of-flight mass spectrometry (GC-TOFMS) combined with headspace solid-phase microextraction (HS-SPME) enables quantitative and qualitative analysis of complex samples in multicomponent mixtures. It is simple, rapid, efficient, and ideal for determining product volatiles (C. Liu et al., 2022; L. Ma et al., 2022; Xia, Zhang, Wang, & Guo, 2014; Yin et al., 2022). Here, we examined the volatile compounds between milk-flavored white tea and Shoumei made from the same grade fresh tea leaves based on HS-SPME and GC-TOFMS analysis. Combined with odor activity value (OAV) and multivariate statistical analysis, the characteristic volatile compounds in milk-flavored white tea were investigated to provide a reference for milk-flavored white tea quality control.

2. Materials and methods

2.1. Tea samples

Seven tea samples were collected in Zhenghe county, Fujian Province, including three milk-flavored white tea samples (MFs) and four traditional Shoumei samples (TFs). The three milk-flavored white tea were provided by Zhenghe Ruiming Tea Co., Ltd. (MF1), Zhenghe Lingyafu Tea Culture Communication Co., Ltd. (MF2) and Shuimunian Tea Co., Ltd. (MF3). Of the four traditional Shoumei tea samples, two were from Zhenghe Ruiming Tea Co., Ltd. (TF1 and TF2), one was from Zhenghe Lingyafu Tea Culture Communication Co., Ltd. (TF3), and another one was from Shuimunian Tea Co., Ltd. (TF4). Fig. 1 depicts the processing of the milk-flavored white tea and Shoumei. These tea samples were processed in May 2022, sealed, and stored at −80 °C for the following GC-TOFMS analysis.

2.2. Chemicals and instruments

Decanoic acid, ethyl ester (chromatographically pure reagent, purity ≥ 99.8%); The standard mixture of *n*-alkanes C_8-C_{30} was purchased from Sigma (Shanghai, China). *α*-ionone, benzaldehyde, methyl salicylate, geranylaceton, nerolidol, and *β*-cyclocitral were from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Octanal, nonanal, phenylactaldehyde, hexanoic acid, and methyl ester were from Shanghai Yi'en Chemical Technology Co., Ltd. (Shanghai, China). 1-Hexanol and heptanal were purchased from Shanghai Zhenzhun Bio-Technology Co., Ltd. (Shang-hai, China).

2.3. Sensory evaluation and quantitative descriptive analysis (QDA)

The sensory assessments were conducted independently by eight reviewers (four men and four women, average age 38 years) with 10 to 20 years of professional experience in tea science, according to the white tea review method in GB/T 23776–2018 "Tea Sensory Review Methods". Weighed 3 g of tea and put it into a 150 mL column cup, brewed in boiling water for 5 min, and then filtered. The panel assessed the type of aroma, concentration, purity, and persistence of the tea samples, gave comments, and scored. Parallel trials were conducted three times.

QDA was conducted with reference to previous studies (Su, He, Zhou, Li, & Zhou, 2022; X. Zheng et al., 2022). The brewed tea sample infusions were coded and evaluated by expert members who rated the tea samples according to their aroma attributes using a scale of 0 to 5, with aroma intensity of 0 being non-existent, 1 being identifiable, 2 being weak, 3 being moderate, 4 being strong and 5 being very strong. Each expert member assessed each sample 3 times at different times.

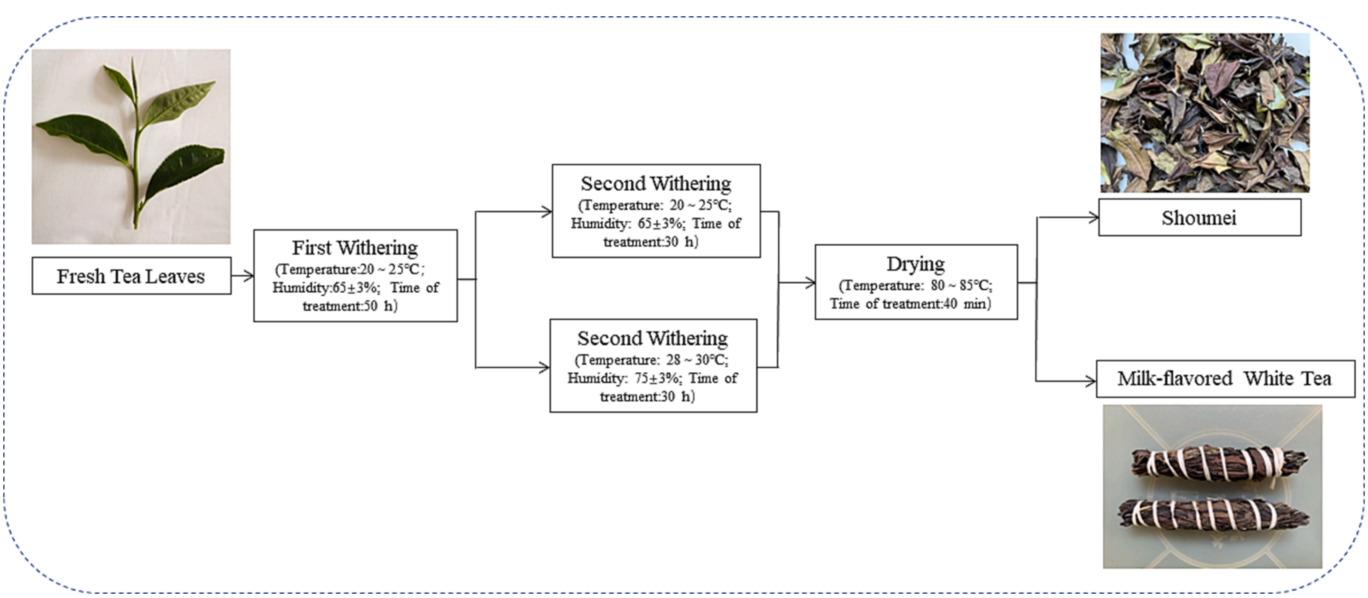

Fig. 1. The processing flow of milk-flavored white tea and the traditional white tea Shoumei.

2.4. Extraction of tea aroma by HS-SPME

Fully grounded samples (2 g) were weighed into a 20 mL headspace flask (accurate to 0.001 g). Then, 1.5 μL of decanoic acid, ethyl ester (internal standard 112 μg/kg) was added by reference to the method of Zeng (Zeng et al., 2022). Equal volumes of each sample were mixed to test the stability of the instrument (ACQUI-TYUPLCI-Class liquid chromatography) in triplicate. HS-SPME was extracted using a PDMS/DVB extraction needle (1 cm, 65 μm, Supelco, Bellefonte, PA, USA). The vials were incubated at 80 °C for 31 min, and then extracted for 60 min, and finally resolved at 250 °C for 3.5 min. The volatiles were then for GC-TOFMS analysis.

2.5. GC–TOFMS analysis of volatile compounds

GC-TOFMS analysis was performed as Zeng described (Zeng et al., 2022). An Agilent 7890B gas chromatograph (Agilent Technologies, USA) interfaced with Pegasus HT time-of-flight mass spectrometer (LECO Corporation, USA) was used for GC-TOFMS analysis. The separation was performed on a Rxi®-5silMS column (30 m, inner diameter 0.25 mm and film thickness 0.25 μm, Restek, Bellefonte, PA, USA) with the inlet temperature set at 250 °C and the transmission line temperature fixed at 275 °C. The carrier gas was helium, and the flow rate was set at 1 mL/min. The programmed ramp-up was set to 50 °C and held for 5 min, followed by a 3 °C/min ramp-up to 210 °C for 3 min and then a 15 °C/min ramp-up to 230 °C. The sample was injected with the splitless flow. For MS analysis, the ionization energy of EI was 70 eV, and the ion source temperature was 250 °C.

Volatiles were identified by matching the peaks of volatile compounds to the National Institute of Standards and Technology mass spectrometry database (NIST) and using the *n*-alkane series (C_8-C_{30}) (Sigma-Aldrich, Shanghai, China) to calculate retention indices (RI) for volatile compounds and comparing them to the RI in the database. Chemical structures, names, and CAS numbers of volatile compounds were determined according to PubChem (https://pubchem.ncbi.nlm.nih.gov/ accessed on August 1st, 2022) and compound odor descriptions were determined through the Flavor Ingredient Library (https://www.femaflavor.org/flavorlibrary/ accessed on August 2nd, 2022) for determination. The relative content of volatiles was calculated based on the internal standard (μg/kg) (Xiao, Cao, Zhu, Chen, & Niu, 2022).

2.6. Odor activity value (OAV) calculation

OAVs were used to assess the contribution of volatile compounds to the aroma of tea samples and were obtained by dividing the calculated concentration of volatile compounds by their odor threshold in water, and volatile compounds with an OAV > 1 were generally considered to make a significant contribution to samples' aroma (Xu et al., 2022). The OAV calculation equation was listed as follows:

$$OAV_i = \frac{C_i}{OT_i}$$

Note: C_i (μg/kg) was the relative content of volatile compounds; OT_i (μg/kg) was the aroma threshold in water for volatile compounds.

2.7. Statistical analysis

Data were processed and diagrams were created using Microsoft Excel 2010. Principal component analysis (PCA) and OPLS-DA were performed via SIMCA 14.1 software (Umetrics, Umea, Sweden). TBtools (https://github.com/CJ-Chen/TBtools accessed on August 18th, 2022) was used for heatmap and hierarchical clustering.

3. Results

3.1. Sensory quality assessment

The traditional sensory quality evaluation was conducted to validate the flavor differences between the milk-flavored white tea (MFs) and the traditional white tea Shoumei (TFs). It revealed that the milky flavor predominated in the three MFs that fluctuated (Fig. 2A), whereas TFs had typically floral, fruity, and green flavor (Fig. 2B). The milk scent in MF1 and MF2 was much stronger and last longer than that in MF3. The sweet aroma was the other key scent in MF1 and MF2. MF3 has a primary sweet flavor. Only MF1 has a faint fruit flavor (Fig. 2A). Compared to the flavors in MFs, the flavors in TFs were thin and were not so lasting (Fig. 2B). All the TFs had a dominant floral and fruity aroma. The green odors in TF1 and TF3 were also obvious. Of the 4 TFs, only TF2 had a little milky aroma (Fig. 2B). These results suggested that the milk-flavored white tea had a distinct milk scent, and the aromas in milk-flavored white tea were thicker and more lasting when compared to the traditional white tea Shoumei.

3.2. Volatile components profiling of milk-flavored white tea and Shoumei via HS-SPME-GC-TOFMS

To explore the volatile components that make milk-flavored white tea taste milky, we performed the volatile components profiling via GC-TOFMS for MFs and TFs (Figure S1-S7). A total of 67 volatile components were identified, including 12 alcohols, 20 esters, 8 ketones, 15 aldehydes, 3 hydrocarbons, 6 acids, 2 heterocycles, and 1 other compound (Table 1, S1). The main volatile components in TFs and MFs were alcohols and esters, with relative levels of 34.51% and 24.90% in TFs and 31.74% and 30.74% in MFs, respectively. Ketones and aldehydes accounted for a higher proportion of 16.84% and 11.20% in MFs and a lower proportion of 12.41% and 8.65% in TFs. However, hydrocarbons were much higher in TFs (16.86%) than those in MFs (0.70%) (Fig. 3A). Based on such classification, the milky scent was much more abundant in MFs than those in TFs, but with much the same roasted, fruity, woody, and green scents (Fig. 3B). The volatile components were obviously divergent in milk-flavored white tea and Shoumei.

The overview of the volatile substances in MFs and TFs was divergent (Fig. 3D). The top 10 most abundant volatile compounds in MFs and TFs were distinctive (Fig. 3C). Among them, dihydro-5-pentyl-2(3H)-furanone, (E)-6,10-dimethyl-5,9-undecadien-2-one, 2-pentyl-furan, *β*-ionone epoxide, 4,4,7a-trimethyl-5,6,7,7a-tetrahydro-2(4H)-benzofuranone, hexanal, (Z)-3,7-dimethyl-2,6-octadien-1-ol, and 3,7-dimethyl-2,6-octadienoic acid, methyl ester were richer in MFs than TFs, whereas phenylethyl alcohol, methyl salicylate, benzyl alcohol, and 3-methyl-tridecane were significantly more abundant in TFs than MFs (Fig. 3C). Dihydro-5-pentyl-2(3H)-furanone and 2-pentyl-furan were related to cheese flavor. Phenylethyl alcohol, methyl salicylate, and 3-methyl-tridecane were floral and fruity flavors. So, analysis of volatile substances exhibited the volatile components defined as cheese or sweet odors accumulated more abundantly in milk-flavored white tea than those in traditional white tea Shoumei.

3.3. Screening for characteristic volatiles

The correlation between the levels of some volatile components and the scents of white tea is insufficient for screening the characteristic volatiles in the milk-flavored white tea. We also calculated OAV to validate the compound contribution to the milk flavor. A total of 31 volatiles with OAV > 1 were identified (Table 2). Then, for these volatile components, we performed an OPLS-DA analysis. The OPLS-DA model performed well without overfitting in terms of model fit ($R^2Y = 0.994$) and predictive power ($Q^2 = 0.991$). The volatile components were separated into two groups (Fig. 4A, B). Normally, volatile substances with VIP (Variable importance in the project) > 1 were regarded as vital

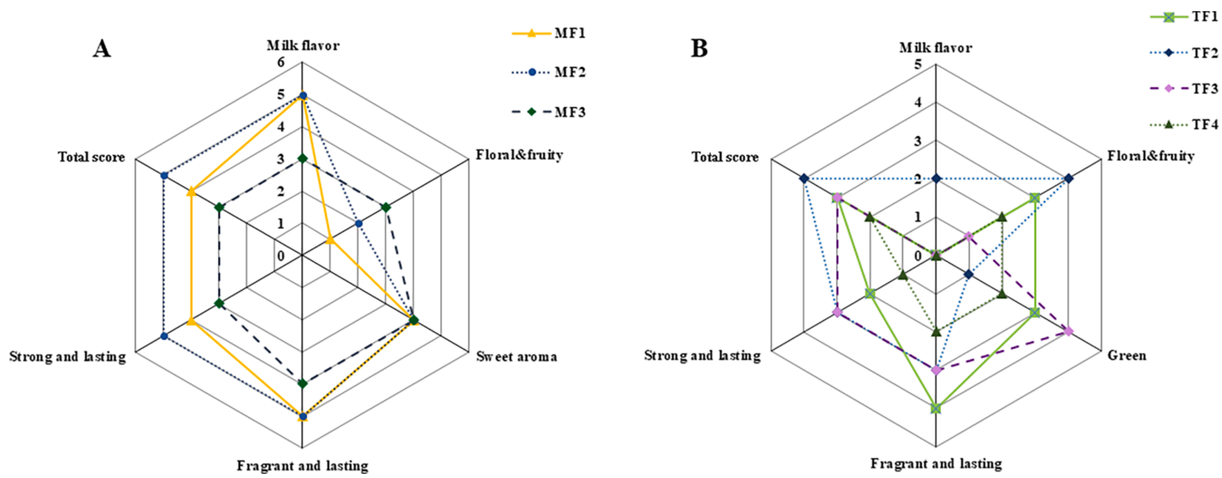

Fig. 2. The traditional sensory evaluation for milk-flavored white tea and the traditional white tea Shoumei. (A) Radar plot of the characteristic aroma profile of milk-flavored white tea samples; (B) Radar plot of the characteristic aroma profile of the traditional white tea samples. MF, milk-flavored white tea; TF, the traditional white tea Shoumei.

components for distinguishing scent differences. To identify the potential volatile markers for MFs, the compounds validated by OPLS-DA were further screened with VIP $>$ 1 and $p < 0.05$. Seven volatiles were finally screened, including benzyl alcohol, phenylethyl alcohol, methyl salicylate, (E)-6,10-dimethyl-5,9-undecadien-2-one, 2-pentyl-furan, dihydro-5-pentyl-2(3H)-furanone, and hexanal (Fig. 4C). Hierarchical clustering revealed that the 7 volatiles were distinctive in MFs and TFs (Fig. 4D). Benzyl alcohol, phenylethyl alcohol, and methyl salicylate were in group I, which were lower in MFs and higher in TFs. However, the volatile substances in Group II, dihydro-5-pentyl-2(3H)-furanone, (E)-6,10-dimethyl-5,9-undecadien-2-one, 2-pentyl-furan, and hexanal are mostly at high levels in MFs but at low levels in TFs. Cheese and coconut flavor compound, dihydro-5-pentyl-2(3H)-furanone, was especially accumulated in MF1 and MF2. 2-Pentyl-furan with a butter, floral and fruity aroma, was also accumulated in MFs. (E)-6,10-Dimethyl-5,9-undecadien-2-one with a strong fruit flavor was also accumulated in TF2, which has a slight milk scent. These results fit well with the sensory assessments.

4. Discussion

4.1. Milk-flavored white tea had an obvious milk scent compared to Shoumei

The withering of white tea affects the endogenous biosynthesis of volatiles and determines tea quality (Chen et al., 2019). Higher temperatures (28 ~ 30 °C) and humidity (80 ~ 85%) during the second withering made milk-flavored white tea taste milky, while the traditional white tea Shoumei tasted more sweet, floral, and fruity (Fig. 1). We conducted the traditional sensory evaluation to confirm the flavor differences between the milk-flavored white tea and Shoumei. Milk-flavored white tea had a dominating milk flavor regardless of the region but under the same withering process. Shoumei had obvious green, floral, and fruity flavors (Fig. 2).

To identify the critical components of milk-flavored white tea, the volatile components were screened for the characteristic components of milk-flavored white tea based on the technique of HS-SPME-GC-TOFMS (Figure S1-S7). A total of 67 volatiles were identified (Table S1). There were 31 volatile substances with OAV >1(Table 2). Among them, 1-heptanol, 1-octanol, 1-octen-3-ol, (E)-6,10-dimethyl-5,9-undecadien-2-one, heptanal, nerolidol and nonanal, which mostly sensed floral, sweet, and fruity (Wang et al., 2022), the average OAV in MFs were greater than those in TFs. The average OAV of 1-hexanol, (E,E)-2,4-nonadienal, (E)-3,7-dimethyl-2,6-octadienal, (Z)-3,7-dimethyl-2,6-octadienal, benzeneacetaldehyde, benzyl alcohol, decanal, heptanoic acid, ethyl ester, hexanoic acid, methyl ester, octanal, octanoic acid, ethyl ester and phenylethyl alcohol with floral and fruity aroma in MFs are lower than those in TFs (Yang et al., 2022; Zeng et al., 2022). Especially, the three volatile components, dihydro-5-pentyl-2(3H)-furanone, 2-pentyl-furan and pentanoic acid, which may contribute to milky aroma, the average OAV in MFs were significantly greater than those in TFs (Li, Ran, et al., 2022). In addition, 2-pentyl-furan and pentanoic acid may be important in the formation of milk flavor as acids from lipolysis and enzymatic hydrolysis were the main aroma substances in milk, cheese, and other dairy products (Liu, Pu, Sun, Shi, Cheng, & Wang, 2022; Ye, Liu, Wan, Liu, Wang, & Chen, 2022). Generally, MFs had a significant milk scent and TFs had a fresh floral and fruity aroma based on the traditional sensory evaluation and the volatile profiles.

4.2. Dihydro-5-pentyl-2(3H)-furanone is essential for the formation of milky aroma

The volatile components were distinctive in MFs and TFs on volatile types and quantities. To screen the characteristic aroma of MFs, the contributions of volatiles to the aroma were evaluated via OAV. Seven volatiles with OAV $>$ 1 and VIP $>$ 1 were screened, which were the candidate characteristic aromas in MFs and TFs (Fig. 4). Green and light fruity scent volatiles, such as methyl salicylate, benzyl alcohol, and phenylethyl alcohol, were richer in TFs than those in MFs. Strong fruity flavors, such as 2-pentyl-furan, (E)-6,10-dimethyl-5,9-undecadien-2-one, and dihydro-5-pentyl-2(3H)-furanone were more abundant in MFs than TFs. These volatiles with different boiling temperatures accumulated probably due to withering temperatures and humidity during white tea processing (Wu et al., 2022). (E)-6,10-Dimethyl-5,9-undecadien-2-one and benzyl alcohol, having a floral and fruity aroma, formed mainly by carotenoid degradation, oxidation of unsaturated fatty acids, or hydrolysis of glycosides (Lin, Ni, Wu, Weng, Li, & Chen, 2021). (E)-6,10-Dimethyl-5,9-undecadien-2-one, usually scented in blueberry and honeydew, was more accumulated in MF3 and TF2 with relatively lighter milk flavors. Benzyl alcohol and phenylethyl alcohol were more accumulated in TF3 and TF4 (Figure 4D). 2-Pentyl-furan, with the highest OAV and VIP value, has a strong fruity and butter aroma and can be produced at high temperatures by a Maillard reaction (Mao, Lu, Li, Ye, Wei, & Tong, 2018). Dihydro-5-pentyl-2(3H)-furanone, with a low threshold, was mainly detected in MFs, especially higher in MF1 and MF2 but lower in MF3, fitting so well with the sensory evaluation

Table 1
Identified volatile compounds in milk-flavored white tea.

No.	Volatile Compounds	CAS Number	RI	ID[a]	Order Type	Odor Description[b]
1	α-Farnesene	502–61-4	1504	MS, RI	Floral&fruity	Citrus, herbal, lavender-like
2	α-Ionone	127–41-3	1420	MS, RI	Woody	Warm woody-like
3	α-Ylangene	3856–25-5	1374	MS, RI	/	/
4	3-Methyl-1-butanol	123–51-3	764	MS, RI	Chemical	Choking alcohol-like
5	2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	432–25-7	1216	MS, RI	/	/
6	1-Heptanol	111–70-6	968	MS, RI	Floral	Fragrant
7	1-Hexanol	111–27-3	863	MS, RI	Fruity	Fruity
8	1-Ethyl-1H-pyrrole-2-carboxaldehyde	2167–14-8	1043	MS, RI	Roasted	Burnt smokey-like
9	1-Octanol	111–87-5	1071	MS, RI	Floral&fruity	Rose-like or lemon-like
10	1-Octen-3-ol	3391–86-4	978	MS, RI	Fruity	Sweet
11	1-Pentanol	71–41-0	779	MS, RI	Chemical	Fusel-like
12	5-Ethyldihydro-2(3H)-furanone	695–06-7	1047	MS, RI	Chemical	Onion-like
13	Dihydro-5-pentyl-2(3H)-furanone	104–61-0	1357	MS, RI	Cheese flavor	Coconut, creamy, waxy with fatty milky notes
14	5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	17092–92-1	1521	MS, RI	Floral	Musky or coumarin-like
15	2(5H)-Furanone	497–23-4	905	MS, RI	Chemical	Rich winey meat-like
16	(E,E)-2,4-Nonadienal	5910–87-2	1213	MS, RI	Floral	Strong floral-like
17	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	1125–21-9	1141	MS, RI	Woody	Woody, sweet
18	(Z)-3,7-Dimethyl-2,6-octadien-1-ol	106–25-2	1224	MS, RI	Floral&fruity	Floral, fruit
19	(Z)-Acetate-3,7-dimethyl- 2,6-octadien-1-ol	141–12-8	1378	MS, RI	Floral&fruity	Floral, fruit
20	(E)-3,7-Dimethyl-2,6-octadienal	141–27-5	1267	MS, RI	Fruity	Lemon-like
21	(Z)-3,7-Dimethyl-2,6-octadienal	106–26-3	1236	MS, RI	Fruity	Lemon-like
22	3,7-Dimethyl-2,6-octadienoic acid, methyl ester	2349–14-6	1320	MS, RI	/	/
23	(Z)-3-Methyl-2-(2-pentenyl)-2-cyclopenten-1-one	488–10-8	1390	MS, RI	Floral	Floral
24	2-Hexenal	505–57-7	846	MS, RI	Fruity	Strong fruity, green, vegetable-like
25	(E)-2-Octenal	2548–87-0	1055	MS, RI	Green	Grass, Green, Spice
26	2-Butyl-2-octenal	13019–16-4	1370	MS, RI	/	/
27	(E,E)-3,5-Octadien-2-one	30086–02-3	1068	MS, RI	Green	Green
28	4-(2,2,6-Trimethyl-7-oxabicyclo[4.1.0]hept-1-yl) −3-buten-2-one	23267–57-4	1480	MS, RI	Fruity	Sweet berry
29	3-Hexen-1-ol	544–12-7	848	MS, RI	Green	Grass, green fruit, green leaf, herb
30	(E)-3-Hexenoic acid, ethyl ester	26553–46-8	1002	MS, RI	/	/
31	3-Octen-2-one	1669–44-9	1036	MS, RI	Fruity	Blueberry-like
32	6,10-Dimethyl-5,9-undecadien-2-one	689–67-8	1447	MS, RI	Fruity	Fruit
33	(E)-6,10-Dimethyl-5,9-undecadien-2-one	3796–70-1	1447	MS, RI	Fruity	Fruit
34	Benzaldehyde	100–52-7	956	MS, RI	Roasted	Characteristic odor or volatile oil of almond
35	Benzeneacetaldehyde	122–78-1	1039	MS, RI	Floral	Lilac and hyacinth-like
36	Benzoic acid, hexyl ester	6789–88-4	1577	MS, RI	Woody	Woody
37	Benzyl alcohol	100–51-6	1032	MS, RI	Floral&fruity	Faint aromatic, fruity odour

(continued on next page)

Table 1 (*continued*)

No.	Volatile Compounds	CAS Number	RI	ID[a]	Order Type	Odor Description[b]
38	Benzyl nitrile	140–29-4	1133	MS, RI	Floral	Aromatic odor
39	2-Methyl-butanoic acid	116–53-0	851	MS, RI	Cheese flavor	Butter, cheese-like
40	2-Methyl-butanoic acid, hexyl ester	10032–15-2	1236	MS, RI	Fruity	Strawberry-like
41	3-Methyl-butanoic acid	503–74-2	837	MS, RI	Cheese flavor	Cheese-like
42	Butyrolactone	96–48-0	906	MS, RI	Cheese flavor	Caramel, cheese, nut
43	*cis*-3-Hexenyl isovalerate	35154–45-1	1230	MS, RI	Fruity	Apple-like
44	Coumarin	91–64-5	1428	MS, RI	Floral&fruity	Vanilla-like
45	Decanal	112–31-2	1205	MS, RI	Floral&fruity	Floral-orange odour
46	2-Pentyl-furan	3777–69-3	987	MS, RI	Fruity &Cheese flavor	Butter, floral, fruit
47	Heptanal	111–71-7	899	MS, RI	Fruity	Penetrating fruity odor
48	Heptanoic acid, ethyl ester	106–30-9	1097	MS, RI	Fruity	Brandy, fruit,
49	Hexanal	66–25-1	799	MS, RI	Green	Green grass odor
50	Hexanoic acid	142–62-1	1006	MS, RI	Cheese flavor	Cheese, oil
51	(Z)-hexanoic acid, 3-Hexenyl ester	31501–11-8	1380	MS, RI	Fruity	Fruity
52	Hexanoic acid, ethyl ester	123–66-0	997	MS, RI	Fruity	Apple peel-like
53	Hexanoic acid, methyl ester	106–70-7	921	MS, RI	Fruity	Pine apple-like
54	Linalool	78–70-6	1099	MS, RI	Floral	floral
55	Methyl salicylate	119–36-8	1189	MS, RI	Green	Green
56	*n*-Decanoic acid	334–48-5	1375	MS, RI	Green	Dust, fat, grass
57	Nerolidol	40716–66-3	1561	MS, RI	Floral&fruity	Faint woody-floral, slightly rose apple aroma
58	Nonanal	124–19-6	1104	MS, RI	Floral&fruity	Rose-orange odor
59	Nonanoic acid, ethyl ester	123–29-5	1296	MS, RI	Floral	Floral
60	Octanal	124–13-0	1001	MS, RI	Floral&fruity	Strong, fruity odor
61	Octanoic acid	124–07-2	1184	MS, RI	Cheese flavor	Cheese, fat
62	Octanoic acid, ethyl ester	106–32-1	1196	MS, RI	Floral&fruity	Apricot, brandy, floral, pineapple
63	Pentanoic acid	109–52-4	889	MS, RI	Cheese flavor	Cheese
64	Pentanoic acid, ethyl ester	539–82-2	899	MS, RI	Floral&fruity	Apple, herb, nut
65	Phenylethyl alcohol	60–12-8	1111	MS, RI	Floral&fruity	Fruit, honey, lilac, rose, wine
66	Methyl-pyrazine	109–08-0	819	MS, RI	Roasted	Nutty, cocoa-like
67	3-Methyl-tridecane	6418–41-3	1372	MS, RI	/	/

'/', information was not found in the literature; a, identification methods; MS, identification based on the NIST14.L; RI, retention index; b, odor description found in the literature with database (Flavornet; https://pubchem.ncbi.nlm.nih.gov/).

(Figs. 2, 4D). In MFs, the average OAV of dihydro-5-pentyl-2(3H)-furanone was 47.69. It was reported to smell milky when strong and floral when mild (Fadel, Mahmoud, Asker, & Lotfy, 2015). It may be the main source of milk flavor in milk-flavored white tea. The formation of milk flavor may be related to fresh tea leaves and process. Fresh tea leaves with different degrees of tenderness contain different substances, and the flavor of the tea made will be different. And it is reported that high temperatures were more favorable for the accumulation of dihydro-5-pentyl-2(3H)-furanone (Lukić, Tomas, Miličević, Radeka, & Peršurić, 2011). The withering and drying process of high temperatures may be the reason for its accumulation. In short, Dihydro-5-pentyl-2(3H)-furanone should be the essential milky aroma, contributing the most to the milky aroma in MFs.

5. Conclusions

In summary, our study characterized the main volatile components in milk-flavored white tea and Shoumei made from the same tea leaves but with a different second withering process. The traditional sensory evaluation exhibited that MFs had an obvious milk scent and TFs had

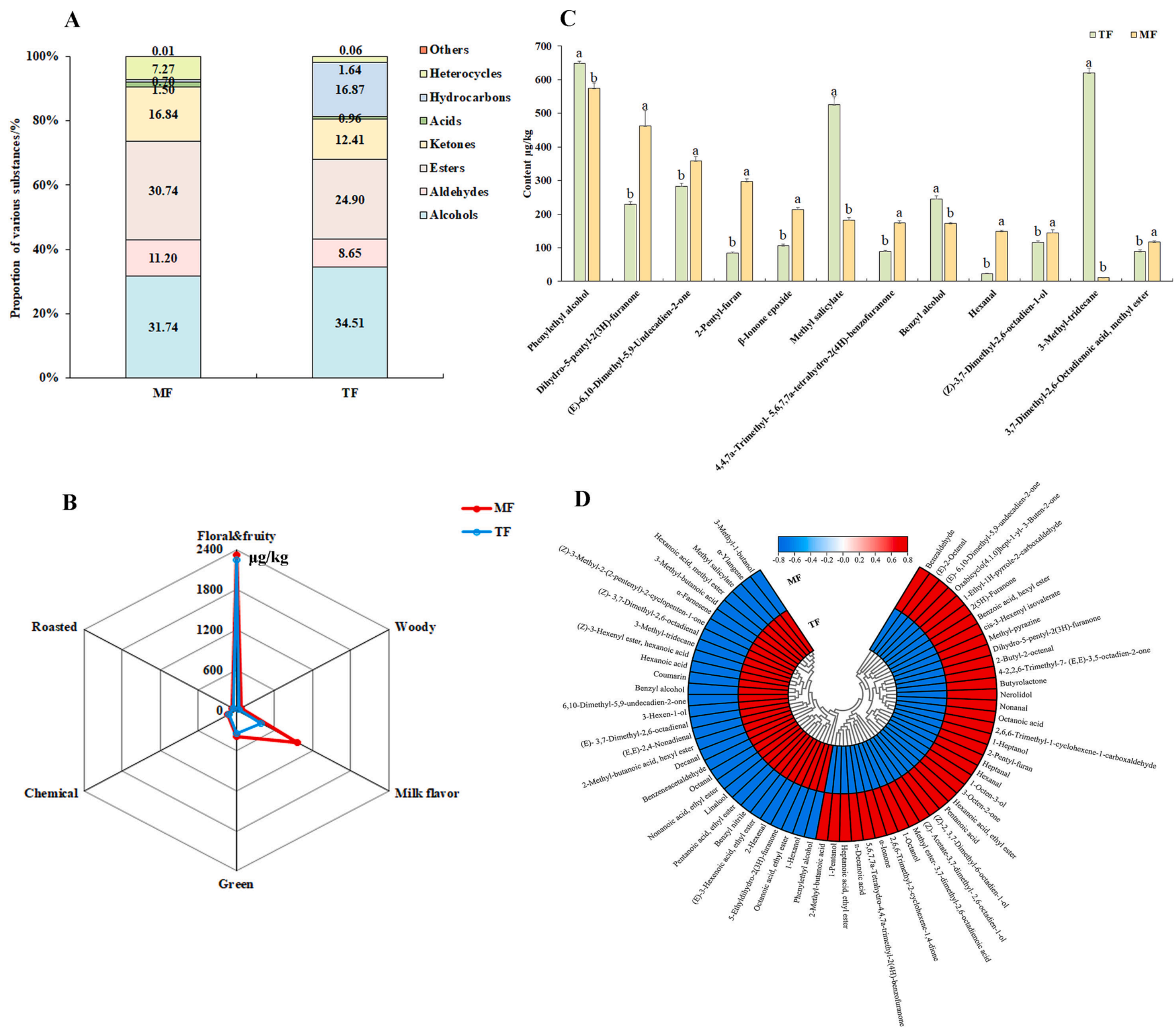

Fig. 3. The proportion and profiles of the volatile components in milk-flavored white tea and Shoumei. (A) The composition proportion of volatile components in MFs and TFs; (B) Radar map of aroma classification in MFs and TFs; (C) The relative contents of the top 10 abundant volatile compounds in MFs and TFs. Value (n = 3) are given as mean ± SD. Different letters indicate a significant difference at $p < 0.05$ level.; (D) Heatmap of the volatile components in MFs and TFs. A color-coded scale grading from blue to red corresponds to the content of volatile compounds shifting from low to high. MF, milk-flavored white tea; TF, the traditional white tea Shoumei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

green, floral, and fruity scents. Sixty-seven volatile components were identified via the HS-SPME-GC-TOFMS, of which 31 compounds with OAV > 1 were further screened via OPLS-DA analysis and were considered as the main aromas to distinguish MFs from TFs. Finally, 7 volatiles (OAV > 1, VIP > 1, and $p < 0.05$) were identified, including benzyl alcohol, phenylethyl alcohol, methyl salicylate, (E)-6,10-dimethyl-5,9-undecadien-2-one, 2-pentyl-furan, dihydro-5-pentyl-2(3H)-furanone, and hexanal. Among these compounds, green and light fruity aromas such as benzyl alcohol, phenylethyl alcohol and methyl salicylate, were more abundant in TFs than MFs, and 2-pentyl-furan, dihydro-5-pentyl-2(3H)-furanone, and hexanal were more abundant in MFs than TFs. (E)-6,10-Dimethyl-5,9-undecadien-2-one was more accumulated in MF3 and TF2, which were with lighter milk flavors. Dihydro-5-pentyl-2(3H)-furanone, verified as the contributor to the coconut and creamy flavor, was highly accumulated in MF1 and MF2, fitting well with the highly milky sensory evaluation. Also, (E)-6,10-Dimethyl-5,9-undecadien-2-one and 2-pentyl-furan, fruity flavors with a relatively high boiling temperature, may contribute to the formation of the milky flavor. In conclusion, the level of dihydro-5-pentyl-2(3H)-furanone was directly related to the milky flavor in MFs, but other aroma substances may also have some synergistic effects on the formation of milk flavor. The mechanism of the milky flavor formation in milk-flavored white tea is yet to be studied. This result enriches the understanding of the critical aroma substances in milk-flavored white tea and provides a reference for the quality control of milk-flavored white tea.

CRediT authorship contribution statement

Zhilong Hao: Conceptualization, Methodology, Writing – review & editing. **Jiao Feng:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft. **Qianlian Chen:** Investigation,

Table 2
The key compounds associated with milk-flavored white tea and Shoumei with significantly high odor-activity values (OAV > 1).

No.	Volatile Compounds	OT* (μg/kg)	OAV						
			MF1	MF2	MF3	TF1	TF2	TF3	TF4
1	α-Ionone	2.60	18.74	26.80	13.72	8.50	16.00	3.52	11.19
2	α-Ylangene	6.00	1.28	1.95	1.08	1.17	3.70	1.25	1.55
3	2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	5.00	6.36	6.88	5.45	4.51	8.08	1.58	4.37
4	1-Heptanol	5.40	1.77	1.94	1.80	1.03	2.61	0.40	0.78
5	1-Hexanol	4.50	16.05	22.69	13.35	18.96	23.17	20.36	15.46
6	1-Octanol	0.80	35.91	38.84	47.40	30.05	52.93	19.31	25.81
7	1-Octen-3-ol	7.00	12.92	15.83	12.74	10.57	19.12	2.92	7.28
8	Dihydro-5-pentyl-2(3H)-furanone	9.70	52.72	63.15	27.21	13.67	28.81	28.04	23.78
9	(E,E)-2,4-Nonadienal	0.16	1.00	1.19	0.44	1.43	2.86	0.00	0.53
10	(E)-3,7-Dimethyl-2,6-octadienal	32.00	0.31	0.72	1.00	1.03	0.84	0.61	1.72
11	(Z)-3,7-Dimethyl-2,6-octadienal	53.00	0.49	0.92	0.96	1.35	1.39	0.93	1.32
12	(E)-2-Octenal	3.00	2.20	1.76	1.41	1.62	3.02	0.00	0.91
13	(E,E)-3,5-Octadien-2-one	0.50	11.47	18.04	18.41	12.93	21.60	2.53	6.83
14	(E)-6,10-Dimethyl-5,9-undecadien-2-one	60.00	4.68	6.28	6.97	3.92	7.49	3.14	4.36
15	Benzeneacetaldehyde	4.00	4.03	4.14	11.56	4.24	6.54	8.80	11.61
16	Benzyl alcohol	100.00	1.59	1.91	1.65	1.63	1.76	2.10	4.35
17	Decanal	9.00	0.94	1.31	1.07	1.20	3.79	0.40	0.70
18	2-Pentyl-furan	6.00	44.33	50.85	52.98	0.00	0.00	16.90	25.49
19	Heptanal	10.00	1.66	2.30	3.05	0.70	0.85	0.71	1.83
20	Heptanoic acid, ethyl ester	2.20	1.60	2.79	4.80	1.78	0.00	0.00	1.79
21	Hexanal	10.00	16.65	20.32	7.47	0.27	0.39	2.22	6.31
22	Hexanoic acid, ethyl ester	1.16	26.10	33.02	46.86	3.59	2.41	19.02	45.55
23	Hexanoic acid, methyl ester	10.00	0.30	0.31	0.24	4.73	6.01	0.22	0.18
24	Methyl salicylate	40.00	1.14	3.41	9.14	0.00	0.00	9.84	16.42
25	Nerolidol	15.00	3.73	4.20	2.19	0.01	0.00	5.44	5.10
26	Nonanal	15.00	2.04	1.98	3.60	0.05	0.10	1.77	4.14
27	Octanal	0.60	26.79	24.92	18.02	48.61	64.07	0.00	14.09
28	Octanoic acid, ethyl ester	12.87	1.92	2.45	2.45	3.12	6.43	0.43	1.05
29	Pentanoic acid	0.36	89.82	112.69	91.25	11.59	0.00	28.36	104.32
30	Phenylethyl alcohol	390.00	1.41	1.51	1.50	0.00	0.01	3.13	3.52
31	Methyl-pyrazine	1.80	2.80	1.96	0.50	1.84	0.00	0.00	0.33

*:OT, odor thresholds in water. Odor thresholds in water were obtained from (Zhu, 2019), (Zeng et al., 2022) & (Feng, 2021).

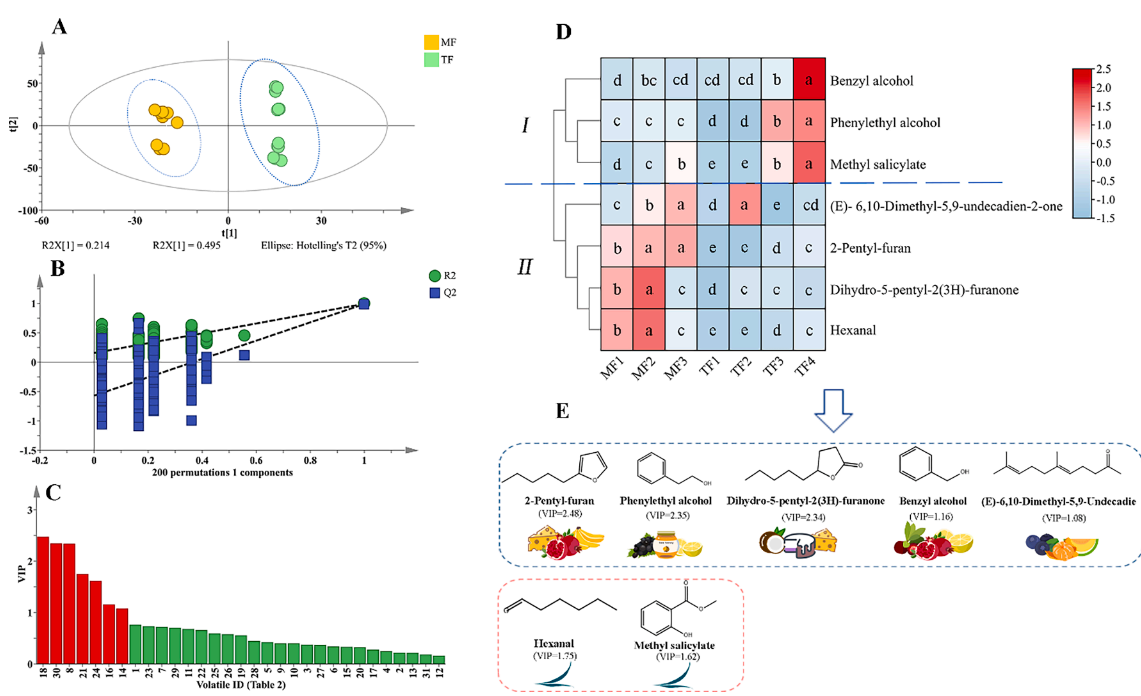

Fig. 4. Characteristic volatiles validation for milk-flavored white tea and Shoumei. (A) The scatter plots of OPLS-DA for MFs and TFs; (B) The validation of the OPLS-DA model. The vector value of R^2 (0.0, 0.142) and Q^2 (0.0, −0.57) from 200 permutations, which indicated that this OPLS-DA model was not overfitting; (C)The variable importance in the project (VIP) of the 31 volatile components in MFs and TFs. The red column highlighted the volatile components with VIP > 1; (D) The heatmap of differentially accumulated volatile compounds in MFs and TFs (OAV > 1 and VIP > 1). A color-coded scale grading from blue to red corresponds to the content of volatile compounds shifting from low to high. Different letters in the same row indicate a significant difference at $p < 0.05$ level; (E) The aroma sketch map of the 7 volatile compounds. MF, milk-flavored white tea; TF, the traditional white tea Shoumei. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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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.

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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.2023.100710.

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