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Aroma effects of critical volatile compounds during thermophilic bacteria pile-fermentation in dark tea using gas chromatography mass spectrometry and odor activity value

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Abstract

Thermophilic bacteria play an important role in aroma formation during pile-fermentation process of dark tea. With the aim to reveal the impact of thermophilic bacteria on volatile compounds in dark tea, *Bacillus licheniformis* (thermophilic bacteria) were inoculated into sun-dried green tea for spontaneous fermentation. In this study, headspace solid phase microextraction combined to gas chromatography mass spectrometry (HS-SPME-GC-MS), odor activity value (OAV), principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) were employed to investigated the characteristics of volatile compounds during thermophilic bacteria pile-fermentation. According to HS-SPME-GC-MS, a total of 64 volatile compounds were identified. PCA revealed that tea samples could be clearly discriminated from each other. Furthermore, sulcatone, hexanoic acid, linalool, 2-methyl-trans-decalin, myrtenal, and α-ionone were found to play an important role in discrimination of tea samples, based on OPLS-DA. In addition, the OAV could effectively characterize the aroma contribution of volatile compounds during thermophilic bacteria pile-fermentation, and (Z)-linalool oxide (furanoid), α-ionone, (E)-2-nonenal, and linalool were the critical volatile compounds of aroma quality in dark tea. This study provides insight into volatile compounds characteristics during thermophilic bacteria pile-fermentation in dark tea.

Keywords: dark tea; thermophilic bacteria; pile-fermentation; HS-SPME-GC-MS; odor activity value.

Practical Application: Critical volatiles during thermophilic bacteria pile-fermentation in dark tea.

1 Introduction

According to the fermentation degree, tea is traditionally categorized into green, white, yellow, oolong, black, and dark tea (Wang et al., 2022b). Due to its various health benefits and quality characteristics, dark teas are gaining popularity worldwide (Xu et al., 2021). There are several famous traditional dark teas in China, such as Yunnan Pu-erh tea, Hunan Fuzhuan tea, Hubei Qingzhuan tea, and Guangxi Liubao tea (Zhang et al., 2021a; Zhou et al., 2022). The primary processes in dark tea production involves fixing, rolling, pile-fermentation and drying (Yan et al., 2021). In the pile-fermentation process, artificially inoculated or naturally bred microorganisms secrete various extracellular enzymes, such as polyphenol oxidase, protease, pectinase, cellulase, and so on (Wang et al., 2022a). These enzymes promote a series of chemical reaction, such as oxidation and condensation under hot and humid conditions. Dark tea is characterized by the special fungal or aged aroma, mellow taste as well as reddish-brown infusion color (Zheng et al., 2015; Lv et al., 2017). In the past decades, many researchers have worked on the volatile compounds, odor-active compounds and formation mechanism of key odor-active compounds of dark teas. However, the interrelationships of these volatile compounds are difficult to be figured out due to their complex nature (Zhang et al., 2021b).

Volatile compounds in teas are only present in minimum quantities of 0.01% of total dry weight (Pripdeevech & Wongpornchai, 2013). However, volatile compounds are the key ingredients that affect the quality of dark tea (Hu et al., 2021). As is known that aroma performance is actually constituted by the mixture of multiple volatile flavor compounds (Ma et al., 2018). Up to now, several studies have investigated the effect of pile-fermentation process on volatile composition of dark teas (Shi et al., 2019). For example, ripened Pu-erh tea, which via pile-fermentation contains more volatile chemical classes than raw Pu-erh (Pang et al., 2019). Lv et al. (2012) employed gas chromatography-olfactometry and determined that 1,2-dimethoxybenzene, 4-ethyl-1,2-dimethoxy-benzene, β-ionone, β-linalool, linalool oxides, and decanal, accounted for the unique flavor of Pu-erh tea. The contents of heptanal, (E)-2-nonenal, and 6-methyl-5-heptene-2-one were increased during pile-fermentation of Qingzhuan tea (Zhang et al., 2021a).

Fermentation is one of the oldest methods to improve food value (Lee et al., 2022). The qualitative characteristics of dark tea, such as its aroma that develop during pile-fermentation process, are dependent on the different populations in the

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microbial community (Yan et al., 2021). In pile-fermentation of Fuzhuan tea, inoculation of *Eurotium cristatum* can accelerate the biotransformation of phenolic compounds, increase the types and contents of volatile compounds such as alcohols, and ultimately improve the tea quality (Xiao et al., 2020). Five volatile compounds that were positively related to the *Byssochlamys*, which were 2-ethylhexanol, safranal, β-dihydroionone, α-ionone, and trans-2-hexenyl ester in the pile-fermentation process of dark tea (Hu et al., 2021). Cao et al. reported that the generation of α-terpineol, β-ionone, and cis-jasmone was attributed to the metabolism of *Eurotium cristatum*, while the accumulation of linalool oxides, β-ionone, and geraniol was related to the metabolism of *Aspergillus niger* (Cao et al., 2018).

Aroma is one of the most important indices to evaluate the quality of dark tea (Zhu et al., 2021b). Microorganisms played an irreplaceable role of aroma developing during pile-fermentation for producing dark teas. Previous study showed that, only a few compounds are the main contributors to the characterized aroma owing to the diverse odor thresholds and concentrations exhibited by the volatiles (Liao et al., 2020). Therefore, in this study, the volatile compounds in aroma quality of thermophilic bacteria pile-fermentation were identified via gas chromatography-mass spectrometry (GC-MS), and OAVs. The critical volatiles compounds contributing to the characteristic aroma of dark tea during thermophilic bacteria pile-fermentation were explored, to ascertain the mechanisms involved in the characteristic aroma formation. The results will provide guidance for the improvement of the quality and expand on our current knowledge of the volatiles properties of dark tea.

2 Materials and methods

2.1 Reagents

Ethyl decanoate (BR; 99%) obtained from Aladdin (Shanghai, China) was used as the internal standard. The internal standard was added in headspace vials for the semi-quantification of volatile compounds. The 20-mL headspace vials covered with magnetic precision screw-thread metal PTFE/ silicone septa caps were purchased from HAMAG Technologies Inc. (Ningbo, China). C_7-C_{40} *n*-alkanes (ANPEL, Shanghai, China) were used to determine linear retention indices. All other reagents with analytical grade were purchased from Sinopharm (Shanghai, China).

2.2 Preparation of thermophilic bacteria strain solution

The thermophilic bacteria strain using for fermentation was isolated from a pile-fermentation industrial production of Qingzhuan tea, which was identified as *Bacillus licheniformis*, and was preserved in our laboratory (Zhang et al., 2019). The strain was preserved on nutrient agar and was sub-cultured every two weeks.

Culture medium (g/L): 5 tryptone, 10 beef extract and 5 NaCl. The culture medium was sterilized at 121 °C for 20 min.

Preparation of thermophilic bacteria strain solution: Two loopfuls of strain which was preserved on a nutrient agar plate was transferred aseptically into a 250-mL Erlenmeyer flask which was contained 150 mL culture medium. The Erlenmeyer flask was incubated aerobically on a shaker of 150 r/min at 55 °C for a period. When the $OD_{600\,nm}$ reached about 0.8, the preparation was completed and waited for subsequent use.

2.3 Samples preparation

The raw material, sun-dried green tea with one bud and five to six leaves, for thermophilic bacteria pile-fermentation were procured from Hubei Dongzhuang Tea Co. Ltd. (Hubei, China). The fresh tea leaves were picked at July, 2018, and were processed by the process technology of sun-dried green tea.

Preparation of samples: enough materials for thermophilic bacteria pile-fermentation were sterilized at 121 °C for 20 min. 20 g sun-dried green tea leaves, initial moisture content about 10% by weight, were mixed with 10 mL distilled water (water content reached about 40%, w/w) or mixed with 3 mL thermophilic bacteria solution and 7 mL distilled water (inoculation amount 10%). Then, these tea leaves were constant cultured at 55 °C for 7 d. Each sample was repeated three times. Inoculated tea samples collected on 7th day were defined as fermentation tea (FT). The non-inoculated leaves collected on 0th and 7th day were defined as control (CK) and high temperature control (FTCK), respectively. Each tea sample was repeated three times. After freeze-drying, the tea samples were ground into powders then stored at -80 °C until extracted before analysis.

2.4 Extraction of volatile compounds by HS-SPME

Extraction of volatile compounds for tea samples were carried out by HS-SPME (headspace solid phase microextraction) method (Zhang et al., 2021a) using a 75-μm carboxen/polydimethylsiloxane fiber (Supelco, St. Louis, MO, USA). The HS-SPME parameters are described in detail as follows: 1 g of homogenized tea powder was weighed (accurate to 0.001 g) to a 20-mL headspace vial, and it was infused with 5 mL boiling water immediately. Subsequently, 2.5 μL ethyl decanoate (0.1 μg/mL) was added into this vial as the internal standard, and it was sealed immediately. The aged extraction head was inserted into this vial through a SPME handheld device, and the vial was equilibrated in the water bath at 60 °C for 10 min. After equilibration, the SPME fiber was pushed out, exposed to the sample head space, and absorbed for 50 min at 60 °C. Then, the fiber was inserted into the GC (gas chromatography) injection port for desorption (5 min, 240 °C) and subsequent analysis. Notably, the SPME fiber had been kept in the GC injection port for 60 min at 250 °C prior to use for preventing contamination.

2.5 Volatile compounds detection by GC-MS

The prepared HS-SPME volatile compounds were analyzed with a Trace 1300 GC equipped with a DSQ II MS (Thermo Fisher Scientific, MA, USA) according to the literature (Qu et al., 2020), with minor modifications. Helium (purity > 99.99%) was employed as carrier gas with a constant flow rate of 1.0 mL/min. After microextraction, desorption of the volatile compounds from the coating fiber was performed in the injection port of the GC apparatus at 240 °C for 5 min in splitless mode. The temperature program was as follows: 40 °C initial temperature held for 2 min, raised to 85 °C at 3 °C/min and held for 2 min, raised to 110 °C at 2 °C/min and held for 2 min, raised to 160 °C at 5 °C/min and held for 1 min, then raised to 220 °C at 5 °C/min and held for 5 min. The ionization mode of MS was EI mode with an ion source temperature of 230 °C and electron energy of 70 eV. The ion mass scanning range was m/z 50 to 650.

2.6 Identification and quantification of volatile compounds

The peaks were obtained from GC-MS analysis, with the total ion current of the peaks were searched through NIST libraries. Volatiles were identified by comparison of retention index (RI) and mass spectra matching in NIST. RIs were calculated after analyzing C7-C40 *n*-alkane series under the same chromatographic conditions (Li et al., 2020). The volatile compounds detected in each sample were collected into a data matrix and the substances with a missing value of < 80% were screened out for subsequent analysis. Ethyl decanoate (internal standard) was used to quantitatively analyze the aroma components by the peak area of the total ion current. The quantitative analysis was performed by dividing the peak area of a compound with the peak area of the internal standard and multiplying the result with the concentration of the internal standard.

2.7 OAV calculation

The odor activity value (OAV) has been proposed to explain how to evaluate the contribution of individual volatile compounds to the overall aroma by the relative concentration of volatile compounds (Zhang et al., 2021b). The OAV was defined as the ratio between the relative concentration of the individual volatile compound and the sensory detection threshold in the literature. Odor thresholds in the water of volatile components were provided in the relevant literature. Volatile compounds with an OAV > 1 can be perceived by the human noses (Wei et al., 2020).

2.8 Statistical analysis

All experimental samples were repeated in triplicate. Data were shown as the mean values ± standard deviation. Primary data were analyzed using Excel software (Excel 2021) (New York, USA). Principal component analysis (PCA) and orthogonal partial least squares discrimination analysis (OPLS-DA) was performed to explore the differences by SIMCA 14.1 (Umetrics, Sweden). The heatmap was performed using the TBtools (Version 1.075).

3 Results

3.1 Qualitative and quantitative analyses of volatile compounds

The volatile compounds from tea samples were analyzed by HS-SPME-GC-MS. A total of 64 volatile compounds were qualitatively identified, which could be mainly classified into eight categories according to the main function groups, comprising alcohols (26.56%), hydrocarbons (20.31%), ketones (18.75%), aldehydes (10.94%), esters (10.94%), ethers (6.25%), nitrogen compounds (1.56%), carboxylic acid (1.56%) and others (3.13%) (Figure 1A). The main volatile compounds categories of the tea samples were alcohols, hydrocarbons and ketones. Consistent

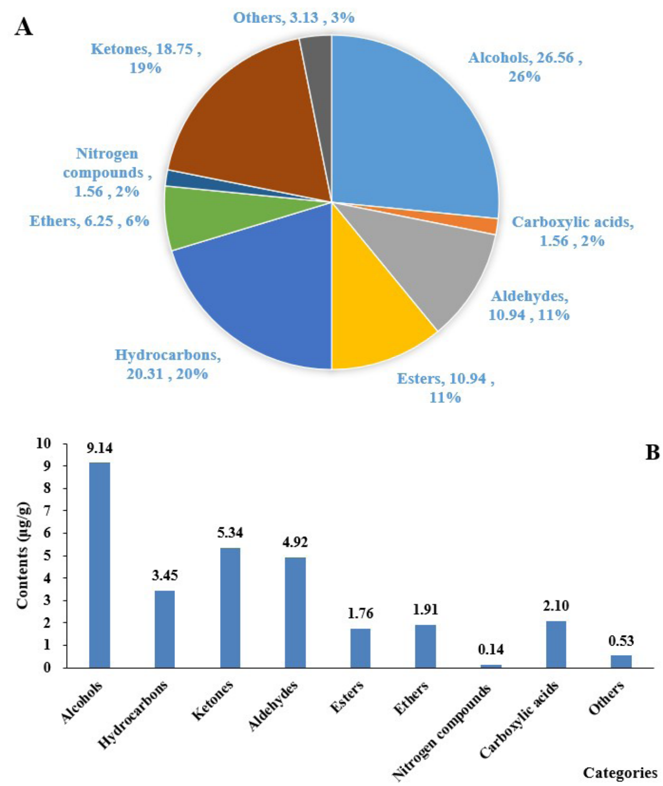

Figure 1. Qualitative and quantitative analyses of volatile compounds in tea samples. A, classification and proportions (%) of volatile compounds; B, categories and contents of volatile compounds.

with this finding, ketones were the main volatile compounds detected in Qingzhuan tea (Zhang et al., 2021a), those of Liubao tea were alcohols (Cao et al., 2018), ketones, esters and alkanes are also the main volatile components detected in Pu-erh tea and Fuzhuan tea (Zhang et al., 2021b).

The quantitative results of volatile compounds in tea samples were shown in Figure 1B. For the total contents of each volatile compounds category, alcohols (9.14 μg/g) had the highest contents in tea samples volatiles of the total compounds, following by ketones (5.34 μg/g) and aldehydes (4.92 μg/g). In contrast, nitrogen compounds (0.14 μg/g) were present at the lowest concentrations. Alcohols and ketones had a greater impact on the aroma characteristics during thermophilic bacteria pile-fermentation, accounting for almost 50% of total volatile compounds. Generally, alcohol compounds emit a floral and sweet odor, and ketone compounds offer floral and woody odors (Zhang et al., 2021b). It was speculated that the aroma formatted during thermophilic bacteria pile-fermentation possible provided a floral, woody and sweet odor.

3.2 Characteristic volatile components screening of tea samples

To visualize overall compound differences among the three groups and the variability between intra-group tea samples, unsupervised PCA was performed in this work. PCA explained 96.8% of the effects of variables on tea samples characteristics, of which PC1 explained 87.0% and PC2 accounted for 9.8%. As shown in Figure 2A, the tea samples of different treatment were clearly separated on the PCA plot. Compared with the distance between samples, the difference between CK and FT was small, but the difference with FTCK was large. OPLS-DA is a new multivariate statistical method for regression modeling, which can accurately determine the characteristic variables that affect the group (Triba et al., 2015). As shown in Figure 2B, OPLS-DA was used to screen further the key volatile compounds that affect the aroma characteristics of tea samples. The model was used to distinguish the differences of tea samples, and the good fitting parameters (R2Y = 0.998, Q2 = 0.991) demonstrated the model's accuracy. The variable importance factor (VIP) can quantify the contribution of each variable to the classification. When the VIP value is >1, the corresponding variable can be defined as the key variable of the discriminant model (Su et al., 2022). In the OPLS-DA model, there 19 volatile compounds (VIP > 1, $p < 0.05$) were screened, including linalool, sulcatone, benzaldehyde, (Z)-linalool oxide (furanoid), furfural, 1-heptanol, lavandulol, dihydroactinidiolide, dehydroelsholtzia ketone, α-ionone, edulan I, (Z, Z)-3, 6-nonadienal, eucalyptol, hexanal, myrtenal, hexanoic acid, 2-methyl-trans-decalin, 2, 5-dimethylcyclohexanol, cis-linaloloxide.

A heatmap was displayed and colored by the relative expression change after the normalization of the key volatile compounds in tea samples. The 19 volatile compounds identified as key volatile compounds were shown in Figure 3. Most of these compounds are aldehydes and alcohols. Overall, the key volatile compounds of three samples (CK, FT, and FTCK) mainly showed downregulation. Among them, benzaldehyde, (Z)-linalool oxide (furanoid), dehydroelsholtzia ketone, (Z, Z)-3, 6-nonadienal, furfural, 1-heptanol, hexanal, lavandulol, dihydroactinidiolide, and edulan I were downregulated in all samples. However, linalool and sulcatone were upregulated in CK and FT samples, respectively. Linalool has a floral and fruity aroma (Zhu et al., 2021a), which was the main contribution to the aroma characteristics of FT.

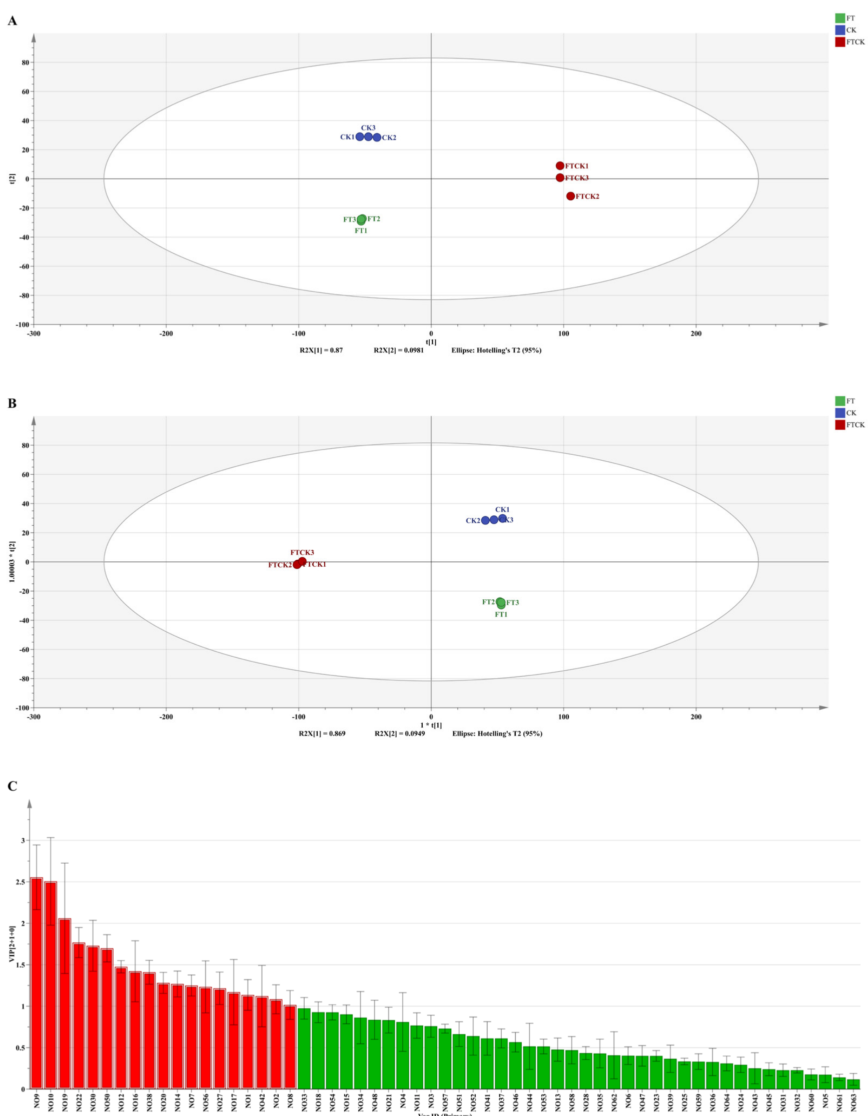

Figure 2. Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discrimination Analysis (OPLS-DA) of three tea samples. A, Score scatter plot of PCA; B, Score scatter plot of OPLS-DA; C, VIP of OPLS-DA.

Table 1. OAVs for tea samples during thermophilic bacteria pile-fermentation.

RT	RI	Compounds	CAS. No.	OD	ODT	RC		OAV	
						FT	CK	FT	CK
17.64	1079	(Z)-Linalool oxide (furanoid)	5989-33-3	flower#	0.03[c]	107.87 ± 3.00	216.19 ± 22.83	3595.67	7206.33
37.86	1410	α-ionone	127-41-3	violet#	0.6[a]	371.60 ± 9.09	157.71 ± 20.18	619.33	262.85
22.81	1167	(E)-2-Nonenal	18829-56-6	cucumber odor#	0.08[b]	34.14 ± 2.92	0.00 ± 0.00	426.75	0.00
18.44	1093	Linalool	78-70-6	flower, lavender#	4[a]	876.12 ± 6.66	591.22 ± 56.23	219.03	147.81
8.61	885	2-Heptanone	110-43-0	slightly spicy odorł	1[a]	42.20 ± 1.81	52.31 ± 16.46	42.20	52.31
12.16	968	1-Heptanol	111-70-6	herb, mushroom, green#	3[b]	46.98 ± 6.64	147.95 ± 22.27	15.66	49.32
14.95	1026	Eucalyptol	470-82-6	mint, sweet#	12[b]	147.94 ± 9.42	339.07 ± 28.48	12.33	28.26
33.78	1340	Caprylyl isobutyrate	109-15-9	fruity, green*	6[b]	67.10 ± 3.99	42.18 ± 8.91	11.18	7.03
5.47	-	Hexanal	66-25-1	grass, tallow, fat#	4.5[b]	16.49 ± 4.89	96.74 ± 22.76	37.70	21.50
39.06	1442	(E)-Geranylacetone	3796-70-1	magnolia, green#	60[a]	109.55 ± 10.78	65.94 ± 7.41	1.83	1.10
11.63	957	Benzaldehyde	100-52-7	almond, burnt sugar#	100[a]	89.28 ± 2.98	235.15 ± 25.89	0.89	2.35
12.83	981	6-methyl-5-heptene-2-one	110-93-0	green citrus-like odourł	50[b]	6.60 ± 0.76	635.34 ± 11.52	0.13	12.50

RT, retention time; RI, retention index; OD, odor descriptor; RC, relative content (μg/L); ODT, odor detection thresholds (μg/L); OAV, odor activity value; -, not available. #Flavornet (2004) *FlavorDB (2017) ł PubChem (2022) [a]Fenaroli's handbook of flavor ingredients. (Burdock, 2009). [b]Odor & Flavor Detection Thresholds in Water (Leffingwell & Associates, 2022) [c]Midwest Grape and Wine Industry Institute (Iowa State University, 2022)

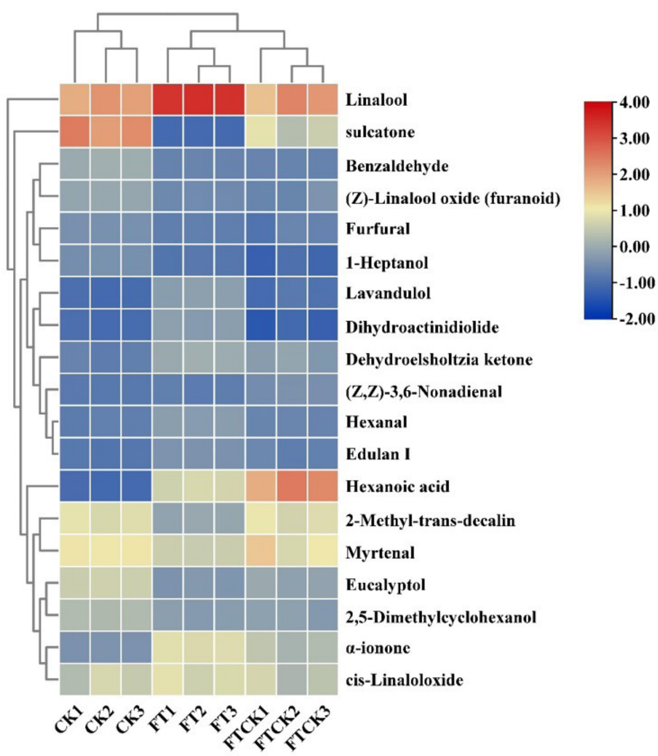

Figure 3. Heatmap of key volatile compounds in tea samples.

3.3 Critical volatile compounds during thermophilic bacteria pile-fermentation

Usually, the importance of a critical aroma-active compound is recognized by the OAV calculation rather than the amount of each compound (Matheis & Granvogl, 2016). When OAV > 1, the volatile component was as the critical volatile compound (Wei et al., 2020). To evaluate the contribution of different volatiles during thermophilic bacteria pile-fermentation to aroma, OAV was calculated for all identified volatile compounds. Table 1 showed the OAVs of important volatile compounds referring to the odor descriptor and odor detection thresholds. A total of 12 critical volatile compounds with an OAV > 1 was identified. The higher the OAV value, the greater the contribution to the aroma characteristics in thermophilic bacteria pile-fermentation. It was observed that (Z)-linalool oxide (furanoid) (CAS 5989-33-3) had the highest OAV, with an OAV of 3595.67 in FT and 7206.33 in CK. Linalool oxide contributes to woody and floral aroma. The contribution of (Z)-linalool oxide (furanoid) to the aroma of dark tea has also been emphasized in a former study.

Although 12 ketones were found during thermophilic bacteria pile-fermentation, only 4 of them revealed OAVs > 1, including α-ionone (CAS 127-41-3), 2-heptanone (CAS 110-43-0), (E)-geranylacetone (CAS 3796-70-1), and 6-methyl-5-heptene-2-one (CAS 110-93-0). Among them, the OAVs of the highest was α-ionone with an OAV of 619.33 in FT and 262.85 in CK. Despite the fact that the total content of aldehydes was high, an OAV < 1 was obtained due to the high odor threshold. Thus, only (E)-2-nonenal, hexanal and benzaldehyde were the critical aroma active compounds in FT and CK. This result showed that the contribution of an odorant could not be determined only by the content, not taking matrix effects and thus, the aroma release into account (Yang et al., 2022).

4 Discussion

Aroma compounds are representative metabolites in tea plants (*Camellia sinensis*) (Zeng et al., 2021). These compounds determine the aroma quality of tea products and have important contributions to tea quality and customer preferences (Yang et al., 2013). Therefore, studies on the formation and regulation of tea aroma compounds have attracted increasing interest in recent years (Ho et al., 2015; Zeng et al., 2019). For example, the characteristic volatiles in raw Pu-erh tea were linalool, tridecane, caffeine, dihydroactinidiolide, β-ionone, 6,10,14-trimethyl-2-pentadecanone, dodecane, etc. (Xu et al., 2021). Previous studies have also shown that the volatile compounds in Qingzhuan tea were mainly hexanal, linalool, nonanal, β-ionone, (E, E)-2,4-heptadienal, 6-methyl-5-heptene-2-one (Liu et al., 2017).

Alcohols were considered to be the main contributors in the formation of the characteristic aroma of dark tea (Lv et al., 2013). As an important class of volatile compounds, alcohols often have special scents, including 'floral', 'sweet', and 'woody' odors (Wang et al., 2022a). Among these identified volatile compounds, linalool was found to be the most abundant in tea samples, with a mean content of 1.03 μg/g, and it contributes to a flower and lavender aroma. It underwent significant oxygenation during the pile-fermentation process (Xu et al., 2016). Linalool oxides were identified to provide'floral'and'woody' odors, which were detected with high concentration in dark teas (Feng et al., 2019). In this study, Cis-linaloloxide (0.52 μg/g) was also present at a high content level. (Z)-linalool oxide (furanoid) promoted the formation of flower odor during thermophilic bacteria pile-fermentation, and it was previously identified as one of the main volatile compounds in black tea (Chen et al., 2019). Therefore, it was evident that linalool and its oxides were the predominant volatile compounds in alcohols group.

Ketones are thought to be a class of compounds that played an essential role in the formation of dark tea aroma because of their relatively low odor thresholds as well as almost each of them emits unique odors (Pang et al., 2019). Generally, ketones in tea infusion are the products of β-carotene oxidative degradation (Liu et al., 2021). The main ketones in dark tea are β-damascenone, α-ionone, and β-ionone, which have rich and complex aromas of violet, wood, and fruit (Lv et al., 2015). In this study, 6-methyl-5-heptene-2-one (0.54 μg/g) was the most abundant compound among ketones, followed by α-ionone (0.45 μg/g). 6-Methyl-5-heptene-2-one provided 'pepper', 'mushroom', and 'rubber' odors, and it was identified as the main compounds to the aroma profile of Pu-erh tea (Wang et al., 2022a). In addition, α-ionone had a wood and violet aroma with a very low threshold (0.6 g/kg), indicating that it was potentially critical to the formation of dark tea aroma quality.

Aldehydes were thought to be mainly produced via lipid oxidation and decomposition, significantly contributing to the entire aroma formation of dark tea because of their relatively low odor threshold values (Wang et al., 2022a). It was worth noting that aldehydes with low boiling point were often volatilized during processing, and some aldehydes presented in tea were synthesized by decarboxylation or deamination of amino acids at the later stage of processing (Zhu et al., 2021b). C_6 aldehydes, such as hexanal, can be generated from α-linolenic acid and linoleic acid via the oxidation reaction initiated by free radicals and lipoxygenase-mediated lipid oxidation; the latter one is the main pathway contributing to the flavor of tea (Ho et al., 2015). (E)-2-Nonenal was identified to provide 'fatty', 'leaf-like', and 'metallic' odors and detected in Pu-erh tea with a high flavor-dilution factor (Pang et al., 2019). In this study, (E)-2-Nonenal was vital to aroma quality during thermophilic bacteria pile-fermentation, although its content was low.

5 Conclusion

Dark tea unique aroma is the comprehensive presentation of various volatile compounds with different concentrations, which is one of the most important factors to measure its quality. In this study, the aroma effects of critical volatile compounds during thermophilic bacteria pile-fermentation of dark tea were analyzed via HS-SPME-GC-MS, as well as the OAV method. HS-SPME-GC-MS identified a total of 64 volatile compounds, and the classification of alcohols, hydrocarbons and ketones better revealed the aroma differences of thermophilic bacteria pile-fermentation. Alcohols (9.14 μg/g) had the highest contents in tea samples, following by ketones (5.34 μg/g). The OPLS-DA (VIP > 1, $p < 0.05$) results indicated that 19 volatile compounds were the key volatile compounds in thermophilic bacteria pile-fermentation. And OAV (> 1) results indicated that 12 volatile compounds were the critical volatile compounds during thermophilic bacteria pile-fermentation. Therefore, (Z)-Linalool oxide (furanoid) (OAV = 3595.67), α-ionone (OAV = 619.33), (E)-2-nonenal (OAV = 426.75), and linalool (OAV = 219.03) were the critical volatile compound of aroma quality during thermophilic bacteria pile-fermentation in dark tea. The study demonstrated that thermophilic bacteria had beneficial effect on the aroma quality of dark tea pile-fermentation, providing insights into the utilization of microbial fermentation.

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