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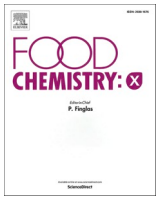

Chemical profile and *in-vitro* bioactivities of three types of yellow teas processed from different tenderness of young shoots of Huoshanjinjizhong (*Camellia sinensis* var. *sinensis*)

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ABSTRACT

In the present study, bud yellow tea (BYT), small-leaf yellow tea (SYT) and large-leaf yellow tea (LYT) were produced from the same local "population" variety Huoshanjinjizhong (*Camellia sinensis* var. *sinensis*), and the effects of raw material tenderness on the chemical profile and bioactivities of these teas were investigated. The results showed that 11 crucial compounds were screened by headspace solid-phase microextraction-gas chromatography–mass spectrometry from 64 volatiles in these yellow teas, among which the heterocyclic compounds showed the greatest variations. In addition, 43 key compounds including organic acids, flavan-3-ols, amino acids, saccharides, glycosides and other compounds were screened by liquid chromatography-mass spectrometry from 1781 non-volatile compounds. BYT showed the best α-glucosidase inhibitory activity and antioxidant capacity among the selected yellow teas, which might be contributed by the higher content of galloylated catechins. These findings provided a better understanding of the chemical profile and bioactivities of yellow teas.

1. Introduction

Yellow tea, a slightly-fermented type of tea, is one of the six traditional Chinese teas. It has a long history in China and is mostly produced in Anhui, Zhejiang, Guangdong, Hunan, and Hubei provinces, of which Anhui province accounts for over half of the total production area and yield distribution in China (Feng et al., 2023). Nowadays, yellow tea has attracted increasingly attention because of its smooth, mellow flavor and multiple health benefits, including antioxidant, anti-metabolic syndrome, anti-cancer and gut microbiota harmony (Kujawska et al., 2016; Tang et al., 2019; Zhou et al., 2018; Zhou et al., 2022). Usually, the manufacturing process of yellow tea mainly includes fixation, rolling, yellowing and drying. It is distinctive from other tea categories by its yellow colour characteristics (yellow dry tea, yellow tea infusion, and yellow infused leaves), which is related to its specific manufacturing process "yellowing".

According to the maturity of raw materials, yellow tea can be classified into bud yellow tea (BYT), small-leaf yellow tea (SYT), and large-leaf yellow tea (LYT) (Guo et al., 2019), in which the raw materials of BYT are the tenderest and LYT are the coarsest (Fig. 1A). The distinctive volatile components in yellow tea worked in combination to form a unique and pleasant flavor quality that favored by many consumers. A study had found that alcohols and esters were the dominant volatiles in BYT and SYT samples and 25 key odorants were characterized as the unique aroma compounds of yellow tea (Shi et al., 2021). Besides, different aroma types of yellow teas were also considered varying differently in the key compounds of their aroma attributes (Hong et al., 2023). For example, the roasty aroma was mainly correlated to pyrazines, while fresh and grassy aromas were mostly associated with alcohols and esters. In addition, yellow tea was considered rich in various metabolites, such as catechins, flavonoids, amino acids, which were important material basis responsible for its special taste and biological activity (Tang et al., 2019). A study have also shown that amino acids, catechins and flavonoid glycosides were strongly correlated with the roasting degree of LYT samples (Li et al., 2023). Although studies have reported the effects of diverse factors, such as growth condition, variety, climate and processing on the composition and content of metabolites in yellow teas, the impact of raw materials, which might highly influence

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the quality of final products deserves in-depth investigation.

Previous research mainly concentrated on the effect of processing on yellow tea metabolite variation, such as the yellowing duration and roasting degrees (Fan et al., 2022; Li et al., 2023; Wei et al., 2021). However, few studies focused on how raw material maturity would influence the chemical profile of yellow tea products, as well as biological activities. Therefore, the volatile and non-volatile metabolites, as well as their potential bioactivities in yellow tea samples made from different plucking standards are worthy to be analyzed. Currently, mass spectrometry combined with metabolomics multivariate analysis became a useful tool for processing multi-dimensional data in complex matrices, which was widely applied to comprehensively investigate the chemical profile of teas (Wen, Sun, et al., 2023). Although there have been some reports on the analysis of volatile and non-volatile compounds of these three types of yellow teas, researchers targeted on yellow tea samples originated from diverse geographical regions or made from various varieties (Li et al., 2024; Shi et al., 2021). In addition, although some studies have reported that yellow teas possessed great *in vitro* activities, such as antioxidative and α-glucosidase inhibitory effects (Tang et al., 2019; Zhou et al., 2018), there is still a lack of systematic comparison between the *in vitro* activities of yellow teas with different material maturities. Meanwhile, the selection of a same tea variety can minimize the effects of other external factors. Therefore, in this study, we picked BYT, SYT and LYT samples from the same variety (Huoshanjinjizhong) at a local tea plantation to explore the effect of raw material maturity on the chemical profile and underlying bioactivities of the yellow teas. Headspace solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC–MS) was applied to study the volatile compounds in these yellow teas. Liquid chromatography-quadrupole time-of-flight mass spectrometry (LC-Q-TOF-MS) combined with widely untargeted metabolomics were used to investigate the non-volatile metabolites. Meanwhile, the *in vitro* bioactivities regarding α-glucosidase inhibitory effect and antioxidant capacity of these yellow teas were also explored.

2. Material and methods

2.1. Samples and chemicals

2.1.1. Tea samples

Three types of yellow tea samples, BYT, SYT and LYT were provided by Yunwufeng Ecological Agriculture Company (Anhui, China). All samples were produced from a same local "population" variety of Huoshanjinjizhong, which were harvested from Huoshan, Anhui, China in April 2023. Finished tea samples were obtained after traditional yellow tea manufacturing process, which mainly includes fixation, rolling, yellowing, drying or roasting, and the manufacturing procedure was similar as described in a previous study (Wei et al., 2021). All samples were lyophilizated and stored at −20 °C before the experiment. The appearance of the dry tea, tea infusion and infused leaves were shown in Fig. 1A.

2.1.2. Standards and reagents

The aroma chemicals were provided commercially and the purity were higher than 95 %: 2-ethyl-3,5-dimethylpyrazine and dimethyl sulfide (Macklin, Shanghai, China); geraniol, hexanal and γ-nonalactone (Aladdin, Shanghai, China). Ethyl decanoate (99 %) was obtained from Aladdin Biochemical Technology (Shanghai, China). Standards of (−)-epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), (−)-epicatechin (EC), (−)-gallocatechin (GC), (+)-catechin (C), gallic acid (GA), caffeine (CAF), and theobromine (THB) were purchased from Shanghai Yuanye Biotechnology Company (Shanghai, China) and the purity were higher than 97 %. L-theanine (Thea), L-serine (Ser), L-glutamic acid (Glu), *L*-phenylalanine (Phe), L-threonine (Thr), L-aspartic acid (Asp), L-tyrosine (Tyr), L-tryptophan (Trp), γ-aminobutyric acid (GABA), L-lysine (Lys), L-valine (Val), L-aspartic acid (Asn), L-glutamine (Gln) were purchased from Sigma-Aldrich Company and the purity were higher than 98 %. α-Glucosidase, 1,1-diphenyl-2-picrylhydrazyl (DPPH), total antioxidant capacity test kits [including 2,2′-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and ferric reducing antioxidant power (FRAP) assays] were purchased from Shanghai Yuanye Biotechnology Company (Shanghai, China).

2.2. Tea sample preparation

2.2.1. Tea infusion preparation for volatile analysis

To prepare tea samples for the analysis of volatile compounds, tea leaves (3 g) were brewed with ultrapure water (95 °C, 150 mL), and after 5 min the tea infusion was quickly filtered according to China national standard GB/T 23776–2018 (Methodology for sensory evaluation of tea). The aqueous extracts were rapidly cooled in an ice bath, prior to

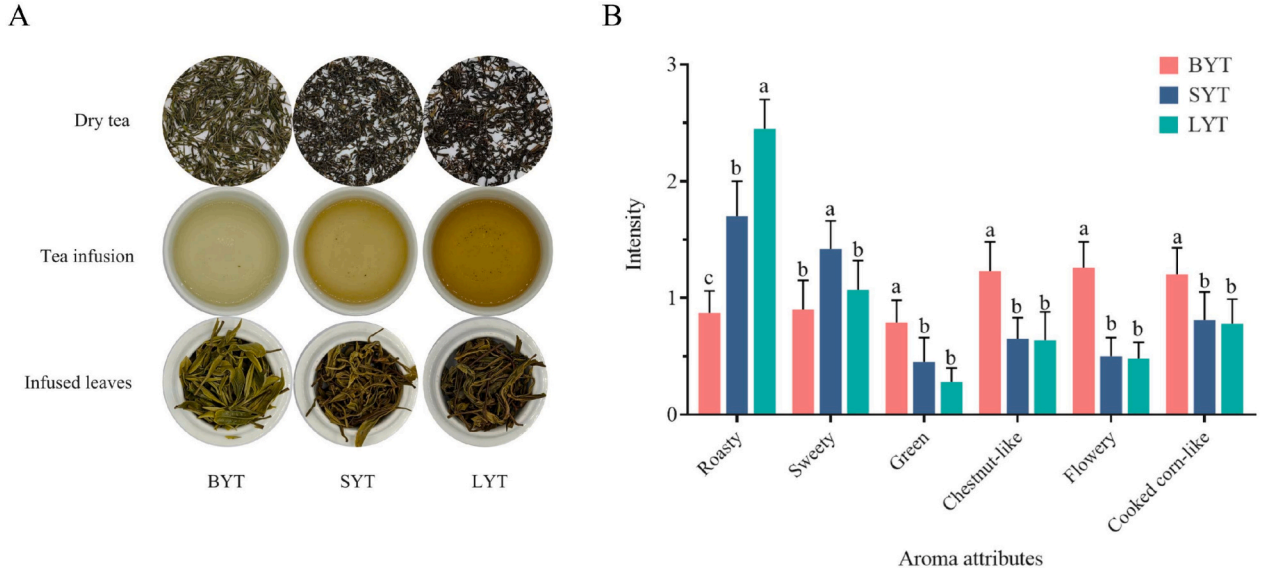

Fig. 1. The appearance (dry tea, tea infusion and infused leaves) (A) and aroma profile analysis (B) of three types of yellow teas. Different letters indicated a significant difference at the $p < 0.05$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analysis.

2.2.2. Tea infusion preparation for non-volatile compounds and bioactivity analysis

Before the analysis of chemical compounds in tea samples, yellow tea samples were ground into tea powder (200 Tyler mesh) before extraction. In brief, 100.00 mg of each tea sample was weighed into a 5 mL centrifuge tube and mixed with 4 mL pure water. The mixture was subsequently extracted in a water bath for 10 min at 100 °C. Then the extract was centrifuged for 10 min at 4293 ×*g* after cooling to room temperature. The extraction procedure was repeated once with 4 mL pure water. Finally, the supernatants were combined and diluted to 10 mL with pure water. The combined sample solution was filtered through 0.22 μm Millipore filter membrane prior to ultra-high performance liquid chromatography (UHPLC), ultra-performance liquid chromatography-triple quadrupole tandem mass spectrometry (UPLC-QQQ-MS/MS), LC-Q-TOF-MS and *in vitro* bioactivity analysis.

2.3. Aroma profile analysis (APA) of yellow teas

Aroma evaluation was conducted in a specialized room by four males and six females (aged from 22 to 28 years), who were weekly trained for at least a half year in olfactory perception with different reference standards and foodstuffs. In the current study, the aroma attributes and the corresponding reference chemicals (or foodstuff) used were roasty (2-ethyl-3,5-dimethylpyrazine), cooked corn-like (dimethyl sulfide), sweety (γ-nonalactone), flowery (geraniol), green (hexanal) and chestnut-like (chestnut). All reference chemicals were dissolved in ethanol and diluted in water at a concentration of 100-fold above the odor thresholds (Wang et al., 2022). For the aroma profile analysis, each aroma attribute was scored from 0 (not perceivable) to 3 (strongly perceivable) in 0.1 unit of intensity.

2.4. Volatile metabolites analysis by HS-SPME-GC–MS

The volatile compounds in yellow tea samples were analyzed using HS-SPME as previously described with slight modifications (Wang et al., 2022). Briefly, stable flex fiber (divinylbenzene/carboxen/polydimethylsiloxane, DVB/CAR/PDMS, 50/30 μm, Supelco, Oakville, Canada) was used to extract the volatile compounds from the headspace of freshly prepared tea infusion mentioned above. Tea infusion (10 mL) and internal standard solution (10 μL, 7.47 μg/mL of ethyl decanoate) with NaCl (2 g) were placed into a 20 mL headspace vial, equilibrated for 15 min, and further extracted for 30 min at 30 °C.

HS-SPME was performed on an Agilent 7890B GC (Agilent Technologies, Palo Alto, CA, USA) equipped with a 5977B mass selective detector (MSD) (Agilent Technologies, Palo Alto, CA, USA). A HP-5MS GC column (30 m × 0.25 mm, 0.25 μm film thickness) was installed and the GC injection was set as splitless mode. Helium (99.999 %) was used as a carrier gas with a linear velocity of 30 cm/s. For HS-SPME analysis, the fiber was desorbed in the GC injection port at 250 °C for 5 min. The temperature program applied for HP-5MS column was as follows: 35 °C for 5 min, ramp of 4 °C/min up to 100 °C, then ramp of 8 °C/min up to 260 °C, and hold for 5 min. The MSD was set in the electron ionization (EI) positive mode with an electron energy of 70 eV and a mass scanning range of *m/z* 30–350.

Volatile compounds were identified based on the National Institute of Standards and Technology (NIST) Chemistry WebBook 2020 database library. The retention indices (RIs) of the compounds were determined using a mixture of *n*-alkane series (C_5-C_{40}) and compared with the theoretical values. RI values were calculated using Eq. (1):

$$RI = 100n + 100 \times \frac{Rt_x - Rt_n}{Rt_{n+1} - Rt_n} \quad (1)$$

where Rt_x is the retention time of each volatile metabolite (*x*), and Rt_n and Rt_{n+1} are the retention times of *n*-alkanes before and after the volatile metabolite (*x*) under the same analytical conditions, respectively.

The quantitative analysis on volatiles was mainly based on their peak areas from the results of GC–MS analysis. SIMCA-P software 14.1 was used for multivariate analysis, including principal component analysis (PCA), hierarchical cluster analysis (HCA) and orthogonally corrected partial least squares discriminant analysis (OPLS-DA) analysis.

2.5. Quantification of catechins, purine alkaloids and gallic acid in yellow tea samples by UHPLC

To determine the contents of catechins (EGCG, GCG, EGC, ECG, EC, GC, C), purine alkaloids (CAF, THB) and gallic acid in three types of yellow tea samples, a UHPLC (Thermo Scientific Dionex UltiMate 3000, USA) system consisted of a degasser, an auto-sampler (WPS-3000RS), a binary pump (HPG- 3400RS), a column compartment (TCC-100) and a diode array detector (DAD, DAD-3000) was used. The determination was conducted according to our previous methods (Wen, Zhou, et al., 2023) with slight modifications. The separation of these compounds was conducted on an Acquity UPLC® BEH Shield RP18 column (2.1 × 50 mm, 1.7 μm, Waters, USA) at 40 °C with a flow rate of 0.25 mL/min. The injection volume was set to 1 μL and the detection wavelength was set to 278 nm. The elution gradient of mobile phase A (0.1 % formic acid–-water, *v*/v) and B (acetonitrile) was as follows: 0–2.5 min, 98 % A; 2.5–3 min, 98–95 % A; 3–8 min, 95–85 % A; 8–14 min, 85–70 % A; 14–16 min, 70–50 % A; 16–18 min, 50–10 % A; 18–20 min, 10–5 % A; 20–22 min, 5–98 % A; 22–26 min, 98 % A.

2.6. Quantification of free amino acids by UPLC-QQQ-MS/MS

To determine the contents of free amino acids in three types of yellow tea samples, a triple-quadrupole linear ion trap mass spectrometer (API 5500 QTRAPTM MS/MS system from AB Sciex, Concord, ON, Canada) equipped with an electrospray (Turbo VTM) ion source coupled to an ExionLC system (AB Sciex) was used. The determination was conducted according to our previous methods (Zhou et al., 2023) with slight modifications. The chromatographic separation was performed on a Comixshell CARP column (2.1 × 50 mm, 1.8 μm) at 35 °C with a flow rate of 1 mL/min. The injection volume was set to 5 μL and the elution gradient of mobile phase A (water, containing 0.02 % formic acid) and B (acetonitrile, containing 0.2 % formic acid) was as follows: 0–2 min, 2 % B; 2–17 min, 2–100 % B; 17–19 min, 100 % B; 19–21 min, 100–2 % B; 21–25 min, 2 % B. Other parameters for mass spectrum analysis including ion spray voltage, turbo spray temperature, nebulizer gas, heater gas, and curtain gas were set at 5500 V, 500 °C, 50 psi, 50 psi, and 30 psi, respectively.

2.7. LC-Q-TOF-MS based untargeted metabolomics analysis

For the analysis of the chemical differences in these yellow tea samples, an Agilent 1290 LC system (Agilent Technologies, Palo Alto, CA, USA) coupled with a time-of-flight mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) was applied. The chromatography conditions were set the same as described in *Section 2.5*. The mass spectrometer was conducted in the negative ionization mode over a full scan range of *m/z* 100–1500 with the following settings: sheath gas temperature, 350 °C; nebulizer, 35 psi; gas flow, 8 L/min; gas temperature, 320 °C; sheath gas flow, 11 L/min.

For the metabolomics analysis, the collected MS data was first inputted into MS-DIAL software (version 5.1) for peak picking, peak alignment, deconvolution, and denoising. Then, the output data matrix from MS-DIAL processing (including mass-to-charge ratio (*m/z*), retention time (t_R), and peak area) was imported into SIMCA-P software (version 14.1, Umetrics, Umea, Sweden) for PCA, HCA and OPLS-DA analysis. VIP values and Loading plot were obtained during OPLS-DA analysis to search for marker compounds in various yellow tea samples.

2.8. In vitro α-glucosidase inhibition assay and antioxidant capacity analysis (ABTS, DPPH, FRAP)

Yellow tea aqueous solutions were diluted to several concentrations (50, 62.5, 75, 100, 200, 250 μg/mL) to assess their inhibition effects against α-glucosidase. The inhibition assays and the inhibition rate calculations were performed similarly as described with our previous reports (Yang et al., 2022).

The total antioxidant capacity of yellow tea samples was analyzed using ABTS, DPPH and FRAP methods. Yellow tea aqueous solutions were diluted to a series of concentrations (0.05, 0.1, 0.25, 0.5, 0.75 and 1 mg/mL for ABTS assay; 0.001, 0.005, 0.01, 0.025, 0.1 and 1 mg/mL for DPPH assay; 0.1, 0.15, 0.2, 0.25, 0.3 and 0.35 mg/mL for FRAP assay) to assess their antioxidant capacities according to our previous study (Yang et al., 2022).

2.9. Statistics analysis

The results were expressed as mean ± standard deviation (SD, $n = 3$). One-way analysis of variance (ANOVA) using Duncan's test was performed in SPSS software (version 25) and $p < 0.05$ indicated statistical significance.

3. Results and discussion

3.1. Aroma profiles of three types of yellow tea infusions

In the present study, APA was conducted to gain a comprehensive understanding of the aroma profiles of three types of yellow teas. Six aroma attributes were selected by the trained evaluation panel based on their frequency of occurrence during preliminary trials. As shown in Fig. 1B, three types of yellow teas showed different aroma profiles. The roasty smell (2.5) was the most intense attribute in LYT infusion among all aroma attributes compared to other yellow tea infusions, which was mainly due to the characteristic roasting manufacturing process. In addition, SYT mainly showed sweety and roasty aromas. On the contrary, BYT had the highest scores on green (0.8), chestnut-like (1.2), flowery (1.3) and cooked corn-like (1.2) aroma attributes compared with SYT and LYT, which might mainly stem from the differences in the tenderness of the raw materials.

3.2. Overview volatile metabolites in three types of yellow teas

3.2.1. The composition of volatile metabolites

According to the results obtained from HS-SPME-GC–MS analysis, a total of 64 volatile metabolites were identified from these three types of yellow teas by comparing the mass spectrometry fragments and RIs (Table 1). The total number of identified aroma compounds in BYT, SYT and LYT were 59, 62 and 62, respectively (Fig. S1A). These metabolites could be tentatively categorized into seven groups, including aldehydes, heterocyclic compounds, ketones, alcohols, esters, alkenes and other compounds. In addition, the relative content of all volatile metabolites was calculated referencing the internal standard compound (ethyl decanoate). The results showed that LYT had the highest concentration (169.71 μg/L) of total volatile compounds among these three types yellow teas (Fig. S1B). Among all these compounds, the content of heterocyclic compounds (such as pyrrole and furan) was found to be lowest in BYT (9.36 μg/L), followed by SYT (36.10 μg/L) and highest in LYT (82.85 μg/L). Previous study have found that heterocyclic compounds were mainly the product of Maillard reaction generated from high-temperature roasting and increased with roasting degree (Li et al., 2023). Moreover, it was reported that compared with small fire and medium fire roasting, old fire roasting produced higher levels of total volatiles and heterocyclic compounds in LYT, which was essential for the formation of crispy-rice-like odor (Guo et al., 2021), while similar discovery was found in oolong tea (Wang et al., 2023).

3.2.2. Multivariate analysis and critical marker compounds of volatile metabolites

To further analyze the variations of volatile metabolites in three types of yellow teas, multivariate analysis was performed based on the quantitative data. As shown in Fig. 2A, PCA analysis could clearly distinguish the three types of yellow teas. HCA analysis (Fig. 2B) revealed that the yellow teas could be further divided into two groups, BYT and SYT being clustered into one group and LYT into another one. Therefore, in the supervised OPLS-DA model (Fig. 2C), we divided these yellow tea samples into two groups as mentioned above, and searched for critical marker compounds *via* VIP values and loading plot. As shown in Fig. 2D and Fig. 2E, 11 volatile metabolites (VIP > 1) including 1-ethylpyrrole, 2-methylbutanal, 1-ethyl-1H-pyrrole-2-carbaldehyde, dimethyl sulfide, toluene, linalool, 2-methylfuran, 3-methylbutanal, 4-methyl-3-penten-2-one, 2-ethyl-5-methylpyrazine and methyl salicylate were identified as critical marker compounds.

In total, 15 aldehydes were identified in these yellow teas. The concentration of aldehydes in these teas decreased from BYT to SYT and increased from SYT to LYT (Fig. S1B). The content of aldehydes in LYT samples were generally higher compared with others. In particular, 2-methylbutanal (VIP 2.70) and 3-methylbutanal (VIP 1.30) were recognized as the key differential compounds between LYT and BYT, SYT, which were more abundant in LYT sample. Study have found that 2-methylbutanal and 3-methylbutanal (with malty smelling) were key odorants in LYT (Zhai et al., 2023). Although the concentration of aldehydes in LYT was higher than that in BYT, it was discovered that the proportion of aldehydes in total volatile compounds were the highest in BYT samples, which may be related to the high raw material tenderness of BYT. In addition, aldehydes were also the key aroma compounds of green tea, and some of them were produced from the Strecker degradation of amino acids during processing (Zhu et al., 2021).

The heterocyclic compounds identified in the present study mainly composed of pyrroles, pyrazines, furans and oxides. Almost all heterocyclic compounds showed the lowest content in BYT and the highest in LYT. Specifically, four heterocyclic compounds including 1-ethylpyrrole (VIP 5.02), 1-ethyl-1H-pyrrole-2-carbaldehyde (VIP 2.37), 2-methylfuran (VIP 1.34) and 2-ethyl-5-methylpyrazine (VIP 1.11) were screened as critical differential compounds. As the compound with the highest VIP value, 1-ethylpyrrole with roasty aroma note was produced from the Maillard reaction of sugar and theanine (Li et al., 2022). Most heterocyclic compounds in LYT including 1-ethylpyrrole and 1-ethyl-1H-pyrrole-2-carbaldehyde increased in content with the increase of the roasting degree (Li et al., 2023). Pyrazines such as 2-ethyl-5-methylpyrazine and 2-ethyl-3,5-dimethylpyrazine were also reported with roasty odor (Zhai et al., 2023), which may be the reason for the high roasty odor attribute of LYT (Fig. 1B). In addition, it was considered that amino acids (especially theanine) participated in the formation of pyrazines during the roasting process of tea leaves, which was closely related to the containing amide group (Li et al., 2022; Sasaki et al., 2017). Furthermore, furans, a kind of *O*-heterocyclic compounds, were mainly produced from thermal processing, such as the thermal reaction between theanine and glucose.

The concentrations of ketones and alcohols were found to be lower in BYT, SYT and relatively higher in LYT. In particular, 4-methyl-3-penten-2-one and linalool were considered critical volatile compounds with VIP values of 1.24 and 1.42, respectively. 4-Methyl-3-penten-2-one was reported as one of the feature components for the differential aroma quality between yellow and green teas (Wen, Zhu, et al., 2023). Furthermore, alcohols were one of the dominant volatiles in 15 kinds of yellow teas (BYT and SYT), in which linalool was emerged as one of the 25 principal key odorants responsible for the distinctive aroma of yellow teas (Shi et al., 2021). Linalool was also considered key aroma compounds in green tea, which was mainly generated from glycosides (Ho et al., 2015; Yu et al., 2023). Although the content of linalool was reported to be higher in tea made from stem than from leaves, its content also increased after the roasting process, in which the cellular tissue of

Table 1
Characterization of volatile components of three types of yellow teas based on HS-SPME-GC–MS analysis.

No.	Compounds[a]	Odor attribute[b]	CAS	RI[c]	RI[d]	Identification[e]	VIP[f]
1	dimethyl sulfide	cooked corn-like	75–18-3	< 600	526	MS, RI	1.85
2	butanal	pungent, green	123–72-8	< 600	595	MS, RI	0.70
3	2-methylfuran	unknown	534–22-5	< 600	598	MS, RI	1.34
4	ethyl acetate	solvent-like, fruity	141–78-6	608	612	MS, RI	0.21
5	3-methylbutanal	malty	590–86-3	642	649	MS, RI	1.30
6	2-methylbutanal	malty	96–17-3	653	659	MS, RI	2.70
7	1-penten-3-ol	pungent, train oil-like	616–25-1	677	683	MS, RI	0.39
8	pentanal	moldy	110–62-3	697	697	MS, RI	0.63
9	2-ethylfuran	butter-like, caramel-like	3208-16-0	702	702	MS, RI	0.62
10	1-methylpyrrole	burnt-like, roasty, nutty	96–54-8	734	743	MS, RI	0.71
11	1H-pyrrole	nutty, sweet	109–97-7	751	755	MS, RI	0.95
12	2-methylpentanal	bitter almond-like, flowery	123–15-9	756	758	MS, RI	0.45
13	toluene	chemical-like	108–88-3	759	760	MS, RI	1.51
14	1-pentanol	fish oil-like	71–41-0	764	765	MS, RI	0.34
15	2-hexanone	fuel-like, fruity	591–78-6	790	790	MS, RI	0.21
16	4-methyl-3-penten-2-one	board-like, nutty, woody	141–79-7	799	798	MS, RI	1.24
17	hexanal	green, grassy	66–25-1	801	801	MS, RI	0.89
18	1-ethylpyrrole	burnt-like, roasty	617–92-5	814	820	MS, RI	5.02
19	butyl acetate	fruity, green	123–86-4	817	812	MS, RI	0.09
20	2,4-dimethyl-1-heptene	unknown	19,549–87-2	840	842	MS, RI	0.19
21	3-methylpyrrole	unknown	616–43-3	842	858	MS, RI	0.32
22	1-hexanol	grassy, marzipan-like	111–27-3	871	867	MS, RI	0.14
23	styrene	solvent-like	100–42-5	888	893	MS, RI	0.34
24	2-heptanone	fruity, green, spicy	110–43-0	892	893	MS, RI	0.46
25	heptanal	green, apricot-like, nutty	111–71-7	902	901	MS, RI	0.48
26	2,5-dimethylpyrazine	roasty, popcorn-like	123–32-0	909	913	MS, RI	0.81
27	α-pinene	fir needle-like, resin-like	7785-70-8	931	935	MS, RI	0.12
28	(*Z*)-3-hexenoic acid methyl ester	unknown	13,894–62-7	935	933	MS, RI	0.16
29	2-ethylpyrrole	unknown	1551-06-0	948	938	MS, RI	0.19
30	(*E*)-2-heptenal	green, fatty	18,829–55-5	957	958	MS, RI	0.14
31	benzaldehyde	bitter almond-like, marzipan-like	100–52-7	958	961	MS, RI	0.77
32	1-octen-3-ol	mushroom-like	3391-86-4	981	981	MS, RI	0.37
33	2,3-octanedione	unknown	585–25-1	986	984	MS, RI	0.24
34	6-methyl-5-hepten-2-one	roasty, popcorn-like	110–93-0	988	988	MS, RI	0.51
35	2-ethyl-6-methylpyrazine	roasty, nutty	13,925–03-6	997	997	MS, RI	0.58
36	2-ethyl-5-methylpyrazine	caramel-like, coffee-like, nutty, roasty	13,360–64-0	999	1000	MS, RI	1.11
37	butyl butanoate	sweet, fruity	109–21-7	997	997	MS, RI	0.05
38	octanal	pungent, fruity, flowery	124–13-0	1003	1003	MS, RI	0.36
39	limonene	citrus-like, green	5989–27-5	1027	1025	MS, RI	0.25
40	2-ethyl-1-hexanol	fruity	104–76-7	1030	1031	MS, RI	0.44
41	2,2,6-trimethylcyclohexan-1-one	pungent, honey-like, citrus-like	2408-37-9	1033	1036	MS, RI	0.61
42	β-ocimene	terpene-like	3338-55-4	1039	1039	MS, RI	0.17
43	phenylacetaldehyde	flowery, cherry-like	122–78-1	1042	1044	MS, RI	0.41
44	2-butenoic acid, butyl ester	unknown	7299-91-4	1045	1046	MS, RI	0.10
45	1-ethyl-1H-pyrrole-2-carbaldehyde	green, fatty	2167-14-8	1048	1046	MS, RI	2.37
46	2-ethyl-3,5-dimethylpyrazine	peanutty, caramel-like, roasty	13,925–07-0	1084	1085	MS, RI	0.74
47	*trans*-linalool oxide (furanoid)	flowery, sweet, woody	34,995–77-2	1087	1088	MS, RI	0.45
48	linalool	citrus-like, flowery	78–70-6	1099	1098	MS, RI	1.42
49	nonanal	citrus-like, soapy	124–19-6	1104	1105	MS, RI	0.34
50	*trans*-linalool oxide (pyranoid)	flowery	39,028–58-5	1177	1178	MS, RI	0.11
51	4-ethylbenzaldehyde	aniseed-like, burnt-like, sweet	4748-78-1	1179	1181	MS, RI	0.36
52	(*E*)-3-hexenyl butanoate	fruity	53,398–84-8	1189	1185	MS, RI	0.33
53	α-terpineol	flowery	98–55-5	1191	1190	MS, RI	0.13
54	methyl salicylate	mint-like, fresh, sweet	119–36-8	1194	1194	MS, RI	1.02
55	decanal	citrus-like, fatty	112–31-2	1206	1206	MS, RI	0.35
56	β-cyclocitral	sweet	432–25-7	1222	1222	MS, RI	0.68
57	(*Z*)-3-hexenyl isovalerate	unknown	35,154–45-1	1235	1238	MS, RI	0.49
58	(*E*)-β-damascenone	cooked, apple-like	23,726–93-4	1388	1386	MS, RI	0.14
59	(*Z*)-jasmone	flowery	488–10-8	1402	1395	MS, RI	0.20
60	α-ionone	flowery, violet-like	127–41-3	1432	1426	MS, RI	0.28
61	geranylacetone	flowery	3796-70-1	1456	1453	MS, RI	0.22
62	(*E*)-β-ionone	violet-like, raspberry-like, flowery	79–77-6	1491	1491	MS, RI	0.67
63	2,4-di-tert-butylphenol	phenolic, leather-like	96–76-4	1514	1513	MS, RI	0.23
64	cedrol	woody	77–53-2	1616	1611	MS, RI	0.61

Note: [a] Compounds were consecutively numbered according to their retention indices on HP-5MS column. [b] Odor attribute found in the literatures (Li et al., 2023; Wang, et al.,2023) and databases (vcf-online, leibniz-lsb and flavornet). [c] Retention indices calculated from the retention times of the compounds and a homologous series of *n*-alkanes. [d] Retention indices of compounds obtained from the literature value with HP-5MS column or NIST 2020 library. [e] Compounds were identified by "MS", mass spectra comparison using NIST 2020 library and "RI", retention indices comparison with RI of published literatures and online library (https://webbook.nist.gov/chemistry/cas-ser/). [f] Variable importance in the projection (VIP) values of OPLS-DA.

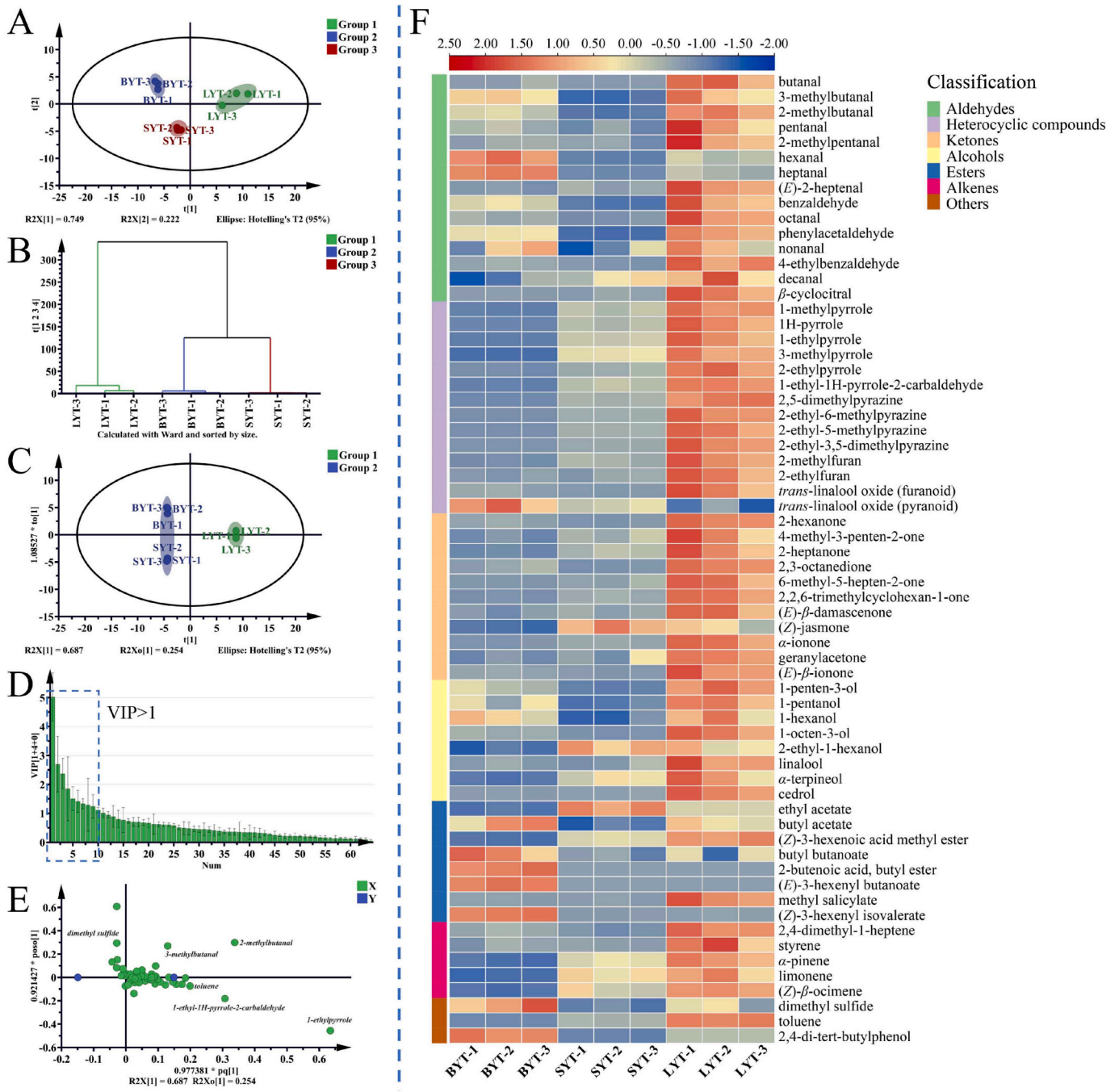

Fig. 2. Analysis of volatile metabolites in three types of yellow teas based on HS-SPME-GC–MS. (A) Scores scatter plot of principal component analysis (PCA). (B) Dendrogram of hierarchical cluster analysis (HCA). (C) Scores scatter plot of orthogonal partial least squares data analysis (OPLS-DA). (D) Variable importance in projection (VIP). (E) Loading scatter plot. (F) The heatmap of marker compounds in three types of yellow teas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the raw materials were damaged (Sasaki et al., 2017), which was similar with the results in this study.

It was reported that esters were the dominant categories in the fresh fragrance types of yellow teas, which played a vital role in the formation of fresh aroma (Hong et al., 2023). In this study, esters including butyl acetate, butyl butanoate, 2-butenoic acid, butyl ester, (*E*)-3-hexenyl butanoate and (*Z*)-3-hexenyl isovalerate were found to be more abundant in BYT. In contrast, methyl salicylate (VIP 1.02) showed a higher concentration in LYT, which was identified as one of the key aroma compounds in floral fragrance type of yellow tea (Hong et al., 2023). Previous study reported that the content of methyl salicylate also increased with the decrease of picking tenderness in green tea (Shao et al., 2022), which was in line with the result from the present study.

In BYT, SYT and LYT samples, alkenes were the least abundant of all volatile compounds, whose concentrations were 0.43, 0.59 and 0.92 μg/L, respectively. Besides, as the only detected sulfide compound, dimethyl sulfide (VIP 1.85) was more abundant in BYT. It was characterized as a key contributor to the top note of yellow tea infusion and its content increased after hot water treatment, which was induced by the thermal degradation of *S*-methylmethionine (Zhai, Wang, et al., 2022). These content differences were possibly caused by the various precursors in raw materials with different tenderness.

3.3. Analysis of catechins, purine alkaloids, gallic acid and free amino acids in three types of yellow teas

3.3.1. Catechins, purine alkaloids and gallic acid

In the present study, the content of major metabolites including catechins, purine alkaloids, gallic acid and free amino acids were determined using UHPLC and UPLC-QQQ-MS/MS. As shown in Fig. 3A, the content of total non-galloylated catechins (C, EC, GC and EGC) in BYT (22.22 mg/g) was significantly ($p < 0.05$) lower than SYT (34.49 mg/g), followed by LYT (36.55 mg/g). However, the levels of overall galloylated catechins (ECG, GCG and EGCG) in BYT (59.55 mg/g) and SYT (56.58 mg/g) were markedly ($p < 0.05$) higher than those in LYT (43.66 mg/g). Especially, EGCG has the highest content of all detected catechins in these yellow teas, accounting for about 50 % of the total

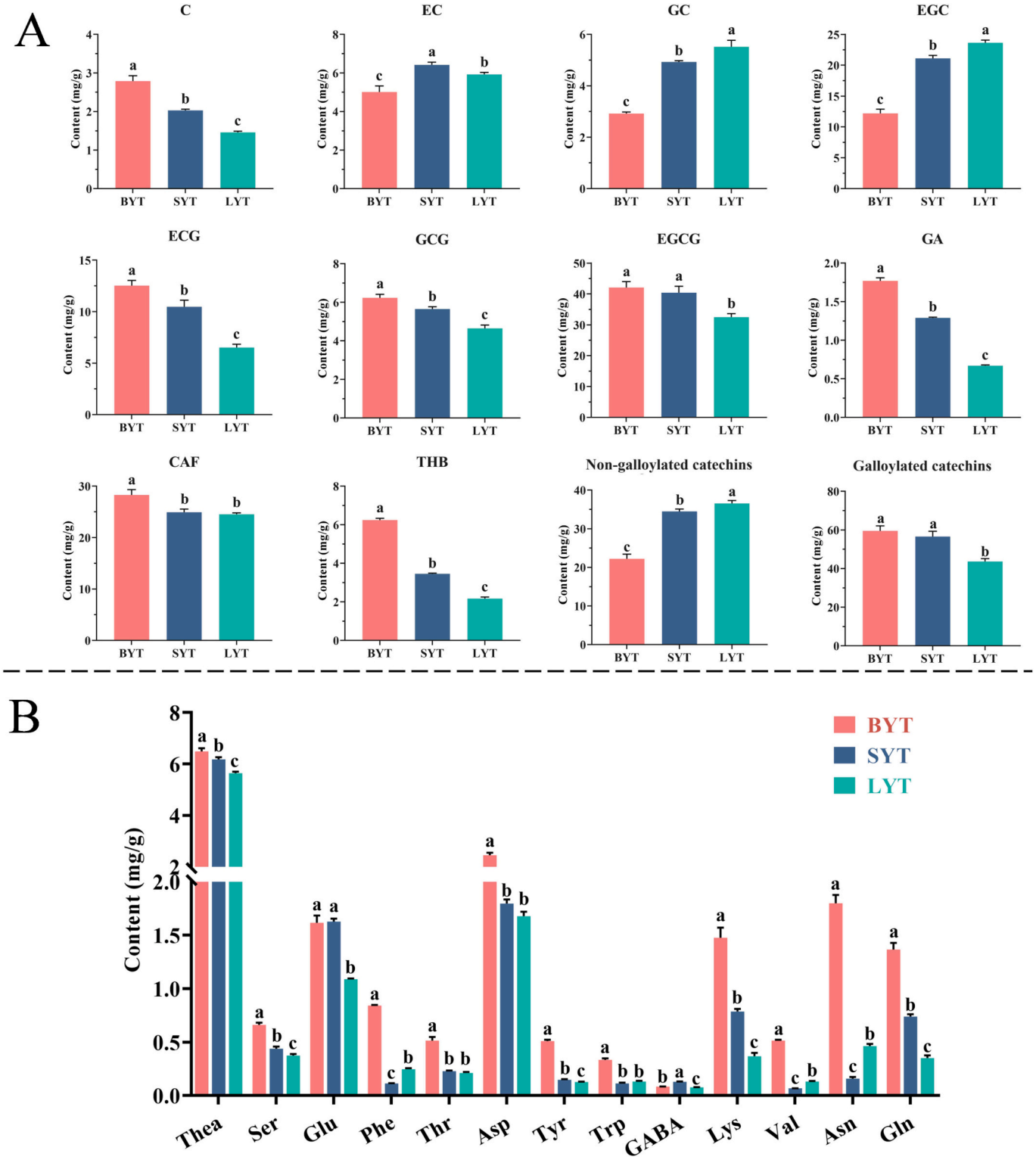

Fig. 3. The contents of catechins, GA, purine alkaloids (A) and free amino acids (B) in three types of yellow teas. Different letters indicated a significant difference at the $p < 0.05$ level. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

catechins (non-galloylated and galloylated catechins). The levels of gallic acid and purine alkaloids (CAF and THB) were higher in BYT than in SYT and LYT.

Researchers have found that the content of galloylated catechins decreased with the increase of tea maturity in green tea, while that of non-galloylated catechins showed a reverse trend (Xu et al., 2021). In addition, during tea processing, metabolites undergo drastic changes, such as epimerization, oxidation, polymerization and degradation reactions of polyphenols. Compared with BYT and SYT, the hydrolysis and isomerization occurred in high-temperature drying process of LYT resulted in the decrease of galloylated catechins content and the increase of non-galloylated catechins content, which was in accordance with the results of this study (Fan et al., 2016; Guo et al., 2021). However, LYT contained lower levels of gallic acid, probably due to its naturally high concentration during the growth and development period and the degradation of galloylated catechins, which was similar with the result of a previous study in green tea (Xu et al., 2021).

3.3.2. *Free amino acids*

As shown in Fig. 3B, a total of 13 free amino acids were determined in BYT, SYT and LYT using UPLC-QQQ-MS/MS method. Basically, marked differences were observed in almost all free amino acids among these three types of yellow teas. The contents of total free amino acids in BYT (18.65 mg/g) were significantly ($p < 0.05$) higher than those in SYT (12.52 mg/g) and LYT (10.89 mg/g). The content of the individual amino acid in these yellow teas showed identical pattern, except for GABA. As for the content of theanine (Thea), a unique and most important non-protein amino acid in tea, was found to be highest in BYT (6.49 mg/g), followed by SYT (6.18 mg/g) and LYT (5.64 mg/g). Apart from theanine, Asp was the most abundant free amino acid in yellow teas, with a content of about 2 mg/g, followed by Glu.

Free amino acids are important indicators of the freshness of yellow tea. Compared with SYT and LYT, BYT was mainly processed with buds and one bud with one leaf, which contained the highest content of amino acids. It was found that most free amino acids showed higher levels in buds than leaves, possibly due to the higher demand of amino acids in buds for chloroplasts biogenesis and growth (Shen et al., 2019). Under the unique high-fire roasting process of LYT, the amino acids undergo decarboxylation, oxidation and thermal reactions, resulting in the decrease of the total free amino acids' content (Guo et al., 2021), which

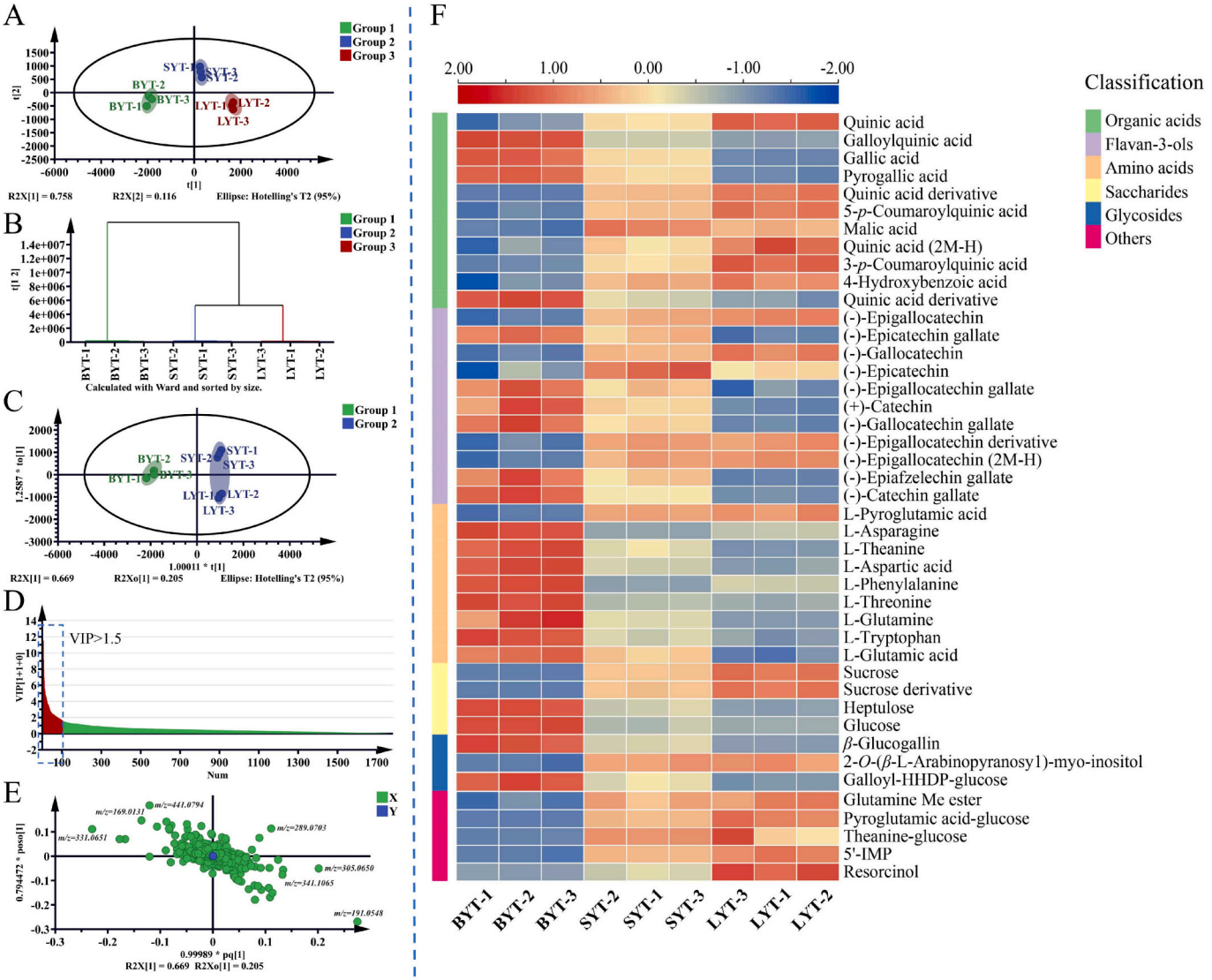

Fig. 4. The non-volatile metabolites in three types of yellow teas based on LC-Q-TOF-MS. (A) Scores scatter plot of principal component analysis (PCA). (B) Dendrogram of hierarchical cluster analysis (HCA). (C) Scores scatter plot of orthogonal partial least squares data analysis (OPLS-DA). (D) Variable importance in projection (VIP). (E) Loading scatter plot. (F) The heatmap of marker compounds in three types of yellow teas. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was the key reason for the low free amino acids' content in LYT. In addition, theanine degraded during the thermal treatment or underwent Maillard reaction with sugars to form pyrazines, pyrroles and furans, which contributed massively to the flavor of yellow teas (Li et al., 2022). Besides, the lower tenderness in the raw material of LYT might also resulted in a lower theanine content. Though free amino acids are a class of metabolites present in trace amounts in yellow tea, they play a vital role in the overall flavor of yellow tea.

3.4. Non-volatile metabolites of different yellow tea samples

3.4.1. Multivariate analysis of LC-MS based untargeted metabolomics

To comprehensively study the marker compounds in these three types of yellow teas, low-molecule-weight metabolites (< 1500 Da) were analyzed using LC-Q-TOF-MS. The differences between yellow teas were compared *via* metabolomics analysis based on mass spectral intensity. As shown in Fig. 4A, PCA result showed that these three yellow teas were clearly distinguished from each other. Besides, HCA result indicated that they could be further categorized into two groups (Fig. 4B), one group was BYT and the other was SYT and LYT. Thus, we divided these tea samples into the above two groups for OPLS-DA analysis (Fig. 4C). VIP values and Loading plot were used for searching the differential metabolites between BYT and SYT, LYT (Fig. 4D and Fig. 4E).

As shown in Table 2, 43 marker compounds (VIP > 1.5) responsible for the classification of yellow teas were screened and listed according to their VIP values. They were tentatively identified by authentic compounds and mass fragments as 11 organic acids, 11 flavan-3-ols, 9 amino acids, 4 saccharides, 3 glycosides and 5 other compounds. For a better overview of the variations in the levels of non-volatile metabolites in these yellow teas, a heatmap was developed based on their abundance as shown in Fig. 4F. It was discovered that the concentration variations of some metabolites including gallic acid, catechins and amino acids were also in line with the results mentioned above.

3.4.2. Key marker compounds of three types of yellow teas

As shown in Fig. 4F and Table 2, organic acids were an important class of key marker compounds among these yellow teas. Most organic acids were more abundant in SYT and LYT compared with BYT, such as quinic acid, quinic acid derivative and 5-*p*-coumaroylquinic acid. Quinic acid had the highest VIP value (11.85), which indicated that it was the most differentiated non-volatile metabolite between BYT and SYT, LYT. On the other hand, the content of other organic acids such as galloylquinic acid, gallic acid and pyrogallic acid were relatively higher in BYT comparing with SYT and LYT.

As an important class of polyphenols, flavan-3-ols contributed greatly to the flavor of yellow tea. For example, galloylated catechins such as EGCG and ECG were considered positively correlated with the bitterness and astringency of yellow tea (Wang et al., 2021). In the

Table 2
The marker compounds responsible for the classification of yellow teas (BYT *vs* SYT, LYT) by LC-Q-TOF-MS.

Var ID	t_R (min)	MS	MS/MS	VIP	Identification	Classification
529	0.773	191.0548	171.0095, 93.0336, 85.0287, 59.0132	11.85	Quinic acid[a]	Organic acids
1110	1.376	331.0651	271.0454, 211.0252, 169.0141, 125.0240	9.37	*β*-Glucogallin[b]	Glycosides
1003	5.967	305.0650	261.0771, 219.0659, 179.0344, 125.0242	8.15	(−)-Epigallocatechin[a]	Flavan-3-ols
1152	2.421	343.0651	325.0478, 259.6110, 191.0575, 169.0143	7.11	Galloylquinic acid[a]	Organic acids
440	1.839	169.0131	125.0245	5.95	Gallic acid[a]	Organic acids
1486	10.724	441.0794	289.0718, 169.0139, 125.0235	5.73	(−)-Epicatechin gallate[a]	Flavan-3-ols
1147	0.710	341.1065	179.0545	5.36	Sucrose[b]	Saccharides
1002	4.323	305.0648	261.0766, 219.0666, 179.0341, 125.0241	5.34	(−)-Gallocatechin[a]	Flavan-3-ols
163	0.965	128.0337	100.0753, 87.0394, 82.0600	4.97	L-Pyroglutamic acid[b]	Amino acids
939	7.486	289.0703	245.0811, 203.0712, 125.0240, 109.0290	4.69	(−)-Epicatechin[a]	Flavan-3-ols
153	1.839	125.0233	108.0206, 79.0182, 67.0182, 51.0233	4.59	Pyrogallic acid[a]	Organic acids
1692	0.749	533.1664	418.0096, 268.0614, 191.0561(quinic acid)	4.37	Quinic acid derivative[b]	Organic acids
1539	9.060	457.0754	305.0661, 169.0141, 125.0235	3.94	(−)-Epigallocatechin gallate[a]	Flavan-3-ols
1132	7.813	337.0908	191.0555, 173.0456, 163.0392, 137.0240	3.72	5-*p*-Coumaroylquinic acid[b]	Organic acids
941	6.294	289.0706	245.0819, 203.0709, 123.0447, 109.0286	3.60	(+)-Catechin[a]	Flavan-3-ols
175	0.684	131.0461	115.0033, 114.0181, 88.0405, 59.0146	3.23	L-Asparagine[b]	Amino acids
1036	0.681	311.0974	179.0556, 161.0449, 125.0242, 87.0084	3.22	2-*O*-(*β*-L-Arabinopyranosy1)-myo-inositol[a]	Glycosides
1538	9.943	457.0747	305.0682, 169.0148, 125.0241	3.15	(−)-Gallocatechin gallate[a]	Flavan-3-ols
184	0.854	133.0131	115.0035, 71.0133	3.01	Malic acid[b]	Organic acids
1306	0.710	387.1112	341.1091, 179.0552	2.95	Sucrose derivative[b]	Glycosides
458	0.798	173.0916	155.0825, 128.0345, 111.0274	2.94	L-Theanine[a]	Amino acids
179	0.710	132.0300	115.0027, 107.0359, 89.0242, 71.0140	2.74	L-Aspartic acid[b]	Amino acids
1297	0.775	383.1198	191.0579	2.64	Quinic acid (2 M-H)	Organic acids
1239	5.965	368.0596	323.0064, 305.0661, 219.0690, 179.0256	2.54	(−)-Epigallocatechin derivative[b]	Flavan-3-ols
1744	5.966	611.1356	305.0670	2.49	(−)-Epigallocatechin (2 M-H)	Flavan-3-ols
1428	12.028	425.0846	273.0751, 255.0673, 169.0120, 123.0087	2.40	(−)-Epiafzelechin gallate[b]	Flavan-3-ols
1755	7.180	633.0685	463.0515, 275.0186, 249.0397, 169.0142	2.39	Galloyl-HHDP-glucose[b]	Glycosides
395	1.322	164.0708	147.0442, 137.0018, 103.0535	2.28	L-Phenylalanine[b]	Amino acids
88	0.688	118.0498	100.0383, 88.0389, 74.0241	2.27	L-Threonine[b]	Amino acids
1128	5.881	337.0898	191.0561, 163.0397, 119.0477	2.25	3-*p*-Coumaroylquinic acid[b]	Organic acids
1485	11.475	441.0792	289.0695, 245.0784, 169.0133, 125.0225	2.22	(−)-Catechin gallate[a]	Flavan-3-ols
245	0.685	145.0620	129.0158, 116.9215, 67.0178	2.18	L-Glutamine[b]	Amino acids
352	0.586	158.9787	130.9831, 114.9880, 102.9858, 86.9926	2.11	Glutamine Me ester[b]	Others
210	5.968	137.0233	124.0154, 109.0284, 97.0293, 83.0134	2.10	4-Hydroxybenzoic acid[b]	Organic acids
944	0.936	290.0852	272.0759, 200.0562, 128.0342, 101.0190	2.10	Pyroglutamic acid-glucose[b]	Others
604	0.780	209.0650	168.0588, 128.0330, 124.0159	1.98	Heptulose[b]	Saccharides
490	0.780	179.0542	85.0287, 59.0130	1.91	Glucose[b]	Saccharides
1117	0.842	333.0589	173.0929, 155.0818	1.90	Theanine-glucose[b]	Others
579	1.055	203.0178	147.0626, 118.8541, 101.0253, 71.0138	1.68	L-Typtophan[b]	Amino acids
1643	0.734	503.1583	488.1354, 405.0992, 256.0753, 191.0547(quinic acid)	1.64	Quinic acid derivative[b]	Organic acids
1166	0.857	347.0586	259.9789, 217.0422, 191.0188, 129.0390	1.60	5'-IMP[a]	Others
34	3.213	109.0282	89.4596	1.53	Resorcinol[a]	Others
247	0.705	146.0443	128.0356	1.50	L-Glutamic acid[a]	Amino acids

Note: [a] Identified by authentic compounds, [b] identified by mass fragment ions.

present study, galloylated catechins were more abundant in BYT, whereas SYT and LYT exhibited a higher content of non-galloylated catechins, which was in line with the quantitative results mentioned above. In addition, free amino acids were mainly produced from protein hydrolysis under the action of humidity and heat during yellow tea processing (Xu et al., 2018). In the current study, 9 free amino acids including L-pyroglutamic acid, L-asparagine, L-theanine, L-aspartic acid, *L*-phenylalanine, L-threonine, L-glutamine, L-tryptophan and L-glutamic acid were screened from the metabolites. It was discovered that the content of these free amino acids (except for L-pyroglutamic acid) were highest in BYT, followed by SYT and LYT, which was in accordance with the quantitative results mentioned above. In addition, high-temperature roasting also led to the decrease in the levels of amino acids in LYT (Guo et al., 2021).

Saccharides were also key contributors to the taste and flavor of yellow tea. Among these critical compounds, the content of sucrose and its derivatives were higher in LYT, while heptulose and glucose were more abundant in BYT. Researchers have found that heptulose and glucose were also the critical compounds between roasted tea and non-roasted tea, and their levels decreased with the increasing roasting temperature (Jiang et al., 2022). In addition, the content of *β*-glucogallin and galloyl-HHDP-glucose were relative higher in BYT compared with SYT and LYT. Glycosides are an important class of non-volatile metabolites in tea, and their hydrolysis contributes significantly to tea flavor (Ho et al., 2015). Some other compounds have also been identified as marker compounds, such as pyroglutamic acid-glucose and theanine-glucose adduct, which were mainly produced by high-temperature roasting process through Maillard reaction and caramelization reaction. According to the obtained results, their contents were the lowest in BYT and the highest in LYT.

3.5. The bioactivities of yellow tea samples regarding to α-glucosidase inhibitory and antioxidant capacity

3.5.1. α-glucosidase inhibition of yellow tea samples

In the present study, the bioactivities of three yellow teas were measured by evaluating the α-glucosidase inhibitory and antioxidant capacity. As depicted in Fig. S2A, it was found that BYT exhibited the best inhibitory effect against α-glucosidase, followed by STY, and LYT. Besides, the inhibitory activity of all three yellow teas against α-glucosidase increased with the increase of sample concentration, which was consistent with a previous study of black tea (Tong et al., 2018). The inhibitory rates (%) of BYT, SYT and LYT reached maximum at the concentration of 250 μg/mL, which were 96.04, 91.75 and 81.51, respectively. Tea has been found to be a natural inhibitor of α-glucosidase due to the presence of a variety of bioactive components, which played an important role in regulating the postprandial blood glucose levels (Gao et al., 2023). A previous study found that EGCG and GCG both showed effective inhibitory activity against α-glucosidase with an IC_{50} value of 5.79 and 2.10 μg/mL, respectively (Zhou et al., 2018). Thus, a better glycosidase inhibitory activity of BYT might be associated with the relatively high content of EGCG and GCG, compared with SYT and LYT.

3.5.2. Antioxidant capacity of yellow tea samples

The antioxidant capacity of these yellow tea samples was conducted in combination with three methods (ABTS, DPPH and FRAP). Briefly, the ABTS method is widely used to evaluate the antioxidant capacity of plants, traditional Chinese medicine extracts, and other substances, whose antioxidant performance is evaluated by their ability to scavenge ABTS free radicals (Tang et al., 2004). The DPPH method is widespread applied to measure the antioxidant activity of different natural samples due to the stable properties of DPPH in organic solvents and its purple colour in alcohol solutions (Xie & Schaich, 2014), while the FRAP method uses ferrous ions (Fe^{2+}) to form complexes with triphenyltriazine (TPTZ) under acid environments, which reflects the total antioxidant capacity of samples by measuring the reducing ability of ferric ions (Fe^{3+}) (Munteanu & Apetrei, 2021). All three methods could be applied to evaluate the antioxidant properties of tea and its extracts through different mechanisms, providing rich information and data supporting for the study of tea antioxidant properties. As shown in Fig. S2B, the ABTS free radical scavenging ability exhibited the results that BYT > SYT > LYT. LYT and SYT showed a relatively higher DPPH free radical scavenging ability than that in BYT, especially in higher concentrations (0.1 and 1 mg/mL) (Fig. S2C). In addition, from the results of FRAP assay, it was also found that BYT possessed the best ferric-reducing antioxidant power, followed by SYT, LYT (Fig. S2D). As tea concentration increased, the scavenging rate (%) and FRAP value of these yellow teas increased. In general, BYT showed the best antioxidant capacity among the three yellow teas. The results of all these three antioxidant experiments showed a concentration-dependent relationship, which was consistent with a previous result of dark tea (Ma et al., 2022). The antioxidant capacity of tea was mostly associated with the composition and content of their bioactive components, such as catechins and gallic acid (Tang et al., 2019). It was also found that the antioxidant capacity of EGCG and GCG (galloylated catechins) was superior to EGC, GC, EC and C (non-galloylated catechins) due to the existence of the galloyl groups (Lang et al., 2024). The quantitative results showed that the content of galloylated catechins, especially ECG and GCG, of BYT was higher than that of SYT and LYT, which might be the main reason for a better antioxidant capacity of BYT.

4. Conclusion

In the present study, three types of yellow teas (BYT, SYT and LYT) made from the same variety with different tenderness of raw materials were systematically investigated for the chemical profile and *in vitro* bioactivity variation. It was discovered that these three yellow teas contained the same types of volatile metabolites, but the total concentration was the highest in LYT compared to BYT and SYT. Among them, heterocyclic compounds were the main differential volatile metabolites in these yellow teas. In addition, it was found that among these three yellow teas, BYT contained higher levels of galloylated catechins, purine alkaloids and free amino acids, but the lowest levels of non-galloylated catechins. Forty-three non-volatile marker compounds were screened from three types of yellow teas, including organic acids, flavan-3-ols, amino acids, saccharides, glycosides and others. Besides, BYT showed the best α-glucosidase inhibitory effect and antioxidant capacity, which was possibly related to the high levels of galloylated catechins. This research enriched the understanding of the effects of different tenderness of raw materials on the chemical compositions and bioactivities of yellow teas. The association between some crucial volatile and non-volatile compounds required further clarification, and the potential *in vivo* activity of BYT deserved further investigation.

Ethical approval

Ethical permission, to conduct a human sensory study, was granted by our institution. And “human sensory ethical inspection” was provided in the supplementary material. Participants gave informed consent *via* the statement “I am aware that my responses are confidential, and I agree to participate in this survey” where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The tea products tested were safe for consumption.

CRediT authorship contribution statement

Chunyin Qin: Writing – original draft, Visualization, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Zisheng Han:** Writing – review & editing, Supervision. **Zongde Jiang:** Methodology, Investigation. **Jia-Ping Ke:** Methodology, Formal

analysis. **Wen Li:** Data curation. **Liang Zhang:** Validation, Resources, Investigation, Conceptualization. **Daxiang Li:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101809.

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