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# Chemical constituents of green teas processed from albino tea cultivars with white and yellow shoots

Jihong Yang [1], Hanchen Zhou [1], Yaqin Liu, Hui Wang, Yujie Xu, Jianqin Huang, Pandeng Lei [*]

*Tea Research Institute, Anhui Academy of Agricultural Sciences, Huangshan 245000, China*

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ABSTRACT

Green tea processed from albino tea varieties often has umami taste and fresh aroma. This study identified green teas made from two types of albino tea cultivar, one having the white shoots (called Naibai, NB) and the other having the yellow shoots (called Huangjinya, HJY). Taste compounds analyses showed that galloylated catechins were highly concentrated in HJY green teas, whereas non-galloylated catechins and amino acids were more abundant in NB green teas. *CsTA* (involved in the catabolism of galloylated catechins) showed high expression in HJY tea shoots, resulting in gallic acid as a precursor for *β*-glucogallin biosynthesis being abundant in HJY. *CsPDX2.1* (responsible for theanine hydrolyzation) had a lower expression level in NB than HJY shoots. Fatty acid–derived volatiles (FADVs), glycosidically bound volatiles (GBVs) and carotenoid–derived volatiles (CDVs) were highly concentrated in HJY green teas, whereas amino acids–derived volatiles were highly concentrated in NB green teas.

## 1. Introduction

Tea plant [*Camellia sinensis* (L.) O. Kuntze] is a perennial woody plant whose leaves are used to produce various kinds of tea products (Huang et al., 2021). Tea genetics plays an important role in sensory properties of tea products, including color, taste and aroma (Zhang et al., 2021). Recently, the albino tea plant with white, yellow or variegated tea leaves, has attracted an increasing attention as raw materials for making tea products. The temperature–sensitive albino tea cultivars (e.g., 'White leaf No.1′ and 'Xiaoxueya') develop white shoots when the temperature is below 20 °C (Li et al., 2011; Du et al., 2008). By contrast, in the light–sensitive albino tea cultivars (e.g., 'Huangjinya', 'Zhonghuang 2′ and 'Yujinxiang') the etiolated phenotype is triggered under intense illumination (>15,000 lx) (Li et al., 2016; Wang et al., 2014; Liu et al., 2017). Recent studies have identified a tea variety that is insensitive to growing conditions and contains green and albino zones in individual leaves (Xie et al., 2021; Lu et al., 2022). Chlorophyll and carotenoids are important photosynthetic pigments, and the genes involved in chlorophyll and carotenoid biosynthesis or degradation have a disorderly expression in albino tea shoots (Zhang et al., 2021). In addition, chloroplast structures (granular stacks and thylakoids) are altered in albino tea leaves compared with normal green tea leaves (Zheng et al., 2021; Jiang et al., 2020).

Tea products processed from the white or yellow tea shoots contain high contents of amino acids and low contents of polyphenols, contributing to low astringency and umami taste of tea infusions (Zhang et al., 2019). In particular, theanine accumulates strongly in albino tea varieties and has multiple benefits to human health, such as improving sleep quality and treating cancer, cardiovascular diseases and obesity (Fu et al., 2020). It was reported that the yellow tea shoots induced by shading contained high concentration of amino acids due to the proteolysis of chloroplast proteins rather than the enhancement of amino acid biosynthesis (Chen et al., 2017). Study on the albino-related yellow tea shoots indicated that high accumulation of theanine was due to weak catabolism rather than the activation of theanine biosynthesis (Cheng et al., 2018; Fu et al., 2020). Compared to green tea shoots, white or yellow tea shoots contain less catechins and caffeine (Feng et al., 2014). iTRAQ (isobaric tag for relative absolute quantitation) proteomic

*Abbreviations:* HS-SPME, headspace solid–phase microextraction; GC–MS, gas chromatography–mass spectrometry; PCA, principal component analysis; VIP, variance importance values; DOT, dose–over–threshold; OAV, odor activity value; *ACI*, aroma character impact; GA, gallic acid; C, catechin; EC, epicatechin; EGC, epigallocatechin; GCG, gallocatechin gallate; EGCG, epigallocatechin gallate; ECG, epicatechin gallate; FADVs, fatty acid–derived volatiles; GBVs, glycosidically bound volatiles; CDVs, carotenoid–derived volatiles.

* Corresponding author.
*E-mail address:* lpteagle@126.com (P. Lei).

[1] These authors contributed equally to this work.

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analyses show that genes involved in flavonoid biosynthesis are down-regulated in yellow tea shoots (Wang et al., 2015).

Compared to normal green tea shoots, white or yellow tea shoots contain lower concentrations of volatile compounds, especially phenylpropanoids/benzenoids and glycosidically bound volatile compounds (GBVs) (Dong et al, 2018). The reason for lower concentrations of GBVs in yellow tea shoots is due to decreased accumulation of geranyl diphosphate (GDP) as the key precursor of terpenoids (Dong et al, 2018). A tea product processed from albino tea variety with yellow shoots had a corn-like aroma (Liao et al., 2020). However, few studies focused on the difference in aroma profile of green teas processed from white and yellow tea shoots. In the present study, two types of albino tea variety with white and yellow shoots were used to make green tea for metabolite analysis.

Albino green teas not only have pleasing flavor, but also contribute to health benefits. There are many studies on albino tea plants, such as characterizing leaf color variation (Wang et al., 2015), determining concentrations of amino acids or polyphenols (Cheng et al., 2018), and profiling volatiles compared with normal green leaves (Dong et al., 2018). In the present study, green teas processed from albino tea cultivar with white and yellow shoots were analyzed comprehensively. The objectives of this study were (i) to profile metabolites of two types of albino green tea, (ii) to identify differential metabolites and (iii) to explore the mechanisms underlying the differences between white and yellow tea shoots.

## 2. Materials and methods

### 2.1. Tea samples

Fresh shoots (one bud and one or two leaves) from two albino tea cultivars were plucked in early spring in Guangde county of Anhui Province, China. Five tea samples used in this study were processed under the same processing and conditions, including NB1 green tea (processed on April 12), NB2 green tea (processed on April 4), HJY1 green tea (processed on April 4), HJY2 green tea (processed on April 6), and HJY3 green tea (processed on April 7). Briefly, fresh leaves were spread out indoor for 5–8 h, then subjected to pan–firing at 300–350 °C for 2–3 min using a fixation machine (Sunyoung Machinery Co., ltd, Quzhou, China). A carding machine (Sunyoung Machinery Co., ltd, Quzhou, China) was used for shaping tea shoots at 250–280 °C for 8–10 min. Subsequently, the drying procedure was conducted at 105–115 °C for 7–10 min and 75–80 °C for 20–30 min. All tea samples were preserved in aluminum foil bags and stored at − 20 °C for further analysis.

### 2.2. Chemicals

The standards for gallic acid (GA), (+)–catechin (C), (−)–epicatechin (EC), (−)–epigallocatechin (EGC), (−)–gallocatechin gallate (GCG), (−)–epigallocatechin gallate (EGCG), (−)–epicatechin gallate (ECG), and caffeine were purchased from Sigma–Aldrich (Darmstadt, Germany). The mixture of amino acids standards was purchased from Sykam (Munich, Germany). *n*–Alkanes were purchased from Sigma–Aldrich (Darmstadt, Germany). Aroma standards including ethyl decanoate (99 %), benzaldehyde (99.5 %), octanal (99 %), benzyl alcohol (99.5 %), phenylacetaldehyde (95 %), linalool (98 %), nonanal (96 %), terpineol (95 %), geraniol (99 %), *(Z)*–3–hexenyl hexanoate (98 %), β–ionone (97 %), cedrol (98 %), and methyl jasmonate (98 %), were purchased from Aladdin (Shanghai, China). Aroma standards including hexanal (98 %), *(Z)*–3–hexenyl acetate (97 %), linalool oxide mixture (97 %), 2–phenylethyl alcohol (98 %), methyl salicylate (99 %), indole (99 %), *(Z)*–jasmone (92 %), α–ionone (90 %), nerolidol (97 %), and *(Z)*–3–hexenyl benzoate (98 %) were purchased from Tokyo Chemical Industry Co., ltd (Tokyo, Japan). Aroma standard ocimene (90 %) was purchased from Yuanye Company (Shanghai, China).

### 2.3. Sensory evaluation

Five green tea samples were evaluated by six skilled experts according to the national standard procedure (GB/T 23776–2018). The tea infusions were filtered after brewing with water (3.0 g, 95 °C, 150 mL) for 4 min. Sensory evaluation of five tea infusions was conducted using a 100–point scale. Based on the description of green tea evaluation in GB/T 23776–2018, green tea infusions with the premium taste traits, such as sweet, fresh, umami, or mellow had a score range of 90–99; the tea samples with thick or mellow traits had a score range of 80–89; the others with thin, thick, and astringent traits had a score range of 70–79. Aroma evaluation of tea infusions was conducted by these aroma characteristics (i.e. fresh and high, green and herbal, floral, fruity, bake-like, over-fired, roasted–nutty). Green tea samples with the premium traits, such as fresh and high, floral, fruity, or roasted–nutty had a score range of 90–99; the tea samples with fresh and bake-like traits had a score range of 80–89; the others with green and herbal, or over-fired traits had a score range of 70–79.

### 2.4. Quantification of catechins and caffeine by HPLC

Tea powder (0.25 g) was extracted with 7 mL of boiling water, followed by shaking at 100 rpm in a 90 °C–water bath for 7 min. The residues were re–extracted three times as described above. The tea infusions were combined and diluted fourfold with water. Before HPLC analysis, the solutions were filtered through a 0.22 μm filter. Each sample was extracted in triplicate.

Catechins and caffeine were analyzed using a HITACHI Chromaster HPLC system equipped with a 5410 ultraviolet (UV) detector (Hitachi, Tokyo, Japan). A reverse–phase C18 column (HITACHI LaChrom C18, 150 mm × 4.6 mm, 5 μm) was used at a flow rate of 1.0 mL/min. An aliquot of 10 μL of the filtrate was injected into the HPLC system for analysis. The mobile phases were 0.04 % (v/v) phosphoric acid (A) in distilled water and 100 % acetonitrile (B), and the gradient elution was as follows: 10 % B (0–1 min), 10–15 % B (1–23 min), 15–85 % B (23.0–23.1 min), 85 % B (23.1–35 min), 85–10 % B (35–55 min), 10 % B (55–65 min). The detection wavelength was set at 280 nm, and the column temperature was 40 °C.

Identification of catechins were performed via comparison with their authentic standards. Quantification (mg/g, dry weight, DW) was based on the calibration curve of analytical standards (Table S1). Three biological replicates were measured.

### 2.5. Identification and quantification of amino acids

The tea infusions for amino acids analysis were prepared as described above for catechin analysis. Triple extractions for each sample were prepared. An automatic amino acid analyzer S–433D (SYKAM, Munich, Germany) coupled with an LCA K07/Li column (SYKAM, Munich, Germany) were used for amino acids analysis. An aliquot of 50 μL of tea infusions was injected into the system. The rate of elution and derivation flow was maintained at 0.45 and 0.25 mL/min, respectively. Solvents A (buffer A–1, 0.12 N, pH 2.9, SYKAM, Munich, Germany), B (buffer B–1, 0.3 N, pH 4.2, SYKAM, Munich, Germany), C (buffer C-4, 0.3 N, pH 8.0, SYKAM, Munich, Germany), and D (500 mM NaOH, 0.68 mM EDTA) were run in a linear gradient. The gradient elution was as follows: 80 % A and 20 % B (0–1 min); 79 % A and 21 % B (1–3 min); 61 % A and 39 % B (3–8 min); 43 % A and 57 % B (8–20 min); 43 % A and 57 % B (20–24 min); 100 % B (24–32 min); 100 % C (32–38 min); 76 % C and 24 % D (38–43 min); 76 % C and 24 % D (43–57 min); 100 % D (57–57.1 min); 100 % D (57.1–64.1 min); 100 % A (64.1–64.2 min); 100 % A (64.2–80 min). Compounds were detected at 570 nm and 440 nm using a visible spectrophotometer.

Identification was performed via comparison with their authentic standards (SYKAM, Munich, Germany). The quantification (mg/g, dry weight, DW) was based on comparison of peak areas in samples and

analytical standards. Three technical replicates were measured.

### 2.6. Quantitative real-time PCR for transcription analysis

Total RNA from white and yellow tea shoots was obtained using a RNAprep Pure Plant Plus kit (Tiangen, Beijing, China). A PrimerScript™ RT kit (Takara Bio Inc, Dalian, China) was used for amplification of first-strand cDNA. The gene specific primers used in this study are listed in Table S2; the *18S* was set as the internal reference gene. The reaction system was prepared using Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China), including 10 μL polymerase mix, 1 μL of 10 μM primer solutions, 2 μL of 100 ng/μL template and 7 μL of RNAase free water. The procedure for polymerase chain reaction was: 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s and 60 °C for 20 s on a Roche LightCycle 480 (Roche, Basel, Switzerland). Three biological replicates were measured. The gene transcript level was calculated using the $2^{-\Delta\Delta Ct}$ method.

### 2.7. Identification and quantification of aroma compounds

Tea aroma compounds were collected by HS-SPME (headspace solid–phase microextraction). According to the previous study (Wang et al., 2017), tea samples (0.5 g) and sodium chloride (15 mg) were placed in a 20 mL vial with boiling water (95 °C, 10 mL). The vial was sealed with a tin foil paper and placed in a water bath to equilibrate at 70 °C for 5 min, followed by absorption using the SPME fiber (50/30 μm DVB/CAR/PDMS, Stable Flex, Supelco, USA) at 70 °C for 30 min.

Volatile compounds were analyzed with an Agilent 7697A gas chromatograph (GC) equipped with 7890A mass selective detector mass spectrometer (MS) system using a column DB–5MS (30 m × 0.25 mm, 0.25 μm, Agilent, California, USA). The SPME fiber was desorbed at 250 °C for 5 min in a split–less mode. The initial GC oven temperature was 40 °C (held for 5 min), then ramped up to 200 °C at 4 °C/ min (held for 2 min), then to 280 °C at 16 °C/ min (held for 3 min). Triplicate injections were analyzed. The carrier gas was helium (>99.99 %) with a constant flow rate of 1 mL/min. The mass spectrometer was operated at an electronic energy of 70 eV, and the mass scan range was 30–600 Amu. The temperature of interface, ion source and quadrupole were set at 280 °C, 230 °C and 150 °C.

The *n*–alkane standards (Sigma–Aldrich, Darmstadt, Germany) were used to calculate the retention indices (RIs) of volatile compounds. Volatile compounds were identified by comparing retention indices (RIs), authentic standards and the NIST database. Six technical replicates were conducted for volatiles analysis. Quantification of volatile compounds (μg/kg) was done according to Zhou et al., 2022.

### 2.8. Odor activity values (OAV)and aroma character impact (ACI) calculations

Contribution of each aroma compound to tea aroma was evaluated by odor activity value, which is the ratio of the concentration of each compound to its detection threshold. Compounds with OAV ≥ 1 are considered as potential contributors to tea aroma profile. Aroma character impact (*ACI*) could be employed to compare aroma contributions of components in a mixture (Yang, 2008). They were calculated as follows:

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

$$ACI_i(\%) = \frac{C_i/T_i}{\sum_k C_k/T_k} \times 100$$

where $C_i$ is the concentration of the odorant and $OT_i$ or $T_i$ is its corresponding odor threshold; $C_k$ and $T_k$ are the concentration of each odorant identified in this study and their corresponding odor threshold.

### 2.9. Chloroplast ultrastructure observation

Chloroplast ultrastructure observation was done according to Huang et al. (2021). The fresh second leaf was cut into pieces (1 × 1 mm) and fixed in glutaraldehyde solution (2.5 %, v/v) at 4 °C for 12 h. The samples were washed using 0.1 M phosphate buffer (pH 7.0) for 15 min and water at 4 °C for 15 min. Dehydration and infiltration were conducted using a gradient acetone and resin mixture, and the specimens were observed using a Hitachi HT–7800 transmission electron microscope (Hitachi, Tokyo, Japan).

### 2.10. Analysis of chlorophyll and carotenoids

Chlorophyll *a*, chlorophyll *b* and total carotenoids were determined using ultraviolet spectrophotometry (TU–1810, Persee, Beijing, China). The amount of 100 mg of fresh tea leaves was extracted with 10 mL of 95 % v/v ethanol for 24 h in the dark and then centrifuged at 8000 rpm for 5 min. The extracts were filtered and analyzed. The absorbance at 664 nm for chlorophyll *a*, 649 nm for chlorophyll *b*, and 470 nm for total carotenoids was measured. The pigments were quantified in accordance with the empirical formula proposed by Lichtenthaler (1987). Two types of tea shoots were tested in three biological replicates.

### 2.11. Statistical analysis

One–way analysis of variance (ANOVA) and Duncan's multiple range tests were performed in SPSS 20.0 (SPSS Inc., USA) to determine significant differences ($P < 0.05$) among samples. The principal component analysis (PCA) and variable importance plot (VIP) value were determined by SIMCA 14.1 (Umetrics Corporation, Sweden).

## 3. Results and discussion

### 3.1. Sensory analysis

Albino tea can be classified into light-sensitive, temperature-sensitive, and ecologically insensitive (Zhang et al., 2019). In the present study, Naibai (NB) and Huangjinya (HJY) albino tea varieties were cultured from the same plantation; NB is a type of temperature-sensitive albino tea variety that has pale or white shoots at temperatures under 20 °C in spring (Fig. 1A), but gradually regreen when the ambient temperature exceeds 23 °C; by contrast, the yellow phenotype of HJY albino tea variety is quite stable without shading, but shoots turn green under reduced light irradiation. As shown in Fig. 1b, NB1 (processed on April 12) and NB2 (processed on April 4) tea shoots show pale leaves with green leaf veins, whereas the color of HJY1, HJY2 and HJY3 tea shoots was greenish–yellow (Fig. 1B).

Sensory evaluation showed that NB1 and NB2 tea infusions had fresh and umami taste, whereas HJY1, HJY2 and HJY3 tea infusions had mellow taste. The taste average score of NB green teas (92.0) was higher than that of HJY green teas (89.6). However, the aroma of HJY green teas (an average score of 94.3) was better compared to NB green teas (an average score of 92.5). Both types of green tea had fresh aroma, but the aroma intensity of HJY green teas was higher than that of NB green teas (Fig. 1C). It is suggested that taste and aroma compounds have different concentrations in white and yellow tea shoots, which impacts on the difference in tea flavor.

### 3.2. Main taste compounds in two types of green tea

The content of catechins (GA, C, EC, EGC, GCG, EGCG, and ECG), caffeine and amino acids were detected in NB and HJY green teas. All standard compounds showed good linearity ($R^2 > 0.9994$) in a relatively wide concentration range (Table S1). Catechins account for approximately 12–24 % of the dry weight of tea leaves and make an important contribution to the taste of green tea. GA is an important catechins in tea

A

Naibai tea variety (April 1) Huangjinya tea variety (May 27)

B

NB1 NB2

HJY1 HJY2 HJY3

C

| Samples | Taste properties | Score | Aroma properties | Score |
|---|---|---|---|---|
| NB1 | Fresh, umami | 91 | Fresh and high, chestnut aroma | 93 |
| NB2 | Sweet, fresh, umami | 93 | Fresh aroma | 92 |
| HJY1 | Fresh, umami, mellow | 94 | Fresh and high | 94 |
| HJY2 | Mellow | 88 | Fresh and high, slightly floral aroma | 95 |
| HJY3 | Mellow | 87 | Fresh and high | 94 |

**Fig. 1.** Phenotype of two albino cultivars and green teas made from them. (A) Phenotype of two types of albino tea varieties. (B) Green teas processed from the white tea shoots (NB1 and NB2) and yellow tea shoots (HJY1, HJY2 and HJY3). (C) Sensory evaluation. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

leaves, not only contribute to the umami taste of tea infusions, but also a crucial precursor of galloylated catechins in tea plants (Zhang, Cao, Granato, Xu, & Ho, 2020). The result shows that the average content of GA in HJY green teas (1.85 mg/g) is 2.6–fold more than in NB green teas (0.70 mg/g) in our study (Fig. 2).

The bitterness and astringency of tea infusions are highly correlated with concentrations of galloylated catechins (EGCG and ECG) compared to non-galloylated catechins such as C, EC, and EGC (Xu et al., 2018). Our study shows that non-galloylated catechins are abundant in NB varieties with white tea shoots, while galloylated catechins such as EGCG and ECG are more highly concentrated in HJY varieties with yellow tea shoots (Fig. 2). Caffeine is a stable compound and accumulates in young tea leaves. A previous study reported that caffeine enhances the bitterness of tea infusions (Zhang, Cao, Granato, Xu, & Ho, 2020). In this study, caffeine in NB and HJY green teas has no differences in concentrations. In addition, the NB1 green tea contains less caffeine compared with other green teas, possibly indicating that caffeine biosynthesis is affected in albinistic stage of albino tea variety with white shoots.

The total amount of amino acids was significantly higher in NB than in HJY green teas (Fig. 2). Amino acids play an important role in umami taste of tea infusions (Zhang, Cao, Granato, Xu, & Ho, 2020). Sixteen amino acids are listed in Table S3. Among them, theanine was present at the highest concentration, followed by glutamic acid, aspartic acid and arginine. Theanine provides a unique 'umami' taste to green tea infusion. Glutamic acid, aspartic acid and proline also contribute to umami taste (Kaneko, Kumazawa, Masuda, Henze, & Hofmann, 2006). In line with sensory evaluation, these amino acids with umami taste traits accumulated highly in NB green teas, contributing to umami taste of NB tea infusions.

Dose–over–threshold (DOT) values (the ratio of the concentration of compound to the taste threshold) for each compound are listed in Table S4. It is considered that compounds with DOTs greater than one have an important impact to tea taste (Yu, Yeo, Low, & Zhou, 2014). The DOT value of EGCG was the highest compared to other taste compounds in this study and the DOT value of EGCG was higher in HJY than in NB tea infusions. The DOT value of ECG in HJY (but not in NB) tea infusions was $> 1$. Combined with sensory evaluation, the data suggested that galloylated catechins enhanced the mellow and thick taste of HJY tea infusions. The DOT value of aspartic acid was greater than one in NB

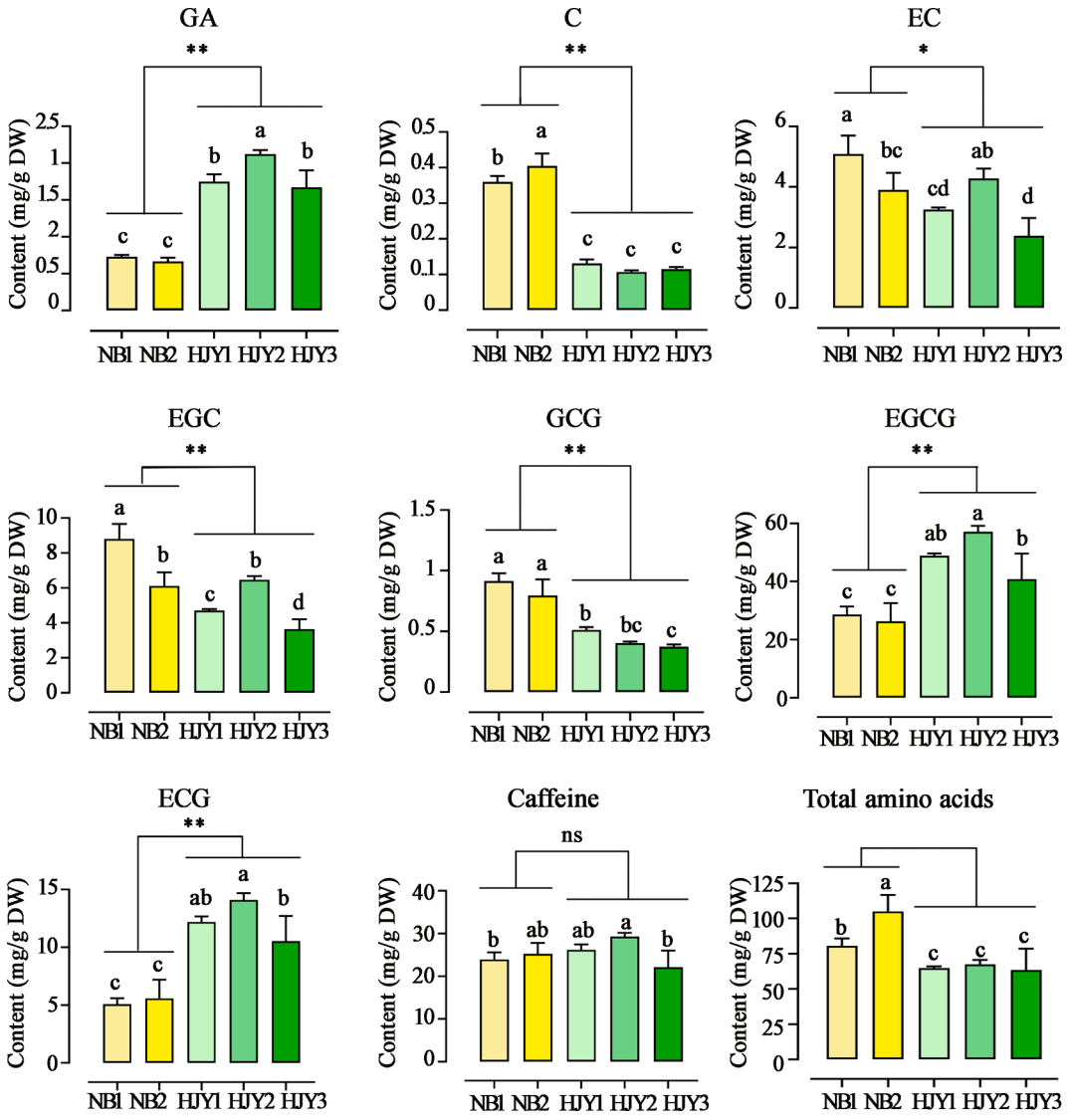


**Fig. 2.** Catechins and caffeine analysis in NB and HJY green teas. Different letters denote significant difference ($p < 0.05$). DW, dry weight. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(but not in HJY). Hence, it had an important impact on NB tea infusions.

### 3.3. Genes involved in biosynthesis and catabolism of catechins and theanine

The pathway and key enzymes for galloylated and non-galloylated catechin biosynthesis in tea are well-understood based on recent studies (Yao et al., 2022; Dai et al., 2020; Cui et al., 2016b; Jun, Lu, Docampo-Palacios, Wang, & Dixon, 2021). The non-galloylated catechins such as (–)–epicatechin, are synthesized from (*2R*, *3S*, *4S*)–leucocyanidin through three catalytic steps mediated by CsANS, CsANR, and CsLAR (Jun, Lu, Docampo-Palacios, Wang, & Dixon, 2021). The recent study showed that galloylated catechins ECG and EGCG are synthesized by CsSCPL4 and its non-catalytic paralog CsSCPL5, with the participation of β-glucogallin and non-galloylated catechins (–)–epicatechin or (–)–epigallocatechin (Yao et al., 2022). It was surprising that the concentrations of non-galloylated catechins were higher in NB green teas, whereas galloylated catechins had a higher concentration in HJY green teas (Fig. 2). Thus, we analyze the genes involved in biosynthesis of non-galloylated and galloylated catechins in NB and HJY tea shoots. The expression of *CsANS* and *CsANR* was higher in NB than in HJY shoots, but *CsLAR* was expressed highly in HJY tea shoots (Fig. 3).

The *CsSCPL4* and *CsSCPL5* also had a higher expression levels in NB than HJY tea shoots. Therefore, *CsTA* (involved in hydrolyzation of galloylated catechins from EGCG to EGC and GA) was also studied. Its expression level was significantly higher in HJY than in NB shoots, which was associated with increased concentration of gallic acid in HJY green tea. The β-glucogallin is an important substrate for biosynthesis of galloylated catechins, which is formed from gallic acid via UGT84A22 (Cui et al., 2016b). The expression of *UGT84A22* was higher in NB than in HJY shoots (Fig. 3). It is hypothesized that gallic acid is the restrictive substrate for biosynthesis of galloylated catechins. Even though biosynthesis of galloylated catechins was active in NB shoots (with high expression of *CsSCPL4* and *CsSCPL5*), the accumulation of gallic acid was lower in NB than HJY tea shoots.

In the previous study, L-theanine accumulation in yellow tea leaves was attributed to the weak catabolism compared to normal leaves (Cheng, et al., 2018). Genes involved in theanine biosynthesis were not upregulated in albino tea variety, but ethylamine, the product of L-theanine catabolism, showed decreased accumulation after supplying [$^2H_5$]-L-theanine (Cheng, et al., 2018), suggesting that the theanine hydrolase activity in albino tea leaves was weak. Hence, the catabolism of some specific metabolites may be inhibited in white leaves compared to yellow leaves due to the variation in hydrolase activity. For example,

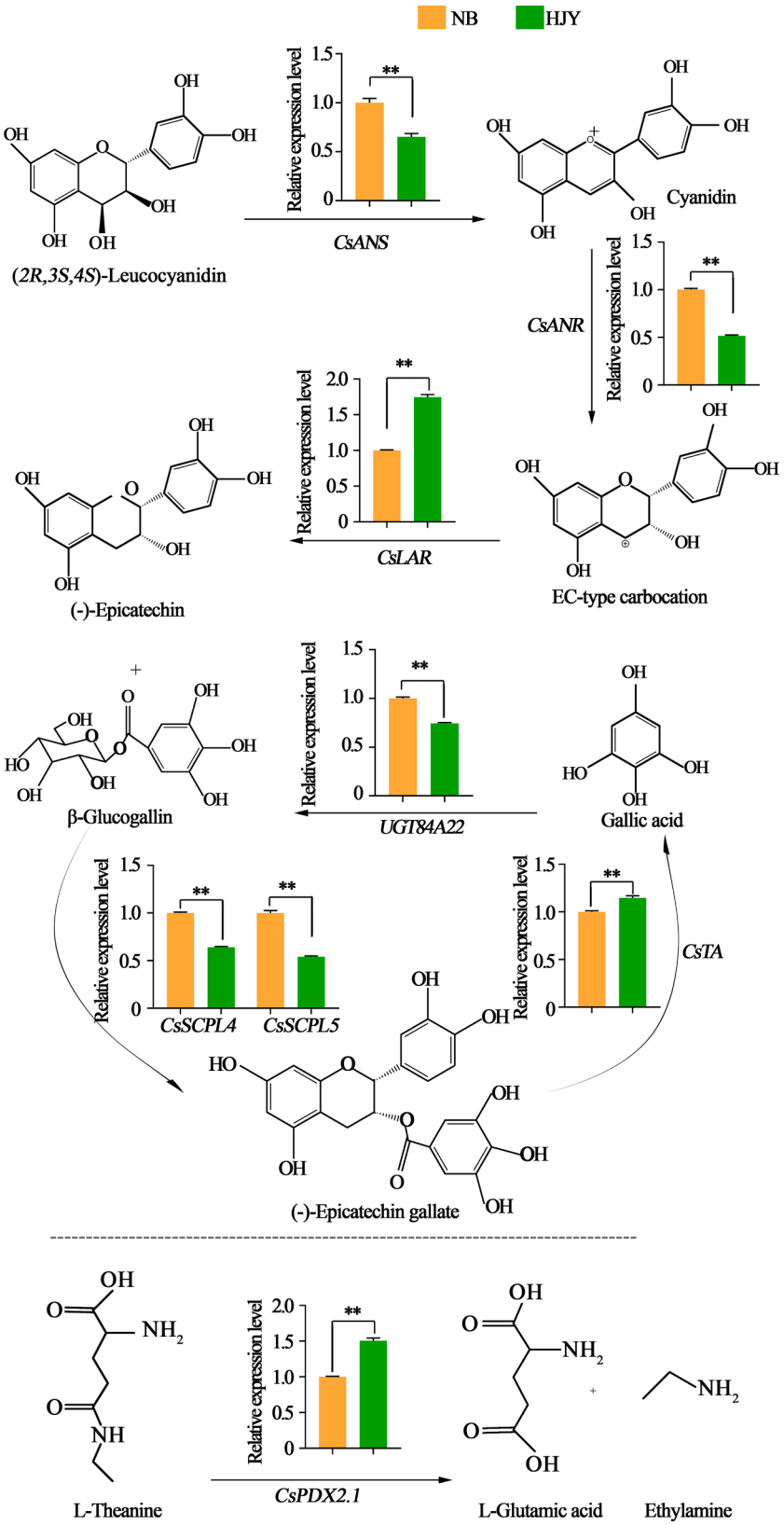


**Fig. 3.** Transcription analysis of genes involved in biosynthesis and catabolism of catechins and theanine.

*CsPDX2.1* (involved in theanine hydrolyzation) had lower expression in NB than in HJY tea shoots (Fig. 3). Interestingly, the catabolism of theanine supports more substrates for its biosynthesis, whereas no more accumulation of theanine was detected in HJY tea shoots. It is well-studied that theanine is primarily biosynthesized from ethylamine and glutamate by theanine synthetase in tea roots (Zhu et al., 2021), and is subsequently transported to tea shoots by the CsAAP transporters (Dong et al., 2020). Even though the hydrolyzation of theanine supplies

ethylamine or glutamate in tea leaves, the biosynthesis of theanine still requires the involvement of ethylamine and glutamate in the roots.

### 3.4. Volatiles profile

Aroma influenced by diverse volatile compounds, is an important criterion in the evaluation of tea quality (Ho, Zheng, & Li, 2015). The aroma profiles of five tea samples were analyzed by HS-SPME-GC–MS. A total of 95 volatile compounds was identified and quantified, including 23 alcohols, 18 aldehydes, 7 heterocyclic compounds, 15 esters, 8 ketones, 8 alkanes, 11 alkenes, one sulfur compound, three aromatic hydrocarbons, one phenol and two others (Table S5). Among them, the volatile compounds with the highest concentrations in albino green teas were benzyl alcohol (5116.95–1377.09 μg/kg), phenylethyl alcohol (573.75–903.46 μg/kg), octanal (335.53–425.24 μg/kg), indole (248.75–344.95 μg/kg), methyl jasmonate (157.98–716.10 μg/kg), and cis–jasmone (91.61–281.01 μg/kg).

The total concentration of volatile compounds in HJY green teas (an average of 9177.74 μg/kg) was significantly higher than that in NB green teas (an average of 4113.40 μg/kg) (Fig. 4A). Alcohols, esters, ketones and alkenes were more abundant in HJY than NB green teas (Fig. 4B). Among alcohols, geraniol was the most abundant volatile in HJY green teas, contributing to flowery aroma. Esters usually contribute to sweet, floral and fruity aroma (Feng et al., 2019). Methyl jasmonate as a floral aroma contributor was highly concentrated in HJY green teas. Similarly, cis–jasmone (also contributing to floral aroma) was more abundant in HJY than NB green teas. This result indicated that biosynthesis of these volatiles might be different in the two types of albino tea varieties.

Principal component analysis (PCA), partial least squares–discriminant analysis (PLS-DA) and orthogonal partial least squares–discriminant analysis (OPLS–DA) were performed for differential metabolites. The PCA and PLS-DA model showed that the five tea samples were clearly classified into two groups (NB and HJY groups) (Fig. 4C and 4D). The cross-validation showed that the PLS-DA model was reliable (Fig. 4E). Fourteen volatile compounds were identified as differing (VIP $> 1$, $P < 0.05$) between the two groups, including benzyl alcohol (VIP = 6.05), methyl jasmonate (VIP = 3.50), indole (VIP = 2.31), octanal (VIP = 2.19), linalool oxide II (VIP = 2.11), phenylethyl alcohol (VIP = 2.01), methyl salicylate (VIP = 1.87), cis–jasmone (VIP = 1.70), geraniol (VIP = 1.44), linalool oxide I (VIP = 1.35), 1–octen–3–ol (VIP = 1.31), nerolidol (VIP = 1.20), hexanal (VIP = 1.17) and linalool (VIP = 1.08) (Fig. 4F).

Odor activity values (OAV) and aroma character impact (*ACI*) are used for identification of important odorants in tea (Liao et al., 2020). Sixteen compounds were considered as the key odorants in albino green

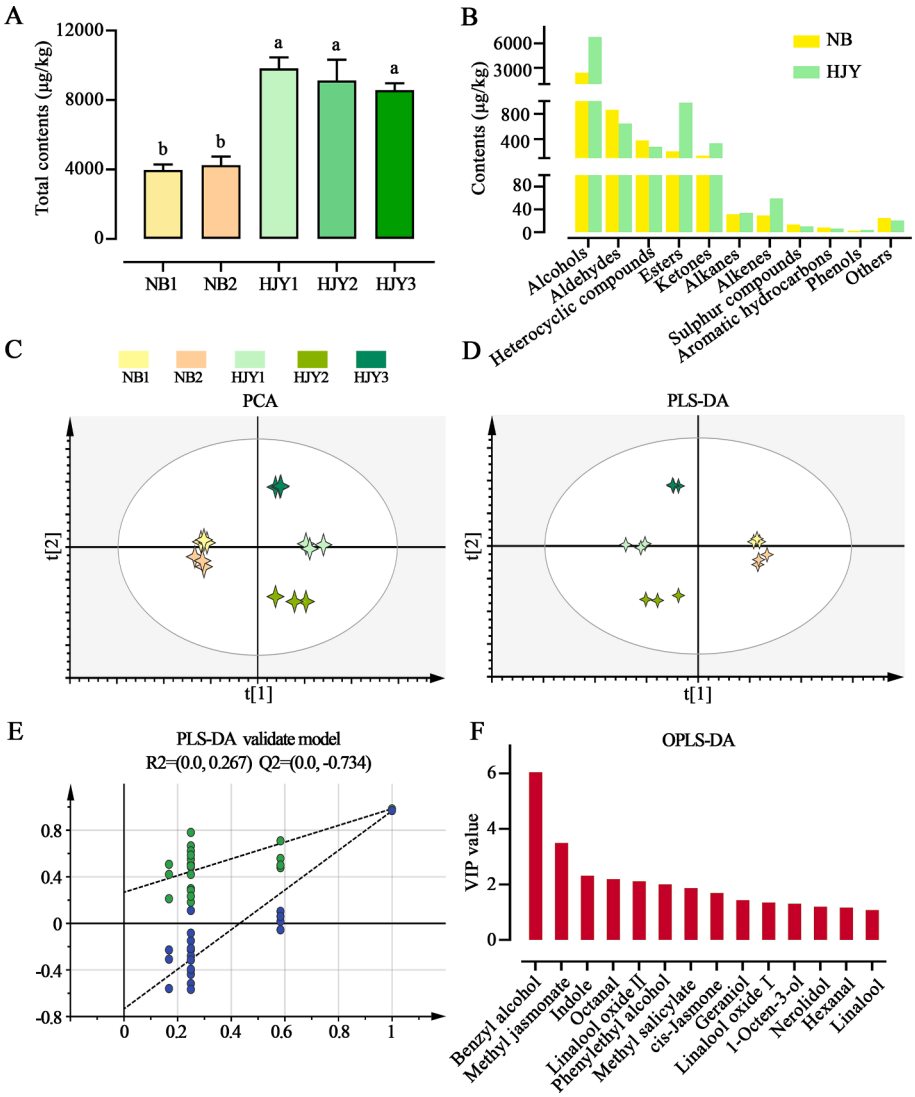


**Fig. 4.** Concentrations, categories and principal component analysis of volatile compounds. (A) Total concentrations of volatiles. (B) Categories of volatile compounds. (C) Principal component analysis. (D) PLS-DA. (E) The validation of PLS-DA model. (F) Differential volatiles with VIPs>1 based on the OPLS-DA model.

teas according to the calculation of OAVs and *ACI* values (Table S6). Octanal, trans–β–ionone, cis–jasmone and dimethyl sulfide were identified as the important odorants in albino green teas due to their high OAVs. According to *ACI* values, these volatile compounds had large contributions to tea aroma, including octanal (17.77–37.45 %), trans–β–ionone (25.11–39.29 %), cis–jasmone (18.56–33.37 %) and dimethyl sulfide (2.84–6.15 %). Octanal and trans–β–ionone were identified as the key odorants in the chestnut–like aroma (Zhu et al., 2018), whereas cis–jasmone was considered to impart a floral odor. Dimethyl sulfide was the dominant odorant in cooked corn–like aroma (Liao et al., 2020). The volatiles profile was consistent with the sensory evaluation.

### 3.5. Differences in aroma formation

During tea manufacturing, carotenoids, fatty acids, glycosides, amino acids, and carbohydrates are important aroma precursors for the formation of the volatile compounds (Ho et al., 2015; Feng et al., 2019). Unsaturated fatty acids are the important precursors for 6–10 carbon aroma compounds that contribute fresh and grass odors in tea infusion. As shown in Fig. 5, most fatty acid–derived volatiles (FADVs) were abundant in HJY green teas, for example *(E)*–2–hexen–1–ol (with fresh and green odor) and jasmine lactone (with jasmine–like floral and fruity odor). Lipoxygenase (LOX) is the key enzyme in biosynthesis of six carbon aldehydes and alcohols from unsaturated fatty acids, located in

| | | NB2/NB1 | HJY1/NB1 | HJY2/NB1 | HJY3/NB1 |
|---|---|---|---|---|---|
| Fatty Acids | *(E)*-2-Hexenal | 3.80 | 1.92 | 11.31 | 2.20 |
| | *(Z)*-3-Hexen-1-ol | 1.33 | 7.63 | 14.89 | 8.20 |
| | cis-Jasmone | 1.15 | 2.85 | 2.60 | 4.43 |
| | Methyl jasmonate | 1.23 | 3.60 | 3.08 | 8.49 |
| | *(E)*-2-Hexen-1-ol | 1.00 | 74.50 | 203.90 | 194.03 |
| | Jasmine lactone | 1.00 | 149.04 | 98.46 | 208.81 |
| | 1-Hexanol | 1.30 | 1.39 | 1.83 | 1.42 |
| | Heptanal | 1.15 | 0.93 | 1.73 | 1.01 |
| | 1-Heptanol | 1.34 | 1.36 | 1.94 | 1.20 |
| | 1-Nonanol | 2.23 | 1.15 | 2.62 | 1.41 |
| | *(E,E)*-2,4-Heptadienal | 6.06 | 1.57 | 1.16 | 1.65 |
| | Octanal | 1.75 | 0.78 | 1.19 | 1.29 |
| | *(E)*-2-Octenal | 3.54 | 0.96 | 1.33 | 1.12 |
| | Decanal | 1.51 | 0.93 | 1.22 | 1.47 |
| | Nonanal | 1.15 | 0.71 | 1.24 | 1.34 |
| | 1-Pentanol | 0.96 | 1.74 | 1.43 | 0.64 |
| | 1-Penten-3-ol | 2.20 | 1.26 | 0.89 | 0.62 |
| | 1-Octanol | 1.23 | 0.88 | 1.17 | 0.97 |
| | 3,5-Octadien-2-one | 2.12 | 1.05 | 1.08 | 0.90 |
| | *(E)*-2-Octen-1-ol | 4.30 | 0.78 | 0.94 | 0.88 |
| | Pentanal | 1.07 | 0.65 | 0.71 | 0.67 |
| | Hexanal | 1.62 | 0.73 | 0.92 | 0.62 |
| | 1-Octen-3-ol | 3.19 | 0.67 | 0.72 | 0.66 |
| | *(E)*-2-Heptenal | 4.19 | 0.93 | 1.01 | 0.00 |
| | *(E)*-2-Decenal | 5.13 | 0.00 | 0.00 | 0.00 |
| Glycosides | Benzyl alcohol | 0.94 | 4.51 | 3.42 | 2.89 |
| | Nerol | 1.00 | 68.18 | 165.98 | 78.71 |
| | Geraniol | 0.80 | 1.69 | 4.46 | 1.67 |
| | Linalool oxide II | 0.95 | 1.70 | 8.96 | 3.28 |
| | Linalool | 1.10 | 1.72 | 3.41 | 2.02 |
| | Phenylethyl alcohol | 1.09 | 1.71 | 1.87 | 1.34 |
| | Methyl salicylate | 0.68 | 1.72 | 8.74 | 1.49 |
| | Linalool oxideI | 2.87 | 0.90 | 2.41 | 1.03 |
| | Linalool oxide III | 0.99 | 0.83 | 2.40 | 1.96 |
| | Linalool oxide IV | 0.91 | 0.70 | 2.23 | 1.32 |
| | Benzaldehyde | 1.19 | 1.27 | 1.08 | 1.05 |
| AA/Carb. | Phenylethyl alcohol | 1.09 | 1.71 | 1.87 | 1.34 |
| | 1-(1H-Pyrrol-2-yl)ethan-1-one | 0.52 | 0.38 | 0.69 | 2.17 |
| | Dimethyl sulfide | 0.51 | 0.25 | 1.22 | 0.29 |
| | 3-Methylbutanal | 1.14 | 0.40 | 0.74 | 0.25 |
| | 2-Methylbutanal | 0.79 | 0.42 | 0.90 | 0.38 |
| | 1-Ethylpyrrole | 0.41 | 0.77 | 0.88 | 1.03 |
| | Benzeneacetaldehyde | 0.90 | 0.47 | 0.81 | 0.38 |
| | 1-Ethyl-1H-pyrrole-2-carboxaldehyde | 0.31 | 0.43 | 0.60 | 0.81 |
| | Indole | 0.49 | 0.72 | 0.20 | 0.69 |
| Carotenoids | Dihydroactinidiolide | 1.81 | 2.28 | 2.64 | 3.93 |
| | *(E)*-β-Farnesene | 1.13 | 3.52 | 1.98 | 4.39 |
| | trans-β-Ionone | 0.82 | 2.21 | 2.17 | 2.95 |
| | β-Ionone epoxide | 0.87 | 3.86 | 2.60 | 4.87 |
| | Nerolidol | 1.02 | 3.95 | 2.06 | 5.06 |
| | α-Ionone | 0.99 | 1.49 | 1.74 | 1.42 |
| | 6-Methyl-5-Hepten-2-one | 1.76 | 0.94 | 1.36 | 1.18 |
| | Safranal | 0.85 | 0.58 | 1.10 | 0.85 |

**Fig. 5.** Volatile categories based on aroma precursors.

chloroplasts (Ho et al., 2015); importantly, the chloroplasts in pale leaves of albino tea varieties have a non-mature structure compared to normal green leaves (Zhang et al., 2019). In addition, the accumulation of fatty acids was closely related to chlorophyll content. It was observed that fatty acid–derived volatiles accumulated more in NB2 compared to NB1 green tea. As shown in Fig. 1, NB1 tea shoots showed stronger albino phenotype compared to NB2 tea shoots.

The chloroplast ultrastructure of the second leaf of NB and HJY tea shoots were observed via a transmission electron microscope (TEM) (Fig. 6A and B). The TEM images indicated that the chloroplast structure in NB and HJY leaves was abnormal (Fig. 6C and 6D), containing fewer grana and thylakoids compared to normal green leaves (Wang et al., 2014). Normal fully developed chloroplasts should have prominent grana and thylakoids with no abnormality in thylakoid membranes and granular stacking (Jiang et al., 2020). It was surprising that chloroplasts in NB leaves contained more thylakoids compared to HJY leaves, and the contents of chlorophyll *a* and b were higher in NB than HJY tea shoots (Fig. 6E). The albino tea variety, 'white leaf No.1′, in the pre–albinistic stage had the chloroplasts with grana, thylakoids and stroma thylakoids, but in the albinistic stage grana were mostly absent (Li et al., 2011). Based on chloroplast structure observed in the present study, NB tea leaves were in the pre–albinistic stage but did not reach the albinistic stage. It is suggested that chloroplasts of albino tea varieties with white tea shoots change significantly during the pre–albinistic, albinistic, and regreening stages, which would strongly influence the formation of volatile compounds.

Glycosidically bound volatiles (GBVs) play important roles in tea aroma formation and are released during withering stage in green tea manufacturing, due to the presence of endogenous glycosidase (Cui et al., 2016a). For glycosidically bound volatiles, terpenoids (such as linalool and its oxides and geraniol) are an important class of odorants. The mevalonic–acid (MVA) pathway and 2–C–methyl–erythritol–4–phosphate (MEP) pathway are responsible for formation of terpenoid precursors; the enzymes of MEP pathway are generally localized to plastids (Nagegowda, 2010). However, defective chloroplasts were observed in yellow leaves of tea plants (Liu et al., 2017). Chloroplasts that are not completely formed may affect the synthesis, accumulation and transformation of some metabolites in the aroma derivation pathway (Wang et al., 2015; Wang et al., 2014; Li et al., 2011). The concentration of geranyl diphosphate, an important precursor for biosynthesis of monoterpenes, was significantly lower in albino than in normal green tea leaves (Dong et al., 2018). In this study, the concentration of most GBVs was higher in HJY than NB green teas. It is proposed that chloroplast structure has a negative effect on GBVs formation in NB tea shoots, and genes involved in biosynthesis of GBVs have a different expression pattern between NB and HJY.

Carotenoid–derived volatiles (CDVs), such as β–ionone and β–damascenone, are formed during tea manufacturing (Feng et al.,

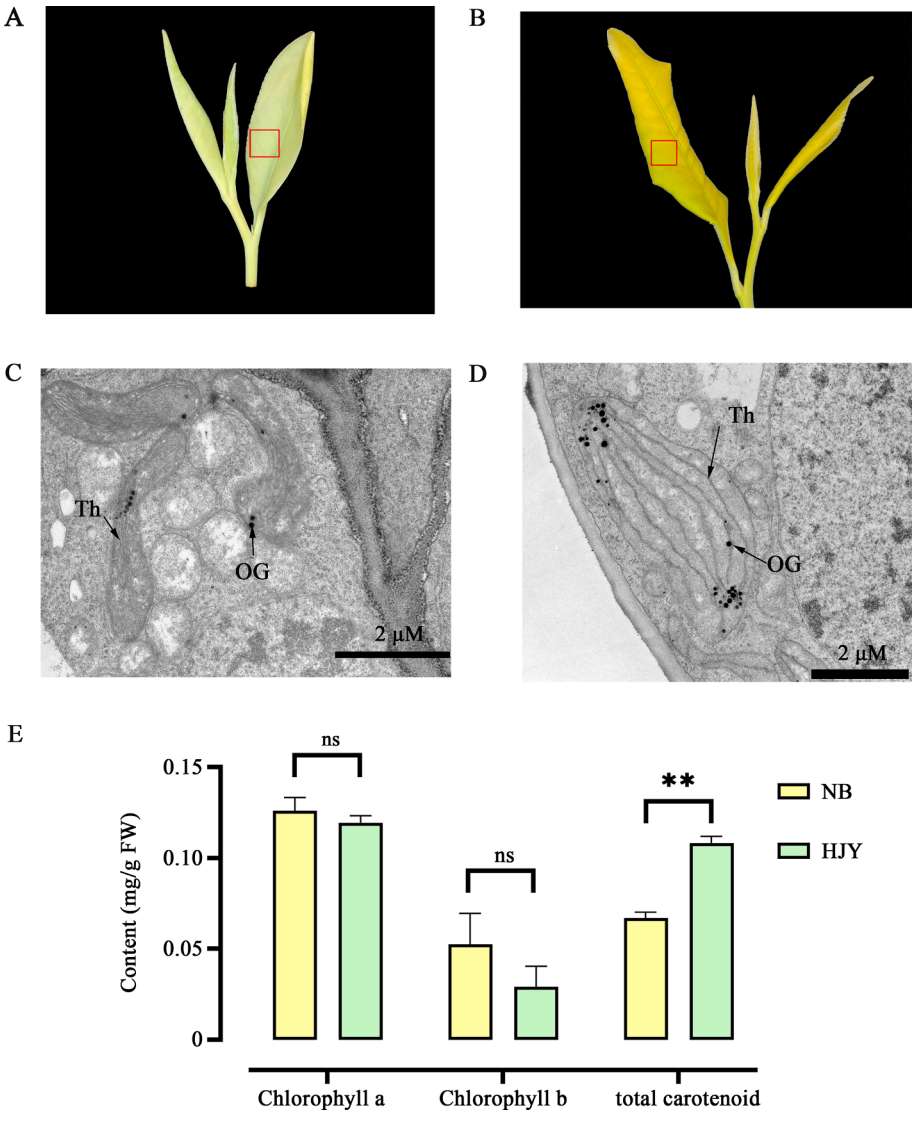


**Fig. 6.** Transmission electron microscope analysis and concentrations of chlorophyll *a* and b and carotenoids. (A) The white shoots of NB tea variety. (B) The yellow shoots of HJY tea variety. (C) Chloroplast structures of NB tea leaf. (D) Chloroplast structures of HJY tea leaf. (E) Concentration of chlorophyll *a* and b and carotenoids. Different letters denote significant difference ($p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2019) and usually contribute to sweet, floral, or fruity odors (Ho et al., 2015). In the present study, CDVs were present in higher concentration in HJY than NB green teas. The concentration of total carotenoids was lower in albino than in normal green tea leaves (Liu et al., 2017), possibly resulted in a difference in formation of carotenoid–derived volatiles. It was reported that carotenoid biosynthesis and accumulation of tea pigments are influenced significantly by light (Fu et al., 2022). The pigment concentrations in white and yellow tea shoots were detected by ultraviolet spectrophotometry (Fig. 6E). Carotenoid concentration was significantly higher in yellow than in white tea shoots, possibly resulting in higher concentration of carotenoid–derived volatiles in HJY than NB green teas.

Amino acids concentration is closely related to the formation of amino acid–derived volatiles (Guo, Ho, Schwab, & Wan, 2021). During tea processing, L–theanine might react in thermal reaction to form nitrogen–containing heterocyclic compounds. In our study, the total concentrations of amino acids and also of amino acid-derived volatiles were significantly higher in NB than HJY green teas, suggesting that high concentrations of amino acids contribute to accumulation of amino acid-derived volatiles during green tea processing.

## 4. Conclusions

Green teas manufactured from albino tea varieties were comprehensively characterized in this study. NB green teas made from white tea shoots contained abundant amino acids, especially theanine, and had less galloylated catechins, contributing to their umami taste. By contrast, HJY green teas made from yellow tea shoots had mellow taste due to high accumulation of galloylated catechins. The strong expression of *CsTA* and high concentration of GA may be the reasons for galloylated catechins being highly concentrated in HJY green teas. Volatile compounds had a higher concentration in HJY than NB green teas and accumulation of those aroma precursors were closely related to formation of volatile compounds during green tea processing. This study defines a metabolite profile of the main taste and aroma compounds in green teas processed from white and yellow tea shoots and showed a different expression pattern of key biosynthetic and catabolic enzymes in two types of albino tea varieties.

## 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.fochms.2022.100143.

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