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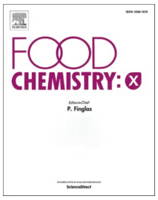

Study on the dynamic change of volatile components of white tea in the pile-up processing based on sensory evaluation and ATD-GC–MS Technology

Hongzheng Lin [a,*], Liangyu Wu [a], Xiaoxi Ou [a], Jingjing Zhou [a], Jiao Feng [a], Wenping Zhang [a], Wanjun Bi [a], Zhilong Hao [a,b,*], Yun Sun [a,b,*]

[a] *College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China*
[b] *Key Laboratory of Tea Science in Universities of Fujian Province, Fuzhou 350002, Fujian, China*

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ABSTRACT

The pile-up processing has a great impact on the flavor of white tea. To investigate the effects of the volatile accumulation of white tea with different piling thickness treatments, tea leaves from different thickness treatments were subjected to sensory quantitative description analysis and ATD-GC–MS detection in this study. As a result, 122 volatile components were identified from white tea with different treatments. A total of 8 key compounds, including isovaleraldehyde, isobutyraldehyde, 2-methyl-butanal, 1-octene-3-ol, linalool, pentanoic acid, hexanal and 1-hexanol were screened out using multivariate statistical analysis, which were characteristic components of grassy, floral-fruity, pekoe aroma and sweet flavors. The results of the selected key characteristic volatile compounds were consistent with the sensory quantitative description. The aroma of mid-pile dried tea (MD) was exhibited a harmonious and pleasant overall flavor. This study provides a novel insight into the accumulation of volatile during the withering step of white tea production.

1. Introduction

White tea is one of the most popular teas due to its floral, refreshing flavor, which is mainly produced in Fuding, Zhenghe, Jianyang, and Songxi in Fujian Province (Zhou et al., 2023). The processes of white tea are composed of leaf picking, withering, pile-up, and drying. Withering is the key process involved in characteristic flavor formation of white tea (Hao et al., 2023). The method of pile-up processing is piling leaves in the late stage of withering to remove grassy odor and improve the quality of white tea (Chen et al., 2018). Previous studies on the impact of pile-up processing on the flavor of white tea have shown that a certain duration of pile-up processing could enhance the sweet aroma and mellow taste but reduce the freshness aroma of white tea (Chen et al., 2018; Wu et al., 2023).

However, limited attention has been paid to elucidate the effects of piling thickness on the volatile accumulation during the pile-up step of white tea production. Usually, the tea shoots subjected to white tea production were organoleptically assessed by tea markers, in order to adjust processing parameters during production process. Nevertheless, this approach is prone to subjectivity due to individual differences in sensory perception and experience. Compared with headspace solid-phase micro-extraction (HS-SPME) (Hao et al., 2023), simultaneous distillation extraction (SDE) (Lin et al., 2021) and other methods (Feng et al., 2019), Tenax-TA adsorption offers an environment-friendly and trace-level detectable approach for the determination of aroma components (Chen et al., 2020a). The automatic thermal desorption-gas chromatography-mass spectrometry (ATD-GC–MS) detection technology is widely used in gas collection (Martin Fabritius et al., 2018), plant fragrance (Qin et al., 2015; Zhu et al., 2011) and food flavor (Cui et al., 2012). This method has been increasingly used to profile the fragrance generated over tea production (Bi et al., 2022; Shao et al., 2022; Wang et al., 2020). This approach was suitable for the determination that performs real time analysis on the dynamic changes of aroma by adsorbing and quantifying the captured volatile on GC–MS platform.

To precisely quantify the volatiles accumulated in different piling treatments, ATD-GC–MS detection was employed in this study, and the sensory quantitative description analysis (QDA) was performed to distinguish the flavor of white tea produced in different piling

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* Corresponding authors at: College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China.
E-mail addresses: linhongzheng2010@126.com (H. Lin), haozhilong@126.com (Z. Hao), sunyun1125@126.com (Y. Sun).

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treatment. In addition, the relative odor activity value (ROAV) and aroma characteristic influence value (*ACI*) were also used to identify the characteristic volatile compounds. The results of this study would provide a better understanding on the formation of the characteristic aroma during the pile-up procedure of white tea manufacture.

2. Materials and methods

2.1. Sample preparation

Fresh shoots (*Camellia sinensis* (L.) O. Kuntze, cv. Fudingdahao) consisted of two leaves with a bud were picked in April 2021. The plucked tea shoots were subjected to sequential manufacturing processes according to the canonical production process including withering, piling, and drying (Feng et al., 2019; Xiang et al., 2023). The different treatments of piling and sampling points were presented in (Fig. 1A). In brief, the picked tea shoots were placed in withering room with the ambient temperature of 24 ± 1℃, relative humidity: 65 ± 5 %, the temperature and humidity were controlled by air conditioning (Gree Electric Appliances, Inc. of Zhuhai, China) and dehumidifier (Dongguan Yixin Electronic Products Co., Ltd., Dongguan, China) respectively to maintain air circulation during the withering process. After withering for 36 h (the moisture content of tea was 17.0–18.0 %), the tea shoots were subjected to pile-up processing. The treatments were divided into low-pile (thickness of 5 cm, termed as L), mid-pile (thickness of 15 cm, termed as M), high-pile (thickness of 25 cm, termed as H), all were piled for 4 h (termed as L1, M1, H1) and 8 h (termed as L2, M2, H2). Finally, the tea shoots were dried at 75 ℃ until the moisture content was less than 7 % (termed as LD, MD, HD).

2.2. Sensory evaluation and quantitative descriptive analysis

Quantitative descriptive analysis (QDA) was applied to depict the odor profiles of tea samples in this study. Briefly, 3 g tea sample from the tea pile was directly taken and put into the 150 mL column cup for aroma evaluation (Fig. 1B). The aroma evaluation panel was consisted of 5 professionals engaged in tea production and organoleptic assessment. The members of panel were composed of 3 males and 2 females. Six attributes were employed to describe the aroma of tea samples, including grassy, floral-fruity, pekoe aroma, sweet, freshness, and strength. The panelists rated the intensity of the attributes of tea samples based on a nine-point scale ranging from 0 (absent) to 9 (extremely strong). The average score of each attribute was used for plotting diagrams. The appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of this research. All panelists were informed and the informed consent was obtained from all participants for this experiment.

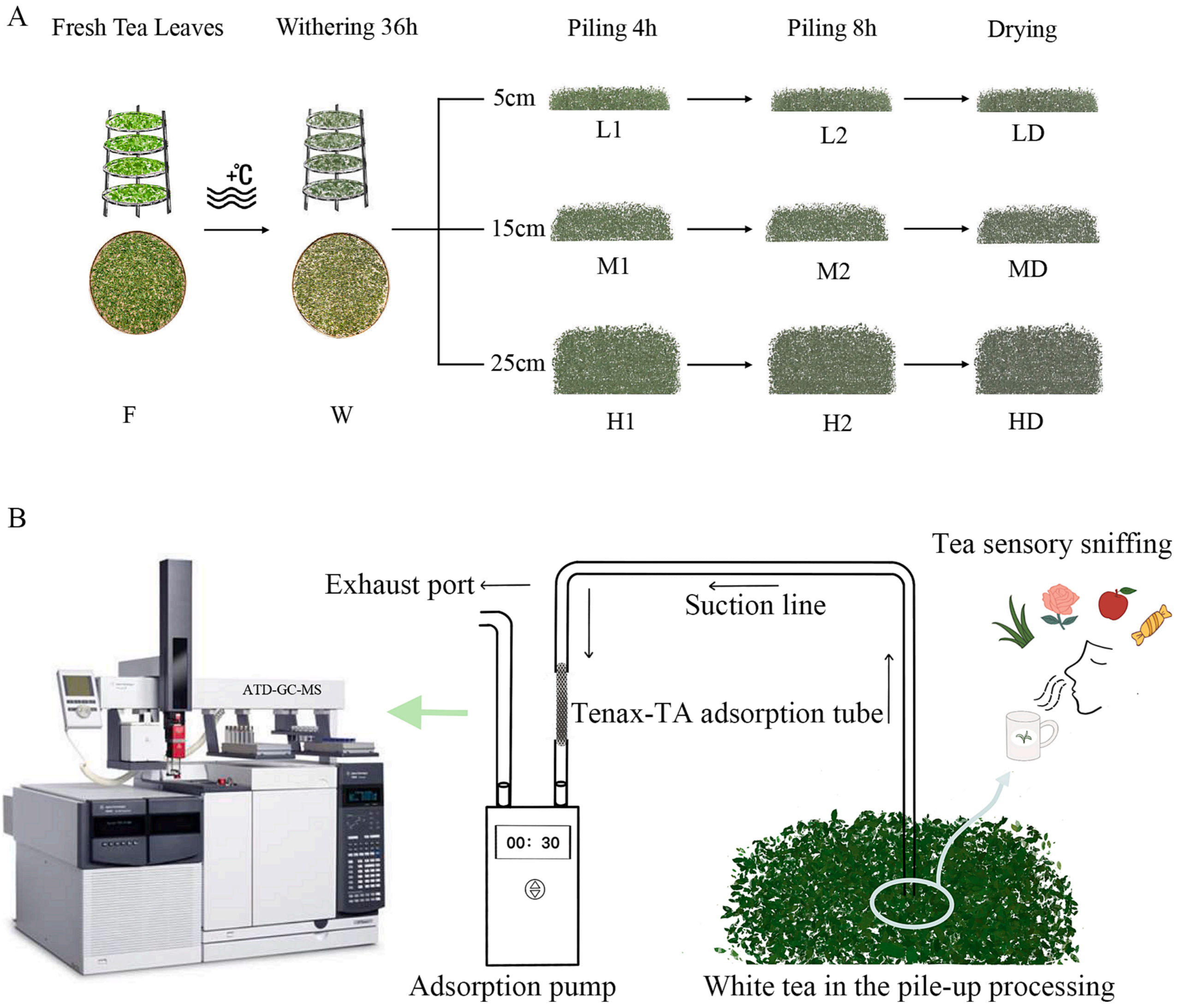

Fig. 1. (A) White Tea in the pile-up processing test plan; (B) Schematic diagram of artificial sensory olfaction and natural volatile gas collection.

2.3. *Collection of volatile components*

The volatiles collection was performed according to the protocol of previous study (Bi et al., 2023). A polytetrafluoroethylene tube connected with a Tenax-TA adsorption tube and a QC-1S air sampling instrument (Beijing Kean Labor Protection New Technology Co., Ltd., Beijing, China) was applied to collect the volatiles, and the suction tube head was inserted into the tea pile center (Fig. 1B). Then the volatile components were collected at 200 mL/min flow rate for 30 min. Three parallel samples were taken simultaneously. The collected volatile samples were immediately transported and stored at −20℃ for further analysis.

2.4. *ATD-GC–MS detection*

Thermal desorption was conducted by automatic thermal desorption (Chengdu Colin Analytical Technology Co., Ltd., Chengdu, China). Volatile compounds were identified using a 2010 GC coupled with an 8040 TQ-MS system (Shimadzu Production Institute, Kyoto, Japan). The automatic thermal desorption detection was carried out according to the previous studies (Wang et al., 2020). The conditions were set as follows: valve temperature 200℃; transport line temperature 200℃; primary desorption temperature 250℃; first stage desorption time 5 min; secondary desorption temperature 300℃; cold trap temperature −29℃; cold trap heating time 3 min; injection time 60 s; cycle time 50 min; carrier gas: high purity helium (purity $\geq$ 99.999 %); carrier gas pressure 60 kPa; driving gas: air.

GC conditions: Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 μm); heating procedure: the initial temperature was maintained at 40℃ for 3 min, and the temperature rises to 120℃ at 5℃/min for 5 min. Then the temperature was increased to 240℃/min at 30℃/min and maintained for 8 min. The flow rate of carrier gas (helium) was 3 mL/min, and the pressure was 49.5 kPa. Shunt ratio: 5:1.

MS conditions: electron bombardment ion source; electron energy 70 eV; transmission line temperature 280℃; interface temperature 280℃; ion source temperature 230℃; the quality scan range was 28 to 500 *m/z*.

2.5. *ROAV and ACI calculation methods*

The relative odor activity values (ROAV) of each volatile components was calculated based on the protocol described in previous reports (Fang et al., 2022; Liang et al., 2023). The ROAV of the component that contributes most to the flavor of the tea sample was defined as 100, and other compounds was calculated as shown in equation (1). Generally, when ROAV $\geq$ 1, the volatile compounds were considered to be the key flavor contributors. When 0.1 $\leq$ ROAV $<$ 1, the compounds were considered to be conducive to the overall flavor of the sample. Aroma character impact value (*ACI*) was used to evaluate the contribution of aroma components (Feng et al., 2020), which was calculated as shown in equation (2):

$$ROAV_i = \frac{C_i}{C_{max}} \times \frac{T_{max}}{T_i} \times 100 \ (1)$$

$$ACI\ (\ \%\ ) = \frac{ROAV_i}{\sum_k ROAV_k} \times 100\ \ (\ 2\ )$$

In which, $ROAV_i$ represents the relative aroma activity value of any volatile compound i; C_i represents the relative content (%) of any volatile component i; C_{max} represents the relative content (%) of the maximum aroma contributing compound; T_i represents the threshold of any volatile component i (μg/kg); T_{max} represents the maximum aroma contribution compound threshold(μg/kg); $\sum_k ROAV_k$ represents the sum of all volatile compound ROAVs.

2.6. *Statistical analysis*

The volatile components were qualitatively analyzed according to the chromatographic retention time, mass spectrometry information and the comparison results of NIST 08 standard spectrum library. The peak area normalization method was used for relative quantification (Du et al., 2014). Data processing and the generation of bar charts and radar charts were performed using Microsoft Excel 2019. Total ion chromatogram and radial stacked bar chart were created using Origin 2021. Significance analysis and Duncan's LSD test were performed using SPSS 19.0 (IBM Corporation). Unsupervised principal component analysis (PCA), hierarchical cluster analysis (HCA), as well as Orthogonal projection to latent structures discriminant analysis (OPLS-DA) were run on SIMCA 14.0 (Umetrics Company, Malmo, Sweden) for the statistical analyses.

3. Results

3.1. *Sensory quantitative description analysis*

The results of QDA showed that the intensities of aroma attributes in withering leaves were dramatically enhanced than those in fresh leaves (Fig. 2A), indicating an accumulation of volatile compounds along with the progress of white tea production. During the piling process, the white tea samples from the group of low-pile 4 h (L1) possessed with strongly floral fragrance, mid-pile 4 h (M1) possessed with strongly floral-fruity scent with pekoe aroma and freshness, and high-pile 4 h (H1) possessed with heavily green odor (Fig. 2B).

With the increasing of the piling time, the green odor of each piled tea samples was weakened. The low-pile 8 h (L2) was stronger in floral-fruity scent, mid-pile 8 h (M2) was stronger in pekoe aroma and floral-fruity, and high-pile 8 h (H2) was stronger in sweet and pekoe aroma (Fig. 2C). After drying, low-pile dried tea (LD) had a sweetly floral-fruity flavor, mid-pile dried tea (MD) had a floral-fruity and pekoe aroma with freshness, and high-pile dried tea (HD) had a ripe and sweet pekoe aroma (Fig. 2D). Comprehensive sensory evaluation, MD (15 cm) had the highest sensory quality, with a harmonious and pleasant overall flavor, which was consistent with the actual production operation. The QDA results showed a dynamic change over the various piling treatments, suggesting the conversion and accumulation of volatile were probably impacted by the piling thickness and duration.

3.2. *Comparison of total volatile components in piled white tea*

Through the analysis of ATD-GC–MS detection results, it can be seen that the profiles of volatile components in the piled white tea samples exhibited significant difference among various samples (Fig. 2E). A total of 122 volatile components were identified and quantified in this study, including 42 hydrocarbons, 25 alcohols, 20 aldehydes, 20 esters, 5 ketones, 3 phenols, and 7 others (Fig. 2F). The peak areas of volatile compounds from piled tea samples were obviously more than those of other samples (Fig. 2G), indicating that piled treatment promoted the production of volatile components. The relative contents of alcohols (45.53 %-55.46 %) and aldehydes (15.54 %-25.15 %) were significantly increased during piling steps (Fig. 2H), suggesting that enhanced floral and fruity aroma in piling samples observed in QDA was probably attributed to the increase of alcohols and aldehydes. After drying, the amount and total peak area of volatile components in LD, MD, and HD decreased significantly. The aldehydes, alcohols, and hydrocarbons accounted for 83.19 % to 85.16 % of the total content. The relative content of alcohols in LD, MD, and HD decreased by 56.14 %, 56.69 %, and 62.44 % compared to L2, M2, and H2. The relative content of aldehydes increased by 3.02, 2.27, and 1.72 times compared to L2, M2, and H2 respectively.

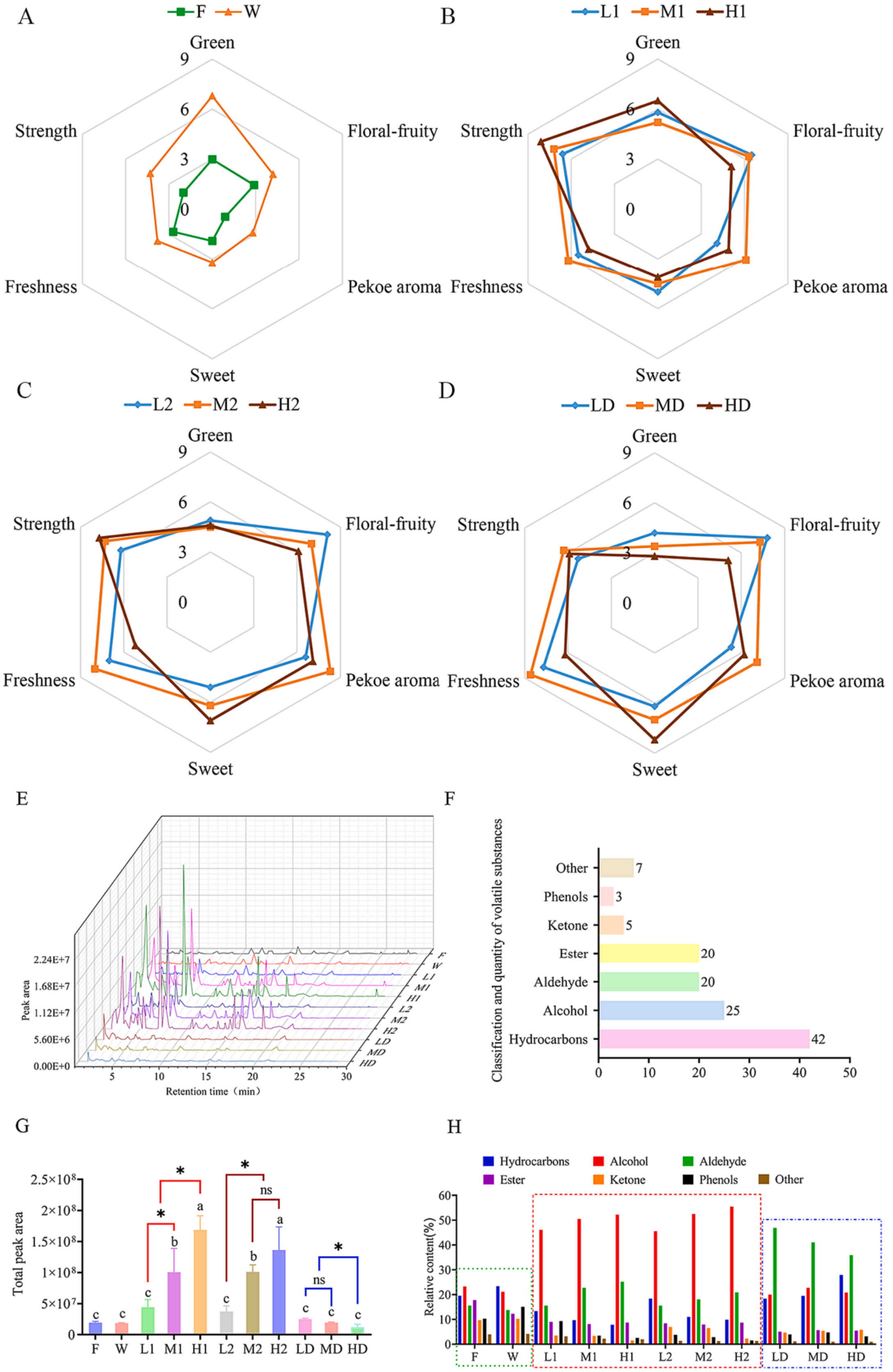

Fig. 2. Sensory quantitative description score radar of tea aroma and volatile fingerprint analysis of white tea during the whole manufacturing process; (A) QDA of fresh leaves and withering leaves;(B) QDA of piling for 4 h; (C) QDA of piling for 8 h; (D) QDA of dried tea; (E) Total ion chromatogram; (F) Quantity of volatile substances; (G) Change in total peak area of each treatment;(H) Changes in the relative content of volatile components. Different letters indicate significant difference at $P < 0.05$ level during white tea processing; '*', indicates significant difference at $p < 0.05$ level between piled tea samples; ns, indicates no significant difference.

3.3. Principal component analysis (PCA) of volatile components in white tea samples

The volatile profiles of each sample were subjected to PCA analysis. As a result, the PC1 and PC2 accounted for 80.8 % of total variance (PC1 = 53.4 %, PC2 = 27.4 %). Moreover, the score plot of PCA showed each sample could be clearly distinguished from others, while the triplicates of each sample were closely clustered (Fig. 3A).

The orthogonal partial least squares-discriminant analysis (OPLS-DA) was used to clearly display the overall variation pattern of volatile components during the piling process (Fig. 3B). Generally, the triplicates of L1, M1 and H1 samples were distributed on negative direction of PC2, whereas the triplicates of L2, M2 and H2 were located on positive direction of PC2, indicating piling duration potentially impacted the accumulation of volatiles. Both of L1 and L2 groups were distributed on positive direction of PC1, while the M and H groups were located on the negative direction, suggesting the thickness of piling also had an influence on the volatile components. The results of hierarchical cluster analysis (HCA) showed that the M and H groups were more similar in volatile profiles than L group (Fig. 3C), which was congruous with the result of PCA. A total of 31 volatile components were obtained using the criteria with variable importance in the projection (VIP) higher than 1.0 (Fig. 3D), including 12 alcohols, 8 aldehydes, 3 esters, 3 hydrocarbons, 1 ketone, 2 phenols, and 2 others, which could be biomarkers for distinction of white tea under different piling treatments.

3.4. Screening key characteristics of volatile components in white tea during the piled processing

To screen out the characteristic volatile components in white tea of different piling treatments, the relative odor activity values (ROAV) of 31 volatile components were calculated (Table 1), among which 13 key flavor volatile components were selected with the criteria of ROAV ≥ 1, including isovaleraldehyde, isobutyraldehyde, linalool, 2-methyl-butanal, 1-hexanol, hexanal, 1-octen-3-ol, benzyl alcohol, ethyl acetate, *cis*-3-hexen-1-ol, methyl salicylate, pentanal, and pentanoic acid, which were key characteristic volatiles imparted grassy, floral, fruity, pekoe aroma, and sweet aroma styles in white tea. Additionally, six volatiles were obtained with the criteria of $0.1 \leq$ ROAV < 1, including 2-pentenal, 1-penten-3-ol, phenylethyl alcohol, toluene, 3-methyl-1-butanol, and 2-ethyl-1-hexanol. The total relative contents of volatile components with ROAV ≥ 0.1 were H1 (71.37 %) > M1 (67.74 %) > L1 (60.01 %), H2 (74.05 %) > M2 (68.83 %) > L2 (65.83 %), suggesting an increasing tendency along with the piling thickness, and piling duration also induced the chronological accumulation of flavoring compounds.

Further analysis showed that the cumulative contribution rates of the top five dominant volatile components in the aroma characteristic impact value (*ACI*) of each treatment range from 72.64 % to 83.15 %, including isovaleraldehyde, isobutyraldehyde, 2-methyl-butanal, 1-octen-3-ol, linalool, pentanoic acid, hexanal, and 1-hexanol in L, M and H groups. The *ACI* of the dominant characteristic components in each piled tea samples were dramatically different, resulting in various characteristic flavors of tea samples (Fig. 3E). The *ACI* of pentanoic acid in L1 was 20.01 %, while the total *ACI* of 1-octen-3-ol and linalool in L1 was 24.63 %, which was significantly higher than other treatments, contributing to the obviously fruity, pungent, and sweet floral aroma in L1. The *ACI* of 2-methyl-butanal in H1 was the highest, leading to the pekoe aroma in H1. The *ACI* of 1-octene-3-ol ranked second in *ACI* of L2, much higher than M2 and H2, exhibiting sweet floral aroma in L2. The *ACI* of linalool and 2-methyl-butanal of M2 was higher than that of L2 and H2, showing floral and pekoe aroma. The *ACI* of isovaleraldehyde and isobutyraldehyde in H2 was higher than that in L2 and M2, which imparted sweetly fruity aroma as the major flavor.

3.5. Screening for key characteristics of volatile components in the dry tea

OPLS-DA model was applied to investigate the difference in the dried teas with various piling treatments. The score plot showed that LD, MD and HD were distributed in different areas (Fig. 4A), indicating that piling treatments had a significant impact on the aroma quality of white tea. A total of 22 volatile components with VIP > 1 were obtained, including 6 alcohols, 6 aldehydes, 6 hydrocarbons, 2 esters, 1ketone and 1 phenol (Table 2, Fig. 4B). 11 volatile components (VIP > 1, ROAV $\geq$ 0.1) were further screened, of which 9 were key volatile components (ROAV $\geq$ 1), and the *ACI* of isovaleraldehyde, isobutyraldehyde, 1-octene-3-ol, and 2-methyl-butanal was higher than 10 %, which was the dominant characteristic volatiles of the dried white tea (Fig. 4C). Isovaleraldehyde and isobutyraldehyde were the two compounds that mainly contributed to the fresh and sweet flavor of dried tea. The *ACI* of isovaleraldehyde and isobutyraldehyde in LD was higher than that of MD and HD, indicating that low-pile was conducive to the fresh flavor of white tea. While 1-octene-3-ol was the major characteristic volatiles in MD according to the *ACI* value, contributing to a remarkable sweet fragrance in MD. The pekoe aroma originated from 2-methyl-butanal was one of the typical flavors in white tea, the *ACI* of 2-methyl-butanal in HD was higher than that in MD and LD, suggesting that thick pile was more conducive to the formation of pekoe aroma. The order of the dominant characteristic compounds with *ACI* $\geq$ 10 % in the piled treatment was LD (92.91 %) > MD (91.14 %) > HD (76.90 %). The test result indicated a greater diversity of volatiles would be generated with the increase of pile thickness resulting in more complex fragrance in dried tea, which was consistent with QDA and previous results (Xiang et al., 2023).

4. Discussion

4.1. Piled treatments have a significant impact on the sensory quality of white tea aroma

The changes of characteristic aroma are one of the key indicators for regulating the operations during tea processing. The grassy odor of white tea was decreased, and the characteristics sweet, floral-fruity and pekoe aroma were gradually enhanced during the piling step, which was similar to the results of the previous studies (Chen et al., 2018; Wu et al., 2023). Low-pile dried tea (LD) had a sweetly floral flavor, but still had a strongly grassy odor. High-pile dried tea (HD) had a strongly sweet, strongly pekoe aroma, moderate floral-fruity aroma, and a slightly grassy flavor. Mid-pile dried tea (MD) had a strongly floral-fruity, pekoe aroma, and sweet aroma, with a slightly grassy, showing an overall harmonious and pleasant flavor. In actual white tea production, tea makers usually use thickness of mid-pile (15 cm) for piling, which can improve the quality of white tea in a short period of time. Therefore, the white tea which manufactured in this way was favored by consumers. This result indicated that an appropriate piling approach could facilitate the formation of characteristic flavor of white tea.

4.2. Piled treatments have obvious enrichment effect on volatile components of white tea

The analysis of the ATD-GC–MS results indicated that the aroma of white tea was significantly enriched during the piling treatment, showing an increasing trend along with the piling thickness. The volatile components of white tea in the piled process were dominated by alcohols (45.53 %~55.46 %) and aldehydes (15.54 %~25.15 %). These components were mainly accumulated during the piling step, as the tea shoots over the piling step were still kept alive, and a large amount of fatty acids and amino acids were degraded into alcohols and aldehydes, resulting in the enhance of floral and fruity aroma (Chen et al., 2020b; Dai et al., 2017). The heating occurred over drying step accounted for the rapid loss of the low-boiling-point volatiles, resulted in the reduction

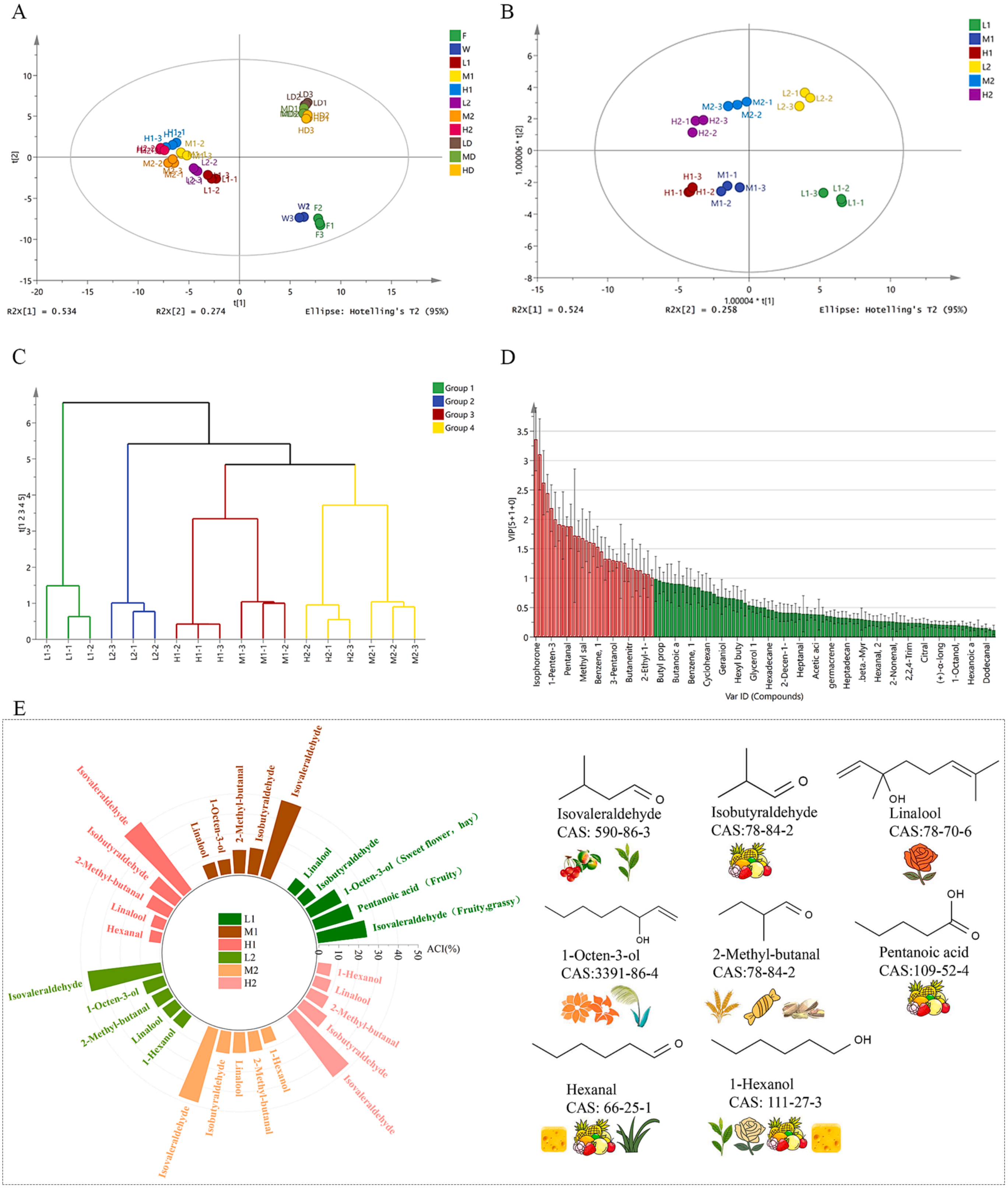

Fig. 3. (A) Score scatter plot for PCA model (total) during white tea processing; (B) Score scatter plot for OPLS-DA model of volatile compounds during white tea in the pile-up processing; (C) Hierarchical cluster analysis for white tea in the pile-up processing; (D) The variable importance in the project (VIP) for white tea in the pile-up processing. The red column highlighted the volatile components with VIP > 1; (E) The top five key characteristics of volatile components in *ACI* after 4 h and 8 h of pile-up processing. The flower, fruit and other shape legends represented for the aroma character of volatile components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
ROAV and *ACI* values of key volatile components in different white tea piling processes.

Compounds	VIP	CAS	Odor description	OT (μg/kg)	ROAV						*ACI*%					
					L1	M1	H1	L2	M2	H2	L1	M1	H1	L2	M2	H2
Isophorone	3.36	78–59-1	Mint	–	–	–	–	–	–	–	–	–	–	–	–	–
Toluene	3.11	108–88-3	Sweet and aromatic taste	200.00	0.37	0.09	0.05	0.90	0.31	0.19	0.09	0.03	0.02	0.33	0.12	0.07
Phenol	2.62	108–95-2	Sweet	–	–	–	–	–	–	–	–	–	–	–	–	–
Cis-3-hexen-1-ol	2.45	928–96-1	Fresh, green	70.00	4.75	3.04	2.78	4.91	4.91	4.24	1.18	1.21	1.18	1.82	1.87	1.63
1-Penten-3-ol	2.19	616–25-1	Fruity	400.00	0.42	0.33	0.34	0.46	0.45	0.44	0.10	0.13	0.14	0.17	0.17	0.17
Ethyl acetate	2.00	141–78-6	Fruity	5.00	8.21	5.93	6.86	16.05	8.71	9.51	2.04	2.35	2.91	5.94	3.32	3.65
2-Methyl-butanal	1.92	96–17-3	Pekoe scent, malt, sweet, fruity, cocoa	2.00	27.99	29.68	29.13	25.06	26.51	26.36	6.95	11.79	12.35	9.27	10.12	10.10
Linalool	1.90	78–70-6	Sweet, tender and fresh floral	3.80	28.95	18.74	15.66	21.26	27.37	21.44	7.19	7.45	6.64	7.87	10.45	8.21
Pentanal	1.88	110–62-3	Almonds, malt, fruity, nutty	12.00	1.30	2.28	2.53	1.46	1.21	1.04	0.32	0.91	1.07	0.54	0.46	0.40
1-Octen-3-ol	1.88	3391–86-4	Sweet flower, hay	1.00	70.19	18.90	8.24	29.40	12.27	15.03	17.44	7.51	3.49	10.88	4.68	5.76
Methyl valeraldehyde	1.72	123–15-9	Peanut, fruity	–	–	–	–	–	–	–	–	–	–	–	–	–
o-Xylene	1.72	95–47-6	Aromatic odor	–	–	–	–	–	–	–	–	–	–	–	–	–
Methyl salicylate	1.68	119–36-8	Holly, mint, sweet flower	40.00	1.55	0.88	0.76	1.21	1.68	1.44	0.38	0.35	0.32	0.45	0.64	0.55
2-*tert*-Butylphenol	1.64	88–18-6	Essence	–	–	–	–	–	–	–	–	–	–	–	–	–
Isobutyraldehyde	1.61	78–84-2	Sweet fruit	1.00	30.13	33.22	37.35	16.73	28.97	37.62	7.48	13.20	15.84	6.19	11.06	14.42
Hexanal	1.60	66–25-1	Fatty, grassy, fruity	4.50	16.08	12.64	13.97	17.64	17.66	17.18	4.00	5.02	5.92	6.53	6.74	6.58
m-Xylene	1.53	108–38-3	Sweet	–	–	–	–	–	–	–	–	–	–	–	–	–
Isovaleraldehyde	1.45	590–86-3	Fruity, grassy	0.40	100.00	100.00	100.00	100.00	100.00	100.00	24.84	39.73	42.40	37.00	38.17	38.32
Diethyl phthalate	1.33	84–66-2	Slightly aromatic	–	–	–	–	–	–	–	–	–	–	–	–	–
Pentanoic acid	1.33	109–52-4	Fruit	0.36	80.54	7.47	1.11	1.28	1.04	1.33	20.01	2.97	0.47	0.47	0.40	0.51
3-Pentanol	1.30	584–02-1	Special smell	4125.00	0.00	0.00	0.01	0.00	0.01	0.01	0.00	0.00	0.00	0.00	0.00	0.00
2-Pentenal	1.29	1576–95-0	Fresh green leaves fragrance	89.00	0.53	0.35	0.36	0.59	0.62	0.58	0.13	0.14	0.15	0.22	0.24	0.22
2-Methyl-1-propanol	1.29	78–83-1	Apple, cocoa	16000.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Phenylethyl alcohol	1.26	60–12-8	Faint rose sweetness	86.00	0.58	0.25	0.19	0.61	0.55	0.38	0.14	0.10	0.08	0.22	0.21	0.14
3-Methylbutanenitrile	1.18	625–28-5	Fruity	–	–	–	–	–	–	–	–	–	–	–	–	–
3-Methyl-1-butanol	1.17	123–51-3	Caramel, cocoa, flower	300.00	0.09	0.07	0.07	0.07	0.11	0.11	0.02	0.03	0.03	0.03	0.04	0.04
2-Methyl-1-butanol	1.14	137–32-6	Green, malt	–	–	–	–	–	–	–	–	–	–	–	–	–
1-Hexanol	1.13	111–27-3	Green, floral, fruity and fatty	4.50	20.27	13.63	12.91	20.58	19.69	17.56	5.03	5.42	5.47	7.62	7.51	6.73
2-Ethyl-1-hexanol	1.08	104–76-7	Sweet, fresh, floral, oily	300.00	0.10	0.02	0.02	0.05	0.02	0.01	0.03	0.01	0.01	0.02	0.01	0.01
Benzyl alcohol	1.07	100–51-6	Floral, rose, balsamic	3.00	10.45	4.17	3.49	11.90	9.85	6.39	2.60	1.66	1.48	4.40	3.76	2.45
Benzaldehyde	1.01	100–52-7	Bitter almonds, fruity	350.00	0.07	0.04	0.03	0.08	0.08	0.07	0.02	0.01	0.01	0.03	0.03	0.03

Note: VIP, Variable Importance in the Projection. Sort by VIP value from highest to lowest; CAS, Chemical Abstracts Service;'0.00′ represents < 0.01;'-' means data was not found in the literature. OT, Odor Threshold (in water), All the odor thresholds were obtained from the references (Chen et al., 2023; Joshi, & Gulati, 2015; Li et al., 2021; Liu et al., 2008; Ou et al., 2022; Shao et al., 2022; Su et al., 2016; Van Gemert, 2018; Zhu et al., 2015). ROAV, the Relative Odor Activity Values; *ACI*, Aroma Character Impact Value.

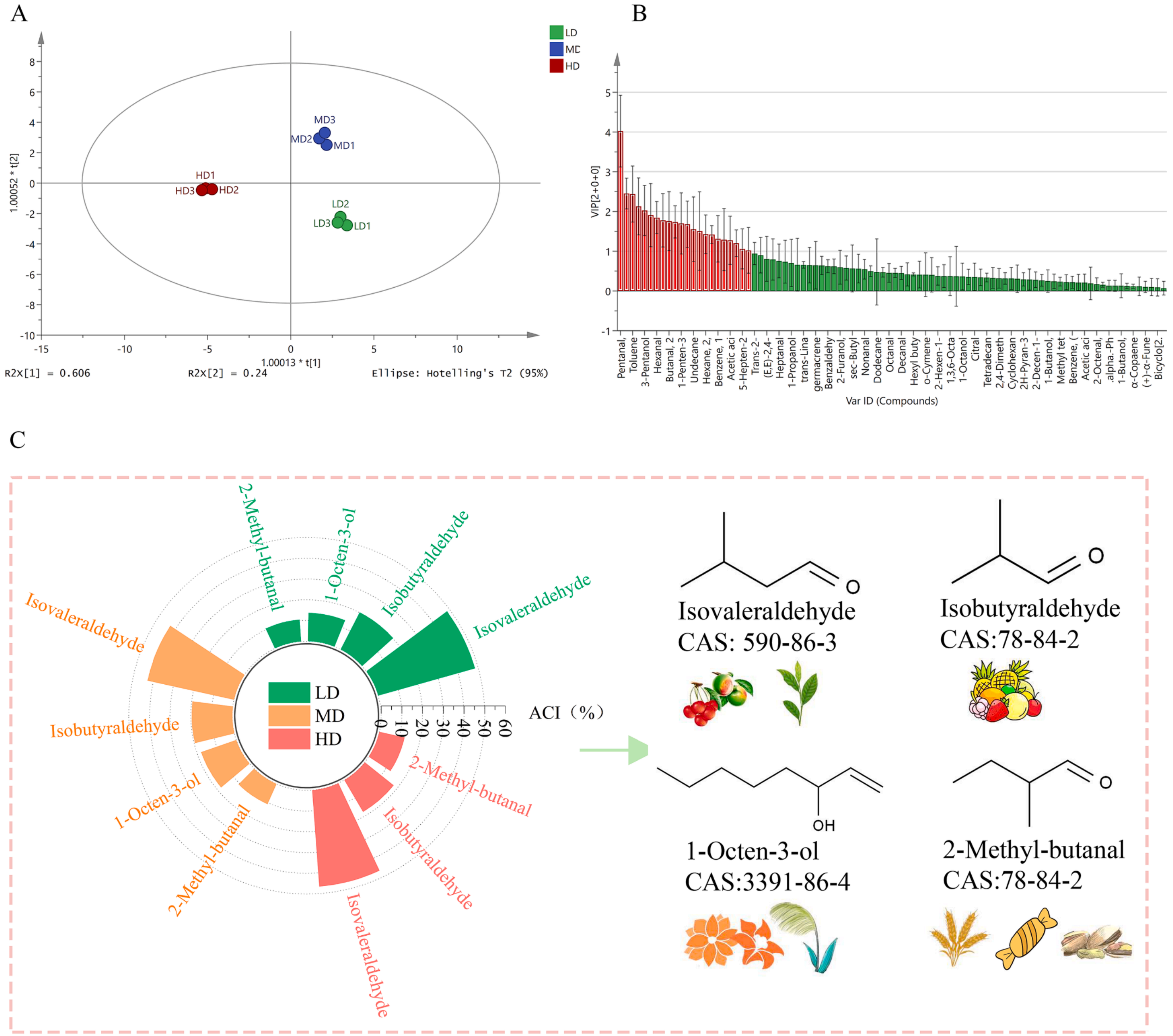

Fig. 4. (A) Score scatter plot for OPLS-DA model of volatile compounds after dried white tea; (B) The variable importance in the project (VIP) of the 22 volatile components in dried white tea. The red column highlighted the volatile components with VIP > 1; (C) Volatile components and aroma characteristics with *ACI* > 10 % in dried white tea. The flower, fruit and other shape legends represented for the aroma character of volatile components. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of total peak areas of LD, MD, and HD. The composition of dried tea was mainly composed of aldehydes, alcohols, and hydrocarbons, since massive alcohols were prone to converted into aldehydes after heating procedure, resulting in the decline of alcohol content and increase of aldehyde content.

4.3. Potential application of key volatiles as on-line intelligent sniffing markers

Dynamic changes of volatile profiles were observed in tea leaves of different processing steps. In this study, the significantly different volatiles, such as isoamylaldehyde, isobutyraldehyde, 2-methyl-butanal, 1-octen-3-ol, linalool, and 1-hexanol, showed dynamic changes along with the processing steps, and these compounds could be promising biomarkers during the white tea production. The previous study on the composition of white tea flavor suggested that characteristic fresh aroma of white tea was derived from high proportion of aldehydes and alcohols, the different composition of these components contributed to the formation of various flavors such as grassy, sweet floral, fruity, and pekoe aroma (Chen et al., 2020b). Isovaleraldehyde was the key component with the highest *ACI* in all piling treatments, imparting fruity and grassy aroma in white tea. Isobutyraldehyde was the major source of fruity-sweet aroma. Isovaleraldehyde and isobutyraldehyde accounted for the material basis of the fresh and sweet flavors of white tea. Additionally, 2-methyl-butanal showed malt (Wu et al., 2022), chocolate-like, and ripe sweet fragrance; linalool showed sweet and tender floral aroma, and was the major contributor to the floral aroma of tea (Wu et al., 2022); hexanal showed young and tender leaf aroma, and the oxidation of hexanal contributes to the fresh and sweet aroma. During the long withering and piling process of white tea, lipid oxidation of unsaturated fatty acids occurred, and the compounds such as alcohols, aldehydes, ketones, and acids were generated by the degradation of the

Table 2
ROAV and *ACI* values of key volatile components in different piled primary tea.

Compounds	VIP	CAS	Odor description	OT(μg/kg)	ROAV			*ACI*%		
					LD	MD	HD	LD	MD	HD
Methyl valeraldehyde	4.02	123–15-9	Peanut, fruity	–	–	–	–	–	–	–
1-Octen-3-ol	2.45	3391–86-4	Sweet flower, hay	1.00	28.20	41.87	19.04	13.69	17.98	8.87
Toluene	2.44	108–88-3	Sweet and aromatic taste	200.00	0.10	0.15	0.42	0.05	0.06	0.20
Isobutyraldehyde	2.13	78–84-2	Sweet fruit	1.00	41.42	45.52	38.23	20.11	19.55	17.82
3-Pentanol	2.03	584–02-1	Special smell	4125.00	0.00	0.01	0.01	0.00	0.00	0.01
Isovaleraldehyde	1.91	590–86-3	Fruity, grassy	0.40	100.00	100.00	100.00	48.54	42.94	46.60
Hexanal	1.84	66–25-1	Fatty, grassy, fruity	4.50	7.73	10.10	9.71	3.75	4.34	4.52
2-*tert*-Butylphenol	1.78	88–18-6	Essence	–	–	–	–	–	–	–
2-Methyl-butanal	1.76	96–17-3	Pekoe scent, malt, sweet, fruity, cocoa	2.00	21.77	24.86	26.79	10.57	10.67	12.48
Linalool	1.74	78–70-6	Sweet, tender and fresh floral	3.80	2.01	2.77	9.75	0.98	1.19	4.54
1-Penten-3-ol	1.70	616–25-1	Fruity	400.00	0.08	0.09	0.09	0.04	0.04	0.04
Ethyl acetate	1.68	141–78-6	Fruity	5.00	2.42	4.33	3.04	1.17	1.86	1.42
Undecane	1.55	1120–21-4	–	–	–	–	–	–	–	–
o-Xylene	1.51	95–47-6	Aromatic odor	–	–	–	–	–	–	–
2, 4-Dimethylhexane	1.43	589–43-5	–	–	–	–	–	–	–	–
Pentanal	1.42	110–62-3	Almonds, malt, fruity, nutty	12.00	0.44	0.94	2.10	0.21	0.40	0.98
m-Xylene	1.31	108–38-3	Sweet	–						
2-Ethyl-1-hexanol	1.29	104–76-7	Sweet, fresh, floral, oily	300.00	0.02	0.02	0.08	0.01	0.01	0.04
Butyl acetate	1.27	123–86-4	Fruity	58.00	0.27	0.23	0.57	0.13	0.10	0.27
p-Xylene	1.20	106–42-3	Sweet and aromatic taste	–	–	–	–	–	–	–
Methylheptenone	1.06	110–93-0	Green, fresh and fruity	–	–	–	–	–	–	–
1-Hexanol	1.02	111–27-3	Green, floral, fruity and fatty	4.50	1.52	1.99	4.77	0.74	0.86	2.22

Note: Sort by VIP value from highest to lowest;'0.00' represents < 0.01;'-' means data was not found in the literature.

unsaturated fatty acids (Deng et al., 2017), the generation of 1-octen-3-ol, hexanal, and 1-penten-3-ol, present a pleasantly floral aroma, fruity aroma, which had an important influence on the fresh and green flavor of white tea (Ho et al., 2015). Interestingly, the ROAV values of most volatiles in mid-pile such as benzyl alcohol, 1-hexanol, phenylethanol, and glutaraldehyde, which presented floral and pekoe aroma, were intermediate between low-pile and high-pile, which explained why the piling process mid-pile was intermediate between the styles in the sensory evaluation.

The different thickness tea piles would construct a micro-environment that possessed with certain temperature and relative humidity, which could influence the withering rate and distributing of water and soluble substances in the tea leaves (Zhang et al., 2022). As the thickness of the tea pile increased, the evaporation rate of the moisture content of the high-pile tea slowed down. Water was an important carrier for the transformation of chemical substances. Within a certain period of time, the moisture content of the high pile tea would be higher than that of the low pile tea, which provided a more suitable chemical reaction condition for the oxidation of white tea (Chen et al., 2018). Therefore, the higher the moisture content of the piled leaves, the more intense the micro-fermentation of white tea. During the long withering and piling process of white tea, lipid oxidation of unsaturated fatty acids occurred, and the compounds such as alcohols, aldehydes, ketones, and acids were generated by the degradation of the unsaturated fatty acids (Deng et al., 2017). The content of most fatty acid compounds in high-pile white tea was higher than that in low-pile, and pile-up treatment will promote the hydrolysis of glycerol esters in the products and produce the accumulation of fatty acids (Xiang et al., 2023). Fatty acids were not only important precursors of the aroma components of white tea (Ma et al., 2017), but also the main components causing tea rancor (Feng et al., 2022). As the piling time increased, the water evaporation occurred, which resulting the reduction of moisture content in tea. This weakened the oxidation and degradation reactions of tea inclusions, leading to a decline in aroma. Previous studies have revealed that a shorter piling period was beneficial for improving the quality of white tea (Chen et al., 2018; Xiang et al., 2023). However, a longer piling period can result in excessive lipid oxidation and unpleasant flavor. Therefore, by employing appropriate piling techniques, the flavor quality of white tea can be enhanced.

In addition, the drying method also played an important role in the formation of white tea aroma (Chen et al., 2019). After drying, the dominant characteristic compounds (*ACI* ≥ 10 %) of dried tea screened included isovaleraldehyde, isobutyraldehyde, 1-octen-3-ol, and 2-methyl-butanal, and these results were similar to the major compositions reported in a previous study (Fu et al., 2020). The highest *ACI* of isovaleraldehyde and isobutyraldehyde were found in LD, which indicated that the thin heap was conducive to the fresh flavor of white tea. The *ACI* of isobutyraldehyde and 2-methyl-butanal in MD were in between those of LD and HD, and these presented a mixed sweet, floral-fruity and pekoe aroma. These volatiles were the basis for the quality of white tea with different treatments. And in this study, the natural volatile aroma during processing were captured directly rather than extracted by other solvent extracts, and the aroma components captured by this method were closer to the QDA result. These key volatile components can be used as biomarkers for online intelligent monitoring quality over the continuous production of white tea.

5. Conclusions

In this study, the profiles and dynamic changes of volatile components in white teas of different piling treatments were determined by using QDA and ATD-GC–MS. Different aroma attributes were observed in various treatments. A total of 122 natural volatile components had been identified during the processing of white tea, the alcohols and aldehydes were the major components generated during the piling process. Eight volatiles including isovaleraldehyde, isobutyraldehyde, 2-methyl-butanal, 1-octen-3-ol, linalool, pentanoic acid, hexanal and 1-hexanol were screened out as the predominant contributors to grassy, floral-fruity fragrance, pekoe aroma, and sweet fragrance during piling process, which were promising biomarkers for online intelligent monitoring quality over the continuous production of white tea. The results of this study indicate that piling is an indispensable process that promotes the chronological accumulation of characteristic aroma during white tea production. This work also provides a novel insight into the elucidation on white tea flavor formation.

Author contributions

H.Z. Lin conceived and designed the experiments. H.Z. Lin, W.J. Bi, L.Y. Wu, Y. Sun and Z.L. Hao performed the experiments. H.Z. Lin, X.X. Ou, J.J Zhou, W.P. Zhang and J. Feng analyzed data. H.Z. Lin and L.Y. Wu prepared the manuscript. Y. Sun and Z.L. Hao revised the manuscript.

CRediT authorship contribution statement

Hongzheng Lin: Conceptualization, Data curation, Methodology, Formal analysis, Investigation, Writing – original draft. **Liangyu Wu:** Investigation, Writing – original draft. **Xiaoxi Ou:** Investigation, Software. **Jingjing Zhou:** Investigation, Supervision. **Jiao Feng:** Supervision, Software. **Wenping Zhang:** Investigation, Supervision. **Wanjun Bi:** Investigation, Software. **Zhilong Hao:** Conceptualization, Methodology, Writing – review & editing. **Yun Sun:** Conceptualization, Supervision, Methodology, 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.2024.101139.

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