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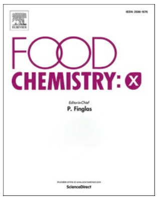

Non-targeted metabolomics characterization of flavor formation of Lichuan black tea processed from different cultivars in Enshi

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ABSTRACT

Nine tea cultivars planted in Enshi were selected and processed into "Lichuan black tea". Sensory evaluation showed that cultivar had the greatest influence on taste and aroma quality, including sweetness, umami and concentration of taste, as well as sweet and floral fragrances of aroma. The non-volatile and volatile components were identified by UPLC-Q-TOF/MS and GC–MS, and PCA analysis showed good separation between cultivars, which could cause the difference in quality. Baiyaqilan, Meizhan and Echa 10 had a floral aroma, with obvious difference in their aromatic composition from other cultivars. Moreover, Echa 10 also had a strong sweet aroma. The key aroma components in Echa 10 (with the largest cultivation area) were further investigated by GC-O-MS combined with odor activity value (OAV) analysis, included β-damascenone, phenylethylaldehyde, nonenal, geraniol, linalool, jasmonone, (E)-2-nonenal, β-cyclocitral, (E)-β-ocimene, methyl salicylate, β-ionone, 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene, citral, β-myrcene, nerol, phenethyl alcohol, benzaldehyde, hexanal, nonanoic acid, and jasmin lactone.

Introduction

As one of the top three non-alcoholic beverages worldwide, tea has always been popular with consumers due to its unique flavor and health benefits, with black tea accounting for over 70% of global tea consumption (Huang, Jiang, Tao, Wen, Xiao, Zhang, et al., 2021). The quality of black tea is an important factor for market consumption, which is mainly reflected by infusion color, taste, and aroma. (Liang, Lu, Zhang, Wu, & Wu, 2003). The quality of black tea can be influenced by many factors, such as cultivars (Chen, Sun, Gao, Peng, Wang, Zhao, et al., 2022), ecological environment (Jayasekera, Kaur, Molan, Garg, & Moughan, 2014), cultivation methods (Mozumder, Hwang, Lee, Kim, & Hong, 2021), processing technology (Li, He, Yu, Zhou, Ran, Chen, et al., 2021), storage (Huang, et al., 2021), etc., with cultivar as a key influencing factor (Chen, et al., 2022).

Tea cultivars vary biochemically, such as large-leaved tea trees with a high content of polyphenols, and albino cultivars with a high content of amino acids (Zhao, Ma, Lou, Zhang, Hu, He, et al., 2022). The content of polyphenols, caffeine, sugars, organic acids, amino acids and volatile compounds can influence the taste, aroma, color, brightness and astringency of tea infusion (Liang, Lu, Zhang, Wu, & Wu, 2003). This suggested that the final quality of black tea depends on the chemical composition of raw tea leaves. Based on previous studies, thearubigins and fructose contribute to the sweet and mellow taste and bright red infusion color of Dianhong tea (Wang, Wang, Yuan, Shen, Li, Hua, et al., 2022), and the sweetness of Huangjincha is related to the content of maltose, theanine and other substances (Penghui, Hao, Xi, Ni, Yushun, & Hongfa, 2021). Similarly, the aroma types of black tea also vary with cultivars. For instance, Keemun black tea processed with *Camellia sinensis cv. Zhuye* has a unique "Keemun aroma", which is related to the aroma compounds 3-methylbutanal, hexanal, β-laurolene and methyl salicylate (Xiao, Cao, Zhu, Chen, & Niu, 2022). Jinmudan black tea exhibits an outstanding floral and fruity aroma, due to its high content of fruit aromatic components such as jasmin lactone (Li, Hao, Jia, Zhang, Wu, Ning, et al., 2022). At present, with the development of global congou black tea market, most of the tea regions in China are producing congou black tea, such as Anhui Qihong, Yunnan Dianhong, Fujian Jinjunmei, Henan Xinyanghong, etc., and most previous studies mainly

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focused on the quality differences of black tea from different regions (Q. Wang, Qin, Huang, Jiang, Fang, Wang, et al., 2022) and the optimization of processing techniques (Zhou, Yu, He, Qiu, Li, Shu, et al., 2020), paying little attention to the quality characteristics of black teas processed from different cultivars. Moreover, due to differences in environmental conditions in China's major tea-producing provinces and in different areas of each province, tea tree cultivars vary in their adaptations, resulting in great differences in their quality (Wang, Gan, Sun, & Chen, 2022). However, the influence of different cultivars on the quality of black tea in specific regions has not been studied in depth.

Enshi, a major tea-producing area in China, belongs to the Wuling Mountains, with a superior ecological environment, where high-quality green tea "Enshiyulu" and black tea "Lichuan black tea" are produced. Lichuan black tea belongs to congou black tea and sells well in China due to its characteristics of agate red, nectar aroma, and sweet mellow taste. Lichuan black tea has a production area of 120,800 ha (ha) and has become a leading industry for local people to increase their income and wealth (Jianliang, 2021). However, our preliminary survey found that a relatively large number of tea cultivars grown in Enshi had led to an uneven quality of the finished teas, thus restricting the economic returns of local tea farmers. Against this background, this study aimed to analyze the quality characteristics of different cultivars of black tea in Enshi Prefecture using different analytical methods. Specifically, nine tea cultivars planted in the local region were selected and processed them into Lichuan black tea using the same manufacturing process, followed analyzing their quality characteristics using LC-MS and GC–MS. Additionally, the key aroma compounds for the "sweet and fruity" flavor of Lichuan black tea processed from the main cultivar Echa 10 were further identified using solvent-assisted flavor evaporation (SAFE) combined with gas chromatography–olfactometry-mass spectrometry (GC-O-MS). This study provides a theoretical basis for quality improvement of Lichuan black tea and facilitates the selection and breeding of high-quality tea cultivars in the region.

Materials and methods

Main chemicals

The main chemicals included ninhydrin, anthrone, concentrated sulfuric acid, potassium dihydrogen phosphate, disodium hydrogen phosphate, stannous chloride, folin phenol, ethanol, methanol, butanol, ethyl acetate, sodium bicarbonate, Sodium chloride, anhydrous sodium sulfate (AR, China national pharmaceutical group, Shanghai chemical reagent Co., Ltd.); methanol (LC, Fisher Scientific, Ireland); formic acid (LC, Tianjin Kemiou Chemical Reagent Co., Ltd.); 7-(2-hydroxyethyl) theophylline (standard for HPLC,≥ 98% Shanghai Ronghe Pharmaceutical Technology Development Co., Ltd.); dichloromethane (GC, Thermo Fisher Scientific Co., Ltd., China); cyclohexanone (standard for GC, ≥ 99.9%, Shanghai Macklin Biochemical Co., Ltd., China).

Tea sample processing

In this study, we used the nine cultivars of Echa 10, Echa 1, Fuyun 6, Fudingdabai, Jinguanyin, Jinxuan, Lenghouhun, Meizhan and Baiyaqilan, which were harvested in April 2021 from the tea plantation of Lichuan Xingdoushan Black Tea Co Ltd, with the standard of single bud (with a small amount of one bud and one leaf at the beginning). The fresh leaves were withered in a 6CWD-200 withering tank (Zhejiang Green Peak Machinery Co., Ltd., Quzhou, China) at a temperature of 32 °C until a moisture content of 58%-60%, with the leaves turned 3–4 times during withering. After withering, the leaves were rolled for 1.5 h in a 6CRT-55 tea twisting machine (Zhejiang Green Peak Machinery Co., Ltd., Quzhou, China) under light-heavy-light pressure. After rolling, the leaves were fermented in a 6CFJ-400 fermenting machine (Zhejiang Green Peak Machinery Co., Ltd., Quzhou, China) for 3.5–4 h at 32 °C and 95% humidity until the leaves exhibited a yellowish-red color and a floral and fruity aroma. After fermentation, the tea leaves were dried initially at 110–115 °C (6CH-20 dryer, Zhejiang Green Peak Machinery Co., Ltd., Quzhou, China) for 10 min until a moisture content of about 30%, followed by spreading them out and dampening for 1 h, then drying them a second time at 100–105 °C until a moisture content of 3%-4%.

Sensory evaluation and routine physical and chemical analysis

Sensory evaluation

Referring to GBT23776-2018 tea sensory evaluation method, each black tea was independently by 5 professional tea tasters on a 100-point scales, with 25% for appearance, 10% for infusion color, 25% for aroma, 30% for taste and 10% for infused leaf.

Quantitative descriptive analysis (QDA)

Additionally, the specific sensory attributes of the nine black teas were evaluated by the quantitative descriptive analysis (QDA) method. Specifically, the appearance indicators included moistness, tightness, fineness, and tippy; the infusion color and infused leaf indicators were redness and brightness; the aroma indicators were floral, fruity, sweet, green, and miscellaneous; the taste indicators included sour, bitter, astringent, umami and sweet and concentrated, with five degrees from 1 to 5 (1 = none/weak, 5 = strong).

Physical and chemical analysis

The content of tea polyphenols was measured according to the colorimetric method of Folin-Phenol in GB/T 8313–2018. The soluble sugar content was determined by the anthrone-sulfuric acid colorimetry. The free amino acid content was determined according to the ninhydrin colorimetric method in GB/T 8314–2013. Theaflavins, thearubigins, and theabrownins were systematically analyzed (Li, et al., 2021). Tea infusion color analysis was performed using the colorimeter method (Li, et al., 2022).

Ultra performance liquid chromatography - tandem quadrupole Rod-Time of flight mass spectrometry (UPLC-Q-TOF/MS) analysis

The non-volatile components of the different cultivars of black tea were determined as previously reported (Y. C. A. Li, et al., 2021). Briefly, 150 mg of ground dry tea sample was weighed into a 10 mL centrifuge tube, followed by adding 7.5 mL of 75% (v/v) methanol solution (containing 150 μL, 250 μg/mL of 7-(2-hydroxyethyl)theophylline as the internal standard), and extraction in a 70 ℃ water bath for 30 min. After cooling to room temperature, the extract was transferred to a 10 mL centrifuge tube and centrifuged at 5000 *g* for 3 min. Finally, the supernatant was filtered through a 0.22 μm membrane, placed in a brown bottle, sealed with parafilm and stored at −20℃ for further analysis.

Metabolomics analysis of each black tea was performed using ultra performance liquid chromatography (UHPLC, Infinity 1290 series, Agilent, California, USA)-tandem quadrupole time-of-flight mass spectrometry (Q-TOF/MS, Q-TOF 6520, Agilent, California, USA) with a Zorbax Eclipse Plus C18 column (100 × 2.1 mm, 1.8 μm, Agilent, California, USA). Chromatographic conditions were as follows: mobile phase A, 0.1% (v/v) formic acid aqueous solution; mobile phase B, methanol; gradient elution program: 0–4 min, 10–15% B; 4–7 min, 15–25% B; 7–9 min, 25–32% B; 9–16 min, 32–40% B; 16–22 min, 40–55% B; 22–28 min, 55–95% B; 28–30 min, 95% B; 30–31 min, 95–10% B; 31–35 min, 10% B; injection volume, 3 μL; column temperature, 35 °C. Mass spectrometry conditions: Mass spectrometry in ESI + mode with capillary voltage of 3.5 kV; drying gas temperature and flow rate of 300 °C and 8.0 L/min, respectively; spray pressure of 3.5 psi, sheath gas temperature and flow rate of 350 °C and 11.0 L/min, respectively; and scan range of 100–1200 Da; non-targeted automatic secondary (Auto MS/MS) scan. The three collision energies were set at 10, 20 and 30 V, respectively. Metabolites were identified according to metabolomics databases established by our previous work with accurate mass, MS^2 spectra and peak time.

Gas Chromatography-Mass spectrometry (GC–MS) analysis

The aroma compounds in each finished tea was determined using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (Zhou, et al., 2020). Extraction of aroma compounds: Before extraction, the PDMS/DVB extraction fibers were conditioned at 250 °C for 1 h at the GC inlet. Meanwhile, each crushed tea sample (1 g) was weighed into a 20 mL headspace flask, followed by adding 5 mL of boiling NaCl saturated aqueous solution and 500 μL of cyclohexanone internal standard solution (0.1 μL/mL), closing the flask tightly immediately and placing the tube at a 60 °C water bath for 1 h. GC–MS analysis was performed under the following conditions: Inlet temperature, 230℃; carrier gas, high purity helium, purity ≥ 99.99%; column flow rate, 1.0 mL/min; temperature program: 45℃ as the initial temperature, up to 80℃ at 7 ℃/min, up to 90 ℃ at 2 ℃/min and hold for 2 min, up to 100℃ at 3℃/min and hold for 2 min, up to 130℃ at 3℃/min and hold for 2 min, then up to 150℃ at 3℃/min, and finally up to 230 ℃ at 10 ℃/min and hold for 5 min; column box temperature, 40 ℃; injection, splitless mode. MS conditions: ion source EI, electron energy 70 eV, ion source temperature 230 °C, and mass scan range m/z 35 ~ 400. Substance characterization was performed based on mass spectra, retention index (RI), and the NIST2014 database.

SAFE for extraction of tea aroma essential oil

Tea aroma essential oil was extracted using the SAFE method (Y. C. Li, et al., 2022). Briefly, 500 mL of boiling distilled water was added to 10 g of each tea sample, followed by soaking the tea for 10 min, straining the tea residue, cooling the tea infusion to room temperature and gradually adding an appropriate amount of tea infusion into the SAFE distillation equipment. In the collection system, 3 g of NaCl was added to facilitate the separation of the organic phase, followed by adding 20 mL of dichloromethane (chromatographically pure) to separate the organic phase from the aqueous phase by ultrasonic extraction for 20 min in an ice-water bath (KQ-300.100 frequency), with the extraction process repeated four times, and the separated organic phase was dried with Na_2SO_4. After standing for several hours to remove the water, the extract was filtered and stored in a sealed container at −20 °C. Finally, the resulting solution was distilled to 10 mL at 40 °C using a Vigreux column, then blown to 0.5 mL in a moderate nitrogen ice bath and used for subsequent sniffing experiments.

Gas chromatography - olfactometry (GC-O) analysis

The black tea concentrate was diluted with methylene chloride at volume ratios of 1:4, 1:16, 1:64, 1:256, 1:1024, and each dilution was stopped when the evaluator could not smell the odor at the end of the sniffing port, and the highest dilution of the substance that the evaluator could smell was defined as the flavor dilution (FD) factor. This evaluation was performed by a team of four members, and each member was required to record the perceived odor, describe the odor characteristics, and record the retention time.

Green tea samples were analyzed by GC–MS using the Thermo Scientific ISQ 7000 mass spectrometer system with a TG-WAXMS column (60 m × 0.25 mm × 0.25um) under following conditions: high purity helium gas; 1 mL/min carrier gas flow rate; an initial temperature of 40 °C and hold for 2 min, and 5 °C/min to 230 °C and hold for 15 min; 1 μL injection amount; splitless injection mode; EI ion source; 70 eV electron energy; 260 °C mass interface temperature; 280 °C ion source temperature; 45–400 m/z mass scanning range.

Results and discussion

Sensory evaluation

The nine different cultivars of Lichuan black tea varied significantly in appearance and quality (Table 1). All black teas had the appearance of wiry; however, there were differences in bloom and the amount of golden pekoe; the more bloom ones are Echa 1, Fuyun 6, Fudingdabai, Jinguanyin, Meizhan, and Lenghouhun, and the more golden pekoe are Echa 1, Echa 10, Fuyun 6, Fudingdabai, Lenghouhun, and Meizhan. The bloom and golden pekoe generally associated with cultivar traits and are regarded as indicators of high-quality black tea (H. Wang, et al., 2022). In infusion color, except for Jinxuan with an orange-red color, the other cultivars of black tea showed different degrees of red and bright color. The results of the sensory evaluation were supported by the color difference analysis of the tea infusion (Fig. 1 C), which showed that Baiyaqilan had the greatest L* and the brightest tea infusion, followed by Lenghouhun, Fudingdabai, Fuyun 6, etc. The lowest L* was present in Echa 1, and the tea's infusion was dull. Echa 1 had much higher a* and b* values than the other cultivars, and the infusion color was the strongest, followed by the higher a* and b* values of Jinguanyin and Echa 10; Lenghouhun had lower a* and b* values, and the infusion color was the lightest.

In terms of aroma (Fig. 1A), the nine black teas showed great difference in floral and fruity aromas as well as sweet aroma. The floral and fruit fragrances were stronger in Baiyaqilan, Meizhan, Jinxuan, Jinguanyin, and Lenghouhun, with reviewers describing Baiyaqilan as having an attractive orchid aroma. Except for Fuyun6, the sweet scent was stronger in all cultivars. Echa10 was also discovered to have a distinct scent quality with fruitiness in addition to a strong sweet odor. In terms of taste (Fig. 1B), the black tea cultivars differ substantially, primarily in sweetness, umami, and concentration. Apart from Fuyun 6 and Echa 1, all of the other cultivars showed good sweetness and umami, while Baiyaqilan lacked sufficient concentration. Overall, the taste quality score exceeded 90 points for Baiyaqilan (93.33), Fudingdabai (92.33), Lenghouhun (92), Meizhan (91.67) and Echa 10 (90.33). For the infused leaf, all the nine black teas showed red brightness, except for Baiyaqilan which was not red enough.

In this experiment, Fudingdabai (92.28), Meizhan (91.93), Echa 10 (91.75), Lenghouhun (91.23), Baiyaqilan (91.23), and Jinxuan (90.3) had a comprehensive quality score above 90, and except for Jinxuan with a significantly lower score, the other cultivars showed no significant difference from one another in total score.

Effect of different cultivars on taste components of black tea

The taste of black tea is related to its chemical compounds, with tea polyphenols, amino acids, soluble sugars and other substances as the basis of forming tea quality characteristics (Y. C. Li, et al., 2022). In order to reveal the influence of different cultivars on the formation of black tea taste components, this study determined the main biochemical components in nine cultivars of black tea and used UPLC-Q-TOF/MS non-targeted metabolomics techniques to detect their non-volatile components. Based on the standards and previous related studies (Dai, Xie, Lu, Li, Lv, Yang, et al., 2017), the ion fragmentation m/z values and retention times of the substance peaks were extracted and 78 substances were identified, including 15 amino acids, 25 flavonoids and flavonoid glycosides, 2 alkaloids, 10 catechins, 9 dimeric catechins, 7 organic acids, 7 aroma glycosides, and 3 others (Table S1). The unsupervised principal component analysis (PCA) of the tested taste components and the score plot showed substantial differences between the nine cultivars in non-volatile compounds (Fig. 2A).

Astringent components

Tea polyphenols, which include catechins, flavonols, flavones and their glycosides, are the key components of astringency and can alter the

Table 1
Sensory evaluation of the nine Lichuan black teas.

	Appearance		Infusion color		Aroma		Taste		Infused leaf		Total score
	Comment	Score	Comment	Score	Comment	Score	Comment	Score	Comment	Score	
Fudingdabai	Wiry, even; black bloom, fairly tippy	93.93 ± 0.12ab	Red and clear	91.67 ± 0.58a	Approaching high sweetness	91.67 ± 0.58d	Fresh and strong	92.33 ± 0.58b	Fine and tender, even ; red and fairly bright; fairly even color	90.1 ± 0.17a	92.28 ± 0.33a
Meizhan	Wiry; red-brown, bloom; fairly tippy	92.1 ± 0.17d	Red, nearly bright	88.83 ± 0.29 cd	Flowery and fruity with sweet, strong and lasting	95.07 ± 0.12a	Fairly fresh and strong	91.67 ± 0.29b	Fine and tender, even; fairly red and bright; even color	88.33 ± 0.58 cd	92.01 ± 0.09ab
Echa 10	Fairly wiry, even; approaching black bloom	93.33 ± 0.58bc	Red, fairly bright	90.17 ± 0.29b	Sweet and fruity aroma, strong and lasting	94.07 ± 0.12b	Mellow and thick, fairly fresh	90.33 ± 0.58c	Fine and tender, even; fairly red and bright; even color	88.67 ± 0.58bc	91.83 ± 0.16b
Baiyaqilan	Fairly wiry, heavy; approaching black bloom	90 ± 0.5f	Fairly red, bright	89.17 ± 0.29c	Elegant orchid, strong and lasting	94.07 ± 0.12b	Fresh and mellow	93.33 ± 0.58a	Fairly fine and tender, fairly even; slightly red, slightly green	83.33 ± 0.58e	91.27 ± 0.09c
Lenghouhun	Wiry, fairly even; approaching black bloom; slightly tippy	91 ± 0.3e	Fairly red, bright	88.67 ± 0.58 cd	Clean flowery, strong and lasting	93 ± 0.5c	Fresh and mellow	92 ± 0.5b	Fine and tender, even; fairly red and bright	87.67 ± 0.58d	91.23 ± 0.3c
Jinxuan	Wiry, fairly even; approaching black bloom	91.17 ± 0.29e	Orange red, fairly bright	85.33 ± 0.58f	Elegant flowery with higher sweetness	92.93 ± 0.12c	Fairly fresh and mellow	88.67 ± 0.29d	Fine and tender, even; fairly red and bright; even color	89.1 ± 0.36b	90.07 ± 0.17d
Jinguanyin	Wiry, even; approaching black bloom	92.17 ± 0.76d	Red, fairly bright	88.17 ± 0.29d	Higher flowery and fruity aroma	91.17 ± 0.29d	Fairly fresh and strong	87.07 ± 0.12e	Fine and tender, even; red and bright; even color	90.33 ± 0.29a	89.8 ± 0.18d
Echa 1	Wiry, even; red brown, bloom; fairly tippy	94.1 ± 0.36a	Red, fairly bright	86.67 ± 0.58e	Fairly strong	88.67 ± 0.58e	Fairly mellow and thick	83.33 ± 0.58f	Fine and tender, even; red and bright; even color	90.83 ± 0.29a	88.44 ± 0.05e
Fuyun 6	Wiry, even; black bloom, slightly tippy	93.17 ± 0.29c	Fairly red, bright	88.33 ± 0.58d	Fairly strong (slightly raw)	85.67 ± 0.58f	Slightly grassy	83.67 ± 0.58f	Fine and tender, even; red and bright	90.83 ± 0.29a	87.73 ± 0.08f

Note: Different small letters in the same column mean the samples are significantly different at $P < 0.05$.

concentration and bitterness of black tea taste (Liang, Lu, Zhang, Wu, & Wu, 2003). According to Table S3, there were significant differences in the amount of polyphenols retained across the various black tea cultivars, with Jinguanyin (12.9%) retaining the most and Fudingdabai (9.57%) the least. These differences are likely due to the catechin composition of the various cultivars' fresh leaves and how those catechins transform during fermentation (Dai, et al., 2017). Catechins are the main polyphenolic compounds in tea and their retention affects the taste quality of black tea. Specifically, ester-type catechins have a strong bitter and astringent taste and are the mainstay of the astringent taste of tea infusion, while non-ester-type catechins have a pleasant aftertaste. The total catechin content for the nine different black tea cultivars was in the following descending order (Fig. 2B): Meizhan (1.60 mg/g), Jinguanyin (1.47 mg/g), Jinxuan (1.15 mg/g), Lenghouhun (0.96 mg/g), Fuyun 6 (0.95 mg/g), Echa 10 (0.92 mg/g), Echa 1 (0.86 mg/g), Baiyaqilan (0.61 mg/g), Fudingdabai (0.53 mg/g). According to further analysis, the ratio of ester-type catechins to non-ester-type catechins was in the following descending order: Jinguanyin (5.21) > Meizhan (4.95) > Echa 1 (4.72) > Jinxuan (4.44) ≈ Echa 10 (4.34) ≈ Baiyaqilan (4.32) ≈ Fudingdabai (4.30) ≈ Fuyun 6 (4.12) > Lenghouhun (3.77). To some extent, the ratio of ester-type catechins to non-ester-type catechins can indicate the irritation of tea infusion (Narukawa, Kimata, Noga, & Watanabe, 2010). These findings confirmed the sensory evaluation, which found Jinguanyin to be thicker while Lenghouhun to be more mellow. Among them, the non-ester type catechin EC may enhance the effect of other astringent compounds (Ding, Kuhr, & Engelhardt, 1992), and the higher content of EC in Jinguanyin and Fuyun 6 may increase the astringency of their tea infusion. Moreover, polyester-type catechins such as Epigallocatechin 3-coumaroate and Epiafzelechin 3-gallate were also detected in this experiment. Theasinensin A was reported to have significant anti-SARS-CoV-2 viral activity, with a potential role in preventing human-to-human transmission of novel coronaviruses (Ohgitani, et al., 2021), and its levels were higher in Baiyaqilan and Meizhan (Table S1).

Theaflavins is the astringent component of the tea infusion and is essential for the quality formation of black tea. The total content of theaflavins in different cultivars varied greatly, with the higher contents of Jinxuan (1.49 mg/g), Baiyaqilan (1.46 mg/g), Meizhan (1.42 mg/g), Echa 10 (1.41 mg/g), and the lowest content of Jinguanyin (1.21 mg/g). In terms of theaflavin fractions, TF showed the least variation among the cultivars (Fig. 2C), suggesting a minimal impact of cultivar on TF. TF-3′-G showed the greatest difference between cultivars (Fig. 2C), with Echa 1 having the highest concentration (0.655 mg/g), followed by Echa 10, and Fuyun 6 having the lowest (0.358 mg/g). For TF-3-G, Echa 1 has much less than the other cultivars. Echa 1 demonstrated a 51.05% decrease in TF-3-G content when compared to Jinxuan. Theaflavins are a major component contributing to the strength and umami of tea infusion as well as its brightness and golden ring. There are four theaflavin fractions with different astringencies (Owuor & McDowell, 1994), and their content and proportion may be the main reason for the differences in the concentration and umami of different cultivars of black tea.

Flavonols and their glycosides have an impact on the taste of tea infusion because they are astringent components with a low threshold of taste presentation, which can increase the bitterness of tea infusion by increasing the bitterness of caffeine (Bai, Wang, Wang, Zheng, Wang, Wan, et al., 2017). Each cultivar's total flavonol and their glycoside

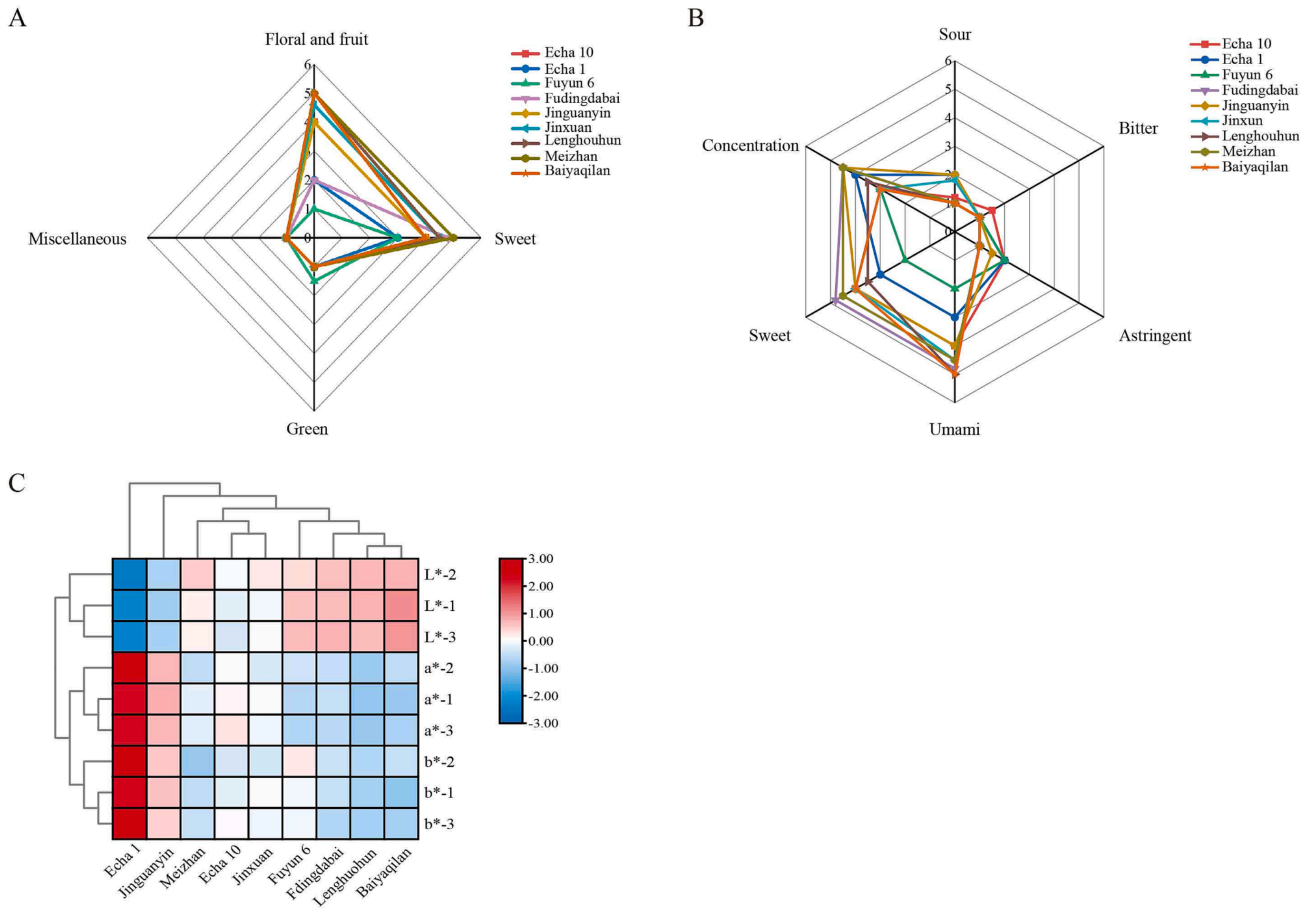

Fig. 1. QDA diagram of aroma (A) and taste (B), with different colored lines for different cultivars. (C) Heat map of the color difference of tea infusion in different cultivars of black tea, with darker red for a larger value and darker blue for a smaller value. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

content (Fig. 2F) fell in the following order: Echa 10 (1.87 mg/g), Baiyaqilan (1.71 mg/g), Fudingdabai (1.61 mg/g), Meizhan (1.34 mg/g), Echa 1 (1.14 mg/g), Fuyun 6 (1.06 mg/g), Jinxuan (1.01 mg/g), Jinguanyin (0.93 mg/g), Lenghouhun (0.59 mg/g). The level of flavonols and flavonol glycosides varies between cultivars, with both flavonols and flavonol glycosides being highest in Echa10, but the former is lowest in Jinxuan, while the latter is lowest in Lenghouhun. Some of these substances can be classified as astringent and bitter based on reports in the literature (Zhang, Cao, Granato, Xu, & Ho, 2020), Fudingdabai had the highest astringent content (60%), with the lowest astringent content (36.2%) in Lenghouhun, while Meizhan had the highest bitter content (12.3%), with the lowest bitter content (5.3%) in Lenghouhun (Fig. 2G). More interestingly, each cultivar contained different kinds of flavonols and flavonol glycosides (Table S1), with the most species (21 species) in Meizhan and Baiyaqilan, and Lenghouhun and Echa 1 had the least species (17 species). For flavonols, kaempferol was not detected in Jinxuan, while quercetin was detected only in Meizhan. For flavonoid glycosides, Quercitrin and Quercetin-7-O-β-D-glucopyranoside were not detected in Echa 1 and Lenghouhun, respectively; quercetin-3-O-rutinose was not detected in Fudingdabai, Fuyun 6, and Lenghouhun; kaempferitrin was only detected in Jinxuan and Baiyaqilan; quercetin-3-O-D-glucosyl-(1–2)-L-rhamnoside was only detected in Fudingdabai and Fuyun 6; glucosyl-vitexin was only detected in Meizhan. These results indicate that the nine different cultivars of black tea varied significantly in the types and contents of flavonols and their glycosides, which may have some influence on their infusion taste. Echa 10 is astringent, whereas Lenghouhun is more refreshing. However, due to the low solubility of flavonols and their glycosides in water, as well as the masking effect of theaflavins, soluble sugars, and sweet and umami amino acids, their effects on taste are not so obvious as those of green tea (Penghui, Hao, Xi, Ni, Yushun, & Hongfa, 2021). Flavonols and their glycosides in tea were shown to have strong antioxidant, anti-inflammatory and anti-cancer activities and also reduce the survival of colon adenoma cells and breast cancer cells (Rha, Jeong, Park, Lee, Sung, & Kim, 2019), thus the intake of such compounds is beneficial to human health and may prevent the occurrence of diseases caused by inflammation. In this trial, Echa 10 was found to have the highest content of flavonols and their glycosides, with the most species in Meizhan and Baiyaqilan, and whether they also have unique antioxidant, anti-inflammatory and anticancer activities need further investigation.

Sweet, umami-tasting substances

Amino acids are important substances for the umami and sweetness of tea infusion (Ho, Zheng, & Li, 2015). As shown in Table S3, the amino acid content of the nine cultivars of black tea ranged from 3.13% to 4.76%, with a relatively high level in Jinxuan and Baiyaqilan while a relatively low level in Echa 1. The 15 amino acids detected were classified by flavor into three types: umami, sweet and bitter. The bitter amino acids are L-valine, L-tyrosine, L-isoleucine, and L-phenylalanine, whose content is higher in Jinxuan and Meizhan while relatively lower in Baiyaqilan, Lenghouhun and Fuyun 6 (Table S1). However, in terms of the proportion (Fig. 2E), the bitter amino acids were shown to be more than 27% in Echa 1, Jinxuan and Meizhan, while$<$20% in Fuyun 6

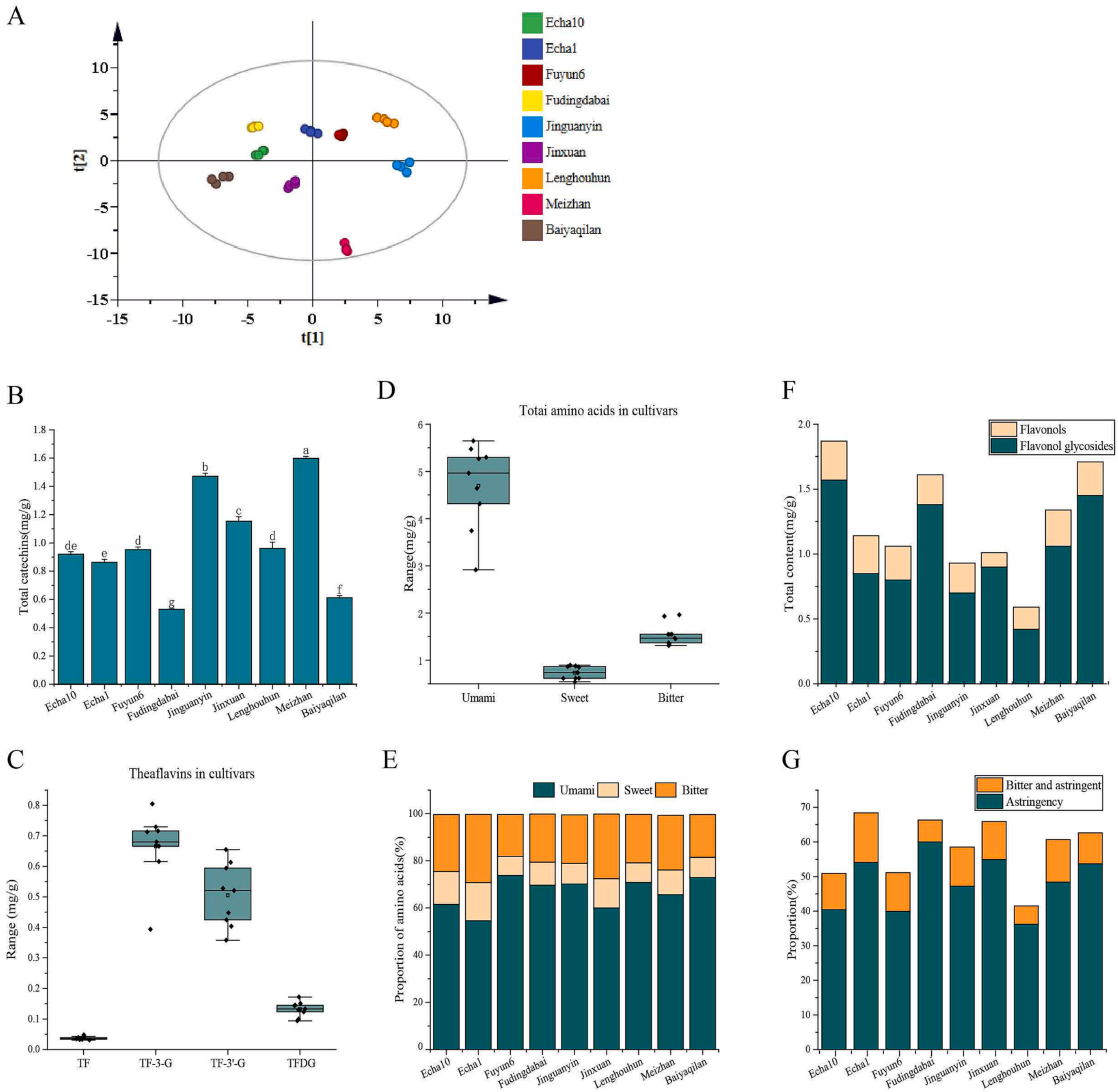

Fig. 2. (A) PCA of non-volatile compounds in the nine cultivars of black tea, with different colored triangles for different cultivars, X-axis for principal component 1 and Y-axis for principal component 2. (B) Total catechin content in black tea of nine cultivars, different small letters above the bar graph represent significant differences between cultivars at $P < 0.05$. (C) Boxplot of the content of theaflavin fractions in the nine cultivars. (D) Boxplot of the total umami, sweet and bitter amino acid content in the nine cultivars. (E) The proportion of total umami, sweet and bitter amino acids in the different cultivars of black tea, with different colors representing different flavor amino acids. (F) Stacked clumn of total flavonol and flavonol glycoside contents in the nine cultivars. (G) Stacked clumn of total flavonols content in nine cultivars with astringent and bitter flavors, respectively.

and Baiyaqilan. The umami amino acids are L-theanine, L-aspartic acid and L-glutamic acid (Yu, Huang, Zhao, Zhong, & Zheng), with the highest variance in total content between cultivars (Fig. 2D), with Fuyun 6, Meizhan, Fudingdabai, and Baiyaqilan having higher content but Echa 1 having lower (Table S1). In terms of proportion (Fig. 2E), umami amino acids were shown to account for more than 70% in Fuyun 6, Baiyaqilan, Jinguanyin, and Lenghouhun, while only 54.6% in Echa 1. L-theanine is the largest contributor to tea infusion umami among the umami amino acids (Zhang, Cao, Granato, Xu, & Ho, 2020), and its accumulation is greatly influenced by cultivars (Z. M. Yu & Yang, 2020). There were considerable variances between the nine cultivars, with Fuyun 6 having the greatest content and Echa 1 having the lowest (Table S1). The sweet amino acids are L-threonine, L-proline, L-tryptophan, L-methionine and L-serine (Yu, Huang, Zhao, Zhong, & Zheng), with the least variation in total content between cultivars (Fig. 2D), with content greater than 0.8 mg/g in Jinxuan, Meizhan, Echa 1 and Echa 10, and the lowest (0.541 mg/g) in Lenghouhun. In terms of percentage (Fig. 2E), it was observed that the sweet amino acids made up more than 10% in Echa 1, Echa 10, Jinxuan, and Meizhan, but only 8.0% in Fuyun 6. It's interesting that L-methionine and L-serine were only found in

Lenghouhun and Meizhan, respectively, and it has to be further investigated whether this affects flavor quality. It should be noted that the cultivars with a higher content and ratio of sweet and umami amino acids have better sweetness and freshness in the tea infusion, such as Jinxuan and Fudingdabai. Whereas Fuyun 6 and Echa 1 have lower sweetness and freshness in the tea infusion, but Fuyun 6 has a high content of umami amino acids and a low content of sweet amino acids, whereas Echa 1 has the opposite trend. The analysis above shows that the composition of amino acids with umami, sweet, and bitter flavors has altered in various cultivars of black tea, and that free amino acids are crucial for rendering sweet and umami flavors as well as for inhibiting the bitterness brought on by caffeine and catechins (Takeo, 1981). The taste of tea infusion can therefore alter due to changes in amino acid content (Zhang, Cao, Granato, Xu, & Ho, 2020), which may be the primary source of the variance in taste style among different cultivars.

Soluble sugars mainly contribute to the sweet and mellow taste of tea infusion, and their content in the nine black teas ranged from 2.31 to 2.84%, with the highest content in Echa10 (2.84%) and the lowest content in Meizhan (2.31%) (Table S3). Soluble sugars were reported to synergize with other substances such as amino acids to influence the taste quality of tea infusion (Z. Wang, Gan, Sun, & Chen, 2022).

Thearubigins are mostly responsible for the sweetness of the flavor and the redness of the infusion, whilst theabrownins are primarily responsible for the darkness of the infusion. Higher levels of thearubigins (Table S3) were found in Echa 10, Jinguanyin, and Fudingdabai, which may explain their sweet and mellow taste. Moreover, Lenghouhun and Fuyun 6, which have lower levels of thearubigins, also have lower sweetness. Consistent with the sensory evaluation, the cultivars with a high content of theabrownins (Table S3) had lower taste scores, such as Echa 1 and Fuyun 6, which had lower sweetness and umami in tea infusion. In previous studies, polysaccharides, proteins and lipids were shown to be involved in the synthesis of theabrownins (Peng, Liu, Liu, Zhou, & Gong, 2013), which may lead to a lighter taste in tea infusion.

Aromatic precursors

The majority of tea aroma compounds exist in fresh tea leaves as glycosides and are released during black tea fermentation through hydrolysis of endogenous enzymes (Supriyadi, Nareswari, Fitriani, & Gunadi, 2021). In the present study, a total of seven aroma glycosides were detected (Table S1), with the highest content of total aroma glycosides in Baiyaqilan, followed by Meizhan. These glycosides were discovered in the retained fractions of the respective finished tea samples, but the other fractions were primarily hydrolyzed by glycoside hydrolases during black tea rolling and fermentation to create the corresponding floral and fruit scent components. For example, benzyl β-primeveroside and Benzyl β-glucoside are hydrolyzed to form benzyl alcohol (Ho, Zheng, & Li, 2015), and linalool oxide primeveroside is hydrolyzed to form linalool oxide IV (Ho, Zheng, & Li, 2015). In this experiment, the content of benzyl β-primeveroside was highest in Jinguanyin, and the highest content of linalool oxide primeveroside was found in Baiyaqilan while the lowest content was found in Lenghouhun. The differences in aroma glycosides in each cultivar of black tea may originate from their different content in fresh leaves (Wu, Chen, Feng, Shen, Wei, Jia, et al., 2022) and transformation mechanisms during processing (Cui, Katsuno, Totsuka, Ohnishi, Takemoto, Mase, et al., 2016), leading to differences in their aroma quality.

GC–MS analysis of volatile components of different cultivars of black tea

In this study, a total of 92 volatile compounds (including 20 alcohols, 17 aldehydes, 14 alkenes,18 esters, 9 ketones, 8 hydrocarbons, 4 carboxylic acids and 2 others) were identified from the 9 cultivars of black tea using headspace solid-phase microextraction combined with GC–MS technique (Table S2), with their number in the order of Echa 10 (70), Jinxuan (61), Fudingdabai (60), Baiyaqilan (59), Meizhan (57), Echa 1 (56), Fuyun 6 (55), Jinguanyin (55), and Lenghouhun (54). Unsupervised principal component analysis (PCA) (Fig. 3A) showed good separation among the nine cultivars of black tea, indicating large differences in their aroma quality. Specifically, Baiyaqilan has a higher separation from the other cultivars, followed by Echa 10 and Meizhan, indicating that the aroma of black tea processed with them is more specific, completely agreeing with the sensory evaluation results, where the corresponding black tea samples were evaluated to possess a strong orchid, fruit sweet and fruit plum aromas, respectively.

Based on Table S2, we calculated the content of different categories of substances and the results are shown as Fig. 3B, with the total volatile compounds in the descending order of Baiyaqilan (1157.74 μg/g) > Meizhan (1039.26 μg/g) > Echa 10 (817.45 μg/g) > Echa 1 (658.44 μg/g) > Lenghouhun (646.63 μg /g) > Jinxuan (621.19 μg/g) > Fuyun 6 (561.34 μg/g) > Fudingdabai (488.75 μg/g) > Jinguanyin (446.63 μg/g). The aroma compounds were dominated by alcohols, accounting for more than 60% (Fig. 3C), followed by alkenes, covering 8.7%-12.9%, and hydrocarbons had a relatively low content, accounting for 0.6%-2.3%. The regularity of aroma categories varied in different cultivars, with alcohols accounting for more than 70% in Meizhan, Baiyaqilan, Lenghouhun and Jinguanyin, in contrast to a relatively low percentage in aldehydes and alkenes, which may be the main reason for the formation of their floral and fruity aromas, because terpene alcohols were reported as the main types of alcohols with good floral and fruity aromas (Supriyadi, Nareswari, Fitriani, & Gunadi, 2021). Studies have shown that geraniol had a rosy and sweet aroma (Q. Wang, et al., 2022). It is the most abundant of the alcohols among the cultivars (Fig. 3D) and is presumed to contribute more to the aroma of black tea. Echa 1, Fudingdabai and Fuyun 6 had a relatively low proportion of alcohols (<70%) and relatively high proportion of aldehydes and alkenes, which may be the main reason for the low floral and fruity aroma of their tea infusions.

In general, the contribution of volatile compounds to tea aroma depends on their concentration in the tea and their own odor threshold, also known as odor activity value (OAV) (Liu, Xu, Wu, Wen, Yu, An, et al., 2021). Only substances with OAV greater than 1 are considered as odor active compounds that contribute to the overall aroma characteristics of tea (Liu, et al., 2021). In order to explore the key odor active compounds in different cultivars of black tea, we measured the OAV values of some major compounds by aroma standards, and the results are shown in Table 2, where the number of aroma compounds with OAV greater than 1 was 18 in Fudingdabai, 17 in Echa 10 and Baiyaqilan, 16 in Echa 1, Jinxuan and Fuyun 6, 15 in Jinguanyin, and 14 in Meizhan and Lenghouhun. Meanwhile, the substances with relatively high OAV values in each cultivar are β-damascenone, phenylacetaldehyde, nonanal, geraniol, linalool, octanal, jasmone, dihydroactinidioli, *trans*-2-nonenal, etc., which can contribute more to the overall aroma of black tea.

The composition of compounds and their OAV values in different cultivars may influence the formation of different aroma types. Compared with other cultivars, Echa 10 was dominated by sweet and fruity aromas, with higher aroma contributions from β-damascenone, phenylethylaldehyde, nonanal, geraniol, linalool, jasmonone, and *trans*-2-nonanal (OAV greater than 100). As shown in Table 2, Echa 10 was higher than other cultivars in nonenal (OAV = 2228.9) and 2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene (OAV = 12.8). Characterized with a strong orchid fragrance, Baiyaqilan contained β-damascenone, geraniol, phenylethylaldehyde, linalool, jasmonone, dihydroactinidioli, and *trans*-2-nonenal with OAV greater than 100, as well as geraniol (3094.2), dihydroactinidioli (236), (E)-β-ocimene (47.6), β-ionone (19.2), and citral (25.4) with OAV values higher than those of other cultivars, which may contribute more to its orchid fragrance. Fudingdabai black tea was only dominated by sweet aroma, and octanal was its unique component with an OAV value of 298, which may contribute to its sweet aroma. The OAV values of *trans*-2-nonenal with a mushroom odor were higher in Fuyun 6 and Echa 1 than in the other cultivars, and

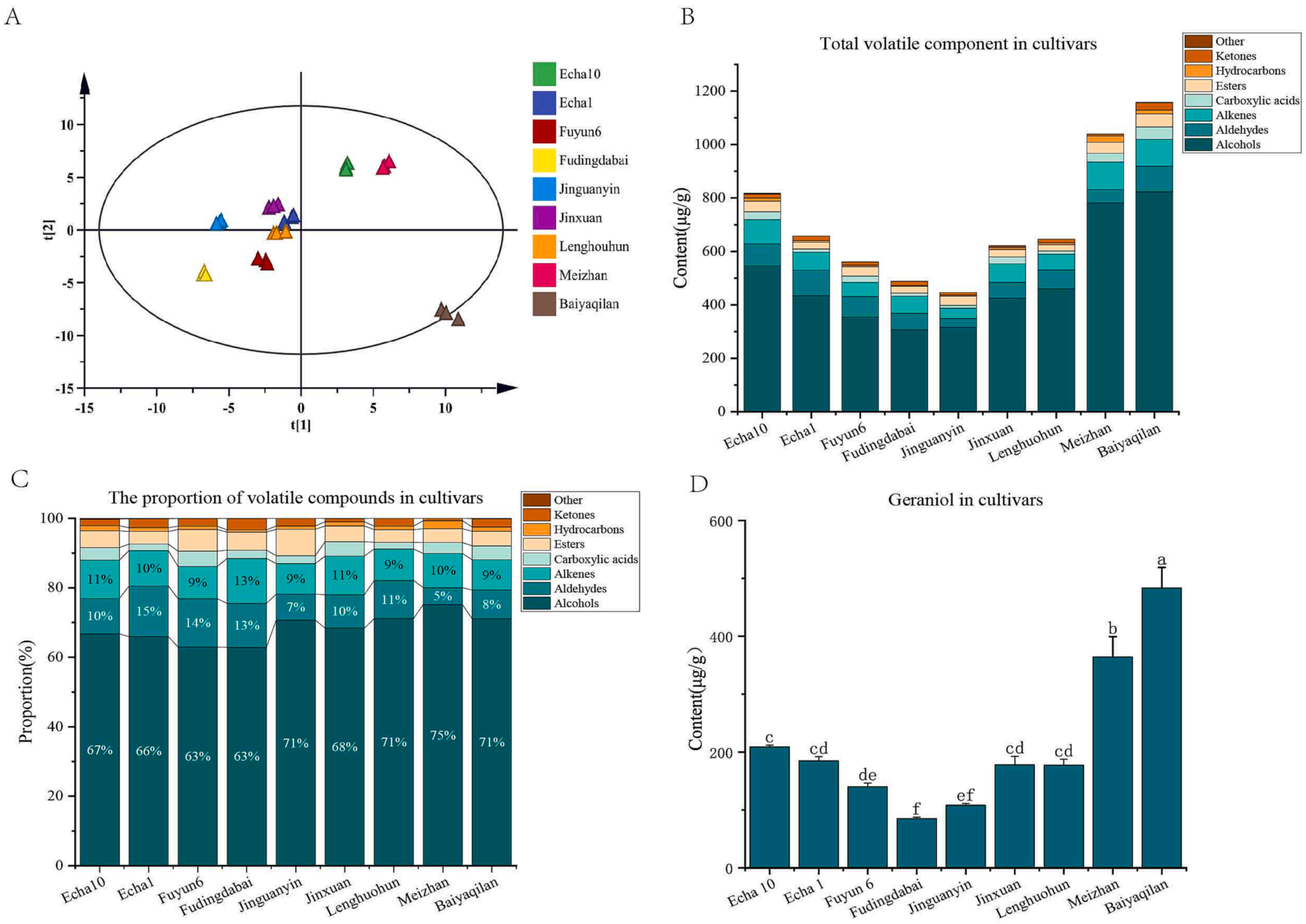

Fig. 3. (A) PCA of volatile compounds in the nine cultivars of black tea, with different colored triangles for different cultivars, X-axis for main component 1, and Y-axis for main component 2. (B) Stacked clumn of the total content of different types of volatile compounds in the nine cultivars of black tea. (C) Percentage graph of total content of different types of volatile compounds in the nine cultivars of black tea. (D) Geraniol content in the nine cultivars of black tea, and different small letters above the bar graph for significant differences between cultivars at $P < 0.05$.

this unpleasant aroma substance may be one of the factors for their lower sensory aroma scores (Gao, Andino, Rivera, & Marquez, 2009). Aside from the above higher OAV compounds with great differences among the nine cultivars, the remaining key aroma active compounds with small OAV values also play a modifying role in their characteristic aroma as important contributors to their aroma differences (Xiao, Cao, Zhu, Chen, & Niu, 2022). For example, β-cyclocitral was not present in Meizhan, but had the highest OAV (61.1) in Baiyaqilan. Additionally, (E)-β-ocimene, an important substance with a floral aroma, had higher OAV values in Baiyaqilan, Meizhan, Jinxuan, and Echa 10 than in the other cultivars, but was not detected in Fudingdabai.

GC-O-MS analysis of Echa 10 black tea aroma

Echa 10 is an asexual cultivar selected from the Enshi local Taizi tea group species, with a cultivation area of more than 20,000 ha in Enshi Prefecture, Hubei Province, thus the dominant cultivar for processing “lichuan black tea”. In the present study, sensory evaluation and PCA analysis of aroma compounds revealed that Echa 10 “lichuan black tea” has a unique sweet and fruity aroma, well differentiating it from other cultivars of black tea. Solvent-assisted flavor evaporation (SAFE) allows for more accurate extraction of trace compounds than HS-SPME. For this reason, we further investigated Echa10 black tea by GC-O-MS analysis with SAFE as a pretreatment coupled with aroma extract dilution analysis (AEDA) to better understand its aroma composition.

This experiment identified a total of 49 compounds that could be perceived by the smellers, including 12 alcohols, 7 aldehydes, 4 alkenes, 6 ketones, 5 esters, 7 hydrocarbons, 4 carboxylic acids, 1 pyridine, 2 pyrazines, and 1 pyrrole (Table 3), where the five compounds with the highest FD factor of 1024 were seen to be hexanal, β-myrcene, phenethyl alcohol, nonanoic acid, and jasmin lactone, indicating that they are the most important contributors to the aroma of Echa 10. Hexanal is the main substance for green and herbaceous aroma of green and white tea (Wu, et al., 2022), nonanoic acid was identified as the contributor to soybean-like aroma of Pu’er tea (Kun, Meng, Wei, Xie, Yan, Ho, et al., 2022), and the remaining substances were dominated by floral and fruity aromas. Especially, phenylethyl alcohol was found to be closely related to sweet aroma (Huang, et al., 2021), which was described as floral aroma by the smeller in the present study, consistent with the characteristic floral and fruity aroma of Echa 10. The FD was 256 for pyrazine, phenylene, benzaldehyde, methyl palmitate, and (E)-geranic acid, whose aroma is mostly sweet and roasted, thus the main contributor to the sweet aroma of Echa 10. According to the olfaction results, most of the substances were described as sweet, which contributed to the sweetness of Echa 10 to a varying degree. Examples include 3-methylidenepentan-2-one, acetophenon, coumarin with FD = 64, and octanoic acid with FD = 16. Note that 1-ethylpyrrole-2-carbaldehyde (tea pyrrole) was previously only identified in oolong tea (X. Y. Guo, Schwab, Ho, Song, & Wan, 2022), but detected in this experiment with FD = 16, which may contribute to the roasted aroma of Echa 10. OAV analysis

Table 2
OAV values of volatile compounds in different cultivars.

Compound	Relative correction factor^{A}	Threshold^{B}(μg/L)	OAV^{C}								
			Echa 10	Echa 1	Fuyun 6	Fudingdabai	Jinguanyin	Jinxuan	Lenghouhun	Meizhan	Baiyaqilan
β-Damascenone	0.035	0.002^{a}	5482.5	5001.2	1547.8	2571.0	1958.5	2843.6	716.1	5675.0	4987.0
Phenylacetaldehyde	4.382	4^{a}	3403.5	4222.1	1918.9	2275.0	1422.5	6250.9	1723.3	1537.6	1281.8
Nonanal	0.443	1.1^{a}	2228.9	2058.9	386.3	694.7	604.5	\	\	\	\
Geraniol	0.240	7.5^{a}	1337.6	1183.8	896.9	544.5	692.1	1141.7	1136.9	2331.4	3094.2
Linalool	0.358	6^{b}	750.0	548.8	495.8	521.7	543.5	590.7	849.7	1142.7	832.0
Octanal	0.217	0.7^{b}	\	\	\	298.0	\	\	\	\	\
Jasmone	0.735	7^{a}	159.5	190.8	175.8	211.6	176.6	60.4	270.4	60.7	258.4
Dihydroactinidioli	1.218	0.5^{c}	\	\	\	136.4	\	\	\	\	236.0
(E)-2-Nonenal	0.093	0.08^{c}	100.1	182.4	220.1	135.5	49.2	87.6	92.9	138.4	119.1
β-Cyclocitral	0.107	3^{a}	41.3	26.6	31.3	38.9	16.1	23.7	23.3	\	61.1
(E)-β-Ocimene	0.239	34^{e}	32.0	14.7	17.4	\	13.9	31.1	18.4	41.0	47.6
Methyl salicylate	0.251	40^{a}	29.0	23.1	33.2	20.6	24.6	19.6	24.4	35.5	22.9
β-Ionone	0.162	8.4^{a}	15.6	5.7	8.5	8.1	2.5	4.8	5.0	7.0	19.2
2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene	0.018	0.2^{d}	12.8	\	7.6	6.5	\	5.3	\	\	\
Citral	0.114	32^{f}	10.8	11.1	12.0	5.2	6.1	11.0	15.2	16.4	25.4
β-Myrcene	0.010	15^{a}	5.4	4.0	4.0	3.3	2.9	4.4	4.3	6.8	7.5
Nerol	0.071	49^{a}	4.4	2.9	2.8	1.9	1.3	3.8	3.9	9.8	10.8
Phenethyl alcohol	0.130	390^{a}	2.3	2.0	2.6	2.4	1.4	1.9	2.9	3.1	2.7
Benzaldehyde	0.890	350^{a}	1.2	1.6	< 1	1.1	< 1	1.1	< 1	1.0	1.5
2-Methyl-hept-2-ene-6-one	0.056	50^{c}	\	< 1	\	\	\	\	\	\	1.4
α-Terpineol	0.103	330^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	1.6	< 1
Jasmin lactone	2.146	300^{a}	< 1	< 1	\	\	\	\	\	\	< 1
Linalool oxide (pyranoid)	0.139	3000^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Neryl acetone	0.118	60^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
α-Ionone	0.099	76^{a}	< 1	< 1	< 1	< 1	\	< 1	\	\	< 1
Benzyl alcohol	0.276	20000^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
Nerolidol	0.017	250^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
(E)-Geranyl formate	0.017	200	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
(Z)-3-Hexen-1-yl caproate	0.016	781^{a}	< 1	\	< 1	< 1	< 1	< 1	< 1	< 1	< 1
(Z)-3-Hexenyl Butyrate	0.010	500^{a}	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1
(E,E)-2,4-Heptadienal	0.166	10000^{a}	\	\	\	< 1	\	\	\	\	\
Dodecane	0.019	10000^{a}	\	\	< 1	< 1	< 1	\	\	\	\
(E)-2-Decenal	0.160	50^{a}	\	\	< 1	< 1	\	\	\	\	\
(Z)-3-Hexenyl (Z)-3-Hexenoate	0.108	10,000	\	\	\	< 1	\	\	\	\	< 1
Hexyl hexanoate	0.131	6400^{a}	\	\	\	\	\	< 1	\	\	< 1
Tetradecane	0.009	10000^{a}	\	\	\		\	\	\	\	\
Phenethyl butyrate	0.230	340^{f}	\	\	< 1	\	\	\	< 1	< 1	< 1
(Z)-3-Hexenyl benzoate	0.386	500^{a}	\	\	\	\	\	\	\	\	< 1

Note: A. The results were calculated by purchasing aroma standards and quantifying them under the same GC–MS instrument conditions.
B. Obtained by reviewing the references: a from (X. Y. Guo, Schwab, Ho, Song, & Wan, 2022);b from (Yang, Zhu, Chen, Xie, Shen, Deng, et al., 2022);c from (Liu, et al., 2021);d from (H. Wang, Hua, Jiang, Yang, Wang, & Yuan, 2020);e from (X. Guo, Ho, Wan, Zhu, Liu, & Wen, 2021);f from (Mao, Lu, Li, Ye, Wei, & Tong, 2018).
C. The average value of the aroma compound content obtained based on the relative correction factor was divided by the aroma compound threshold.

(OAV > 1) and GC-O-MS results (FD > 1024) revealed the key aromatic substances of Echa 10 "lichuan hong" as β-damascenone, phenylethylaldehyde, nonenal, geraniol, linalool, jasmonone, *trans*-2-nonenal, β-Cyclocitral, (E)-β-Ocimene, methyl salicylate, β-Ionone, 2,6,10,10-Tetramethyl-1-oxaspiro[4.5]dec-6-ene, citral, β-Myrcene, Nerol, phenethyl alcohol, benzaldehyde, hexanal, nonanoic acid, and jasmin lactone.

Conclusions

Different cultivars of raw materials have a significant impact on the quality of black tea, particularly its aroma and taste quality. The variations of flavors in different cultivars of black tea are attributed to the differences in the composition and content of catechins and their oxides, amino acids, soluble sugars, flavonols and flavonoid glycosid, which mainly affect the sweetness, umami and concentration of tea infusion. The present results demonstrate that Baiyaqilan and Lenghouhun have special floral aroma as well as fresh and mellow taste, which are suitable for processing floral aroma Lichuan black tea; Meizhan, Jinguanyin and Echa 10 have floral and sweet aroma and taste, which are suitable for processing floral sweet aroma Lichuan black tea; Fudingdabai has sweet aroma and taste, which is suitable for processing sweet aroma Lichuan black tea. Based on their unique aroma characteristics, Echa 10, Baiyaqilan, Lenghouhun, Meizhan and Jinguanyin are recommended for processing Lichuan black tea. The special fruity sweet aroma of Echa10 black tea was presumably contributed by the key compounds such as β-damascenone, phenylethylaldehyde, nonenal, geraniol, linalool, jasmonone, (E)-2-nonenal, β-cyclocitral, (E)-β-ocimene, methyl salicylate, etc. This study facilitates understanding the aroma quality characteristics of different cultivars of Enshi black tea and the selection and breeding of high-quality tea cultivars.

CRediT authorship contribution statement

Xinxue Qin: Investigation, Methodology, Software, Writing – original draft. **Jingtao Zhou:** Software, Data curation. **Chang He:** Methodology, Software. **Li Qiu:** Resources. **De Zhang:** Visualization. **Zhi Yu:**

Table 3
GC-O-MS sniffing results of Echa 10 black tea.

RT	CAS	RI A	Compound	Odor description B	FD C
12.59	66-25-1	1083 ± 8	Hexanal	Green, grassy, metallic	1024
13.93	4359-77-7	1133 ± 5	3-Methylidenepentan-2-one	Floral, sweet	64
14.33	616-25-1	1159 ± 10	1-Penten-3-ol	Green fragrance	16
14.75	123-35-3	1161 ± 7	β-Myrcene	Lemon, woody, floral	1024
14.99	600-36-2	1172 ± 15	2,4-Dimethyl-3-pentanol	Potato and sweet aroma	16
15.24	110-86-1	1185 ± 10	Pyridine	Sweet, floral	16
15.67	123-51-3	1209 ± 9	3-Methyl-1-butanol	Grassy and coarse green air	4
15.78	5989-54-8	1199 ± 6	(S)-(-)-Limonene	Lemon	4
15.95	290-37-9	1212 ± 12	Pyrazine	Caramelised and toasted	256
16.37	505-57-7	1213 ± 7	2-Hexenal	grassy	64
16.69	3338-55-4	1235 ± 8	(Z)-β-Ocimene	Sweet	4
17.37	100-42-5	1261 ± 10	Phenylethylene	Woody	256
18.54	629-50-5	1300	Tridecane	Sweet	1
19.45	13925-00-3	1337 ± 12	2-Ethylpyrazine	Nutty	4
19.56	110-93-0	1338 ± 9	6-Methyl-5-hepten-2-one	Citrus, grassy	1
19.69	629-33-4	1352 ± 5	Formic acid, hexyl ester	Fruity, green leafy	16
20.27	123-42-2	1358 ± 14	4-Hydroxy-4-methyl-2-pentanone	Sweet, floral	4
21.23	629-59-4	1400	Tetradecane	Green and floral	4
21.42	142-83-6	1400 ± 8	(E,E)-2,4-Hexadienal	Grassy	4
22.38	5989-33-3	1444 ± 19	(Z)-Linalool oxide (furanoid)	Toasty and sweet aroma	1
22.79	98-01-1	1461 ± 11	Furfural	Bread, almond, sweet	4
23.13	34995-77-2	1452 ± 11	(E)-Linalool oxide (furanoid)	Toasty and sweet aroma	4
23.3	104-76-7	1491 ± 5	2-Ethylhexanol	Greenish air, leaves	16
24.55	100-52-7	1520 ± 14	Benzaldehyde	Almond and caramel	256
24.77	78-70-6	1547 ± 7	Linalool	Floral, lavender scent	16
26.3	544-76-3	1600	Hexadecane	Floral	64
26.66	2167-14-8	1610 ± 0	1-Ethylpyrrole-2-carbaldehyde	Toasty, nutty	16
27.42	122-78-1	1640 ± 13	Phenylacetaldehyde	Sweet and fragrant honey	1
27.65	98-86-2	1647 ± 13	Acetophenone	Sweet, floral	64
28.66	629-78-7	1700	Heptadecane	Sweet	4
29.43	5392-40-5	1718 ± 16	Citral	Intense lemon scent	4
29.88	39028-58-5	1739 ± 12	(E)-Linalool oxide (pyranoid)	Floral	1
30.55	119-36-8	1765 ± 21	Methyl salicylate	Mint, holly flavor	1
30.68	106-25-2	1797 ± 11	Nerol	Floral	1
30.94	593-45-3	1800	Octadecane	Sweet	64
31.69	106-24-1	1847 ± 10	Geraniol	Rosy, floral	64
32.35	100-51-6	1870 ± 14	Benzyl alcohol	Sweet, floral	1
33.15	60-12-8	1906 ± 15	Phenylethyl Alcohol	Floral	1024
34.16	13419-69-7	1967 ± 11	(E)-Hex-2-enoic acid	Special oil fragrance, sweet	1
34.54	1139-30-6	1989 ± 19	(-)-Caryophyllene oxide	Lilac	16
35.12	112-95-8	2000	Eicosane	Sweet	1
35.68	7212-44-4	2034 ± 14	Nerolidol	Fruity	4
35.98	124-07-2	2060 ± 15	Octanoic acid	Creamy, sweet	16
38.01	112-05-0	2171 ± 17	Nonanoic acid	Fatty	1024
39.24	112-39-0	2208 ± 10	Methyl palmitate	Sweet and toasty	256
40.26	25524-95-2	2257 ± 16	Jasmin lactone	Jasmine, fruity	1024
41.16	4613-38-1	2328 ± 12	(E)-Geranic acid	Floral, fruity and sweet	256
44.24	91-64-5	2454 ± 6	Coumarin	Sweet	64
50.79	84-74-2	2680 ± 13	Dibutyl phthalate	Sweet	64

Note: A. Reference to NIST 2014 database;
B. The aroma was smelled by the smeller and the rest was found through the website https://www.thegoodscentscompany.com/;
C. Under dilution settings, the maximum dilution gradient at which an aroma component may be smelled by the smeller.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2023.100809.

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