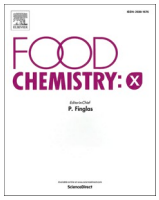
Food Chemistry: X 24 (2024) 101794

Contents lists available at ScienceDirect

Food Chemistry: X

journal homepage: www.sciencedirect.com/journal/food-chemistry-x



Effect of tea stems on the quality formation of large-leaf yellow tea: Sensomics and flavoromics approaches

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ARTICLE INFO

Keywords:
Large-leaf yellow tea
Tea stems
Flavor quality
Sensomics
Flavoromics

ABSTRACT

In this study, the stems (ST) and leaves (LT) isolated from Large-leaf yellow tea (LYT) were used for sensory evaluation and quantitative analysis of flavor metabolites by sensomics and flavoromics. The results showed that the flavors of ST and LT in LYT were significantly different, and ST had stronger roasty and nutty aroma and sweet taste, which was mainly due to the accumulation of higher theanine and soluble monosaccharides in ST, and provided more substrates for the production of more pyrazine by the Maillard reaction; whereas LT contributed to the mellow and thick taste quality of LYT, and the abundance of catechins and caffeine were the main reason. The metabolic patterns of flavor metabolites indicated that the flavor differences between ST and LT were mainly due to biological metabolism in tea plants. This study provides the selection of raw materials for LYT in the future and product development of tea stems.

1. Introduction

Large-leaf yellow tea (LYT) belongs to the yellow tea among the six tea types, mainly produced in the Dabie mountain region of China (Zhou et al., 2019), and is increasingly popular among consumers for its unique flavor quality and excellent health benefits (Han et al., 2016; Zhao et al., 2023). In 2023, the yield and output value of yellow tea in China increased by 78.4 % and 61.4 %, respectively, and it is widely recognized by consumers. LYT is made from the new shoots of high maturity (one bud and four or five leaves), which is processed through the process of fixation, rolling, first drying, yellowing, second drying, and over-fired drying (Zhou et al., 2019). Because of its coarse raw materials and unique processing technology, LYT has the qualities of full shoot appearance, orange-red infusion color, rice-crust aroma, and mellow and thick taste (Li et al., 2024). A study showed that contributing to the rice-crust aroma of LYT characteristic aroma of are 17 highest active odorants such as 2-methylbutanal, (*E*, *E*)-2,4-heptandienal, 2-methylpropanal, 2,3-diethyl-5-methylpyrazine and *β*-ionone (Zhai et al., 2023). The main reason for this characteristic aroma is due to the prolonged high temperatures that LYT undergoes during the over-fired drying process (Yin et al., 2023; Zhou et al., 2019), which exacerbate the Maillard reaction and the degradation of fatty acids. However, the over-fired drying did not produce the rice-crust aroma in another tea (Zhang et al., 2023), which may be due to that LYT contains more tea stems in addition to the variety of tea plants. LYT is always full shoot during processing from picking, the stems and leaves are connected as one, and the leaves naturally fall off from the stems due to the reduction in overall moisture after over-fired drying. Despite this, LYT is sold and brewed in its full form with a mixture of stems and leaves. However, the stems are often considered to be a sign of coarse and poor quality in most teas, which affects the appearance of the tea, and are often artificially removed before processing in some famous teas and high grade teas (Zeng et al., 2017), which not only adds more processing operations, but also is very detrimental to efficient production and utilization.

The unprocessed stems are the stalks of the tea plants new shoots, which play a role in connecting, supporting, and transporting between the roots and the leaves (Melkikh & Sutormina, 2022). Due to the different sites for the biosynthesis of metabolites and the different efficiencies of their transportation in the tea plants, the accumulation of metabolites in the stems and the leaves of the tea plants has a large difference (Zeng et al., 2017). Compared to tea leaves, tea stems have lower concentrations of catechins and caffeine and higher concentrations of soluble sugars, free amino acids, and volatile monoterpenes. In addition, as the tenderness of tea stems decreases, secondary

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https://doi.org/10.1016/j.fochx.2024.101794
Received 7 August 2024; Received in revised form 26 August 2024; Accepted 27 August 2024
Available online 2 September 2024

metabolites other than cellulose are reduced to varying degrees (Zeng et al., 2017; Zhang, Guo, et al., 2022). The composition and concentration of metabolites are responsible for determining the flavor formation of tea (Yu et al., 2014). Regular LYT has a high stem content (0.2 g/g), and thus the contribution of stems to the flavor of LYT has attracted the attention of both producers and consumers. Tea makers have attempted to separate the tea stems and tea leaves from the LYT that are naturally shed after over-fired drying, they have found that the flavors of tea stems and tea leaves alone have great differences and that the flavor of LYT becomes incomplete with the loss of the tea stems, but the contribution of the stems to the formation of the characteristic flavor of LYT is still not clear. Here, in order to explain the above problem, different parts of LYT (stems and leaves) were separated, and three samples of tea stems, tea leaves, and intact LYT (as a reference) were obtained, and then the flavor metabolites in the three samples were quantified by sensomics and flavoromics, and the metabolic patterns of the flavor metabolites in the biosynthetic pathway were established, and the correlation network between sensory quality and flavor metabolites was finally constructed to comprehensively explain the contribution of the tea stems and tea leaves to the characteristic flavor of LYT and the reasons for it. This study systematically investigated the flavor differences between stems and leaves of LYT to clarify the contribution of stems to the overall flavor of LYT, which provided the choices of both raw materials in the future and directed flavors of consumers for LYT.

2. Materials and methods

2.1. Chemicals

1-Ethyl-1*H*-pyrrole, 2,4,5-trimethyloxazole, 2,5-dimethylpyrazine, benzaldehyde, 1-octen-3-ol, octanal, (*E*, *E*)-2,4-heptadienal, benzeneacetaldehyde, 1-ethyl-1*H*-pyrrole-2-carboxaldehyde, 2-acetylpyrrole, (*Z*)-linalool oxide (furanoid), 2-ethyl-3,6-dimethylpyrazine, 2-ethyl-3,5-dimethylpyrazine, (*E*)-linalool oxide (furanoid), benzoic acid methyl ester, linalool, (*E, Z*)-2,6-nonadienal, 3,5-diethyl-2-methylpyrazine, 2,3,5-trimethyl-6-ethylpyrazine, (*Z*)-linalool oxide (pyranoid), methyl salicylate, geraniol, indole, 3-methyl-indole, (*E*)-*β*-ion sodium chloride one, and *DL*-4-chlorophenylalanine were purchased from Macklin Biochemical Co., Ltd. (Shanghai, China). *α*-Ionone was purchased from ChemFaces Biotechnology Co., Ltd. (Wuhan, Hubei, China). Hexanal and ethyl decanoate were purchased from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). All reference odorants had purity >98 % for GC.

n-Alkanes (C_6–C_{40}), methoxyamine hydrochloride, pyridine, and all free amino acid standards were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). Isopropanol was purchased from Merck Co., Ltd. (Darmstadt, Germany). Sodium chloride (NaCl) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Sugar standards were purchased from Anpel Co., Ltd. (Shanghai, China), ZZBIO Co., Ltd. (Shanghai, China), and TCI Development Co., Ltd. (Shanghai, China). Caffeine, epigallocatechin gallate, epigallocatechin, epicatechin gallate, gallocatechin gallate, epicatechin, and catechin were purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Acetic acid, acetonitrile, and methanol were purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Sulfosalicylic acid was purchased from Shanghai Adamas-Beta Chemical Reagent Co., Ltd. (Shanghai, China). Deionized water was produced using a Milli-Q water purification system (Millipore, Billerica, MA, USA).

2.2. Tea samples

In June 2022, LYT samples were purchased from Hengda Tea Factory, Huoshan County, Anhui Province, China. The samples are processed by local traditional craftsmanship and have the typical flavor characteristics of LYT. The processing includes fixation, rolling, first drying, yellowing, second drying, and over-fired drying. The LYT samples were divided into stem and leaf parts to obtain leaf tea (LT) and stem tea (ST) samples, respectively. Unseparated samples are labeled LYT.

2.3. Sensory evaluation and quantitative descriptive analysis (QDA)

The tea infusion of three samples (LYT, LT, and ST) was analyzed for traditional sensory evaluation and QDA according to the relevant Chinese national standard (GB/T 23776–2018). Briefly, the tea infusion of each sample was obtained by thoroughly mixing tea leaves (3.0 g) and boiling water (150 mL) for 5 min, followed by filtration. A total of 11 trained personnel (23–45 years old, 5 males and 6 females) from Anhui Agricultural University participated in our traditional sensory evaluation (1 male and 2 females) and QDA (5 males and 5 females). The traditional sensory evaluation was harmonized to describe the different components of LYT according to DB 34/T 3020-2017. QDA for aroma and taste were subsequently conducted. Participants had undergone monthly at least one year of sensory training according to the methods reported in our previous studies (Huang et al., 2022; Huang et al., 2024). For the QDA of aroma, participants provided the four most compatible descriptions based on the aroma profiles of the three LYT samples, and the top four descriptions with the highest frequency of occurrence were ultimately used as the scoring items and quantified. For the QDA of taste, participants quantified four taste attributes, namely bitterness, astringency, umami, and sweetness, of the three sample tea infusions. The QDAs were scored on a 10-point scale (precision 0.1), and the mouths were rinsed by drinking purified water once in between each two evaluations. The sensory evaluation protocol was reviewed and approved by the Human Sensory Ethics Inspection Committee of Anhui Agricultural University. Participants were informed of the rules and risks of the sensory experiment in advance and all their information was fully protected. Participants provided informed consent by affirmatively replying to the statement “I am aware that my responses are confidential, and I agree to participate in this sensory evaluation.” They were able to withdraw from the evaluation at any time without providing a reason.

2.4. Stir Bar Sorptive Extraction (SBSE)

The method used by SBSE followed the description in the study of Wang et al. (2020) with some modifications. 150 mL of boiling pure water was added to 3 g of tea sample, and the tea infusion was filtered out after a sealed maceration for 5 min. 10 mL of tea infusion was pipetted into a 20 mL vial, and 3 g of NaCl and the internal standard solution (ethyl decanoate) were added, which was mixed thoroughly, and then added into the Twister (polydimethylsiloxane, 10 mm length, 0.5 mm film thickness; Gerstel, Germany). The Twister was adsorbed in a water bath at 40 °C for 90 min, after which the Twister was removed and dried, and then transferred to a glass desorption tube, which was placed on a separate sealed sample tray. The temperature of the thermal desorption unit was set as follows: an initial temperature of 30 °C, then 240 °C at 100 °C/min for 5 min. A cooling injection system (CIS-4, Gerstel, Germany) was used to maintain the temperature at −100 °C using liquid nitrogen (99.99 %). After the desorption of volatile compounds, the temperature of the CIS-4 was ramped up from −100 °C (hold 1 min) to 280 °C (hold 3 min) at a rate of 12 °C/s, followed by GC–MS/O analysis.

2.5. Identification of odorants through gas chromatograph – mass spectrometer/olfactometry (GC–MS/O)

Volatile compounds were separated using an Agilent 5977B GC equipped with an 8890 A MS (Santa Clara, California, USA) and an olfactory detector port (ODP4, Gerstel, Germany). Volatile compounds were separated on an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) with a carrier gas of high purity helium (purity >99.999 %).

The oven temperature was set as follows: the initial temperature was 40 °C (hold 5 min), then warmed up to 100 °C at 3 °C/min, then to 130 °C at 2 °C/min, and then to 250 °C at 10 °C/min (hold 5 min). The mass spectrum was obtained in the range of 30–350 *m/z* at 70 eV in the electron ionization mode with an ion source temperature of 230 °C. Retention indices (RI) were determined for the volatile compounds by a homologous series of n-alkanes (C_7-C_{40}).

The volatile mixture was split into two portions, one going directly to the mass spectrometry detector and the other to the sniffer port. The gas chromatograph – olfactometry injector and transfer line temperatures were maintained at 230 °C and 250 °C, respectively. Five members (2 males and 3 females, 20–25 years old) with aroma training experience captured odors at the sniffing port and recorded their aroma quality and quantified the perceived aroma intensity (AI, 4-point scale (0, none; 1, weak; 2, medium; 3, strong; 4, very strong)) value, they were trained weekly at least one year of training experience, using a common aroma language to identify entity foodstuffs and aqueous solutions of reference odorants with characteristic odor qualities. Each odorant was confirmed to have been perceived by at least three people sniffing it, and the AI value being expressed as a mean value for all (Huang et al., 2024). Volatile compounds were compared to mass spectra via RI and the national institute of standards and technology (NIST 20) of U.S. Department of commerce database, and olfactory and reference compounds were further confirmed and quantified by internal standards.

2.6. Determination of free amino acids

0.1 g of tea powder was added to 4 mL of 4 % sulfosalicylic acid solution (sulfosalicylic acid/water, *v*/v, 1/24), and extracted by ultrasonication for 30 min, followed by centrifugation at 12000 r/min for 30 min, after which the precipitate was extracted again by the above method. Subsequently, the combined supernatants from the two centrifugations were analyzed by passing them through a 0.22 μm membrane. Free amino acids were analyzed using a high-speed amino acid analyser (L-8900, Hitachi, Tokyo, Japan), which used lithium citrate as the mobile phase at a flow rate of 0.35 mL/min and derivatization reagents at a flow rate of 0.3 mL/min. Detection wavelengths of 570 nm and 440 nm were used. The detection wavelengths were 570 nm and 440 nm, ultraviolet visible wavelengths. The temperature of the column was set at 38 °C, the temperature of the post-column reaction equipment was maintained at 130 °C, and the temperature of the autosampler was 4 °C. The qualitative and quantitative analysis was performed by mixing the reference compounds, and the injection volume of both the sample and the reference compounds was 20 μL.

2.7. Determination of catechins and caffeine

0.2 g of tea powder was added with 5 mL of 70 % methanol (70 °C, methanol/water, *v*/v, 7/3), incubated fully in a 70 °C water bath for 10 min (stirring every 5 min), cooled to room temperature and centrifuged at 3500 r/min for 10 min, and the precipitate was extracted repeatedly, and the combined supernatant of the two extracts was fixed with 70 % methanol to 10 mL. Subsequently, the extract was diluted 5-fold with stabilizing solution (250 mg Ethylenediaminetetraacetic acid disodium salt (EDTA-2Na), 250 mg ascorbic acid, 50 mL acetonitrile and water fixed to 500 mL) and finally filtered through a 0.22 membrane in a vial. Catechins and caffeine were detected using an Agilent 1260 high-performance liquid chromatography (HPLC) system (Agilent Technologies, Palo Alto, CA, USA). HPLC equipment parameters were the same as in the previous study (Fang et al., 2019).

2.8. Extraction and detection of sugars

Sugars were extracted with reference to the method of (Sun et al., 2016) et al. Briefly, 20 mg of lyophilized tea powder was added with 500 μL of methanol: isopropanol: water (3:3:2, *v*/v/v), vortexed for 3 min followed by ultrasonic extraction for 30 min, and centrifuged (4 °C) at 12,000 r/min for 3 min. Subsequently, 50 μL of supernatant was pipetted and mixed with 20 μL of internal standard (1000 μg/mL) and evaporated under a stream of nitrogen. After evaporation, the sample was transferred to a lyophilizer for lyophilization and then used for further derivatization. Then, methoxyamine hydrochloride was added to and mixed with 100 μL of pyridine (15 mg/mL). After incubation for 2 h at 37 °C, 100 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) was added, vortexed, and mixed at 37 °C for 30 min. The mixture was then diluted to the appropriate concentration and analyzed by gas chromatography–mass spectrometry.

Sugars were analyzed using an Agilent 8890GC equipped with a 5977B MS. Sugar compounds were separated on a DB-5MS column (30 m × 0.25 mm × 0.25 μm; J&W Scientific, USA) with helium as the carrier gas (flow rate of 1 mL/min). The injection was performed in split mode (split ratio of 5:1) with a 1 μL volume of injection liquid, and the temperature program of the column was as follows: starting from 40 °C (held for 1 min), the temperature was increased to 200 °C at 6 °C/min, then to 270 °C at 10 °C/min, and then to 300 °C at 5 °C/min, and finally to 370 °C at 20 °C/min. C/min to 320 °C (held for 5.5 min). The temperature of the ion source and transmission line were 230 °C and 280 °C, respectively, and all samples were analyzed by selected ion monitoring.

2.9. Determination of non-volatile compounds by liquid chromatography – orbitrap – mass spectrometry (LC – Orbitrap – MS)

0.4 g of tea powder was added with 8 ml of 70 % methanol solution (methanol: water, 7:3, *v*/v, containing 0.2 mg/ml of 4-chloro-DL-phenylalanine), sonicated for 30 min, and then left to stand for 4 h (with shaking every 1 h). Subsequently, the sample was sonicated again and allowed to stand for 4 h. The supernatant after centrifugation (12,000 r/min, 10 min) was diluted 40-fold with 70 % methanol and then passed through a 0.22 μm membrane for LC–MS analysis.

Non-volatile metabolites were analyzed using an ultra–performance liquid chromatography (UPLC) system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) equipped with a mass spectrometer (Q-Exactive Focus, Thermo Fisher Scientific, Waltham, MA, USA). Metabolites were separated on an Acquity UPLC HSS T3 column (1.8 μm, 100 mm × 2.1 mm) with mobile phases of 0.05 % formic acid in water (A) and acetonitrile containing 0.05 % formic acid (B) at an injection volume of 2 μL and a flow rate of 0.2 mL/min. The samples were scanned in a negative ion mode in the range of 100–1500 *m/z*. The MS/MS The scanning mode was set to data-dependent ms2 scanning with a resolution of 17,500 m/z, and high collision-induced dissociation was set to 10, 20, and 60 eV in step mode (Xu et al., 2019).

2.10. Data analysis

The method of data analysis is consistent with our previous study (Huang et al., 2022). The raw LC–MS data were first converted into ABf format and subsequently, all features were exported via MS-DIAL ver 3.82. Matches were obtained in MSFINDER ver 3.04 based on the MS/MS of each feature. Compounds that could be identified with standard compounds were visualized through XReport. Compounds were characterized by matching references, databases (Human Metabolome Database), and mass spectral fragments. Analysis of Variance (ANOVA) and Pearson's correlation analyses were performed using SPSS (version 26; IBM, Armonk, NY, USA), $p < 0.05$ indicates a statistical difference in ANOVA (Duncan's test). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed using Simca 14.1 (Umetrics Corp., Umea, Sweden). Figure visualization was performed using Origin 2021 and TBtools. Network analysis was realized by Cytoscape. Each test was performed with three replications and the results are expressed as mean ± standard deviation.

3. Results and discussion

3.1. Sensory evaluation of different parts of LYT

The evaluators first conducted a traditional sensory evaluation of the three samples (LT, LYT, and ST) in terms of infusion color, aroma, and taste (Fig. 1), and found that the quality characteristics of LYT were orange-red and lighter bright color, rice-crust aroma as well as mellow and thick taste (Fig. 1A), which were related to the processing techniques of the yellowing and over-fired drying of LYT (Li et al., 2024). The quality of LT was more similar to that of LYT, which may be mainly due to the higher leaf content of LYT (about 0.8 g/g), and hence the greater contribution of LT to the quality of the LYT. In addition, LT and ST provided different characteristics to the quality of LYT. Specifically, in terms of infusion color, ST had orange and bright characteristics, while LT was orange red, and slightly bright (Fig. 1B), and the presence of a small amount of ST contributed to the increase in the brightness of LYT infusion color. Not only that, ST and LT also have large differences in aroma and taste, ST has an over-fired aroma with a sweet aroma as well as a mellow, sweet, and smooth taste, which contributes to the unique over-fire aroma of LYT; at the same time, it also attenuates the astringent taste of LYT tea infusion, and increases the sweet and smooth taste of the tea.

QDA quantified the main attributes of aroma and taste, and the results showed that ST had a strong roasty and nutty aroma (Fig. 1C), while LT had a prominent cooked corn-like aroma, and the rice crust-like aroma of LYT could be a specific combination of roasty, nutty and cooked corn-like aroma. On the other hand, the taste difference between ST and LT was obvious (Fig. 1D), and LT had a strong bitter taste, which was consistent with the results of the sensory evaluation. The color and taste of tea infusion are the result of the combination of the type and concentration of water-soluble inclusions, e.g., flavonols and flavonoid glycosides, etc. (Jiang et al., 2015; Scharbert & Hofmann, 2005), and it was found by the comparison of water extracts content (Table S1) that LT was significantly higher than that of ST, which might have led to the darker color and bitter taste of LT tea infusion.

3.2. Aroma analysis of different parts of LYT

The aroma quality of tea is the result of the comprehensive effect of active volatiles and their concentrations. SBSE is a solvent-free extraction method relying on the Twister adsorption layer, and the large adsorption layer and the active stirring of internal magnetism provide this extraction method the advantages of high throughput and sensitivity, which are widely used in the capture of tea infusion aroma and trace volatiles (Xiao et al., 2022; Yang et al., 2013). To further obtain the reasons for the different contributions of ST and LT to the aroma of LYT, a total of 39 aroma active regions were obtained by SBSE-GC–MS/O combined with the AI values (Table 1 and Fig. S1), and their concentrations were available within the detectable range, including 6 alcohols, 7 aldehydes, 4 esters, 3 ketones, 18 heterocycles, and one other. The aroma of LYT was mainly composed of pyrazine-dominated heterocyclic compounds, which is in agreement with the findings of Zhai et al. (2023), which was attributed to the intense Maillard reaction that occurs during the over-fired drying in LYT (Yin et al., 2023). For LYT, the higher AI (AI >2.5) of 2,5-dimethylpyrazine (**A5**, 2.67), 1-ethyl-1*H*-pyrrole-2-carboxaldehyde (**A14**, 2.67), 2-ethyl-3,6-dimethylpyrazine (**A17**, 3.00) and 2-ethyl-3,5-dimethylpyrazine (**A18**, 3.00), these active odorants have roasty and nutty aromas. Roasty and nutty aromas are the key profiles that make up the quality of the rice-crust aroma of LYT, and thus these highly active odorants are important in the formation of the flavor of LYT.

Notably, there was a significant difference in the concentration of pyrazine detected in the LT and ST. The concentrations of odorants with roasty and nutty aromas were all higher in ST, these odorants were more than 50 % higher in ST than in LT and were perceived in sensory sniffing of GC-O, and they may be the main reason for the higher over-fired aroma of ST. For example 1-ethyl-1*H*-pyrrole-2-carboxaldehyde (**A14**, roasty, 261 %), 2-ethyl-3,6-dimethylpyrazine (**A17**, nutty, 64 %), 2-ethyl-3,5-dimethylpyrazine (**A18**, nutty, 70 %), 3,5-diethyl-2-methylpyrazine (**A24**, roasty, 66 %), 2,3,5-trimethyl-6-ethylpyrazine (**A25**, earthy, 81 %) and 2-isoamyl-6-methylpyrazine (**A31**, earthy, 80 %). Other compounds (namely alcohols, aldehydes, esters, and ketones), which are mainly produced by the enzymatic reaction phase of tea leaves after harvesting (Dudareva et al., 2013), have a floral, fruity, and sweet aroma (Zhai et al., 2022), they are the opposite of heterocyclic compounds. More accumulated due to higher substrate concentration and enzyme activity of the leaves in the tea plant, their concentration in LT is higher than that of ST, which is sensory as a result of the combined effect of higher fired rice aroma of LT and lower roasty aroma. In particular, the floral odorants linalool and geraniol were more abundant in the ST, and higher levels of geraniol and linalool in green tea stems than in green tea leaves were also reported by Hara et al. (1993). In addition, linalool and geraniol as terpenes are able to increase the

(A) Sensory evaluation

	Liquor color	Aroma	Taste
LT	Orange red and slightly bright	Fired rice aroma with over-fired aroma	Mellow and thick, slightly astringent
LYT	Orange red and lighter bright	Rice crust-like aroma high and brisk	Mellow and thick, smooth
ST	Orange and bright	Over-fired aroma with sweet aroma	Mellow, sweet and smooth

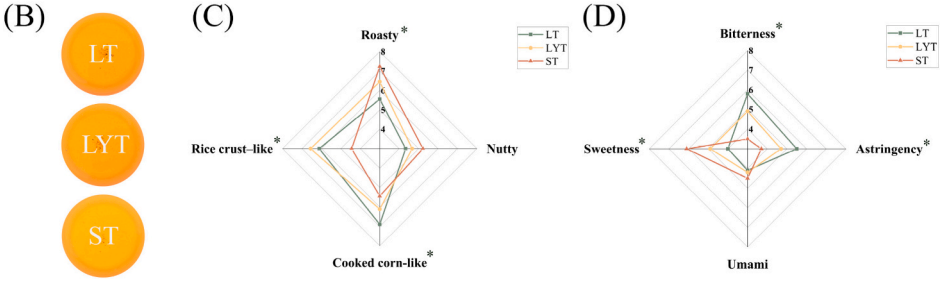

Fig. 1. Infusion color and flavor quality of large-leaf yellow tea (LYT) and its stems (ST) and leaves (LT) evaluated by traditional sensory evaluation and quantitative descriptive analysis. (A) Traditional sensory evaluation of LYT and its ST and LT; (B) Infusion color; (C) quantitative descriptive analysis of aroma; (D) quantitative descriptive analysis of taste. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Odorants in large-leaf yellow tea infusion by SBSE-GC-O.

No	RI[a]	RI[b]	Odorants[c]	Odor quality[d]	Relative concentration (μg/L)[e]			AI[f]			Identification[g]
					LT	LYT	ST	LT	LYT	ST	
A1	800	801	Hexanal	green, grassy	1.11 ± 0.05a	0.99 ± 0.06a	0.32 ± 0.28b	1.50	1.00	0.50	MS,RI,O,Std
A2	821	828	1-Ethyl-1H-pyrrole	rubber,burnt	56.27 ± 0.08a	34.5 ± 0.07b	26.75 ± 0.03c	1.25	1.00	1.00	MS,RI,O,Std
A3	852	856	2,4,5-Trimethyloxazole	burnt, nutty, hazelnut-like	15.02 ± 0.03a	11.85 ± 0.14b	6.31 ± 0.08c	1.67	1.67	1.67	MS,RI,O,Std
A4	865	868	*p*-Xylene	plastic, pungen	5.80 ± 0.11a	4.34 ± 0.21b	1.42 ± 0.23c	2.33	2.00	1.67	MS,RI,O
A5	917	928	2,5-Dimethylpyrazine	earthy, nutty	95.07 ± 0.04a	95.48 ± 0.03a	97.53 ± 0.03a	2.67	2.67	2.75	MS,RI,O,Std
A6	962	963	Benzaldehyde	bitter almond-like	3.36 ± 0.03a	2.73 ± 0.07b	1.33 ± 0.02c	1.75	nd	nd	MS,RI,O,Std
A7	965	971	5-Methyl-2-furancarboxaldehyde	sweet, bitter almond-like	8.01 ± 0.08a	6.71 ± 0.04b	3.87 ± 0.07c	1.83	1.67	1.67	MS,RI,O
A8	980	978	1-Octen-3-ol	mushroom-like	8.13 ± 0.07a	7.08 ± 0.03b	1.62 ± 0.11c	2.67	2.17	2.00	MS,RI,O,Std
A9	995	995	2-Ethyl-6-methylpyrazine	roasty, nutty	13.4 ± 0.04a	13.44 ± 0.02a	13.67 ± 0.04a	nd	1.00	1.00	MS,RI,O
A10	1002	1000	2-Ethyl-5-methylpyrazine	roasty, nutty	31.34 ± 0.03a	31.28 ± 0.02a	30.81 ± 0.01a	2.00	2.00	2.00	MS,RI,O,Std
A11	1004	1004	Octanal	citrus-like, green	1.55 ± 0.09a	1.38 ± 0.05a	0.39 ± 0.05b	2.33	2.33	2.17	MS,RI,O,Std
A12	1012	1012	(*E,E*)-2,4-Heptadienal	fatty, floral	2.64 ± 0.07a	2.24 ± 0.07b	0.77 ± 0.09c	2.00	2.00	1.67	MS,RI,O,Std
A13	1045	1044	Benzeneacetaldehyde	floral, honey-like	6.48 ± 0.04a	6.53 ± 0.06a	7.05 ± 0.05a	2.50	2.50	2.67	MS,RI,O,Std
A14	1046	1046	1-Ethyl-1H-pyrrole-2-carboxaldehyde	roasty, nutty	77.61 ± 0.14c	119.53 ± 0.19b	280.35 ± 0.10a	2.50	2.67	3.00	MS,RI,O,Std
A15	1064	1060	2-Acetylpyrrole	honey-like	5.70 ± 0.25a	3.02 ± 0.12b	2.58 ± 0.02b	2.33	2.33	1.83	MS,RI,O,Std
A16	1074	1070	(*Z*)-Linalool oxide (furanoid)	sweet, floral, creamy	4.29 ± 0.02a	4.17 ± 0.06a	4.03 ± 0.04a	2.00	2.00	2.00	MS,RI,O,Std
A17	1079	1080	2-Ethyl-3,6-dimethylpyrazine	roasty, nutty	35.42 ± 0.04c	45.05 ± 0.13b	58.00 ± 0.05a	3.00	3.00	3.17	MS,RI,O,Std
A18	1083	1083	2-Ethyl-3,5-dimethylpyrazine	roasty, nutty	11.03 ± 0.04c	13.13 ± 0.07b	18.72 ± 0.06a	2.83	3.00	3.50	MS,RI,O,Std
A19	1086	1088	(*E*)-Linalool oxide (furanoid)	sweet, floral, creamy	5.28 ± 0.14a	4.91 ± 0.12ab	4.10 ± 0.03b	2.33	2.17	2.00	MS,RI,O,Std
A20	1094	1099	Benzoic acid methyl ester	sweet, fruity	1.53 ± 0.01b	1.67 ± 0.02a	1.68 ± 0.03a	2.33	2.33	2.33	MS,RI,O,Std
A21	1099	1100	Linalool	citrus-like, floral	2.64 ± 0.01b	2.80 ± 0.08b	4.42 ± 0.03a	3.67	3.67	3.67	MS,RI,O,Std
A22	1149	1147	5H-5-Methyl-6,7-dihydrocyclopentapyrazine	nutty, roasty	1.57 ± 0.17b	1.99 ± 0.04a	2.24 ± 0.02a	1.00	1.00	1.25	MS,RI,O
A23	1155	1155	(*E,Z*)-2,6-Nonadienal	cucumber-like	1.95 ± 0.01b	1.96 ± 0.01b	2.04 ± 0.04a	1.50	1.50	1.50	MS,RI,O,Std
A24	1162	1162	3,5-Diethyl-2-methylpyrazine	bell pepper-like, roasty	5.98 ± 0.02c	6.59 ± 0.06b	9.93 ± 0.04a	2.17	2.33	2.50	MS,RI,O,Std
A25	1163	1163	2,3,5-Trimethyl-6-ethylpyrazine	earthy	3.23 ± 0.03b	3.64 ± 0.14b	5.85 ± 0.01a	2.17	2.33	2.33	MS,RI,O,Std
A26	1173	1167	(*Z*)-Linalool oxide (pyranoid)	earthy	2.17 ± 0.09a	1.94 ± 0.08a	1.40 ± 0.12b	2.25	1.00	nd	MS,RI,O,Std
A27	1178	1176	Benzeneacetic acid methyl ester	fruity, floral	25.43 ± 0.06a	25.31 ± 0.06a	23.95 ± 0.03a	1.50	1.33	1.33	MS,RI,O
A28	1187	1182	1-(2-Furanylmethyl)-1H-pyrrole	roasty,nutty	38.22 ± 0.01a	31.13 ± 0.03b	25.84 ± 0.05c	2.17	2.17	2.17	MS,RI,O
A29	1192	1190	Methyl salicylate	mint-like	53.17 ± 0.05a	50.43 ± 0.10a	49.93 ± 0.09a	2.33	2.17	2.17	MS,RI,O,Std
A30	1207	1208	2,5-Dimethyl-3-(2-methylpropyl)-pyrazine	nutty, roasty, sweet	11.72 ± 0.01b	12.03 ± 0.01b	14.70 ± 0.07a	1.50	1.50	2.00	MS,RI,O
A31	1246	1242	2-Isoamyl-6-methylpyrazine	earthy, pea-like	2.43 ± 0.05c	2.73 ± 0.07b	4.38 ± 0.01a	1.00	1.00	1.25	MS,RI,O
A32	1255	1256	Geraniol	rose-like, citrus-like	5.50 ± 0.03b	5.52 ± 0.01b	6.97 ± 0.06a	2.00	2.00	2.00	MS,RI,O,Std
A33	1262	1258	Benzenepropanoic acid methyl ester	floral, fruity	0.65 ± 0.04a	0.65 ± 0.03a	0.66 ± 0.02a	1.50	1.50	1.50	MS,RI,O
A34	1293	1293	Indole	fecal, mothball-like	7.09 ± 0.01a	6.85 ± 0.04a	6.64 ± 0.05a	2.25	2.00	2.00	MS,RI,O,Std
A35	1304	1308	2,5-Dimethyl-3-(3-methylbutyl)-pyrazine	roasty, sweet	4.84 ± 0.02c	5.39 ± 0.04b	7.09 ± 0.03a	1.50	1.50	1.83	MS,RI,O
A36	1384	1388	3-Methyl-Indole	fecal, mothball-like	4.90 ± 0.20a	4.20 ± 0.02a	3.79 ± 0.02a	2.33	2.17	2.17	MS,RI,O,Std

(*continued on next page*)

Table 1 (*continued*)

No	RI[a]	RI[b]	Odorants[c]	Odor quality[d]	Relative concentration (μg/L)[e]			AI[f]			Identification[g]
					LT	LYT	ST	LT	LYT	ST	
A37	1405	1400	Jasmone	floral	0.75 ± 0.06a	0.72 ± 0.02a	0.53 ± 0.04b	2.00	1.50	1.50	MS,RI,O
A38	1435	1426	*α*-Ionone	floral, raspberry-like	0.21 ± 0.06a	0.21 ± 0.06a	0.11 ± 0.14b	1.67	1.00	1.00	MS,RI,O,Std
A39	1490	1489	(*E*)-*β*-Ionone	floral, violet-like	0.91 ± 0.09a	0.74 ± 0.15a	0.37 ± 0.14b	2.67	2.33	2.00	MS,RI,O,Std

a, Retention index (RI) calculated from the retention times of the compounds and a homologous series of n-alkanes (C_7-C_{40}) separated separately by the HP-5MS capillary column;
b, RI referenced by HP-5MS capillary columns available in the NIST database(https://webbook.nist.gov/chemistry/);
c, Odorants were smelled and identified in the three LYT infusion;
d, Odor quality of each odorant at the sniffing port;
e, Relative concentrations of odorants in LYT and its stems (ST) and leaves (LT), different letters indicate significant differences at 0.05 level.
f, AI, aroma intensity value of each odorant at the sniffing port; nd, Odor not detected at the sniffing port.
g, Methods of identification: MS, odorants were identified by mass spectra; RI, retention indices; O, olfactometry; and Std, reference compounds.

heating process through thermal decomposition (Wang et al., 2000), and thus ST may lead to the formation of linalool and geraniol in large quantities in over-fired drying. As a result, the full characteristic aroma of LYT is a combination of LT providing the dominant cooked-corn and sweet aroma and ST providing the roasty and nutty aroma.

3.3. Non-volatile compounds analyzed in different parts of LYT

Sensory evaluation combined with GC-O analysis we found out that ST and LT played different roles in composing the aroma quality of LYT, the roasty and nutty aroma provided by ST contributing significantly to the formation of the key rice-crust aroma of LYT. On the other hand, ST and LT showed greater differences in taste quality (Fig. 1). Amino acids, soluble sugars, alkaloids, and tea polyphenols are considered to be the major non-volatile metabolites in tea, contributing to its rich taste quality (Huang et al., 2022), where tea polyphenols, a class of polyphenol-containing mixtures that includes catechins, flavonoids and flavonoid glycosides, anthocyanidins and leucoanthocyanidins, and phenolic acids, account for 18–36 % of the dry weight of tea (Wan, 2003). To obtain more information about the composition and concentration of non-volatile compounds in tea, ST and LT of LYT were analyzed by target and non-target metabolomics approaches (Fig. S2), and 39 compounds in tea were accurately matched and quantified using reference compounds (Table 2), the other compounds were matched with Human Metabolome Database (https://hmdb.ca/) by MS/MS fragments of LC-MS. The raw data of LC-MS were initially filtered by QC samples with a relative standard deviation lower than 30 % and signal-to-noise ratio greater than 10, and the filtered 2154 features were analyzed by PCA (Fig. S3A) and HCA (Fig. S3B). The LT and ST of LYT were clearly differentiated, and the QC samples were in the middle of the two-dimensional coordinates, which indicated the credibility of our test. Moreover, the closer Euclidean distance between LYT and LT suggests that the non-volatile compound composition of LT is more similar to LYT, which is consistent with the results of the sensory evaluation. Furthermore, 23 additional metabolites with peak signals were identified by LC-MS as metabolite complements (Table S2), and a total of 62 compounds were categorized according to their chemical structures into free amino acids (**T1** ~ **T17**), soluble sugars (**T18** ~ **T32**), caffeine (**T33**), catechins (**T34** ~ **T44**), flavonoids (**T45** ~ **T59**), and organic acids (**T60** ~ **T62**) (Table 2). Except for GCG, all the compounds detected in LYT were able to be detected in LT and ST.

The free amino acids mainly enriched in LYT were theanine (**T3**, 21.0–4.5 %), aspartic acid (**T4**, 16.9–25.9 %), glutamic acid (**T1**, 7.6–10.1 %), and arginine (**T17**, 7.8–13.3 %), which were dominant among the free amino acids. Most of the amino acids accumulated more in LT, significantly higher than ST, such as **T4**, **T6**, **T9** ~ **T17**, and they are protein amino acids, which may be since leaves undergo more intense photosynthesis and require more amino acids to provide nitrogen source and energy for photosynthesis (Zhang, Guo, et al., 2022). In contrast, the total free amino acid content in ST reached 2.865 mg/g, which was 20 % higher than that of LT, and this contrast was mainly contributed by theanine, which was 142 % higher in ST (1.217 mg/g) than in ST (0.503 mg/g), and was the major amino acid in ST. Theanine is a non-protein amino acid that has been found to be synthesized from glutamic acid and ethylamine catalyzed by the theanine synthase in the roots of the tea plant, and the continuously synthesized theanine is transported upward through the stems into the leaves (Fu et al., 2021; Yu & Yang, 2020). In addition, there is an efficient conversion of ammonium nitrogen to glutamic acid in tea plant roots, but it does not accumulate in the form of glutamate, but quickly converts glutamic acid to amides such as theanine (Zhu et al., 2021).

Sugars are important substances that provide energy for the growth and development of the tea plant, of which the soluble part is the main sweetener in the tea infusion (Ren et al., 2022). The concentration of soluble sugars and sugar alcohols in LYT reached 5.995–7.801 mg/g, compared with other teas, the lower concentration of sugars in LYT was due to the large content of sugars involved in the Maillard reaction during the over-fired drying (Yin et al., 2023). The total content of sugars in LT was higher than that in ST, and sucrose (85.8 % ~ 87.5 %) was the highest sugar in both LT and LYT, followed by lactose (4.3 % ~ 7.4 %), glucose (2.3 % ~ 4.4 %), and fructose (1.1 % ~ 2.7 %), which amounted to more than 97 % of the total sugars, and were the main sweeteners in LYT. It is worth noting that LT and ST have different types of sugars, with more disaccharides in LT and monosaccharides in ST. Disaccharides, such as sucrose, are mainly produced in leaf cells by the condensation of two monosaccharides, and disaccharides are transported to stems and other parts through the phloem, where the excess sucrose produced during transport is hydrolyzed to glucose (Ren et al., 2022). In addition, different soluble sugars have different sweetness and contribute differently to the taste of tea infusion. The sweetness of the main four sugars in LYT showed fructose (1.50) > sucrose (1.00) > glucose (0.76) > lactose (0.40) (Magwaza & Opara, 2015), and higher monosaccharides in ST may potentially contribute to the sweet and mellow taste quality of tea infusion.

Caffeine **(T33)** is found in high levels in tea, accounting for 1 % to 4 % of the dry weight of tea, and is considered to have a strong bitter taste (Zhang et al., 2020). The content of caffeine in LYT was 26.932 mg/g, and there was a large difference between ST (15.567 mg/g) and LT (31.174 mg/g), with the higher amount of caffeine in LT providing a significant sensory bitter stimulation of the tea infusion. Moreover, caffeine is a relatively stable secondary metabolite in tea, and most studies concluded that the effect of changing processing parameters during postharvest processing on the content of caffeine is not significant (Huang et al., 2022; Mao et al., 2018), and its content is mainly determined by secondary metabolism in the tea plant, which implies that it is very difficult for the presence of caffeine to be improved by

Table 2
Nonvolatile compounds in large-leaf yellow tea infusion.

No.	Compounds[a]	Concentration (mg/g)[b]			Sourse[c]	Identification[d]
		LT	LYT	ST		
T1	Glutamic acid	0.182 ± 0.004c	0.201 ± 0.007b	0.288 ± 0.002a	Amino acid analyser	Std
T2	Glutamine	0.037 ± 0.001b	0.038 ± 0.001b	0.051 ± 0.001a	Amino acid analyser	Std
T3	Theanine	0.503 ± 0.003c	0.704 ± 0.005b	1.217 ± 0.007a	Amino acid analyser	Std
T4	Aspartic acid	0.619 ± 0.012a	0.558 ± 0.008b	0.485 ± 0.007c	Amino acid analyser	Std
T5	Threonine	0.050 ± 0.001b	0.050 ± 0.001b	0.070 ± 0.002a	Amino acid analyser	Std
T6	Serine	0.098 ± 0.002a	0.085 ± 0.002b	0.067 ± 0.001c	Amino acid analyser	Std
T7	Glycine	0.010 ± 0.000a	0.010 ± 0.000a	0.010 ± 0.000a	Amino acid analyser	Std
T8	Alanine	0.110 ± 0.000a	0.108 ± 0.001a	0.109 ± 0.002a	Amino acid analyser	Std
T9	Cysteine	0.123 ± 0.001a	0.115 ± 0.001b	0.092 ± 0.002c	Amino acid analyser	Std
T10	Valine	0.077 ± 0.001a	0.070 ± 0.001b	0.067 ± 0.004b	Amino acid analyser	Std
T11	Isoleucine	0.044 ± 0.001a	0.036 ± 0.000b	0.028 ± 0.001c	Amino acid analyser	Std
T12	Leucine	0.033 ± 0.000a	0.027 ± 0.000b	0.024 ± 0.001c	Amino acid analyser	Std
T13	Tyrosine	0.039 ± 0.001a	0.034 ± 0.000b	0.033 ± 0.001b	Amino acid analyser	Std
T14	Phenylalanine	0.063 ± 0.001a	0.053 ± 0.000b	0.051 ± 0.000b	Amino acid analyser	Std
T15	Lysine	0.067 ± 0.002a	0.055 ± 0.000b	0.037 ± 0.002c	Amino acid analyser	Std
T16	Histidine	0.017 ± 0.001a	0.015 ± 0.000b	0.013 ± 0.000c	Amino acid analyser	Std
T17	Arginine	0.319 ± 0.003a	0.236 ± 0.002b	0.223 ± 0.005c	Amino acid analyser	Std
T18	Trehalose	0.028 ± 0.000a	0.023 ± 0.000b	0.017 ± 0.001c	GC–MS	MS, Std
T19	Maltose	0.027 ± 0.000a	0.023 ± 0.003ab	0.020 ± 0.003b	GC–MS	MS, Std
T20	Lactose	0.539 ± 0.020a	0.446 ± 0.015b	0.260 ± 0.027c	GC–MS	MS, Std
T21	Sucrose	6.823 ± 0.303a	5.197 ± 0.099b	5.143 ± 0.349b	GC–MS	MS, Std
T22	Cellobiose	0.052 ± 0.003a	0.042 ± 0.003b	0.038 ± 0.004b	GC–MS	MS, Std
T23	D-Xylulose	0.001 ± 0.000a	0.001 ± 0.000a	0.001 ± 0.000a	GC–MS	MS, Std
T24	D-Sorbitol	0.002 ± 0.000c	0.003 ± 0.000b	0.005 ± 0.000a	GC–MS	MS, Std
T25	L-Rhamnose	0.005 ± 0.000a	0.005 ± 0.000a	0.005 ± 0.000a	GC–MS	MS, Std
T26	D-Mannose	0.020 ± 0.001b	0.019 ± 0.000b	0.038 ± 0.001a	GC–MS	MS, Std
T27	D-Glucose	0.177 ± 0.001b	0.174 ± 0.001b	0.264 ± 0.007a	GC–MS	MS, Std
T28	D-Galactose	0.011 ± 0.001a	0.011 ± 0.000a	0.011 ± 0.000a	GC–MS	MS, Std
T29	D-Fructose	0.087 ± 0.002b	0.083 ± 0.001b	0.163 ± 0.011a	GC–MS	MS, Std
T30	D-Arabinose	0.008 ± 0.000a	0.009 ± 0.000a	0.009 ± 0.000a	GC–MS	MS, Std
T31	D-Arabinitol	0.015 ± 0.000a	0.016 ± 0.000a	0.015 ± 0.000a	GC–MS	MS, Std
T32	Xylitol	0.005 ± 0.000a	0.005 ± 0.000a	0.006 ± 0.000a	GC–MS	MS, Std
T33	Caffeine	31.174 ± 2.884a	26.932 ± 0.498b	15.567 ± 0.551c	HPLC	Std
T34	Epigallocatechin gallate	18.859 ± 0.485a	10.73 ± 0.770b	0.753 ± 0.088c	HPLC	Std
T35	Epigallocatechin	9.197 ± 2.043a	8.475 ± 1.163a	3.381 ± 0.088b	HPLC	Std
T36	Epicatechin gallate	4.542 ± 0.481a	2.342 ± 0.258b	0.215 ± 0.022c	HPLC	Std
T37	Gallocatechin gallate	4.886 ± 0.889a	2.308 ± 0.376b	nd.	HPLC	Std
T38	Epicatechin	2.719 ± 0.287a	2.371 ± 0.071a	1.979 ± 0.048b	HPLC	Std
T39	Catechin	1.719 ± 0.157a	1.478 ± 0.018a	1.499 ± 0.095a	HPLC	Std
T40	Assamicain A	6.708 ± 0.162a	4.563 ± 0.230b	0.385 ± 0.008c	LC-MS	MS
T41	(−)-Epigallocatechin sulfate	0.191 ± 0.012a	0.161 ± 0.003b	0.070 ± 0.003c	LC-MS	MS
T42	(−)-Epiafzelechin 3-gallate	1.194 ± 0.010a	0.938 ± 0.014b	0.175 ± 0.004c	LC-MS	MS
T43	Epigallocatechin-(4beta- > 8)-epicatechin 3-O-gallate	0.136 ± 0.010a	0.101 ± 0.003b	0.092 ± 0.013b	LC-MS	MS
T44	Epigallocatechin-(4beta- > 8)-epigallocatechin-3-O-gallate	0.151 ± 0.008a	0.142 ± 0.008a	0.023 ± 0.037b	LC-MS	MS
T45	Myricetin	0.293 ± 0.008a	0.257 ± 0.011b	0.199 ± 0.005c	LC-MS	MS, Std
T46	Kaempferol	1.580 ± 0.068a	1.187 ± 0.046b	0.464 ± 0.025c	LC-MS	MS, Std
T47	Quercetin	0.101 ± 0.006a	0.088 ± 0.009b	0.029 ± 0.000c	LC-MS	MS, Std
T48	Eriodictyol	0.435 ± 0.015a	0.409 ± 0.012b	0.070 ± 0.004c	LC-MS	MS
T49	Naringenin	0.094 ± 0.006c	0.108 ± 0.010b	0.129 ± 0.004a	LC-MS	MS, Std
T50	Kaempferol 3-O-arabinoside	0.201 ± 0.010a	0.184 ± 0.012a	0.144 ± 0.003b	LC-MS	MS, Std
T51	Quercetin 3-galactoside	0.169 ± 0.007a	0.130 ± 0.003b	0.048 ± 0.003c	LC-MS	MS, Std
T52	Myricetin 3-galactoside	0.752 ± 0.016a	0.598 ± 0.009b	0.179 ± 0.008c	LC-MS	MS, Std
T53	Genistin	0.085 ± 0.002a	0.047 ± 0.008b	0.040 ± 0.003b	LC-MS	MS
T54	Genistein 7-O-(2-p-coumaroylglucoside)	0.541 ± 0.022c	0.765 ± 0.031b	0.846 ± 0.025a	LC-MS	MS
T55	Naringin	0.037 ± 0.005c	0.088 ± 0.002b	0.349 ± 0.006a	LC-MS	MS, Std
T56	Kaempferol 7-(6″-galloylglucoside)	0.087 ± 0.005b	0.127 ± 0.004a	0.132 ± 0.004a	LC-MS	MS
T57	Quercetin 3-(3-p-coumaroylglucoside)	0.173 ± 0.004c	0.198 ± 0.006b	0.235 ± 0.002a	LC-MS	MS
T58	Apigenin 7-(3″-acetyl-6″-*E*-p-coumaroylglucoside)	0.314 ± 0.006c	0.597 ± 0.013b	1.395 ± 0.023a	LC-MS	MS
T59	Procyanidin C1	0.043 ± 0.003c	0.056 ± 0.003b	0.078 ± 0.005a	LC-MS	MS
T60	Shikimic acid	0.266 ± 0.007b	0.284 ± 0.004b	0.381 ± 0.015a	LC-MS	MS, Std
T61	Quinic acid	34.397 ± 0.944c	44.88 ± 1.341b	82.517 ± 0.598a	LC-MS	MS, Std
T62	Gallic acid	3.981 ± 0.104a	3.391 ± 0.021b	0.937 ± 0.026c	LC-MS	MS, Std

a, Non-volatile compounds detected in LYT and its stems (ST) and leaves (LT).
b, Concentrations of non-volatile compounds in LYT and its stems (ST) and leaves (LT); different letters indicate significant differences at 0.05 level; nd, not detected in ST sample.
c, Methods of detection: compound detected by Amino acid analyser, gas chromatography–mass spectrometry (GC–MS), high performance liquid chromatography (HPLC) or liquid chromatography-mass spectrometry (LC-MS).
d, Methods of identification: MS, compounds were identified by mass spectra; Std, reference compounds.

postharvest processing. Caffeine was concentrated in the buds and leaves of the new shoots, less in the older leaves, and less in the stems (Zhang, Guo, et al., 2022).

Six catechin monomers (**T34** ~ **T39**) and five catechin polymers (**T40** ~ **T44**) were detected in LYT, and all of them showed a highly uniform distribution of LT content significantly higher than ST. Catechins were mainly concentrated in the vigorously growing parts of the tea plant, such as the new shoots, and were less abundant in the old leaves and stems in the same way as caffeine. Stronger photosynthesis promoted carbon metabolism and inhibited nitrogen metabolism in tea leaves. The biosynthesis and esterification of catechins are related to photosynthesis, and light promotes the conversion of theanine to catechins, which are highly accumulated in the LT, which may be another reason for the lower theanine content in the LT. Catechins were mainly accumulated as monomers (82.4 % ~ 91.3 %) as shown in epigallocatechin gallate (EGCG, **T34**, 8.8 % ~ 37.5 %), epigallocatechin (EGC, **T35**, 18.3 % ~ 39.4 %), epicatechin gallate (ECG, **T36**, 2.5 % ~ 9.0 %), gallocatechin gallate (GCG, **T37**, nd ~ 9.7 %), epicatechin (EC, **T38**, 5.4 % ~ 23.1 %), and catechin (C, **T39**, 3.4 % ~ 17.5 %). It is noteworthy that the greatest difference in catechin monomer content between LT and ST was found for ester catechins, i.e., EGCG (LT, 18.859 mg/g; ST, 0.753 mg/g), ECG (LT, 4.542 mg/g; ST, 0.215 mg/g) and GCG (LT, 4.886 mg/g; ST, nd), whereas for non-ester catechins (EGC, EC and C), which accumulated mainly in ST, were the top three catechins (17.5 % to 39.4 %). This suggests that the content of catechins in LYT has a different spatial distribution due to secondary metabolism in the tea plant, and the monomeric-dominated catechins accumulated in the LT. On the other hand, ester catechins were mainly accumulated in the leaves, while non-ester types dominated in the ST.

There are many types of flavonoids, mainly flavonols and flavonoid glycosides, most of them are yellow and have a bitter and astringent taste, which is an important natural colorant for the yellow color of LYT tea infusion, and also one of the reasons for the mellow and thick taste of LYT. Fifteen flavonoids were detected in LYT, including flavonols (**T45** ~ **T48**), flavonoid glycosides (**T49** ~ **T58**), and anthocyanins (**T59**). There was no apparent consistent pattern of flavonoid accumulation in LT and ST. Flavonols are synthesized by naringenin (**T49**) catalyzed by different enzymes and then combined with different sugars to form flavonoid glycosides. There is a rich variety of flavonoid glycosides due to different sugars and different binding sites. Flavonols and flavonoid glycosides also undergo changes during post-harvest processing, flavonoid glycosides are hydrolyzed to remove the glycosidic ligands into flavonols in the presence of heat and enzymes (Bozzo & Unterlander, 2021).

3.4. Metabolic pathway analysis related to the flavor compounds of LYT

Quantification of volatiles and non-volatiles in tea by metabolomics approach revealed that LT and ST in LYT differed significantly in compound content. Thus, quantitative data from volatomics and non-volatomics were combined to enrich the metabolic pathways involved in flavor formation in LYT. The metabolic pathways associated with these flavor metabolites were selected and then mapped to the above mentioned volatile compounds (Table 1) and non-volatile compounds (Table 2), namely sugar and free amino acids biosynthesis and the Maillard reaction (Fig. 2), flavonoid biosynthesis (Fig. 3A) and caffeine biosynthesis (Fig. 3B), to further analyze the formation and metabolism of flavor metabolites in ST and LT.

Free amino acids and soluble sugars are potential substances contributing to the umami and sweetness of tea infusion, however, because of the lower concentration and higher threshold of free amino acids and soluble sugars in LYT, it is the higher concentration of theanine, glutamic acid, glucose, and fructose that can be recognized by the senses as the tastant, which have a positive effect on alleviating the bitterness and astringency increasing the umami and sweetness of tea infusion. The growth and metabolic characteristics of the tea plant enrich the ST with more theanine, glutamic acid, and monosaccharides, which is one of the main reasons for the sweet and mellow taste of tea infusion. In addition to their role as tastant, free amino acids and monosaccharides are closely linked to metabolism in tea plants, disaccharides are hydrolyzed to monosaccharides and then amino acids are synthesized through a series of enzymatic reactions (Fig. 2) (Kc et al., 2018). During the over-fired drying stage of LYT, a large number of soluble sugars and free amino acids are consumed in a Maillard reaction to produce heterocyclic compounds such as pyrazines and pyrroles, most of which likewise exhibit higher concentrations in ST. The GC-O combined with AT results showed that these products of the Maillard reaction have strong roasty and nutty aromas in the sensory profile, especially **A5** (earthy and nutty, 2.67), **A14** (roasty and nutty, 2.67), **A17** (roasty and nutty, 3.00) and **A18** (roasty and nutty, 3.00), they are probably the main reason for the higher roasty and nutty aroma profiles in ST and are important odorants contributing to the rice-crust aroma of LYT.

Flavonoids include catechins, flavonols, and flavonoid glycosides etc., which are polyphenols that are responsible for the color and bitter and astringent taste of tea infusion. Catechins and flavonols are synthesized through the flavonoid biosynthetic pathway (Fig. 3A), and phenylalanine is synthesized through a series of enzymatic reactions to produce naringenin (Wang et al., 2021), which is an important intermediate in the direct or indirect synthesis of dihydrocannabinol,

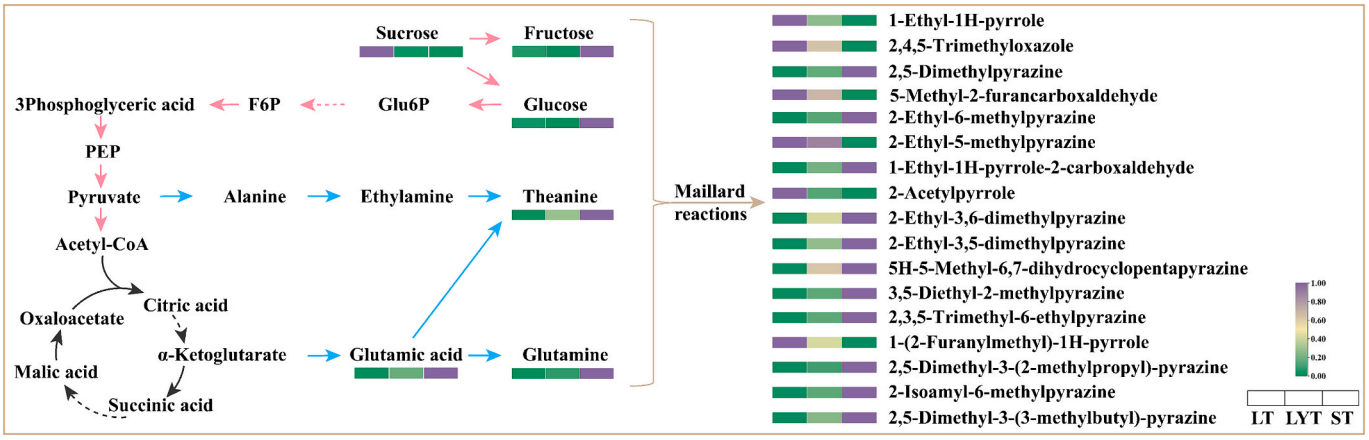

Fig. 2. A Simplified model of the biosynthesis pathways of partial soluble sugars and free amino acids and the Maillard reaction in LYT, and a heat map of the content of the relevant compound of large-leaf yellow tea (LYT) and its stems (ST) and leaves (LT). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

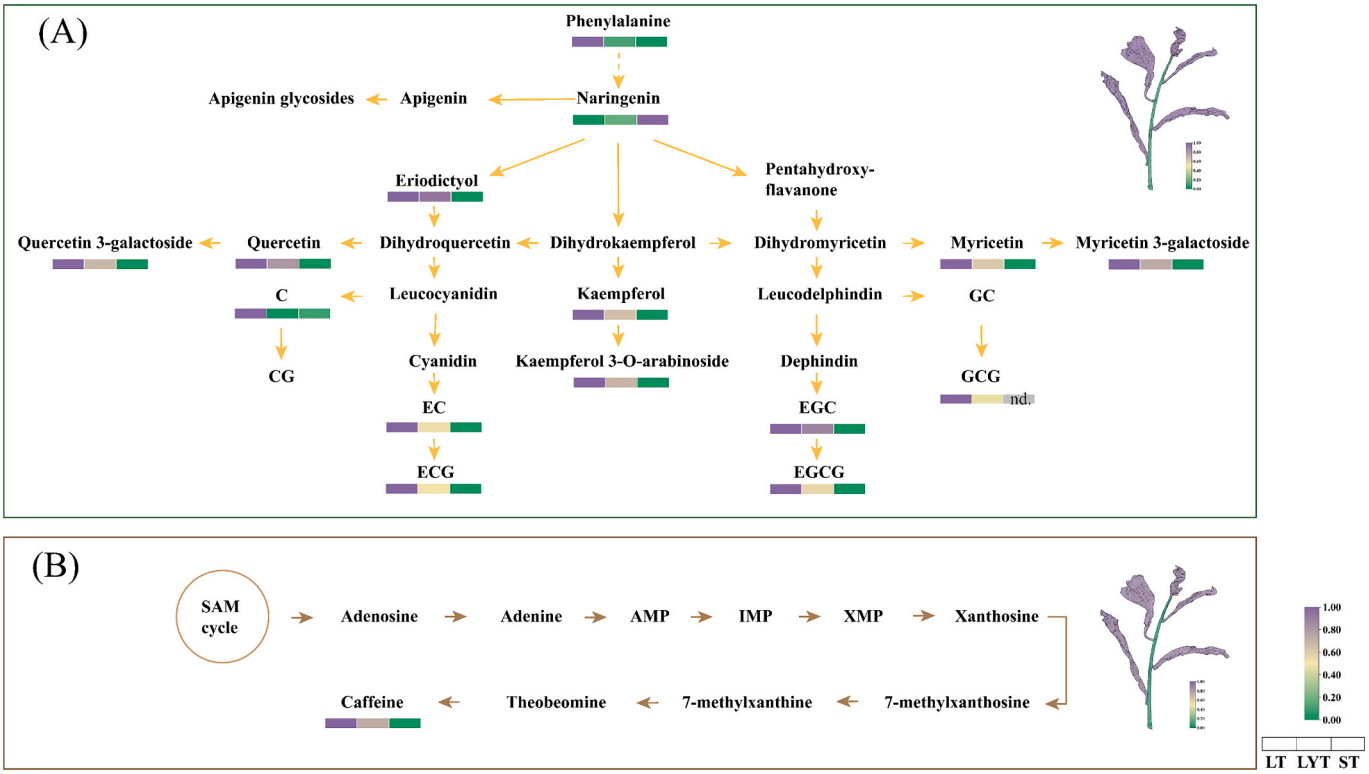

Fig. 3. A Simplified model of the biosynthetic pathway of (A) flavonoids and (B) caffeine in LYT, and the heat map of the content of the relevant compound of large-leaf yellow tea (LYT) and its stems (ST) and leaves (LT). The figure in the upper right panel shows the mapping of LYT phenotype to the content of (A) total catechins and (B) caffeine. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dihydroquercetin, and dihydromyricetin. It then through several enzymatic reactions and modifications to produce flavonols (kaempferol, quercetin, and myricetin) and non-ester catechins (EC, EGC, C, and GC). The flavonols are specifically combined with some sugars to produce corresponding flavonoid glycosides, and non-ester catechins are esterified with gallic acid (GA) to produce ester catechins (ECG, EGCG, CG, and GCG) (Liu et al., 2021). Polyphenols are the most abundant class of compounds in tea infusion other than water and determine the color and taste quality of the tea infusion. There was a highly significant difference in polyphenols concentrations between LT and ST in LYT (Table 2 and Fig. 3A). A range of detected polyphenols downstream of naringenin exhibited significantly higher concentrations of LT than ST, with a consistent distribution in the metabolism of flavonoids.

Caffeine biosynthesis in tea plants is relatively independent of other flavor substance metabolic pathways. Briefly, it is mainly synthesized from adenine through a series of cyclization and methylation (Fig. 3B) (Zhang, Fu, et al., 2022), and its more stable and high content is used as an important reference in tea processing. Caffeine has a strong bitter taste, which not only increases the bitter taste of tea infusion but also synergizes with tea polyphenols such as EGCG to enhance the astringent taste of tea infusion and reduce the umami sweet taste of tea infusion (Xu et al., 2018; Yu et al., 2014).

The pathways of both the Maillard reaction and flavonoid biosynthesis suggest that the differences in flavor substances in different parts of LYT are mainly due to biometabolism in the tea plant, and that its unique flavor characteristics (e.g., roasty aroma in ST and cooked corn-like aroma in LT) are the result of a combination of both the material basis and processing.

3.5. Correlation network analysis between flavor profiles and metabolites of LYT

The sensory evaluation and metabolite analysis showed that the unique quality of LYT is jointly determined by LT and ST, and is the result of the combined expression of the quality differences between LT and ST. Differences in the content and percentage of rich metabolites in LT and ST comprise the differences in multidimensional perception of tea infusion. In order to further clarify the specific contribution of metabolites to the flavor of tea infusion, the QDA results of aroma and taste were correlated with volatile and non-volatile compounds were established separately (Table S3 and Table S4), and the correlation (r) greater than 0.8 are shown in Fig. 4.

For aroma (Fig. 4A), we divided all odorants into two categories according to the synthesis pathway, namely Maillard reaction and others. It was found that almost all of the odorants from the Maillard reaction were significantly positively correlated with roasty and nutty aromas and significantly negatively correlated with rice crust-like and cooked corn-like aromas, which may be due to the fact that more pyrazine and pyrrole are responsible for contributing to roasty and nutty aroma. On the other hand, most of the products of the non-Maillard reaction, including glycoside hydrolyzates (**A1** and **A16**), carotenoid degradation (**A38** and **A39**), and fatty acid degradation (**A1**, **A11**, **A12**, **A8,** and **A37**) showed the opposite results, and they mainly had floral, fruity, and sweet aroma, which showed a significant negative correlation with roasty and nutty aroma. Thus, rice crust-like of the characteristic aroma of LYT, which is due to the combined expression of roasty and nutty aromas contributed by ST and cooked corn-like aroma contributed by LT.

For the color and taste of tea infusion (Fig. 4B), catechins and caffeine showed a significant positive correlation with bitter and astringent tastes and a significant negative correlation with umami and sweet tastes. This is due to the high concentration of both catechins and caffeine, which not only contributed to astringent and bitter tastes, respectively but also interacted with each other resulting in enhanced tastes. The strong correlations between umami and sweet taste were found to be soluble monosaccharides, organic acids, and theanine.

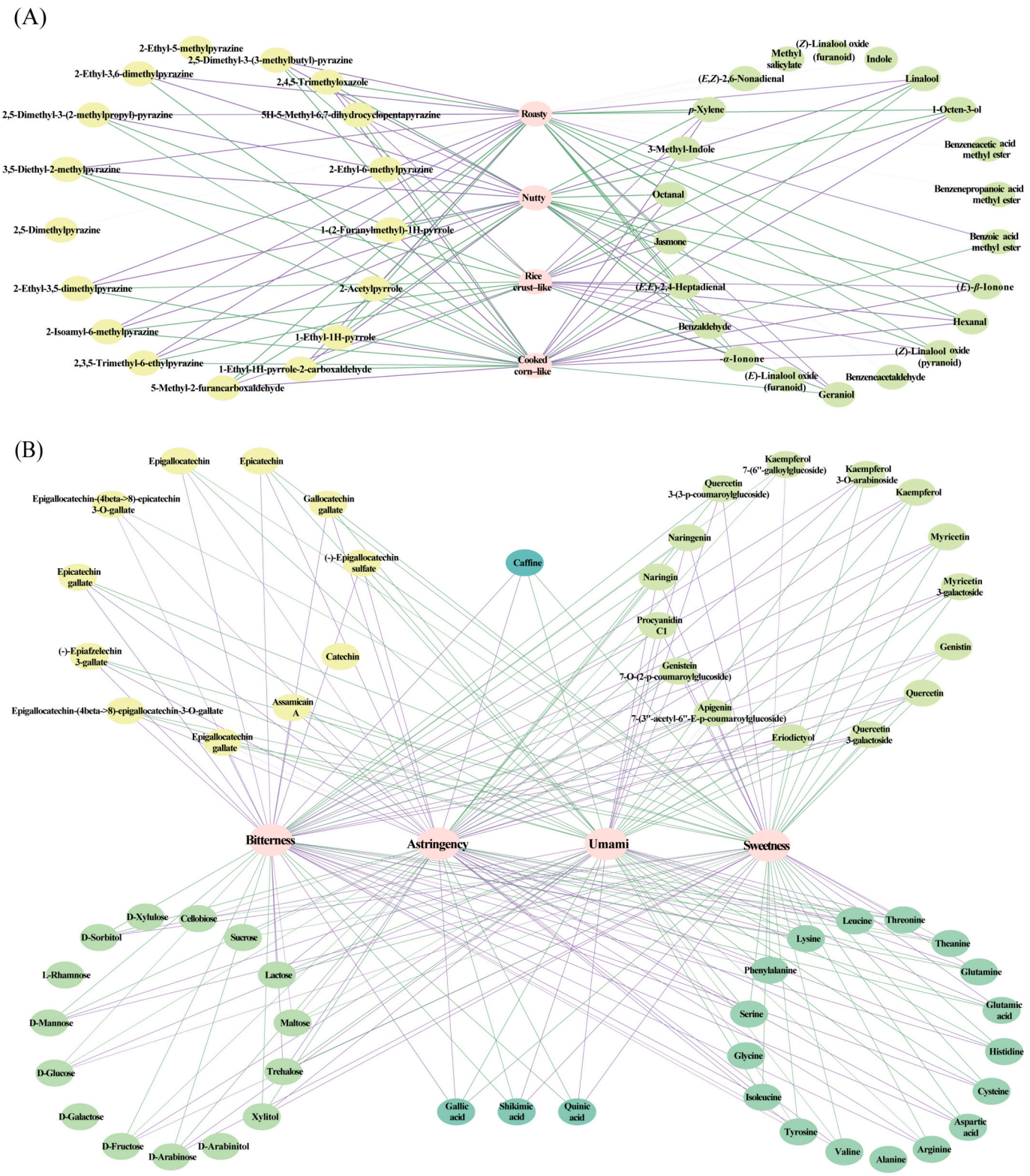

Fig. 4. Correlation analysis (| r | > 0.8) network of flavor profiles and metabolites. (Purple color lines indicates significant positive correlation and green color lines indicates significant negative correlation). (A) Correlation network between results of quantitative descriptive analysis of aroma and odorants; (B) correlation network between the results of quantitative descriptive analysis of taste and non-volatiles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Therefore, higher concentrations of soluble monosaccharides, theanine, and organic acids as well as lower concentrations of catechins and caffeine have obvious advantages in enhancing the umami and sweet tastes and reducing bitter and astringent tastes of tea infusion, which ST has compared to LT. Thus, LT is predominantly necessary to make up the flavor of LYT, and the addition of moderate amounts of ST is effective in diluting bitter and astringent taste (Fig. 1A and C), which is more desirable to consumers in terms of taste quality and health.

In summary, active odorants, free amino acids, soluble sugars, catechins, caffeine, and flavonoids are all important metabolites in the overall flavor of LYT. Amino acids and soluble sugars not only function as flavor tastant for umami and sweet taste but also undergo Maillard reactions contributing to the roasty and nutty aroma of LYT. Caffeine, catechins, and flavonoids independently and synergistically provide the bitterness and astringency of LYT tea infusion, and their lower concentrations are also able to provide a positive influence on the enhancement of perceived sweetness. ST and LT play different roles in contributing to the flavor quality of LYT. LT lacks the important roasty and nutty aroma of LYT and exacerbates the bitter and astringent taste of LYT, but the higher concentration of caffeine and catechins is the main reason for contributing to the bitter and astringent as well as mellow and thick taste of LYT. Whereas the ST lacks the cooked corn-like aroma and reduces the mellow and thick taste, but the higher amount of Maillard reaction products in the ST is the main reason contributing to the roasty and nutty aroma of the LYT. Therefore, the moderate blending of ST and LT is the key to make up the complete flavor quality of the LYT. Most of the flavor metabolites were strongly correlated with sensory quality, suggesting both direct and indirect flavor-contributing roles in LYT, and the larger concentration differences in ST and LT conferred a more important role to these metabolites. However, the cross-influences and interactions of odorants for taste as well as tastants for aroma are intricate and need to be refined by further studies.

4. Conclusion

In this study, we quantified the sensory qualities and flavor compounds of LYT and its different components (ST and LT) using a combined sensomics and flavoromics approach. Subsequently, the metabolic patterns of the flavor metabolites in the biosynthetic pathway were established, and the correlation network between sensory quality and flavor metabolites was constructed. ST provided a stronger roasty and nutty aroma and sweet taste to LYT, this was mainly due to the higher accumulation of theanine and soluble monosaccharides in ST, which provided more substrates for the generation of more pyrazines by the Maillard reaction; whereas LT contributed to the mellow and thick taste quality of LYT, and the abundant catechins and caffeine were the main reasons for the mellowness and more astringency of LT. The difference in metabolite concentrations between ST and LT is due to the biological metabolism in the tea plant. Neither ST nor LT alone can form a complete LYT flavor, and the base of the raw material with full shoot is the key to the formation of LYT flavor. This study reveals the contribution of ST and LT to LYT flavor formation and the reasons for it and provides production guidance and a theoretical basis for the selection of raw materials for future processing of LYT and consumer demand for LYT directed flavors.

CRediT authorship contribution statement

Wenjing Huang: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Qiuyan Liu:** Visualization, Validation, Supervision, Software, Methodology, Investigation, Data curation. **Jingming Ning:** Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

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.

Acknowledgements

This work was supported by the National Key Research & Development Plan (2021YFD1601102).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101794.

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