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Exploring the effect of greenhouse covering cultivation on the changes of sensory quality and flavor substances of green tea

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ABSTRACT

To protect tea plant (*Camellia sinensis* (L.) O. Kuntze) from freezing injury, plastic greenhouse covering is widely used in northern tea areas of China. Currently, there was few researches about the effect of greenhouse covering on tea quality. Our results showed greenhouse covering increased tea yield, changed leaf phenotype and decreased green tea quality. Further analysis revealed greenhouse increased the content of soluble sugars and decreased the content of EGCG and 14 amino acids. Besides, there were 223 differential volatile components were identified in green tea produced by fresh leaves with plastic greenhouse covering (GT) and green tea produced by fresh leaves without plastic greenhouse covering (TT). 81 key aroma components were contributors to the bean-like aroma of TT. 98 key aroma components contributed to the clean aroma of GT. Based on these results, the flavor wheels were constructed, providing a visual presentation of flavor between TT and GT.

1. Introduction

Green tea has attracted an increasing number of consumers worldwide due to its multiple health benefits. China has the largest green tea consumption, green tea output as well as tea plantation area in the world (Hao et al., 2018). In China, due to the freezing and windy winter in tea region of north Yangtze River, tea plants are vulnerable to suffer from freezing and drought damage, leading to a sharp decrease in yield (Wang et al., 2021). For this reason, plastic greenhouse covering is usually used as one of the key techniques to protect tea plants from the adverse effects of climate in north China (Yin et al., 2012). Studies showed that plastic greenhouse covering can improve the growth environment for tea plants by increasing ambient temperature and relative humidity, as a result, raise tea production and bring economic benefits (Kong et al., 2023). Nevertheless, with the increasing of domestication time, some tea plants can survive in winter of north China without any covering. So far, two types of green teas, namely 'greenhouse tea' and 'field tea', have been in an intense competition in the northern market. Shown by a large number of studies, moderate abiotic stress, such as cold stress and drought stress, plays an important role in improvement of tea aroma and taste (Chen, Wu, et al., 2021; Li, 2023; Kong et al., 2023). For example, moderate low temperature could enhance the expression of aroma synthetic genes, resulting in the abundant accumulation of characteristic aroma compounds in tea leaves (Zeng et al., 2018). A certain degree of drought stress produced significant effect on the contents of sugar, sugar alcohols and amino acids (Shen et al., 2022). It is widely known that the temperature and humidity for tea plant growth can be improved by plastic greenhouse covering. But what exactly the impact of greenhouse covering on green tea quality is still unknow.

The worldwide popularity of green tea is mostly due to its unique color, aroma and taste (Wang et al., 2022; Xue et al., 2023). According to the methodology for sensory evaluation of tea (national standard of China, GB/T 23776–2018), taste and aroma accounts for 25 % and 30 % of total quality respectively. Thus, good taste and pleasant aroma is considered as an important indicator of high-quality green tea (Yu & Zhou, 2014; Ho et al., 2015). Until now, few studies focus on the impact of plastic greenhouse covering on tea quality. In order to clarify the quality difference between the green tea produced by fresh leaves with plastic greenhouse covering (GT) and the green tea produced by fresh leaves without plastic greenhouse covering (TT), the quality-related

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Abbreviation: GFL, fresh tea leaves with plastic greenhouse covering; TFL, fresh tea leaves without plastic greenhouse covering; GT, green tea produced by fresh leaves with plastic greenhouse covering; TT, green tea produced by fresh leaves without plastic greenhouse covering..

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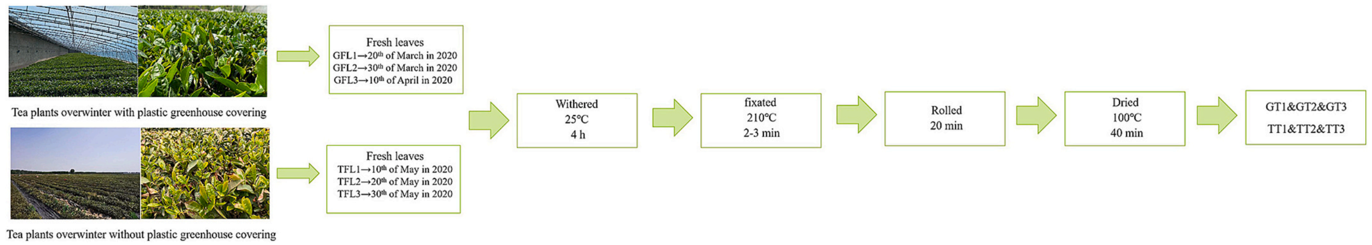

Fig. 1. Tea processing model diagram.
Note: GFL: fresh tea leaves with plastic greenhouse covering; TFL: fresh tea leaves without plastic greenhouse covering; GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

constituents were analyzed by gas chromatography–mass spectrometry (GC–MS) and ultra-performance liquid chromatography-mass spectrometry (UPLC-MS). Ultimately, a flavor wheel was constructed to exhibit the flavor characteristics of GT and TT, which was of great significance for guiding production of high-quality green tea.

2. Materials and methods

2.1. Greenhouse covering

Each mu of tea garden was builded with 3 greenhouses. Each greenhouse had the span of 5 m, the length of 35 m, and the height of 2.5 m. They are covered with Polyvinyl chloride film for winter protection. The coverage period lasted from November to April of the following year.

2.2. Sample collection and preparation

Fresh one bud and two leaves were picked from *Camellia sinensis sv.* 'Huangshanzhong' of 6-year-old in the same tea garden of Qingdao, Shandong, China (N36°16′11.95″ E120°37′58.57). When the germination rate of tea plants exceeded 10 % in spring, the picking was started. And three batches of samples were collected in total. The fresh leaves of GT (GFL) was picked at 20th, 30th of March and 10th of April in 2020. The fresh leaves of TT (TFL) was picked at 10th, 20th, 30th of May in 2020. Accordingly, the samples were divided into two portions. One portion was immediately frozen in liquid nitrogen and stored at −80 °C, the other was processed to green tea as follows: fresh leaves were firstly withered at 25 °C for 4 h, and then fixated at 210 °C for 2–3 min. The enzyme-denatured leaves were rolled for 20 min, and finally dried at 100 °C for 40 min until the moisture of tea was within 6 %. Dried teas were grounded to powders and stored at −20 °C for further analysis. The collection and preparation of tea samples was shown in Fig. 1.

2.3. Quadrat survey of tea yield and leaf phenotype

Sampling method was based on previous study with little modification (Sano et al., 2018). Totally, 20 branches were collected from each quadrat. And each experimental field was divided into three quadrats. One bud and two leaves were separated for determination of 100-bud weight. The length of internode I between bud and 1st leave and internode II between 1st leave and 2nd leave as well as the stem thickness was measured by using vernier caliper. The areas of 1st leave and 2nd leave were measured by using leaf area meter (Yaxin-1241, Yaxin, Beijing, China). Leaf color was analyzed by the contents of chlorophyll and carotenoid. The determination of chlorophyll and carotenoid was based on previous studies (Sano et al., 2018; Chen, Xie, et al., 2021). The fresh leaves which were ground into powders in liquid nitrogen and extracted with acetone, ethanol, water (4.5:4.5:1). Chlorophyll contents were calculated from the readings of absorption at 663 nm and 645 nm using microplate reader with multi-wavelength measurement system (HBS-1101, Droide, Shanghai, China). The calculation formula was as follows:

Content of chlorophyll a (C_a, mgL^{-1}) = $12.72A_{663}–2.95A_{645}$,
Content of chlorophyll b (C_b, mgL^{-1}) = $22.88A_{645}–4.67A_{663}$,
Content of total chlorophyll (C_T, mgL^{-1}) = $C_a + C_b$.

where A_{663} and A_{645} were the absorbance at 663 nm and 645 nm respectively.

Finally, the total chlorophyll content was calculated as mg/g dried sample.

2.4. Sensory evaluation

The sensory quality of green tea was blindly evaluated by five professional tea assessors (three males and two females). The method of sensory evaluation was based on previous study (Yang et al., 2020). The sensory evaluation was performed in compliance with relevant laws and institutional guidelines and conducted with ethical approval of Institute of Qingdao Agricultural University. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this sensory evaluation" where an affirmative reply was required to enter the sensory evaluation. Meanwhile, participants could withdraw from the sensory evaluation at any time without giving a reason. Before brewed, 200 g dried tea was placed in a white plate for appearance evaluation. 3.0 g dried tea was brewed with 150 mL boiled water for 4 min in a white porcelain cup. Then the infusion was filtrated into a white porcelain bowl. The professional assessors were instructed to evaluate liquor, aroma, taste and infused leaves in turn. The total sensory quality was scored by using a 100-point scale, and the weight coefficients of appearance, liquor color, aroma, taste and infused leaves were 25 %, 10 %, 25 %, 30 % and 10 % respectively. All samples were evaluated three times.

2.5. Electronic nose analysis

The electronic nose analysis of tea aroma was based on previous study (Wang et al., 2022). 3.0 g green tea was brewed with 150 mL boiled water for 4 min. After filtration, tea infused leaves were immediately detected by using an electronic nose (Pen3, Air-sense, Germen). The parameters of electronic nose were set as follows: 120 s of sensor automatic cleaning, 10 s of re-set, 5 s of sample preparation, 120 s of sampling and analyzing, 1 s of interval between sampling, 400 mL/min of flow rate. Ten odor sensors were installed in electronic nose for ordor recognition (Table S1). All samples were detected three times.

2.6. Non-volatile components analysis

2.6.1. Water extracts, tea polyphenols and water soluble carbohydrate

The content of water extracts was determined by GB/T8305–2013. Tea polyphenol content was determined by Folin-Ciocalteu method (Chen et al., 2023). Water soluble carbohydrate content was determined by anthrone-sulfuric acid colorimetric method (Qu et al., 2020).

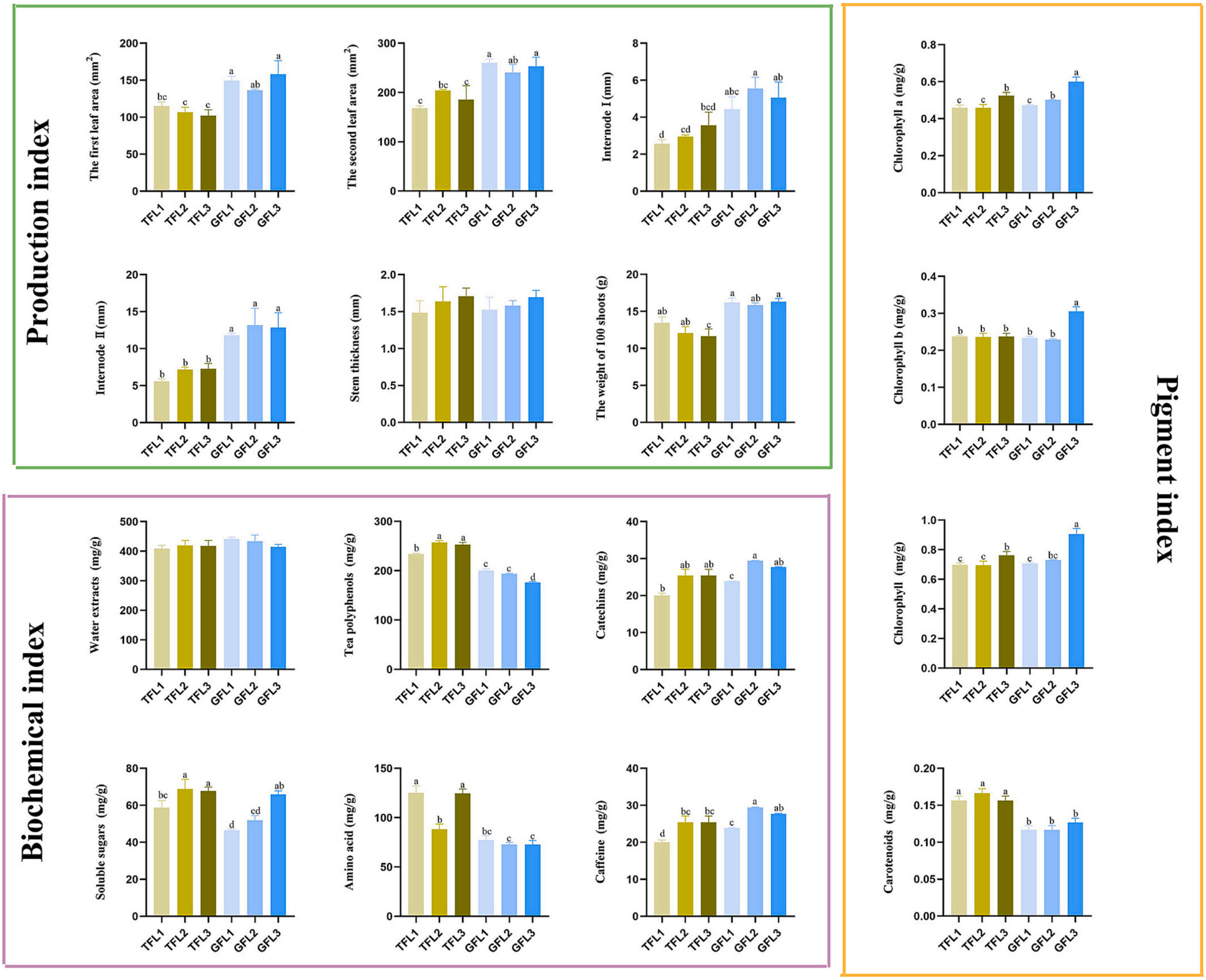

Fig. 2. The physiological and biochemical traits of the fresh tea leaves.
Note: Values with different letters are significantly different at $p < 0.05$; GFL: fresh tea leaves with plastic greenhouse covering; TFL: fresh tea leaves without plastic greenhouse covering.

2.6.2. Catechin mononers and caffeine

The content of catechin monomers and caffeine was determined by ultra-high performance liquid chromatography (UPLC) (ACQUITY UPLC H-Class, Waters, Denmark). Samples were extracted with 70 % methanol according to GB/T 8313–2018. UPLC condition was as follows: AC QUITY UPLC HSS T3 chromatographic column (2.1 mm × 100 mm); mobile phase A (pure water with 0.4 % acetic acid), mobile phase B (acetonitrile); elution program 0–12 min, 5–30 % B, 12–13 min, 30–50 % B, 13–14 min, 50–5 % B, 14–16 min, 5–5 % B; flow rate 0.2 mL/min; injection volume 5 μL; column temperature was 35 °C. Catechins and caffeine were identified by using the PDA detector at a continuous wavelength of 280–330 nm (Liu, Wang, et al., 2023). The contents of catechin monomers and caffeine were quantified by standard curve.

2.6.3. Amino acid compositions

The content of amino acid compositions was determined by UPLC-MS (Ma et al., 2022). 50 mg of samples was extracted with 500 μL −20 °C pre-cooled 70 % methanol for 3 min. After centrifugation at 12000 r/min for 10 min, the supernatant was collected for analysis. The data acquisition instrument system includes Ultra Performance Liquid Chromatography (ExionLC™ AD,Waters, Denmark) and Tandem Mass Spectrometry (QTRAP^{R} 6500+, Waters, Denmark).

UPLC condition: ACQUITY BEH Amide chromatographic column (1.7 μm, 100 mm × 100 mm); flow rate 0.4 mL/min; column temperature 40 °C; injection volume 2 μL; mobile phase A (ultrapure water with 2 mM ammonium acetate and 0.04 % formic acid), mobile phase B (acetonitrile with 2 mM ammonium acetate and 0.04 % formic acid); elution program 0–1.2 min 10 % A, 9 min 40 % A, 10–11 min 60 % A, 11.01–15 min 10 % A.

MS condition: electrospray ionization temperature 550 °C; mass spectral voltage 5500 V in positive ion mode; mass spectral voltage −4500 V in negative ion mode; curtain gas 35 psi. Each ion pair was scanned for detection based on optimized declustering potential and collision energy. The contents of amino acid were quantified by standard curve (Table S2).

2.7. Volatile components analysis

2.7.1. HS-SPME/GC–MS analysis

Volatile components were extracted by solid phase micro-extraction (SPME) method. 1.0 g of samples was added into a 20 mL headspace vial, followed by adding 2 mL NaCl and 10 μL (50 μg/mL) internal standard

Table 1
The sensory quality of GT and TT.

	Appearance(25 %)		Liquor color(10 %)		Aroma(25 %)		Taste(30 %)		Infused leaf (10 %)		Total score
	Remarks	Score	Remarks	Score	Remarks	Score	Remarks	Score	Remarks	Score	
TT1	Tightly with pekoe, neatly,yellow and bright	95.93 ± 0.52^{ab}	Yellow and bright	91.27 ± 0.86^{c}	Bean-like aroma	95.63 ± 0.68^{a}	Fresh and mellow	95.73 ± 0.42^{a}	Yellowish green, bright, fairly tender, fairly uniform	94.8 ± 0.54^{a}	95.22 ± 0.50^{a}
TT2	Tightly,neatly, yellowish green	93.1 ± 0.28^{bc}	Yellow and bright	92.27 ± 0.87^{bc}	Bean-like aroma	95.4 ± 0.95^{ab}	Mellow and thick	92.77 ± 0.39^{b}	Yellowish green, bright, fairly uniform	92.63 ± 0.48^{b}	93.45 ± 0.30^{b}
TT3	Tightly,neatly, yellowish green	92.97 ± 0.63^{bc}	Yellow and bright	92.47 ± 0.62^{bc}	Bean-like aroma	94.77 ± 0.62^{ab}	Mellow and heavy	90.97 ± 0.62^{bc}	Yellowish green, bright, fairly uniform	91.63 ± 0.4^{bc}	92.63 ± 0.48^{b}
GT1	Tightly with pekoe, fairly netly, yellowish green with grey	94.67 ± 0.45^{ab}	Light yellow and bright	95.3 ± 0.71^{a}	Clean aroma with little chestnut aroma	92.93 ± 0.85^{bc}	Fresh and fairly brisk	89.8 ± 0.41^{c}	Yellowish green, fairly bright, fairly uniform	89.67 ± 0.45^{cd}	92.34 ± 0.23^{b}
GT2	Tightly,fairly neatly, yellowish green	92.23 ± 1.05^{c}	Light yellow and bright	93.73 ± 0.33^{ab}	Clean aroma with little chestnut aroma	90.77 ± 0.62^{cd}	Fairly fresh and brisk	87.8 ± 0.71^{d}	Yellowish green, fairly bright, passable uniform	88.87 ± 0.69^{de}	90.35 ± 0.71^{c}
GT3	Tightly,fairly neatly, yellowish green	89.77 ± 0.39^{d}	Light yellow and bright	94.07 ± 0.21^{ab}	Clean aroma with little chestnut aroma	89.77 ± 0.62^{d}	Fairly fresh and brisk	86.9 ± 0.7^{d}	Yellowish green, fairly bright, passable uniform	86.93 ± 0.4^{e}	89.05 ± 0.57^{c}

Note: Values with different letters are significantly different at $p < 0.05$; GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering.

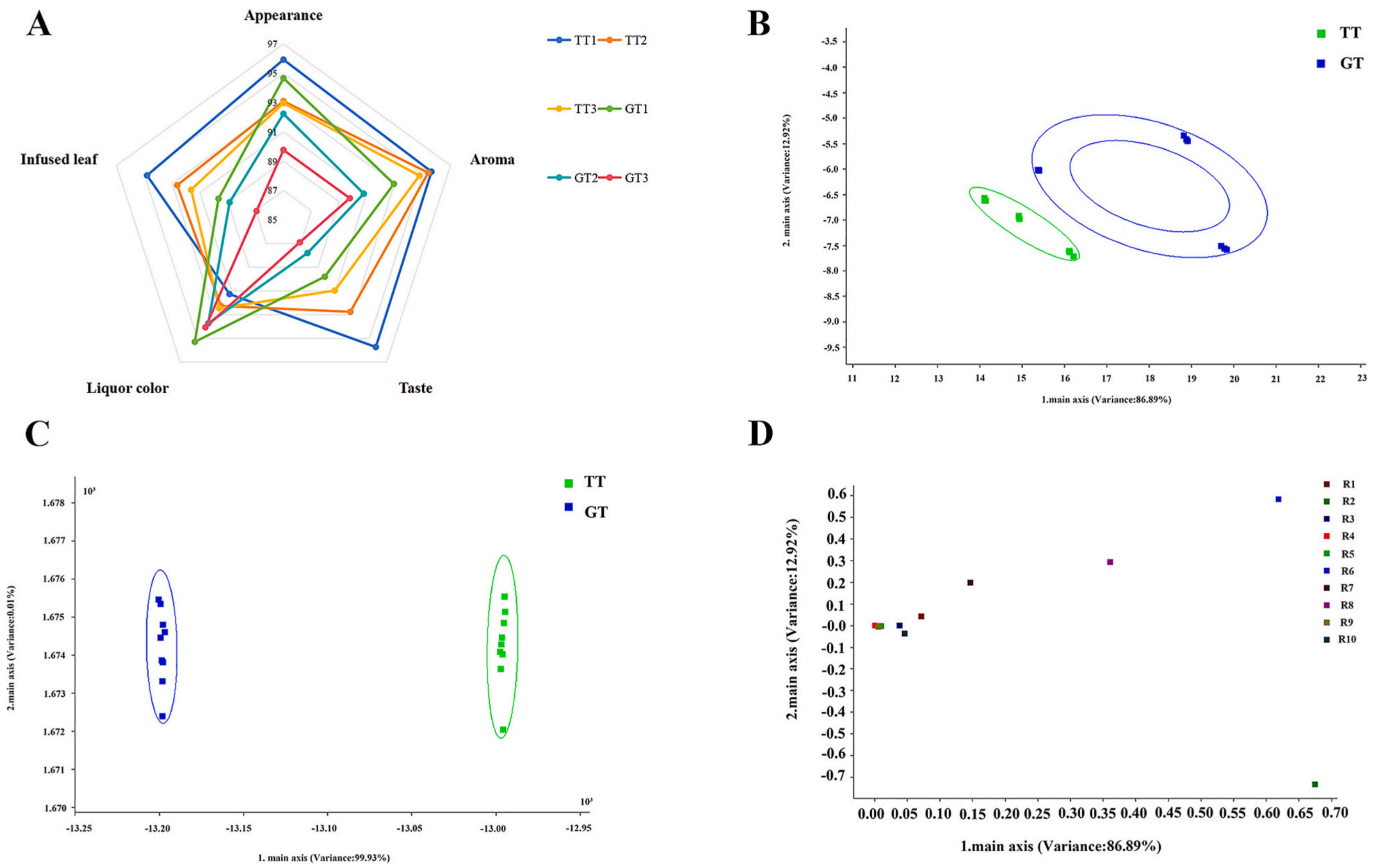

Fig. 3. A)The sensory quality analysis of GT and TT B)The PCA analysis of electronic nose of GT and TT C)The LDA analysis of electronic nose of GT and TT D)The Loadings analysis of electronic nose of GT and TT.
Note: GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

solution 3-Hexanone-2,2,4,4-d4. The headspace vial was immediately heated in water bath at 60 °C for 10 min. Then the DVB/CAR/PDMS microextraction fiber (65 μm) was inserted into the vial for 20 min.

Volatile components were determined with GC–MS (7890B—7000D, Agilent). GC–MS condition: 30 m*0.25 mm*1.0 μm DB-5MS (5 % phenyl-polymethylsiloxane) capillary column; injector temperature 250 °C, 5 min in splitless mode; column temperature program: 40 °C hold for 3.5 min, increasing to 100 °C at 10 °C/min, increasing to 280 °C

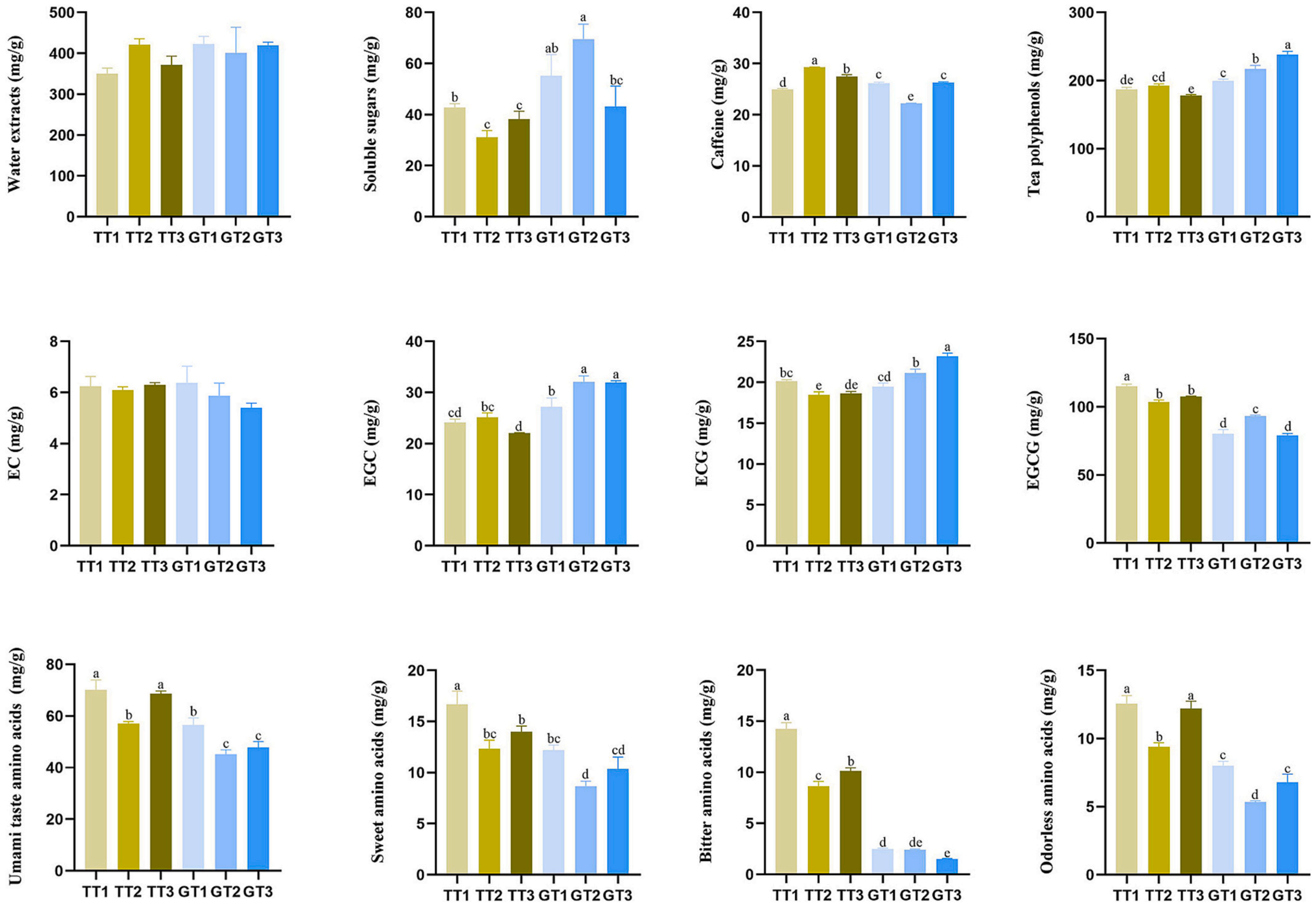

Fig. 4. The taste components of GT and TT.

Note: Values with different letters are significantly different at $p < 0.05$; GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

at 25 °C/min and hold for 5 min; helium as carrier gas with a flow rate of 1.0 mL/min. Mass spectra was recorded in electron impact (EI) ionization mode at 70 eV, and the temperature of the quadrupole mass detector, ion source and transfer line was 230 °C, 150 °C, 280 °C respectively. Mass spectra scanned at 1 s interval in the range of m/z 30–50 amu. The method of SPME and GC–MS was based on the previous study with a little modification (Wang et al., 2022).

2.7.2. Characterization and quantification

Volatile compounds were characterized by National Institute of Standards and Technology (NIST) mass spectrometry database and retention indices (RI, determined by n-alkanes C_7-C_{40}).

The RI was calculated as follows:

$$RI = 100Z + 100\frac{TR(X) - TR(Z)}{TR(Z+1) - TR(Z)}$$

where TR(x), TR(z), and TR(z + 1) represent the retention temperature of the constituent and the carbon number Z, Z + 1, respectively, and TR(Z) < TR(X) < TR(Z + 1).

The relative content was calculated as follows:

$$C_i = \frac{(C_{is} \times A_i)}{A_{is}}$$

where C_i was the concentration of volatile component, C_{is} was the concentration of internal standard, A_i was the peak area of volatile component, A_{is} was the peak area of internal standard.

2.7.3. Odor activity value analysis

Odor activity value (OAV) can be used to analyze the contribution of volatile component. The calculation formula was as follows:

$$OAV = \frac{c}{OT}$$

where c was the concentration of volatile component, OT was the threshold of volatile component in water.

2.8. Statistical analysis

All the data was expressed by mean ± standard deviation. Statistical analysis was performed by SPSS (Chicago, IL, USA) and $p < 0.05$ was considered to be significantly different. Principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) were conducted by SIMCA-P software (Version 14.1, Umetrics, Umea, Sweden). All experiments were repeated thrice.

3. Results

3.1. Tea yield and leaf phenotype

The plastic greenhouse covering had a significant effect on tea yield and shoot phenotype (Fig. 2). The area of 1st leave and 2nd leave, the

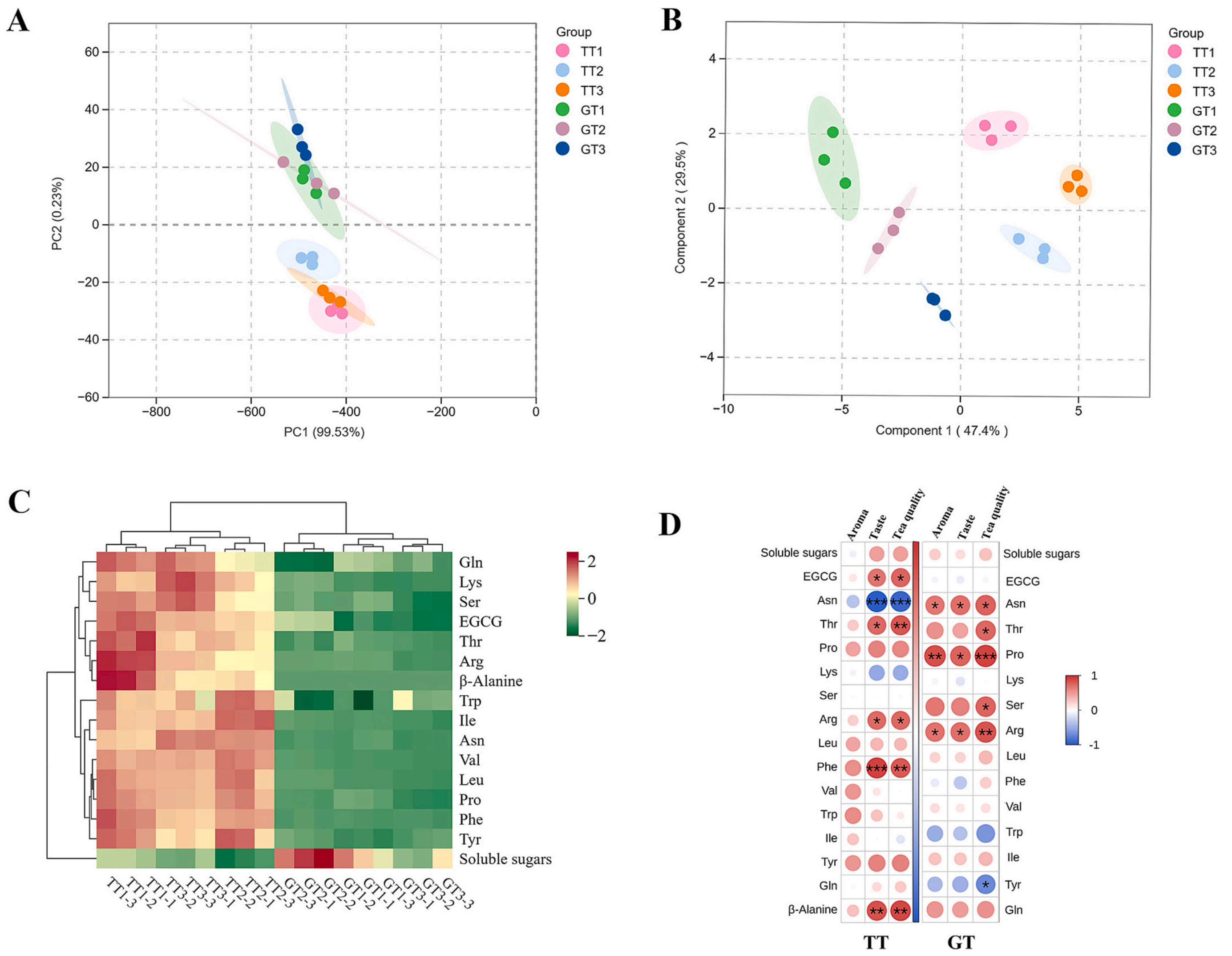

Fig. 5. (A)The PCA analysis of differential taste components of GT and TT (B)The OPLS-DA analysis of differential taste components of GT and TT (C)The heat map of differential taste components of GT and TT (D)The correlation diagram of differential taste components of GT and TT.
Note: * is significantly different at $0.01 < p < 0.05$; ** is significantly different at $0.005 < p < 0.01$; *** is significantly different at $p < 0.005$; GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

length of internode I and internode II of fresh tea leaves with plastic greenhouse covering (GFL) was significantly larger than that of fresh tea leaves without plastic greenhouse covering (TFL). Also, the fresh weight of 100 shoots of tea plants was significantly increased after covering, indicating that plastic greenhouse covering cultivation could effectively improve tea yield. However, no remarkable change was obversed in sterm thickness between TFL and GFL. Moreover, the pigments of tea leaves were also determined in this study. Fig. 2 showed the contents of chlorophyll a, chlorophyll b and total chlorophyll of GFL were significantly higher than those of TFL, while the contents of carotenoid of GFL were significantly lower than those of TFL. Detailed data is shown in Table S3.

3.2. Sensory quality

To figure out the quality difference between GT and TT, the sensory quality of GT and TT was evaluated as shown in Table 1. Results showed the sensory quality of GT and TT was significantly different. The color of appearance and infused leaves of GT was greener than that of TT. And the liquor color of TT was darker yellow than that of GT. Furthermore, the major difference between GT and TT was the type of aroma and taste. The scores of aroma and taste of TT were significantly higher than those of GT. The aroma type of GT was clean with little chestnut-like, while the aroma type of TT was bean-like. TT tasted mellower and thicker, while GT tasted fresher. Overall, the quality of TT was superior to GT.

For further analysis of tea sensory quality, electronic nose was conducted in this study. Electronic nose analysis (Fig. 3) showed the aroma of GT and TT was completely separated by PCA nd LDA analysis, indicating the aroma type between GT and TT was remarkably different. In the loadings analysis, the contribution rate of the first principal component (PC1) and the second principal component (PC2) was 86.89 % and 12.92 % respectively. Sensor R5 (W5C) contributed most to PC1, sensor R6 (W1S) contributes most to PC2, which meant the major differential compounds were alkanes, aromatic compounds.

3.3. Non-volatile components

3.3.1. Comparison of non-volatile components between GFL and TFL

Fig. 2 showed that greenhouse covering had a significant effect on non-volatile components of tea leaves. The contents of tea polyphenols, soluble sugars and amino acids of GFL were significantly higher than

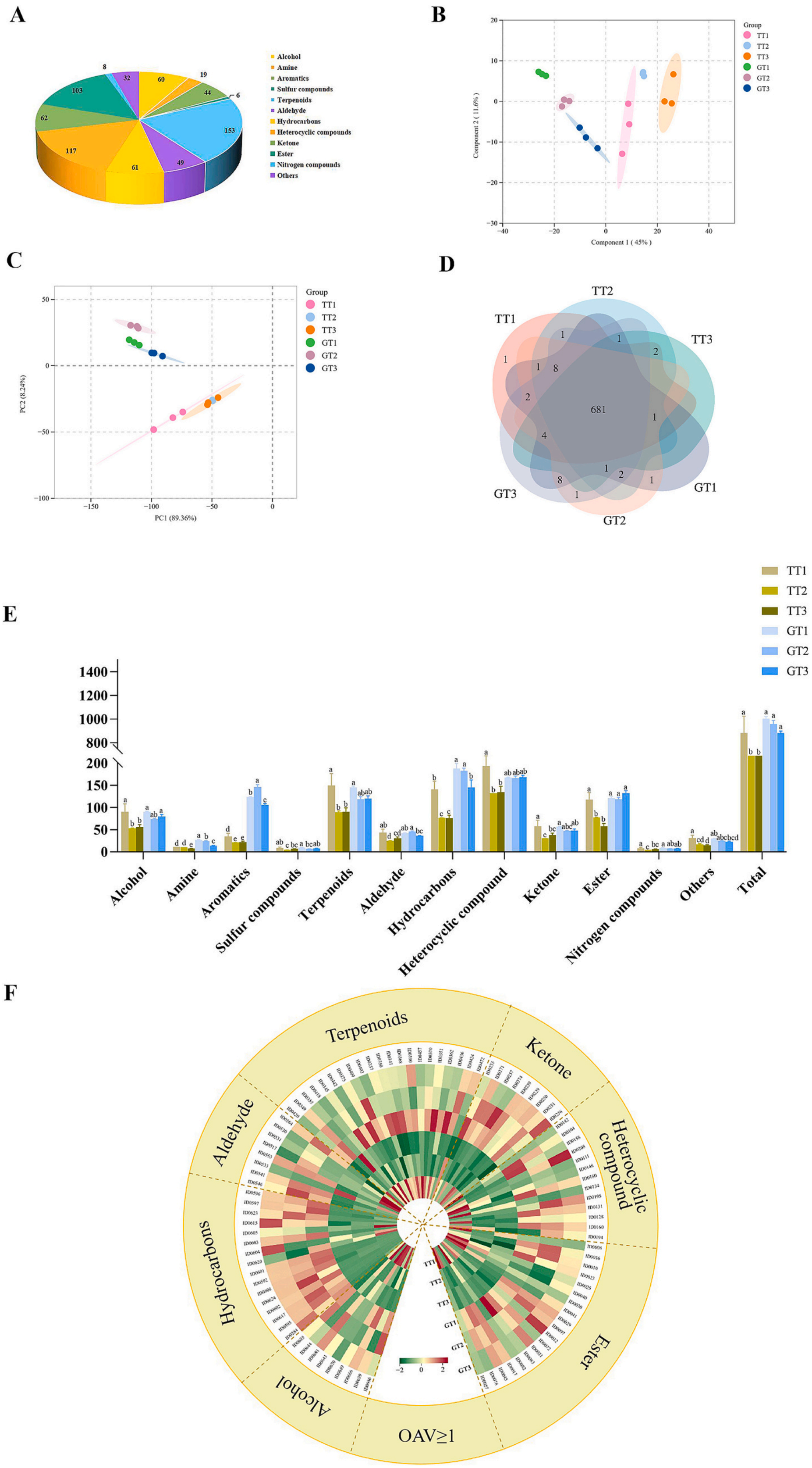

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Fig. 6. (A)The pie chart of volatile components of GT and TT (B)The PCA analysis of volatile components of GT and TT (C)The OPLS-DA analysis of volatile components of GT and TT (D)The Venn of volatilecomponents of GT and TT (E)The volatile components of GT and TT (F)The heat map of key volatile components of GT and TT.

Note: Values with different letters are significantly different at $p < 0.05$; GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

TFL. The change of catechins, caffeine and water extracts in tea leaves was not obvious after covered by plastic greenhouse. Despite no significant change in total catechins, the contents of catechin monomers like ECG, EGC and EGCG were found to be differential between GFL and TFL. TFL had higher contents of ECG and EGC, while GFL had higher contents of EGCG. Based on these results, we inferred that the plastic covering cultivation might have remarkable influence on the taste of tea. Detailed data was shown in Table S3.

3.3.2. *Comparison of non-volatile components between GT and TT*

As shown in Fig. 4, the effect of greenhouse covering on the change of soluble sugars, polyphenols, ECG, EGC and EGCG in tea products was consistent with those in fresh tea leaves. GT had higher contents of soluble sugars, polyphenols, ECG, EGC, and lower contents of EGCG and caffeine than TT. Most notably, the contents of amino acids of TT were significantly higher than GT, including umami taste amino acids (L-theanine, L-glutamic, L-aspartate, L-asparagine), sweet amino acids (L-serine, L-threonine, L-lysine, L-alanine, L-proline, γ-aminobutyric-acid), bitter amino acids (L-arginine, L-leucine, L-valine, L-tyrosine, L-tryptophan, L-isoleucine, L-hisidine, L-phenylalanine) and other odorless amino acids (L-glutamine, β-alanine). The greenhouse covering seemed to have no effect on the content of water extracts and EC in tea. Detailed data was shown in Table S4.

3.3.3. *Key differential components between GT and TT*

To further screen the key differential components between GT and TT, the analysis of PCA and OPLS-DA were conducted in our study. Fig. 5A showed the non-volatile compounds of TT and GT were separated by PCA. In PCA model, the contribution rate of the first principal component (PC1) and the second principal component (PC2) was 49.40 % and 15.10 % respectively, the total contribution was 64.50 %, indicating the reproducibility between each group of samples was good. In OPLS-DA model, TT and GT were distributed in different quadrants, and all samples were basically within the 95 % confidence intervals (Fig. 5B). It provided visual evidence of the difference between the tea samples with greenhouse covering or not. The differential non-volatile components between GT and TT were further screened by the variable importance in projection (VIP) >1.0 and fold change ≥2.0 or ≤ 0.5. Fig. 5C revealed that there were 16 differential non-volatile components between GT and TT. Greenhouse covering produced significant impact on decreasing the contents of glutamic acid, lysine, serine, EGCG, threonine, arginine, β-alanine, tryptophan, isoleucine, asparagine, valine, leucine, proline, phenylalanine and tyrosine, but increasing the contents of soluble sugars of tea, thus giving the green tea a weak taste. The correlation between differential taste components and sensory quality of GT and TT was shown as Fig. 5D. Asparagine, proline and arginine was found to be positively correlated with taste quality of GT, while EGCG, threonine, arginine, phenylalanine and β-alanine showed positive correlation with taste quality of TT.

3.4. *Volatile components*

3.4.1. *Comparison of volatile components between GT and TT*

To clarify the impact of greenhouse covering on aroma of tea, HS-SPME and GC–MS were applied to analyze the difference of volatile components between GT and TT. Totally, 714 volatile components were identified in GT and TT, including 153 terpenoids, 117 heterocyclic compounds, 103 esters, 62 ketones, 61 hydrocarbons, 60 alcohols, 49 aldehydes, 44 aromatics, 19 amines, 19 acids, 9 phenols, 8 nitrogen compounds, 6 sulfur compounds, 2 ethers and 2 others (Fig. 6A). Fig. 6E showed the relative contents of amine, aromatics, hydrocarbons, esters, and total volatiles in GT were much higher than those of TT. Fig. 6B and C indicated that there was a huge difference in aroma components between GT and TT. In PCA model, the contribution rate of PC1 and the PC2 was 46.80 % and 14.80 % respectively, and TT and GT was distributed in different quadrants (Fig. 6B). OPLS-DA analysis showed GT and TT was completely separated which was in consistence with the result of PCA (Fig. 6C). From Fig. 6D, 681 volatile components were both found in GT and TT. GT had 8 particular components including 3,6-dimethyl-2,3,3a,4,5,7a-hexahydrobenzofuran, 1-(3-ethoxyphenyl) acetone, eucalyptol, D-verbenone, 4-hydroxy-benzaldehyde, 4-methoxy-benzaldehyde, 1-oxide-2-methoxy-1-propyl-diazene and α-2,6,6-tetramethyl-1-cyclohexene-1-propanol. The above results suggested that it is not the higher the content of volatile components that leads to better aroma quality of tea.

3.4.2. *Differential volatile components*

In order to figure out the reason why TT got superior aroma than GT, the differential volatile components between GT and TT were further identified by the variable importance in projection (VIP) >1.0 combined with fold change ≥2.0 and fold change ≤0.5. Results showed here were 223 differential volatile compounds between GT and TT, including 37 esters, 34 heterocyclic compounds, 19 ketones, 21 hydrocarbons, 28 terpenoids, 21 alcohols, 18 aldehydes, 24 aromatics, 4 amines, 6 acids, 2 phenols, 6 nitrogen compounds, 1 sulfur compounds, 2 phenols , 1 ethers and 1 other. Compared with GT, the relative content of 200 volatile compounds including ethyl ester benzoic acid, pentyl octanoate, 2-undecanone and β-pinene was higher in TT, and the relative content of 1H-pyrazole, cyclohexanone, heptanal and other 23 volatile compounds were lower in TT, leading to the different aroma types of GT and TT.

3.4.3. *Key aroma compounds identified by OAV*

OAV was used to analyze the contribution of individual volatile component to the aroma of tea. The volatile component with OAV >1 was usually considered as the key component contributing to tea aroma. Fig. 5F summarized the content of volatile components with OAV > 1 in GT and TT. It revealed that there were 81 key aroma components including dodecanenitrile (OAV = 4296), dodecanal (OAV = 4153), dicyclopentadiene (OAV = 2666), furaneol (OAV = 983), benzeneacetaldehyde (OAV = 378) and other 76 components with OAV > 1 in TT (Table S5); 98 key aroma components (OAV > 1) were screened in GT (Table S6), like dicyclopentadiene (OAV = 12333), dodecanal (OAV = 11974), dodecanenitrile (OAV = 9481), naphthalene (OAV = 4662) and 1-methyl-naphthalene (OAV = 2383). Therefore, the bean-like aroma of TT was owing to 2-pentyl-furan, 2-ethyl-5-methyl-pyrazine, dodecanenitrile, dodecanal, dicyclopentadiene and other key aroma components. β-Ionone, indole, naphthalene, 1-methyl-naphthalene and other key aroma components contributed the clean with little chestnut-like aroma of GT.

3.5. *The flavor wheel construction of GT and TT*

The application of flavor wheel makes the standardization of sensory evaluation, localization of tea and is also an important strategic product control to achieve tea quality (Wang et al., 2024). Our work visualized the ingredients that contribute significantly to the taste and aroma of GT

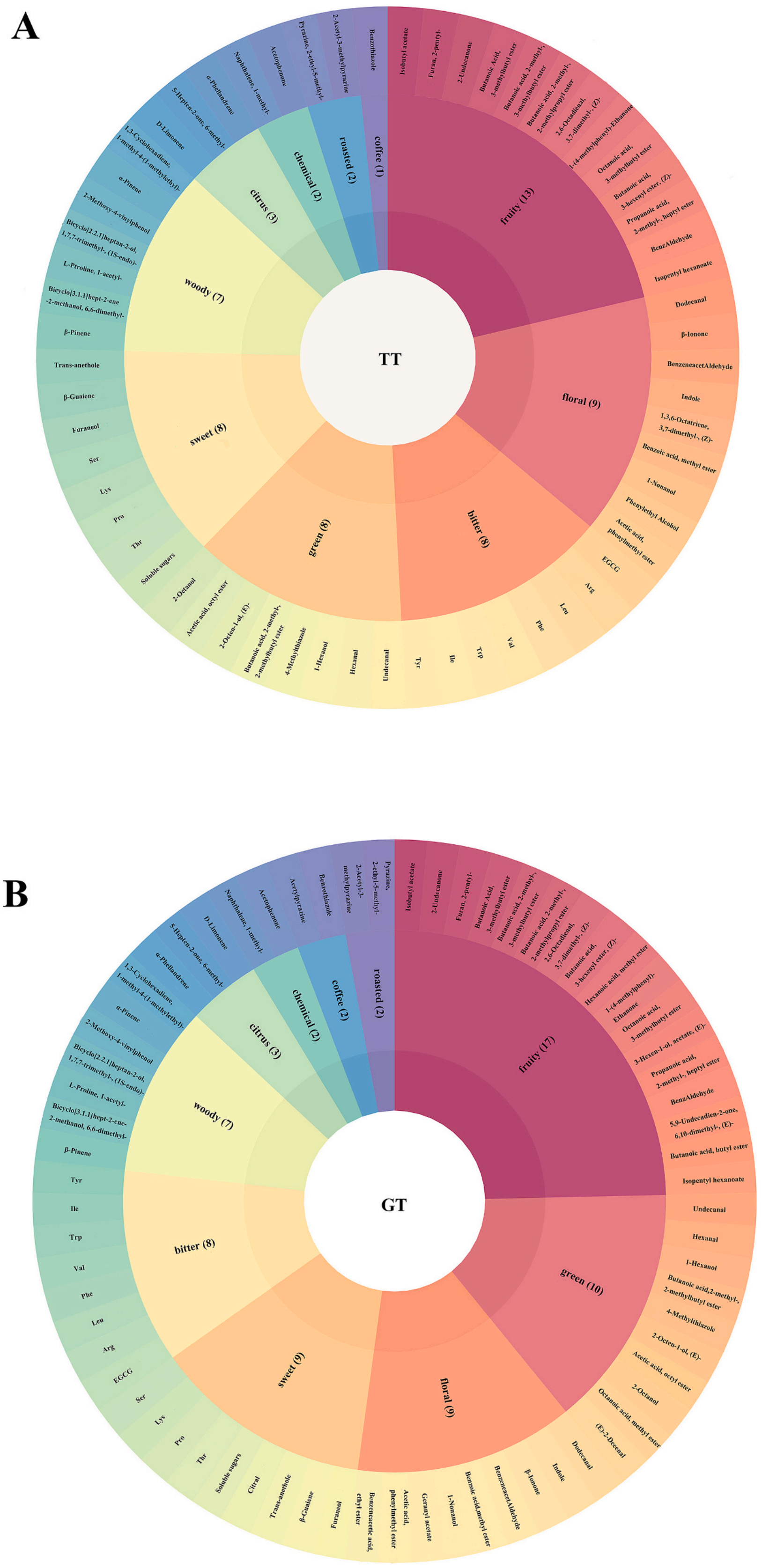

Fig. 7. (A) The flavor wheel of TT (B) The flavor wheel of GT.
Note: GT: green tea produced by fresh leaves with plastic greenhouse covering; TT: green tea produced by fresh leaves without plastic greenhouse covering. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

and TT, and selected 10 descriptors, including sweet, floral, bitter, fruity, green, woody, citrus, chemical, coffee and roasted to characterize sensory qualities (Fig. 7). The flavor wheel of TT and GT provided theoretical basis for scientific description of tea quality.

4. Discussion

It is well known that low temperature and drought are important factors affecting plant growth and development. And extreme cold temperatures and drought can cause irreversible physiological damage to tea plants (Çetinbaş-Genç et al., 2020; Jin et al., 2023; Wang et al., 2023). Greenhouse covering provides a controllable and ideal condition for tea plants growing, creating more tea yield and superior phenotype. Our results revealed that the leaf area, internode length, 100 shoots weight and chlorophyll contents of tea plants were obviously promoted by the greenhouse covering. Many studies have proved that shading can increase chlorophyll contents but decrease leaf thickness, internode length as well as tea yield (Chen et al., 2023; Sano et al., 2018). Based on our results, we propsed a novel cultivation technique, that greenhouse covering combined with black net shading would not only increase chlorophyll contents but also raise tea yield.

The chemical composition of fresh leaves determines the quality of green tea. In our study, the major differential non-volatile components between GT and TT was EGCG, soluble sugar and 14 amimo acids. In comparison with open field cultivation, the greenhouse cultivation markedly increases the air temperature, ground temperature, effective accumulated temperature and decreases the temperature difference between day and night (Li et al., 2016; Li et al., 2022). Moderate temperature difference between day and night effectively improve the content of tea in total free amino acid, caffeine and tea polyphenol (Li, 2023). That is why the contents of amino acids and EGCG in TT were higher than GT. EGCG contributes to the astringency of green tea, and caffeine promots bitterness of green tea (Narukawa et al., 2010; Xie et al., 2024). Amino acids give green tea umami taste by inhibiting the bitter and astringent taste presented by catechins (Liu, Ran, et al., 2023). Soluble sugar contributes to the sweetness of green tea (Wang et al., 2020). Therefore, the green tea produced by fresh leaves without greenhouse covering had thicker and mellower taste than greenhouse tea.

Tea aroma is a comprehensive reflection of the components and content of valtiles in tea, which is an important indicator for evaluating tea quality. Green tea presents various aroma types such as clean, chestnut-like, floral and bean-like aroma (Ho et al., 2015; Zhu et al., 2018). Our results showed that the greenhouse covering could change the aroma types of green tea from bean-like to clean. Further analysis found that the major difference of the aroma type between TT and GT was not the kinds of volatiles but the relative contents of key aroma components which OAV > 1. Dodecanal (waxy, citrus, green), furaneol (sweet), benzeneacetaldehyde (green floral) and other key aroma components were major contributors to the bean-like aroma of TT. Undecanal (floral, green, fresh), β-ionone (sweet, fruity), hexanal (fresh, green) and other key aroma components contributed to the clean with little chestnut-like aroma of GT. Among which, hexanal, octanal, nonanal with fresh, clean and green aroma has been proved to play a vital role in chestnut-like aroma of green tea (Zhu et al., 2018). It is widely believed that environmental factors exert a substantial influence on the variability of volatile flavor compounds in plants (Ge et al., 2024). Previous study found that cold stress could increase the expression of genes related to volatile components, as a consequence, promoting the accumulation of aroma components in tea leaves (Zeng et al., 2018). Therefore, the aroma qualtiy of TT was superior to GT.

5. Conclusion

Greenhouse covering produced remarkable impact on quality of green tea. Based on the above results, the flavor wheel of TT and GT was constructed (Fig. 7). EGCG, serine, lysine, proline and other 11 amino acids contributed to the sweetness and thickness of TT taste. The bean-like aroma of TT was owing to 83 key aroma components including dodecanal, furaneol, benzeneacetaldehyde, 2-pentyl-furan, 2-ethyl-5-methyl-pyrazine and other key aroma components. Soluble sugars played an important role in GT taste. And the clean with little chest-like aroma of GT was determined by 98 volatile components including β-ionone, indole, naphthalene, 1-methyl-naphthalene and other key aroma components. Our study clarified the effect of greenhouse covering on the quality of green tea and provided a theoretical basis for scientifically evaluating the quality of TT and GT.

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Author contributions statement

Qian Wang performed the experimental work, validation, and formal analysis, wrote the original version, edited the final version, and prepared illustrations; Meng Li performed the experimental work; Jie Wang and Xueming Ma performed the statistical analysis, and also contributed to the interpretation and global integration of the results, validation; Lei Liu provide technical guidance for tea garden management; Jianhui Hu responsible for organizing sensory evaluation of tea; Xinfu Zhang and Peiqiang Wang performed the experimental work, and review and editing of the final version, provided a funder for this study; Fengfeng Qu was responsible for conceptualization, writing, review and editing of the final version, supervision, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

CRediT authorship contribution statement

Qian Wang: Writing – review & editing, Writing – original draft, Validation, Data curation. **Meng Li:** Data curation. **Jie Wang:** Validation. **Xueming Ma:** Validation. **Lei Liu:** Resources. **Peiqiang Wang:** Writing – review & editing, Funding acquisition. **Jianhui Hu:** Validation, Supervision. **Xinfu Zhang:** Writing – review & editing, Funding acquisition. **Fengfeng Qu:** Writing – review & editing, Validation, Funding acquisition, Conceptualization.

Declaration of competing interest

All authors declared that they had no known competitive financial interests or personal relationships that could have appeared to influence the study reported in this paper.

Data availability

Data will be made available on request.

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