



Metabolomics and electronic-tongue analysis reveal differences in color and taste quality of large-leaf yellow tea under different roasting methods

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ABSTRACT

Roasting is a key process in the production of large-leaf yellow tea (LYT). In this study, we synthesized metabolomics and electronic-tongue analysis to compare the quality of charcoal-roasted, electric-roasted and drum-roasted LYT. Charcoal-roasted LYT had the highest yellowness and redness, drum-roasted LYT had a more prominent umami and brightness, and electric roasting reduced astringency. A total of 48 metabolites were identified by metabolomics. Among these, leucocyanidin, kaempferol, luteolin-7-lactate, and apigenin-7-O-neohesperidoside might affect the brightness and yellowness. Theanine, aspartic acid, and glutamic acid contents significantly and positively correlated with umami levels, and the high retention of flavonoid glycosides and catechins in drum-roasted LYT contributed to its astringency. These findings elucidate the contribution of the roasting method to the quality of LYT and provide a theoretical basis for LYT production.

1. Introduction

Yellow tea is a unique tea that originated during the Ming Dynasty and was regarded as a symbol of noble dignity in ancient China. It is primarily produced in the Anhui, Zhejiang, Hunan, Hubei, Guizhou, and Sichuan provinces of China (Xu et al., 2018). Although yellow tea accounts for the smallest share of production among the six major tea types, it has gained prominence owing to its sweetness, mellow taste, and attractive aroma (Feng et al., 2023). Yellow tea can be divided into four categories: bud, bud leaf, multi-leaf and compact. Multi-leaf yellow tea is also known as large-leaf yellow tea (LYT). LYT processing includes fixation, rolling, first yellowing, first drying, second yellowing, second drying, third yellowing, and roasting (Zhai et al., 2023). Roasting is a key step that reduces the water content of tea and preserves it, while also significantly affecting its color, taste, and aroma.

Color and taste are two key factors in the evaluation of tea quality. The effects of roasting on the taste of Wuyi rock tea, LYT, and Lu'an Guapian tea were demonstrated. For example, Zhang et al. (2024) compared the nonvolatile compounds in Lu'an Guapian tea before and after overfired drying and showed that overfired drying promoted the transformation of some catechins, amino acids, and flavonoids to reduce

bitterness. Jiang et al. (2022) used metabolomics to analyze the metabolites of roasted and unroasted yellow tea and oolong tea and reported that roasting significantly reduced gallatechin, caffeine, and astringent flavonoid glycoside content. These studies suggest that the taste of tea, particularly its bitterness and astringency, is affected by roasting.

Traditionally, LYT is roasted over charcoal. This method requires 1–2 people to follow the entire process and control the tea quality. High-quality charcoal is required; thus, the production costs are high. With societal development, the degree of mechanization is increasing, and electric and drum roasting are also being used in the LYT roasting process. In electric roasting, air is heated to dry tea, causing its internal water to evaporate slowly; and the heat is primarily transferred through convection. However, hot-air drying was prone to browning of the product in a study on the effect of drying method on daylily quality (Chu et al., 2023). During drum roasting, tea leaves come in contact with the inner wall of a high-temperature drum and are heated through conduction. Drum roasting is used to dry several types of teas. Zhang et al. (2023) studied the effects of different drying methods on the quality of Lu'an Guapian tea, and reported that drum roasting strengthened its roasted aroma. Because all three methods were applied to roasted LYT,

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there were differences in the quality of the resulting tea. However, no study has analyzed the causes of these in-depth differences.

Metabolomics is a new science and technology after genomics, transcriptomics, and proteomics. It is efficient and comprehensive and has therefore attracted significant attention from researchers in recent years. Shen et al. (2022) used targeted and non-targeted metabolomic methods to reveal the potential compounds produced in An tea during storage. Wei et al. (2023) used metabolomic analysis to identify the mechanism through which an optimized yellowing method improved the color and taste of yellow tea. The electronic-tongue (e-tongue) is a device that mimics human taste perception (Sliwinska et al., 2014), and can objectively evaluate the taste of various samples. E-tongues have the advantages of being fast, stable, and sensitive and have been widely used in the food industry (Escuder-Gilabert & Peris, 2010). Therefore, in this study, targeted and nontargeted metabolomic methods and an e-tongue were used to reveal the metabolites and taste differences in LYT after charcoal, electric, or drum roasting. Moreover, the color appearance and infusion color of LYT were detected using computer vision technology. Finally, the potential nonvolatile metabolites responsible for the color and taste differences were determined using correlation analyses. These results will help practitioners select a suitable roasting method for producing high-quality LYT.

2. Materials and methods

2.1. Tea samples

Tea samples were obtained on June 20, 2023, after they had undergone a third yellowing stage at the Yunwufeng Eco-Agriculture Company in Lu'an City, Anhui Province, China. The tea samples were processed from the same batch of fresh leaves. They were divided equally into three groups, each of which had the same tea mass. All roasting processes were implemented for over 95 min with a leaf loading of 10 kg. For charcoal roasting (CR), the roasting cage had a surface temperature of $145 \pm 5^\circ\text{C}$ and the leaf temperature was maintained at $125 \pm 10^\circ\text{C}$. For electric roasting (ER), the dryer temperature was set to 145°C and the temperature of the leaves was maintained at $125 \pm 5^\circ\text{C}$. Finally, for drum roasting (DR), the inner wall of the drum was heated to 145°C before loading the tea, and the leaf temperature was maintained at $90 \pm 5^\circ\text{C}$.

All roasted samples are in triplicate and have a moisture content $<3\%$ (Fig. 1). For each roasted sample, 1 kg was sealed in an aluminum foil pouch and set aside for cold storage at 4°C for subsequent analysis.

2.2. Chemicals and materials

The chemicals and materials used in the study are listed in Table S1.

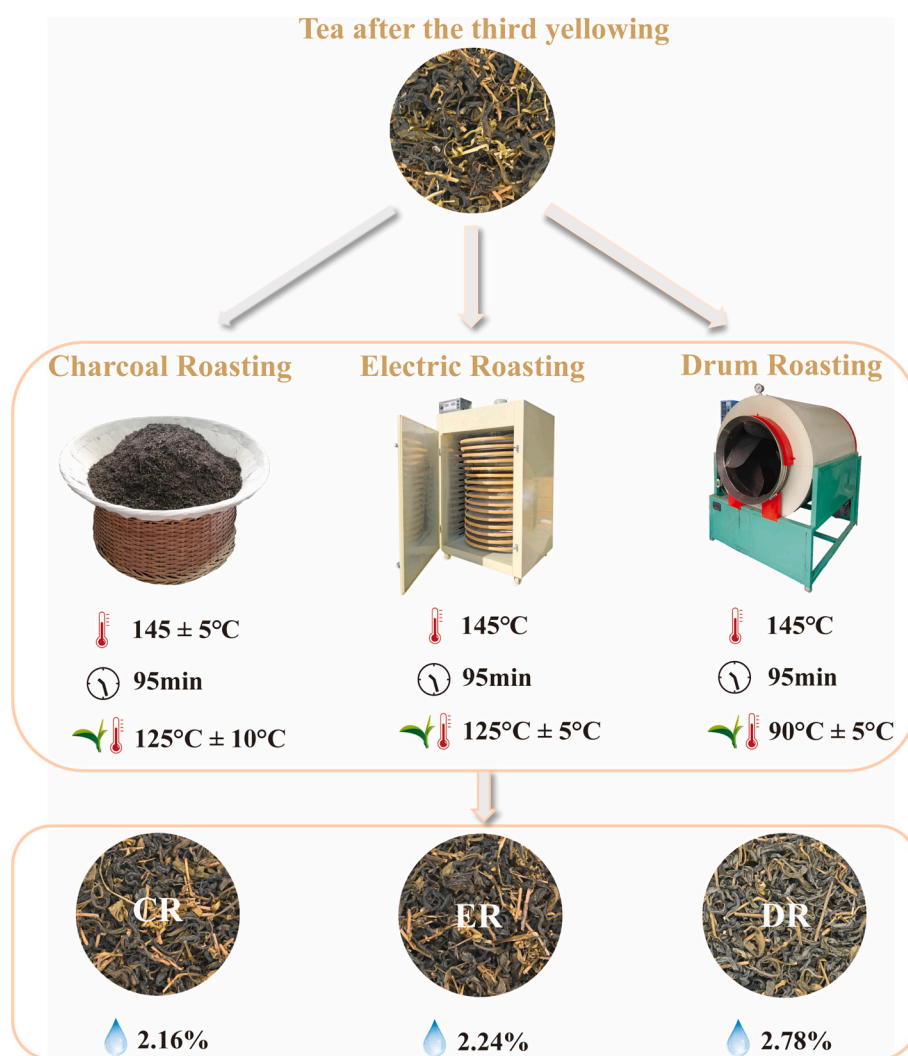


Fig. 1. Parameters for each large-leaf yellow tea roasting method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.3. Color measurement

Referring to the methods described by Wei et al. (2020), the colors of the tea leaves and infusions were analyzed using computer vision. A digital camera (NIKON Z 50, Japan) was used to capture images with the following parameters: focal length, 40 mm; ISO sensitivity 100, aperture, f/8.0; shutter speed, 1/100 s; white balance, 5000 K. The image colors were analyzed using ImageJ software (version 1.51, National Institutes of Health, Bethesda, MD, USA) and MATLAB 2014a (MathWorks, Natick, MA, USA), and the brightness L^* , red/green content a^* , and blue/yellow content b^* of each image were determined. The browning index was calculated as follows (Aghajanzadeh et al., 2023):

$$BI = \frac{100 \times \left(\frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012 \times b^*} \right) - 0.31}{0.17}$$

2.4. Determination of 5-hydroxymethylfurfural

The method described by Udomkun et al. (2015) was used to detect 5-hydroxymethylfurfural, with some modifications. First, a 0.25 g tea sample was mixed with 5 mL of 90% ethanol and swirled for 1 min, followed by centrifugation at 3500 rpm for 10 min. Two milliliters of the supernatant were transferred to a test tube, and 2 mL of 12 g/100 mL trichloroacetic acid and 2 mL of 0.025 mol/L thiobarbituric acid were added. After full mixing, the test tube was set in a water bath at 40 °C for 50 min. The samples were cooled and the absorbance was measured at 443 nm using an ultraviolet-visible (UV-VIS) spectrophotometer (U-5100 HITACHI Japan).

2.5. E-tongue analysis

The tea sample was brewed in accordance with the Chinese standard (GB/T, 2018), and the tea infusion was filtered into an evaluation bowl with gauze while hot, but cooled to room temperature before analysis. Prior to data acquisition, the e-tongue was activated and calibrated to ensure data stability and reliability. For testing, 35 mL of the tea infusion was transferred to an e-tongue cup, and sample testing (120 s), after taste testing (40 s), and cleaning testing (10 s) were performed. The data obtained during a testing period of 110–120 s were recorded. Each sample was subjected to four rounds of testing and stable data from the last three tests were selected for subsequent analyses (Huang et al., 2022).

2.6. Determination of gallic acid, catechins and caffeine

An extraction was performed on 0.20 g of tea powder with 5 mL of 70% aqueous methanol solution (70 °C) for 10 min with shaking every 5 min. The solution was allowed to cool and centrifuged at 3500 rpm for 10 min, and the supernatant was transferred to a volumetric bottle. This process was repeated once, the supernatants were combined, and 10 mL of the resulting liquid was used as the mother liquor.

The concentrations of gallic acid, catechins and caffeine were determined using an Agilent 1260 high-performance liquid chromatography (HPLC) system (Agilent, USA) equipped with a Waters Symmetry® C18 HPLC column (4.6 × 250 mm, 5 μm). Specifically, 2 mL of the mother liquor was added to a stable solution (250 mg EDTA-2Na, 250 mg ascorbic acid, 50 mL acetonitrile, and 500 mL fixed-volume water) and then filtered with a 0.22-μm Millipore filter for the HPLC analysis. The elution process was as reported by Fang et al. (2019). Gallic acid, catechin and caffeine were quantified using standard curves and the contents were expressed in mg/g (Table S2).

2.7. Determination of free amino acids

0.10 g tea powder was ultrasonically-extracted with 4 mL

sulfosalicylic acid (4% concentration) at room temperature for 30 min (shaken every 5 min), and after standing for 10 min, 1.5 mL supernatant liquid was centrifuged (12,000 r/min, 30 min), and then put into small bottles through 0.22-μm Millipore filter for detection. The samples were tested using a high-speed amino acid analyzer (L-8900, Hitachi). According to the method of Lu et al. (2019), the standard and sample injection volume was 20 μL, the mobile phase flow rate was 0.35 mL/min, and the column temperature was 38 °C. All experiments were repeated in triplicate. The amino acid concentration was calculated by relative quantification based on the ratio of the peak area of the sample to that of the standard (Lu et al., 2019).

2.8. Determination of soluble sugars

The method of Metware Biotechnology Co. Ltd. (<http://www.metware.cn>, Wuhan, China) was used, and the soluble sugar content was measured by gaseous chromatography-mass spectrometry (GC-MS) (8890-5977B, Agilent, Santa Clara, USA). The chromatographic MS collection conditions were as follows: The column model was a DB-5MS system (30 m × 0.25 mm × 0.25 μm), a split ratio of 5:1 was used, and the sample size was 1 μL. Heating was performed and the temperature was maintained at 160 °C for 1 min, increased to 200 °C at 6 °C/min, then to 270 °C at 10 °C/min, and then to 320 °C at 20 °C/min for 5.5 min. The ion source temperature was 230 °C, the helium carrier gas flow rate was 1 mL/min, the MS scanning range was 30–400 m/z , and the ionization energy was 70 eV. The MS scanning mode was selective ion monitoring mode (SIM). The soluble sugars were quantified using an external standard curve method (Table S3).

2.9. LC-Orbitrap-MS analysis

The metabolites were extracted as described by Shen et al. (2022). A total of 0.40 g of tea powder was weighed and extracted with 8 mL of a methanol-water mixture (7/3 v/v) with a DL-4-chlorophenylalanine internal standard. The solution was then ultrasonicated for 30 min and allowed to rest for 4 h. After shaking thoroughly, the solution was ultrasonicated for 30 min and allowed to rest for 4 h. The solution was centrifuged at 12000 rpm for 10 min and filtered for LC-Orbitrap-MS analysis. Tests for each sample were conducted in triplicate.

An ultra-performance LC (UPLC) system (Ultimate 3000, Dionex, Sunnyvale, CA, USA) coupled to a mass spectrometer (Q-Exactive Focus, Thermo Fisher Scientific, Waltham, MA, USA) was used to analyze the metabolites. Separation was performed using an Acquity UPLC HSST3 column (100 mm × 2.1 mm × 1.8 μm, Waters, USA). The flow rate was set to 0.2 mL/min and the injection volume was 2 μL. The mobile phases consisted of A and B, where mobile phase A was configured by adding 0.5 mL of formic acid (1 L of pure water, and mobile phase B by adding 0.5 mL of formic acid to 1 L of acetonitrile). The samples were run in negative ionization mode. The negative (–) ESI mode parameters were set as follows: spray voltage 3000 V, capillary temperature 320 °C, resolution 70,000 and mass scan range 100–1500 m/z for the full scan analysis. Tandem mass spectrometry (MS/MS) was a data-dependent ms2 (dd-ms2) scan at a resolution of 17,500, and the energy for high collision-induced dissociation was set to 10, 20, and 60 eV in step mode (Xu et al., 2019).

2.10. Data analysis

Raw data for non-targeted metabolomic analyses were converted to mz/ML and ABf formats using MS-Convert and Analysis Base File Converters, respectively. The ABf file was imported into MS-Dial (version 3.82) software. Noise filtering, peak identification, overlapping peak analysis, peak alignment, and peak filling were performed using the corresponding parameters. MS1 and MS2 characteristic tables, m/z values, residence times (Rt), ID, and relative MS peak intensities were obtained. Principal component analysis (PCA), hierarchical cluster

analysis (HCA), and partial least squares discriminant analysis (PLS-DA) of the metabolites were performed using SIMCA-P (version 14.1). Differential metabolites in the three samples were identified using variables important for project (VIP) values. Metabolite identification was performed using MSFINDER ver 3.04 by comparing their retention times and MS2 information with standards or with those in databases (MoNA, HMDB, and PubChem, Table S5).

SPSS 24.0 software was used for analysis of variance (minimum significance difference test) and pearson correlation analysis. All images were visualized using Origin 2024 and TBTools. Plot the network in Cytoscape. All trials were repeated in triplicate.

3. Results and discussion

3.1. Difference in LYT color between roasting methods

The color parameters of LYT for the three roasting methods are shown in Fig. 2A–C. The L*, a*, and b* values denote brightness (+)/greenness (–), and yellowness (+)/blueness (–), respectively. Regarding tea appearance, DR had the highest L* value (47.20 ± 0.62), which means that drum roasting favors the brightness of tea. However, the a* value of DR is negative (-0.45 ± 0.03), indicating that the color is greenish, while the characteristic of yellow tea is “three yellows” (yellow dry tea, yellow tea infusion and yellow brewed tea leaves) (Feng et al., 2023), which is not in line with the basic characteristics of yellow tea. In drum roasting, prolonged friction due to repeated contact between the tea leaves and the heated drum wall may have led to cell wall rupture, chlorophyll overflow, or thermal degradation, which led to the appearance of gray-green tea (Hua et al., 2018).

Compared with the ER and DR infusions, the CR infusion was 1.51- and 2.82-fold more yellow higher, respectively, and 1.14- and 1.2-fold redder, respectively; these differences were significant. However, DR tea infusion was the brightest. The primary contributors to the color of tea infusions are the water-soluble pigments in tea, including the oxidation products of flavanols and flavonoids (Wan, 2003). Compared

with the ER and DR infusions, the CR infusion was 1.51- and 2.82-fold more yellow higher, respectively, and 1.14- and 1.2-fold redder, respectively; these differences were significant. However, DR tea infusion was the brightest. The primary contributors to the color of tea infusions are the water-soluble pigments in tea, including the oxidation products of flavanols and flavonoids (Van Boekel, 2006). A study of black tea colorants revealed that theanine–glucose and pyroglutamic-acid–glucose Amadori products modulate the yellow tone of black tea (Long et al., 2024). In LYT and Wuyi rock tea, the Maillard reaction causes the color of the tea broth to change from yellow to slightly reddish (Su et al., 2024). Therefore, the CR LYT infusion may have had the highest b* value in this study because the most intense Maillard reaction occurred during CR. An intermediate of the Maillard reaction, 5-hydroxymethylfurfural, has been used as an indicator of the occurrence of this reaction in several studies (Udomkun et al., 2015). Fig. 2D shows the colorimetric detection results for 5-hydroxymethylfurfural; the absorbance was significantly higher for CR than for DR and slightly higher than for ER, suggesting that the Maillard reaction was the primary cause of the redder color of the CR tea infusion.

During the roasting process, tea undergoes a browning reaction due to the Maillard reaction, caramelization reaction, and ascorbic acid browning of the tea contents, which in turn leads to a decrease in the brightness value (L*) of the product (Ren et al., 2021). As shown in Fig. 2E, the browning values of all three tea samples for both tea appearance and tea infusion were significantly different and showed the same result, that is, the DR samples had the lowest browning index; therefore, the DR samples had high brightness values in terms of both tea appearance and tea infusion.

3.2. E-tongue characterization of LYT taste

An objective taste analysis was performed using an e-tongue (Table 1). The three samples exhibited significantly different in their levels of bitterness, astringency, and umami attributes. Specifically, CR was the most bitter ($1.22\times$ and $1.33\times$ higher than for ER and DR); the

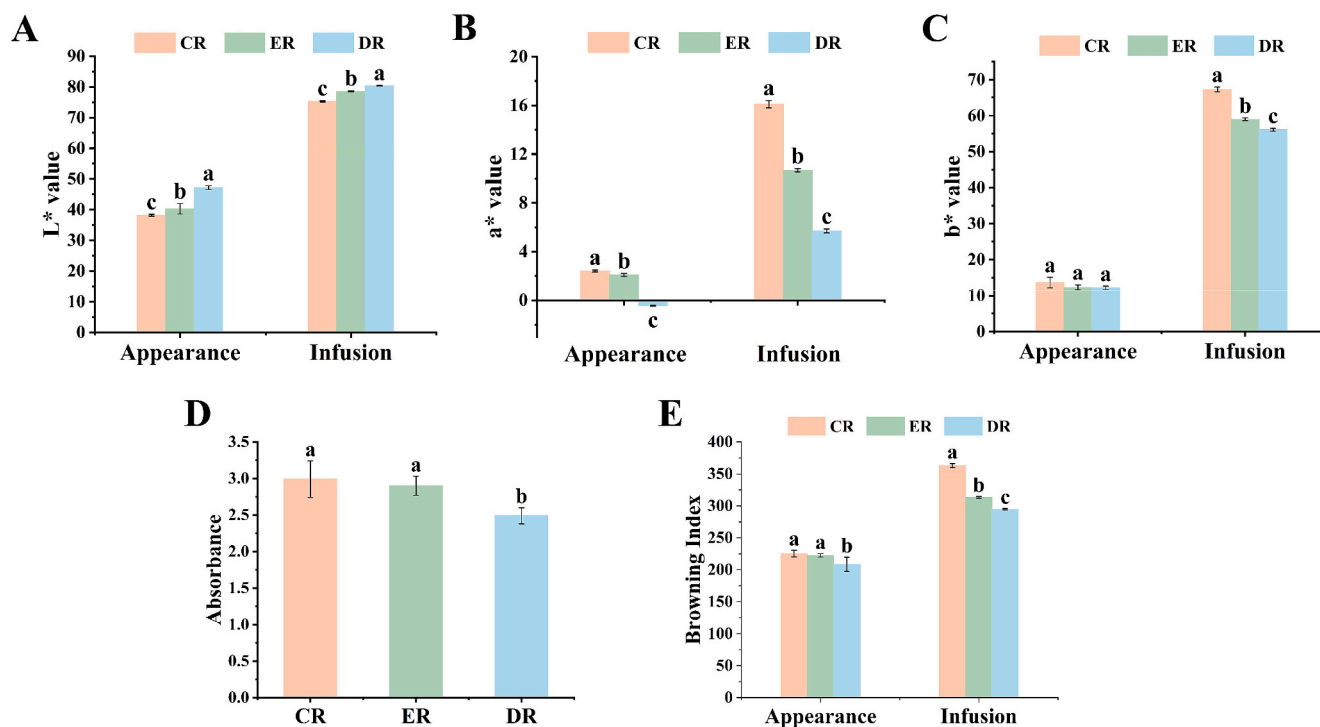


Fig. 2. (A–C), Appearance and infusion color parameters of charcoal-roasted, electric-roasted and drum-roasted large-leaf yellow tea; (D), Absorbance results of 5-hydroxymethylfurfural; (E), Appearance and infusion browning indices of three samples of large-leaf yellow tea. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
E-tongue quantitative taste evaluation results.

	CR	ER	DR
Bitterness	3.10 ± 0.12 ^a	2.54 ± 0.17 ^b	2.32 ± 0.23 ^b
Astringency	10.43 ± 0.06 ^b	9.61 ± 0.14 ^c	13.07 ± 0.24 ^a
Umami	11.49 ± 0.06 ^b	11.38 ± 0.02 ^c	12.96 ± 0.06 ^a
Sweetness	1.36 ± 0.21 ^a	1.37 ± 0.28 ^a	1.20 ± 0.23 ^a

Note: CR, charcoal roasting; ER, electric roasting; DR, drum roasting. Different letters indicate a significant difference at the 0.05 level.

DR sample had the lowest bitter and highest umami values; ER significantly reduced the astringency of LYT. This may be attributed to the effect of roasting on the content of various nonvolatile metabolites. However, sweetness values of the three samples were not significantly different ($p > 0.05$).

3.3. Analysis of the main taste components

3.3.1. Analysis of gallic acid, catechin and caffeine results

Catechin and caffeine contents are the most fundamental indices for evaluating tea quality; these compounds are primarily responsible for the bitterness and astringency of tea infusions (Wan, 2003). During processing, catechins can undergo reactions such as differential isomerization, hydrolysis, and polymerization at high temperatures, which may affect the taste of tea infusions. Six catechins and gallic acid (GA) were quantified by HPLC (Table S4); the content of most catechins differed significantly between the tea samples. Epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) constitute half of the total catechin content and significantly affect the astringency of tea infusions. The DR samples contained the highest concentrations of EGCG and ECG (particularly EGCG). The average EGCG content of 25.25 mg/g was 13.48% and 8.32% higher than those of ER and DR, respectively; this higher EGCG content may explain the higher astringency of the DR sample. Epigallocatechin (EGC), catechin (C), and epicatechin (EC) are non-galloylated catechins that are not as bitter and astringent as EGCG and ECG. Interestingly, unlike other catechins, the contents of GA and galocatechin gallate (GCG) were highest in CR tea and lowest in DR tea. At high temperatures, such as CR, galloylated catechins undergo thermal cleavage to produce GA, and EGCG undergoes differential isomerization to form GCG. Specifically, the substituent at the C₂ position of the catechin B ring is flipped and becomes spatially different from the substituent at the C₃ position, which produces GCG (Wan, 2003). In this study, the roasting time was the same for all three methods; thus, we hypothesized that the differences in catechin production were related to differences in the degree of roasting.

Caffeine is the most abundant alkaloid in tea and is considered to significantly affect its bitterness. Table S4 indicates that the differences in caffeine content among the three samples were not significant, likely because caffeine is stable and not easily affected by processing. Therefore, differences in bitterness are likely attributable to differences in amino acid or flavonoid content.

3.3.2. Effect of roasting method on free amino acids content

Free amino acids are among the five taste compounds found in tea (Feng et al., 2023), and the 18 common amino acids can be divided into umami, sweet, and bitter amino acids (Huang et al., 2022). As indicated in Table 2, the amino acid content differed significantly between the roasting methods. All amino acids were most abundant in DR, and these differences were significant among the three samples for nine of the amino acids. In particular, the contents of the four amino acids that significantly affected the umami taste of the tea infusions (Asp, Glu, Gln, and Thea) differed between the samples. DR had the highest content of umami amino acids, followed by ER, which may have contributed to the prominence of the umami taste of the DR infusion. However, although CR had the lowest umami amino acid content, its umami value was

Table 2
Free amino acids content in three samples.

Free amino acids(μg/g)	CR	ER	DR
Umami AAs			
Asp	1042.66 ± 6.56 ^c	1141.05 ± 15.22 ^b	1575.69 ± 14.95 ^a
Glu	529.11 ± 8.08 ^c	620.48 ± 3.60 ^b	1837.39 ± 24.66 ^a
Gln	65.32 ± 0.20 ^b	80.10 ± 3.31 ^b	584.03 ± 19.13 ^a
Thea	3395.59 ± 38.12 ^c	3895.49 ± 8.25 ^b	10,560.35 ± 54.25 ^a
Sweet AAs			
Thr	114.33 ± 1.41 ^c	124.58 ± 0.90 ^b	200.65 ± 3.84 ^a
Ser	182.31 ± 2.50 ^c	192.89 ± 0.38 ^b	349.25 ± 1.01 ^a
Gly	30.35 ± 0.44 ^b	31.38 ± 0.19 ^{ab}	36.05 ± 0.26 ^a
Ala	160.17 ± 4.95 ^c	171.47 ± 1.36 ^b	208.00 ± 0.77 ^a
Cys	115.98 ± 2.57 ^c	126.06 ± 1.09 ^b	242.74 ± 1.61 ^a
Pro	33.41 ± 3.29 ^a	37.73 ± 0.11 ^a	43.84 ± 6.78 ^a
Bitter AAs			
Val	74.95 ± 0.88 ^b	62.66 ± 1.00 ^c	103.38 ± 2.00 ^a
Ile	61.13 ± 0.72 ^b	59.92 ± 0.26 ^{ab}	112.42 ± 3.67 ^a
Leu	50.78 ± 0.43 ^b	53.04 ± 0.46 ^b	86.66 ± 1.35 ^a
Tyr	74.19 ± 0.74 ^b	75.05 ± 2.10 ^b	114.09 ± 3.04 ^a
Phe	137.11 ± 6.88 ^b	131.98 ± 7.36 ^b	249.22 ± 12.53 ^a
Lys	62.40 ± 0.34 ^b	61.26 ± 0.79 ^b	105.92 ± 1.71 ^a
His	nd	nd	40.42 ± 0.10 ^a
Arg	467.49 ± 2.04 ^c	564.11 ± 8.23 ^b	840.85 ± 13.81 ^a
Total	6597.29 ± 49.85 ^c	7429.24 ± 23.11 ^b	17,300.79 ± 92.29 ^a

Note: Aspartic acid (Asp), glutamic acid (Glu), glutamine (Gln), theanine (Thea), threonine (Thr), serine (Ser), glycine (Gly), alanine (Ala), cysteine (Cys), proline (Pro), valine (Val), isoleucine (Ile), leucine (Leu), tyrosine (Tyr), phenylalanine (Phe), lysine (Lys), histidine (His), arginine (Arg). CR, charcoal roasting; ER, electric roasting; DR, drum roasting. Different letters indicate a significant difference at the 0.05 level.

between those of ER and DR. This may be attributed to synergistic and antagonistic interactions between the taste compounds. This interaction may also occur between bitter amino acids and other compounds, because the DR Sample had the highest amount of bitter amino acids but the lowest bitterness value. Zhou et al. (2023) reported that Thea, Asp, and Glu can reduce the bitterness of galloylated catechins, which may have contributed to the low bitterness of DR infusion. In addition, the differences in tea infusion bitterness may be ascribed to differences in flavonoid compound retention.

3.3.3. Effect of roasting method on soluble sugars content

Soluble sugars in tea are the primary components affecting sweetness. Soluble sugars are predominantly monosaccharides and disaccharides, in addition to small amounts of trisaccharides and tetrasaccharides (Wan, 2003). A total of 18 soluble sugars (1 trisaccharide, 4 disaccharides, and 13 monosaccharides) were detected in the three samples using GC-MS; their standard curve equations and contents are displayed in Table S3. During high-temperature tea roasting, the sugars undergo Maillard and caramelization reactions, and differences in the tools and heat transfer modes of the three roasting methods result in differences in the soluble sugar content. Fig. 3A presents a heat map of the 18 soluble sugars. Trisaccharide raffinose is thermally stable and participates minimally in Maillard reactions at high temperatures (Tang et al., 2008); therefore, its content did not differ significantly among the three samples. DR tea had high retention of most disaccharides and monosaccharides. Categorization of the monosaccharides (Fig. 3B) revealed that the content of all reducing monosaccharides was the highest in the DR samples. Reducing sugars have carbonyl and aldehyde groups and therefore frequently undergo Maillard reactions with amino acids at high temperatures. Previous studies have shown that reducing monosaccharide content typically decreases with increasing roasting

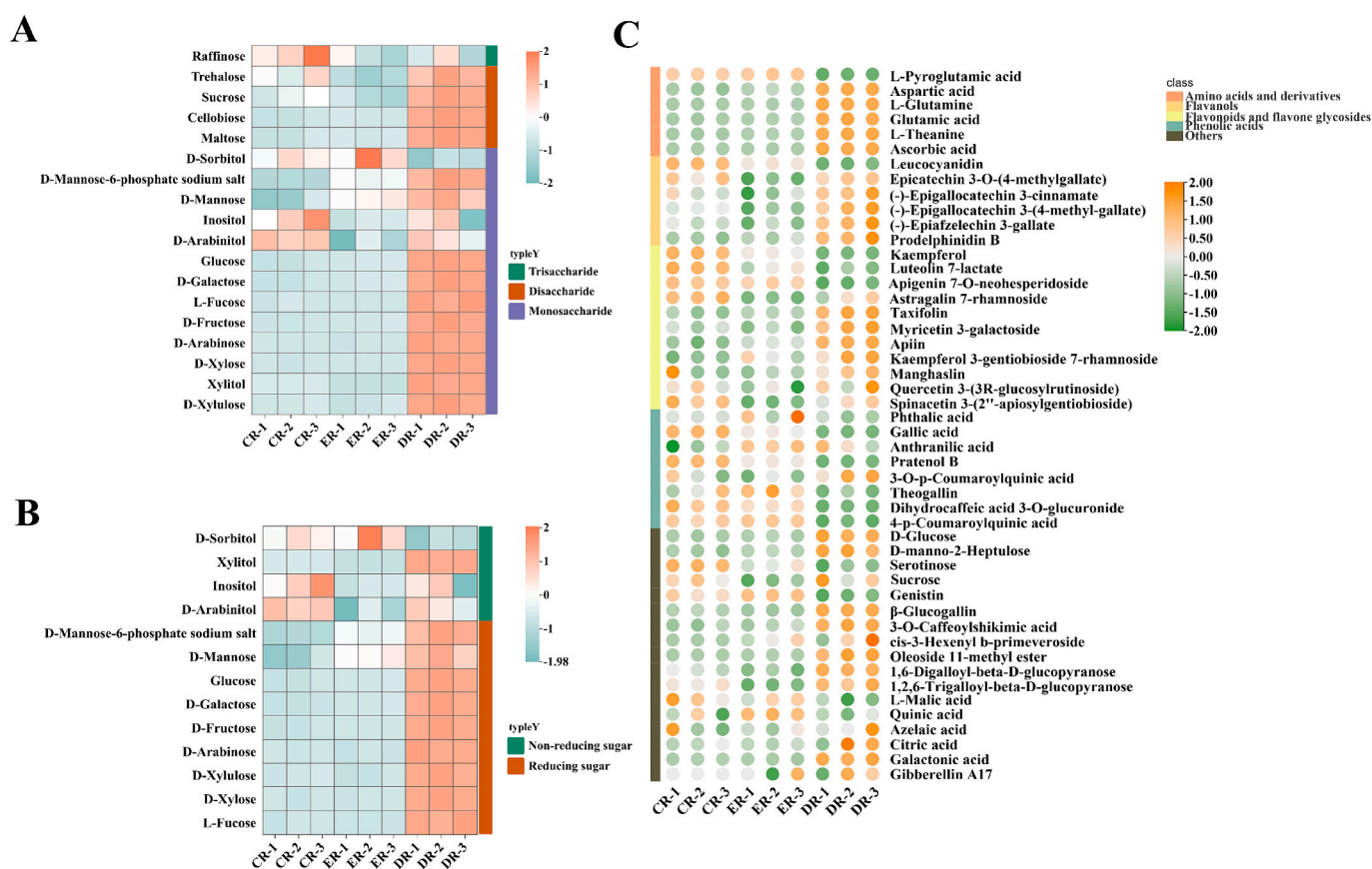


Fig. 3. (A), Heat map of the content of 18 soluble sugars identified in the three samples; (B), Heat map of the content of 13 soluble monosaccharides; (C), Heatmap of 48 identified differential metabolites in the three samples. A color-coded scale grading from green to orange corresponds to the content of differential metabolites shifting from low to high. CR, charcoal roasting; ER, electric roasting; DR, drum roasting. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

temperature (Yin et al., 2023). In this study, the degree of the Maillard reaction was lower and more reducing monosaccharides were retained than in ER and CR because of the repeated tossing of tea leaves into the air during drum roasting, where the heating time with the inner wall of the drum was shorter than the actual roasting time. However, there was no significant difference in sweetness values among the three samples. This may be due to the fact that the concentration of sweetening compounds is so low that the dose-over-threshold factor is <0.1 , and therefore does not make a major contribution to typical flavors (Zhang et al., 2020).

3.4. LC-MS analysis

To comprehensively understand the differences between the nonvolatile metabolites of LYT for different roasting methods, non-targeted metabolomic analysis was performed using LC-Orbitrap-MS. After peak extraction, alignment, and filtering, a total of 3566 characteristic ions were detected in the three samples, which were analyzed using multivariate statistical analysis. First, the PCA (Fig. S1A) results showed that the first two principal components explained 61.2% and 20.9% of the total variance, respectively. The first principal component indicated that the metabolites of CR and ER were similar but clearly distinguishable from those of DR. The second principal component reflected the variability between CR and ER. Collectively, the unsupervised PCA model suggested that the different roasting methods led to significant variations in the nonvolatile compounds of LYT. The HCA (Fig. S1B) results were consistent with the PCA results in that the three samples were categorized into two classes at Euclidean distances >6000 ; CR and ER comprised one class, and DR was alone in the other class. The

supervised PLS-DA model showed similar results; for 200 cross-validated permutation tests, Q^2 was -0.0861 , indicating that the PLS-DA model was not overfitted (Fig. S1C-D). Finally, to identify the key differential metabolites, the Variable Importance in the Projection (VIP) values of all metabolites were ranked, and compounds with $VIP > 1.2$ were selected as marker compounds and identified.

A total of 48 differential metabolites were identified, comprising 6 amino acids and their derivatives, 6 flavanols, 11 flavonoids and flavonoid glycosides, 8 phenolic acids, and 17 other metabolites (Table S5). The compounds are visualized as heat maps (Fig. 3C). The results for the amino acids and their derivatives were almost identical to the target quantification results in Section 3.4.2. Notably, the L-pyroglutamic acid content showed a completely opposite result to the other amino acids, which is due to the fact that it is generated by cyclization of glutamic acid at high temperatures (Wang et al., 2022). Therefore, its content is negatively correlated with that of glutamate.

In addition to catechins, flavonoids and flavone glycosides are also key contributors to the bitter and astringent flavors of tea infusions. LC-MS results revealed significant differences in the flavonoid and flavone glycoside contents of the three LYT samples. CR tended to increase the flavonoid content, whereas DR favored the retention of flavone glycosides. Flavone glycosides are formed through combinations of flavonoids and sugars in tea, and flavone glycosides differ in the positions and connections of the combined sugars (Wan, 2003). During heat treatment, the flavone glycoside content decreased because of the destruction of glycosidic bonds. In the present study, DR and CR had the highest and lowest flavone glycoside contents, respectively. There are two possible explanations for this. First, unlike the ER and CR, in the DR, the intermittent contact between the leaves and drum decreased the actual

roasting time to less than the nominal roasting time. Moreover, this process results in uneven heating and incomplete chemical reactions. Second, in the CR, heat is primarily transferred through thermal radiation. Because both the temperature and moisture are transferred in the same direction, the heat transfer efficiency is high. Moreover, the covalent ether bonds connecting flavonoids and sugars in flavonoid glycosides may be decomposed by the far-infrared rays emitted by heated charcoal, further promoting the decomposition of flavonoid glycosides (Ren et al., 2021). This ultimately leads to an increase in flavonoid content and a decrease in flavonoid glycoside content in CR.

Phenolic acids are aromatic compounds containing carboxyl and hydroxyl groups. In particular, the phenolic acid GA is an umami-enhancing compound in green tea that can increase the umami intensity of L-glutamate (Kaneko et al., 2006). The heat map revealed that the GA content was the highest in the CR and lowest in the DR, which is consistent with the HPLC results in Section 3.4.1. This may explain why the umami value was higher in CR than in ER, despite the higher umami amino acid content in CR. Some phenolic acids (other than GA) that were positively correlated with bitterness and astringency in tea infusions also had higher contents in CR than in ER; this may be related to the different degrees of stress on the tea leaves due to the roasting methods. Because phenolic acids typically exist as cell wall compounds connected to polysaccharides through ester or ether bonds, they accumulate in large quantities under external stress (Udomkum et al., 2015). Therefore, the accumulation of phenolic acids was likely higher for CR because the leaf temperature reached 125 °C in these processes, which is notably higher than that during drum roasting.

3.5. Correlation analysis between substances affecting the color of tea infusions and chroma values

The primary substances affecting the color of a tea infusion are

water-soluble pigments in raw tea leaves (natural pigments) and pigments formed during processing (theaflavin, thearubigin, and theabrownin). Because the LYT in this study had already undergone fixation, polyphenol oxidase and peroxidase in the tea leaves were no longer active. Thus, the primary substances affecting the color of tea infusions in this study were natural pigments, including the oxidation products of flavonoids, anthocyanins, and catechins. A Pearson's correlation analysis was performed for these substances with the colorimetric values of the tea infusions, and the results are shown in Table S6. Seven compounds significantly affected the color of the tea infusions: one anthocyanin and six flavonoids. These were identified as leucocyanidin (ID5234), prodelphinidin B (ID12990), kaempferol (ID4002), taxifolin (ID4513), luteolin-7-lactate (ID6021), apin (ID11629), and apigenin-7-O-neohesperidoside (ID11941). In general, high anthocyanin content leads to a brownish and dark infusion color (Wan, 2003). Thus, leucocyanidin likely reduces the brightness of the tea infusion, and its content IS significantly and negatively correlated with the L^* values of the tea infusions. In addition, the contents of ID4002, ID6021, and ID11941 were also negatively correlated with L^* . However, because these substances are yellow pigments, they also significantly affect the a^* value of tea broth.

3.6. Correlation analysis of non-volatile compounds and taste characteristics

Partial least squares regression analysis (PLSR) and Pearson's correlation analysis were used to explore the relationship between tea taste characteristics and nonvolatile compound content. In the PLSR plot (Fig. 4A), the two variables were positively correlated if they were connected to the origin at an angle $<90^\circ$. The metabolites of origin have a more significant contribution to taste. Most of the substances positively correlated with the tea infusion umami taste were the umami

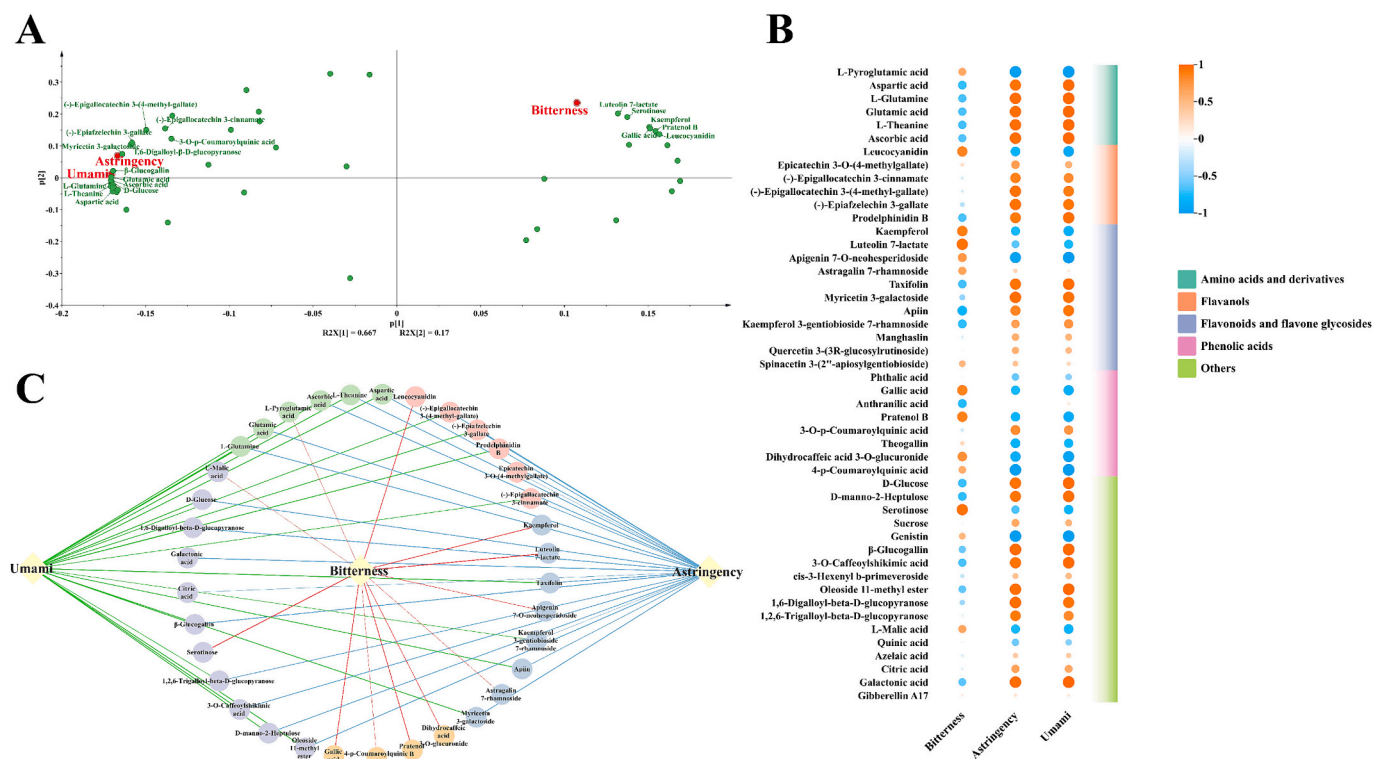


Fig. 4. (A), Partial least squares discriminant analysis between taste attributes and metabolites of large-leaf yellow tea. Red indicates taste attributes, green indicates metabolites. (B), Pearson correlation analysis between taste attributes and metabolites. (C), Association networks of metabolites with taste attributes. Rhombic nodes represent taste characteristics; circular nodes represent metabolites, and different colored circles indicate different classes of metabolites, with green representing amino acids and their derivatives, red representing flavanols, blue representing flavonoids and flavone glycosides, yellow representing phenolic acids, and purple representing others. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

amino acids Glu, Gln, Asp, and Thea. Up to 70% of the umami intensity of green tea is attributed to amino acids, especially L-glutamic acid and L-glutamine (Yu et al., 2014). L-theanine comprises approximately half of the total amino acid content and is considered the primary umami-enhancing compound in high-grade powdered green tea in Japan (Kaneko et al., 2006). In our study, the substances closely related to the bitterness and astringency of tea were primarily flavanols, flavonoids, flavone glycosides, and phenolic acids, which is consistent with previous findings (Shan et al., 2024). Most people believe that astringency is a textural feature perceived by the tactile system, rather than a taste (Brossard et al., 2021). This is because astringent compounds have numerous phenyl hydroxyl groups that bind to proline-containing proteins in the saliva through intermolecular forces and hydrogen bonding (Horne et al., 2002). Compounds that are positively correlated with astringency include (–)-epigallocatechin-3-(4-methyl-gallate), (–)-epi-afzelechin-3-gallate, (–)-epigallocatechin-3-cinnamate, myricetin-3-galactoside, 3-O-*p*-coumaroylquinic acid, and 1, 6-digalloyl- β -D-glucopyranose. Flavone glycosides and phenolic acids have extremely low taste thresholds and are the key contributors to tea astringency. *P*-coumaroylquinic acid is also strongly and positively correlated to astringency and has an even smaller threshold than EGCG of only 38 μ mol/L. In addition, one study noted that flavone glycosides have a lower taste threshold than epicatechins and proanthocyanidins (Sáenz-Navajas et al., 2012). Six compounds affect the bitter taste of tea: kaempferol and proanthocyanidins. However, the contribution of the products of the Maillard reaction to the bitter flavor should not be overlooked, as compounds such as reduced ketones and furans produced by the Maillard reaction may exhibit a bitter flavor (Chu et al., 2024).

Fig. 4B shows the Pearson correlation results for key differential metabolites and flavor characteristics, which were highly consistent with those of the PLSR. Correlation network analysis was performed to determine the compounds associated with taste (Fig. 4C). We found that some umami compounds significantly affected the astringency of tea, and some bitter compounds were also positively correlated with umami due to interactions between these compounds. Liu et al. (2023) reported that umami amino acids (Thea, Glu, and Asp) can inhibit the bitterness and astringency of ester catechins; moreover, ester catechins can also enhance the umami intensity of the umami amino acids at various concentrations. Moreover, taste interactions do not only occur between umami and astringent compounds; promotion and inhibition can occur between bitter and astringent compounds, astringent and sweet compounds, and sweet and umami compounds. For example, the taste threshold for sweet compounds is much higher than that for astringent compounds; thus, the presence of astringent compounds may inhibit the perception of sweetness (Huang et al., 2022). Fig. 4B shows the Pearson correlation results for key differential metabolites and flavor characteristics, which were highly consistent with those of the PLSR. Correlation network analysis was performed to determine the compounds associated with taste (Bandyopadhyay et al., 2012). Collectively, the complex interactions between taste compounds warrant further investigation.

4. Conclusions

In summary, this study comprehensively and objectively analyzed the metabolites of LYT using three roasting methods, and identified their relationships with tea quality using computer vision technology, e-tongue, and metabolomics. Color analysis revealed that DR resulted in little browning and high brightness for both appearance and infusion; however, the tea had a green color. For the CR, the tea tended to be yellower and redder than the other samples. The e-tongue results indicated that ER helps to reduce astringency, DR helps to reduce bitterness, and has the highest umami value. A total of 48 key differential metabolites were identified using metabolomics, including six amino acids and their derivatives, 6 flavanols, 11 flavonoids and flavone glycosides, 8 phenolic acids, and 17 other substances. The contents of the umami

amino acids Asp, Glu, Gln, and Thea were highest in DR tea, favorably contributing to its umami taste. However, low leaf temperatures during DR resulted in low flavonoid degradation and, thus, stronger astringency. On the other hand, the high retention of catechin in the drum roasted sample is also the primary reason for its astringency. CR LYT has a high flavonoid content, and CR promotes the Maillard reaction, both of which increase the bitterness of the tea. This study identified the differences in the color and taste of LYT with different roasting methods, providing a theoretical basis for the processing of high-quality LYT in the future.

CRedit authorship contribution statement

Caiyan Sheng: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Mingxia Lu:** Software, Methodology, Formal analysis. **Jixin Zhang:** Writing – review & editing, Methodology, Data curation. **Wei Zhao:** Methodology, Investigation, Data curation. **Yanqun Jiang:** Methodology, Investigation, Data curation. **Tiehan Li:** Writing – review & editing, Validation, Supervision. **Yujie Wang:** Writing – review & editing, Validation, Supervision. **Jingming Ning:** Resources, 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.2024.101721>.

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