Dynamic changes of key metabolites in Longjing green tea during processing revealed by widely targeted metabolomic profiling and sensory experiments

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Abstract:

In this study, widely targeted metabolomics and chemometrics were utilized to comprehensively analyse the formation of taste substances in Longjing green tea. A total of 580 non-volatile metabolites were identified using ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry, and alterations in three metabolic pathways were investigated. Notably, the fixation process reduced phosphatidic acid levels, resulting in the formation of lyso-phosphatidylcholine and lyso-phosphatidylethanolamine, as well as the release of esterified polyunsaturated fatty acids. Baiye No.1 had high levels of L-glutamic acid and L-glutamate, while Longjing 43 showed elevated levels of flavones. Correlation analysis and sensory verification indicated that an appropriate concentration of L-aspartic acid increased the stringency of the tea. These findings advance our understanding of Longjing green tea quality improvement and cultivar development.

Keywords: Longjing tea; Widely targeted metabolomics; Processing; Taste; Cultivar

1. Introduction

Green tea, a non-fermented tea, is the most produced and consumed tea in China. It is renowned for its health-enhancing properties, including antioxidant, anti-inflammatory, and anticancer effects (Musial et al., 2020). Manufactured from the fresh new shoots of tea plant (*Camellia sinensis*), the typical green tea process involves picking, fixation, rolling, and drying. Sensory evaluations of green tea reveal a variety of aromas attributes, including faint, floral, chestnut-like, and other categories. The tea infusion exhibits a green and bright colour and the taste is usually composed of bitterness, astringency, sweetness, and umami. Importantly, taste is a key determinant of consumer preference and acceptance, making it a critical aspect of the sensory characteristics of green tea. The quality of tea depends on the secondary metabolites it contains. For instance, the astringency of tea is primarily due to the presence of polyphenols, alkaloids, and catechins. Anthocyanins contribute significantly to its bitter taste (Ye et al., 2022). Amino acids constitute approximately 70% of the umami taste intensity of green tea (Nakagawa, 1975), while soluble sugars are the primary source of sweetness (Yue et al., 2017).

Taste compounds in tea leaves are influenced by the quality of fresh tea leaves (varieties, growing conditions, and picking tenderness), processing technique, and storage (Zeng et al., 2022). Firstly, the quality of tea is closely associated with the tea cultivar (Wang et al., 2021). For instance, Longjing 43 is considered the most suitable cultivar for producing Longjing tea (Dragon well tea), primarily due to its chlorophyll *b* content that contributes to the formation of a brown-beige colour in dry tea (Wang

& Ruan, 2010). The fresh and mellow taste of Anji bai tea processed from Baiye No.1 is related to its high L-theanine content and low tea polyphenol levels (Zeng, Lin, Liu & Liu, 2019). Secondly, tea processing technologies substantially impact metabolites in the final tea product. Many bioactive compounds, which are present in the final tea product and contribute to its quality or functional properties, are produced during the tea manufacturing process (Liao, Zhou, & Zeng, 2020). In the fixation or roasting process, amino acids can react with carbonyl compounds to form Strecker aldehydes that contribute to the formation of the tea aroma (Rizzi, 2008). The fixation stage is primarily associated with chlorophyll decomposition, phosphatidic acids reduction and glycolipids degradation (Li et al., 2021). Finally, the storage year is one of the quality evaluation criteria for tea, especially dark tea and white tea (Zhou., et al 2023). However, green tea is usually recommended to be consumed without long-term storage because its taste and aroma deteriorate quickly. Therefore, comprehensive studies are necessary to explore the dynamic changes of green tea used fresh tea leaves from different cultivars as raw materials during the entire manufacturing process.

In recent years, there has been significant progress in the development and refinement of precision instruments, enabling the application of accurate and powerful techniques for food analysis. Metabolomics studies in the field of tea research have utilized various approaches, including targeted, untargeted, and widely targeted metabolomics. These methodologies have been employed in investigating different aspects of tea, such as evaluating the grade of *Tieguanyin* tea (Zeng et al., 2023),

assessing the impact of processing on green tea (Shi et al., 2022), and discriminating tea cultivars in oolong tea (Zeng et al., 2022). Targeted metabolomic investigations have traditionally involved the identification and quantification of a predefined set of analytes, enabling the monitoring of metabolic changes over time. This approach has been extensively utilized for several decades in the field of metabolism research. In contrast, untargeted metabolomics represents a comprehensive and unbiased analytical approach that aims to detect metabolic disturbances without relying on a predefined list of analytes. However, the identification of metabolites in untargeted metabolomics can be complex and time-consuming (Hertzog et al., 2022). A recent advancement in metabolomics is the widely targeted approach, which combines the advantages of untargeted and targeted methods. Widely targeted metabolomics integrates the generality of untargeted metabolomics with the accuracy of targeted metabolomics, offering a valuable tool for comprehensive detection of metabolites (Zhou et al., 2022). Therefore, in this study, we employed the widely targeted metabolomics approach to analyse the metabolites.

The objective of this research is to investigate the influence of cultivars and processing on the metabolite profile of Longjing green tea, utilizing ultra-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (UPLC-ESI-MS/MS) and chemometrics. Specifically, three tea cultivars were selected, which were the albino tea cultivar "Baiye No.1," the traditional Longjing cultivars "Longjing 43" and the variety "Quantizhong." Fresh tea leaves from these cultivars underwent the same manufacturing process to become Longjing green tea samples. Our primary

goal is to elucidate the dynamic changes in metabolites that occur during processing and establish a sensory evaluation based on these metabolite variations. The findings of this study are expected to contribute to the theoretical understanding of Longjing green tea quality improvements and the development of high-quality tea varieties.

2. Materials and methods

2.1. Chemicals

Pure water was from HangZhou Wahaha Group Co., Ltd. (Hangzhou, China). Acetonitrile (ACN, HPLC grade), methanol (MeOH, HPLC grade), and glacial acetic acid (HPLC, ≥99.9%) were from Merck (Darmstadt, Germany). Formic acid (FA), L-aspartic acid, epigallocatechin (EGC), dihydromyricetin, and L-leucine were from Aladdin (Shanghai, China). Ethyl caprate (99%) and ethanol (99%) were from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China).

2.2. Tea samples

In 2020, young shoots consisting of one bud and two leaves from *Camellia sinensis* cultivars 'Longjing 43 (LJ)', 'Longjing Quntizhong (QTZ)', and 'Baiye No.1 (BY)' were collected from Pang'an County in Zhejiang Province, China. These leaves were utilized in Longjing tea processing experiments. The tea processing procedure involved sequential steps, starting with the picking of fresh leaves and followed by natural withering at temperatures ranging from 18 to 24 °C for a duration of 8 hours. Subsequently, fixation was carried out using a roller-hot air fixation machine at temperatures between 180 and 195 °C for a period of 1 hour, with a rotational speed of 20 rpm and a leaf load of 80-100g. After fixation, the roasting process was

conducted for 30 min in the same roller-hot air fixation machine with a temperature of 90 °C. Finally, the leaves were dried at a temperature of 200 °C for 1 min. Samples were collected and preserved for analysis during the processing stages, including fresh tea leaves (FTL), withering (Wi), fixation (Fix), roasting (R), and the final tea product (T). The samples were carefully obtained and stored to ensure their integrity before being subjected to further analysis.

2.3. *Sensory evaluation*

Each tea infusion was prepared by brewing 3.0 g of dried tea leaves with 150 mL of boiling water for a duration of 4 min at room temperature (RT, 25 ± 2 °C), according to the standard method for Longjing green tea brewing outlined in Chinese standard GB/T 23776-2018. The evaluation and scoring of the tea infusion were conducted by a trained panel of experts from the Tea Research Institute, Chinese Academy of Agricultural Sciences. The panellists, aged between 25 and 48 years, possessed certifications for tea quality evaluation issued by the Tea Scientific Society of China. Prior to the evaluation, all panellists signed a consent form and willingly underwent comprehensive training to develop their ability to discern various taste attributes, including bitterness, astringency, umami, and total score. Each member of the panel assigned scores to the different taste attributes, using a 0-10 scale to indicate the intensity. The scoring scale ranged from 0-2 (very weak/just perceptible) to 8-10 (very strong intensity). The mean values of the scores were calculated and reported (Zeng et al., 2023).

2.4. Sample preparation and extraction

2.4.1. Dry sample extraction

Biological samples were subjected to vacuum freeze-drying using a Scientz-100F lyophilizer. Subsequently, the dried samples were ground to a powder form using a Retsch grinder (MM 400) operating at a frequency of 30 Hz for 1.5 min. For further analysis, 50 mg of the powdered sample was weighed using an MS105DM electronic balance. Subsequently, 1200 μL of a 70% methanolic aqueous internal standard extract, pre-cooled to -20 ^{o} C, was added to the sample. The sample and extract were vortexed once every 30 min for a duration of 30 seconds, repeating this process six times. Following centrifugation at a rotation speed of 12000 rpm for 3 minutes, th

e supernatant was aspirated, and the sample was filtered using a microporous membrane with a pore size of 0.22 μm. The filtered sample was then stored in an injection vial for subsequent UPLC-MS/MS analysis.

2.4.2. UPLC Conditions

The sample extracts were subjected to analysis using a UPLC-ESI-MS/MS system (UPLC, ExionLC™ AD) coupled with tandem mass spectrometry. The analytical conditions included an Agilent SB-C18 UPLC column (1.8 μm particle size, 2.1 mm × 100 mm); the mobile phase consisted of solvent A, which was composed of pure water with 0.1% formic acid, and solvent B, which was composed of acetonitrile with 0.1% formic acid. Sample measurements were carried out using a gradient program. Initially, the composition was 95% A and 5% B. Within 9 minutes, a linear gradient to 5% A and 95% B was applied, and this composition was

maintained for 1 minute. Subsequently, within 1.1 minutes, the composition was adjusted to 95% A and 5.0% B, and this composition was maintained for 2.9 minutes. The flow velocity was set at 0.35 mL per minute, and the column oven temperature was maintained at 40 ° C. The injection volume was 2 μL. The effluent from the UPLC system was directed to an ESI-triple quadrupole-linear ion trap (QTRAP)-MS for analysis.

2.4.3. ESI-Q TRAP-MS/MS

The electrospray ionization source operation parameters were set as follows: the source temperature was maintained at 500 ° C and the ion spray voltage (IS) was set to 5500 V for positive ion mode and -4500 V for negative ion mode. The ion source gases I (GSI) and II (GSII) and the curtain gas (CUR) were set at 50, 60, and 25 psi, respectively. The collision-activated dissociation (CAD) was set to high. Quantitative multiple reaction monitoring (MRM) scans were acquired using a triple quadrupole mass spectrometer, with the collision gas (nitrogen) set to medium. The declustering potential (DP) and collision energy (CE) for each MRM transition were optimized through further DP and CE optimization. A specific set of MRM transitions was monitored for each period based on the eluted metabolites within that period.

2.4.4. Principles of metabolite qualitative and quantitative analysis

In our study, we employed the self-built Metware Database (MWDB) for substance qualification, utilizing secondary spectral information. During the analysis, we implemented a filtering process to eliminate duplicate signals originating from isotopes, as well as ions such as K^+, Na^+, NH_4^+, and fragment ions that are inherent

components of larger molecular weight substances. For metabolite quantification, we utilized the MRM mode of a triple quadrupole mass spectrometer. In this mode, the quadrupole initially screened the precursor ions (parent ions) specific to the target substance, thereby excluding ions corresponding to other molecular weight compounds, and effectively minimizing interference. Subsequently, the precursor ions were induced to undergo ionization within the collision chamber, resulting in fragmentation into multiple ion fragments. These fragment ions were further filtered through the triple quadrupole to select a characteristic fragment ion, thereby eliminating non-target ion interferences. This approach significantly enhanced the accuracy and reproducibility of quantification.

Upon acquisition of mass spectrometry data for metabolomic analysis from diverse samples, peak area integration was performed for all chromatographic peaks corresponding to the compounds of interest. Subsequently, integration correction was applied to the mass spectrometry peaks of the same metabolite across different samples. Metabolites exhibiting a matching score of 0.7 or higher for retention time and spectral fragmentation ions in the database were selected and retained for further analysis.

2.5. Sensory verification experiment

We prepared 450 mL of tea according to the method described in section 2.3 of the study. We divided the tea into 10 equal parts, with each part containing 40 mL, while reserving 50 mL as the control sample (CK). Design different concentration gradients for the sensory verification experiment based on the desired concentrations

of L-aspartic acid, L-phenylalanine, EGC, dihydromyricetin, and L-leucine in the tea. The additive amount for each compound should be as follows:

L-aspartic acid: 1.2mg and 2.8mg

L-phenylalanine: 8mg and 24mg

EGC: 8mg and 24mg

Dihydromyricetin: 200mg and 400mg

L-leucine: 12mg and 36mg

Add the appropriate amount of each compound, according to the designed concentration gradients, to the respective 40 mL portions of tea. Ensure thorough mixing to achieve homogeneity. Ask panellists to evaluate the corresponding taste attributes of the tea samples. Each member will receive a sample with a specific concentration of the compound(s) for evaluation. The taste attributes to be assessed include bitterness, astringency, umami, and the overall flavour score. The CK should serve as a reference for comparison. Collect the evaluation scores and feedback from panellists for further analysis and interpretation.

2.6. Data processing and statistical analysis

Partial least squares discriminant analysis (PLS-DA) modelling was conducted using SIMCA 13.0 software (Umetrics, Sweden). Heat map analysis was performed using TBtools v2.003 software (China). Hierarchical cluster analysis and radar map visualization were conducted using Origin 2023b software (USA). Pearson correlation analysis between taste attributes and chemical compounds was performed using SPSS software (version 20.0), and the resulting network diagram was generated using

Cytoscape software (version 3.9.1). K-means clustering analysis was performed using R software (version 3.5.1).

3. Results and discussion

3.1. Overall determination of non-volatile metabolites in three varying varieties

A total of 2616 ion features were obtained after peak-picking and alignment in the analysis. Subsequently, 580 non-volatile metabolites were screened out based on their retention time and spectral fragmentation ions. These metabolites comprised various classes, including 36 alkaloids, 64 amino acids and derivatives, 138 flavonoids, 29 lignans and coumarins, 78 lipids, 27 nucleotides and derivatives, 33 organic acids, 70 phenolic acids, 22 tannins, 19 terpenoids, and 64 others (Figure 1A).

To gain a better understanding of the changes in non-volatile metabolites during the processing of Longjing tea, a hierarchical cluster was performed on three different varieties (Figure 1B). The analyses revealed significant differences among the three varieties, indicating distinct metabolic profiles. When comparing variations in processing within the same variety, it was observed that samples taken before and after fixation showed noticeable discrepancies. Additionally, by examining the processing stages, four different change tendencies were identified for these metabolites (Figure 1C). For instance, 184 metabolites exhibited an initial increase in the withering stage followed by a subsequent decrease in their levels.

3.2. Alteration of metabolites by fixation

The hierarchical cluster analysis results demonstrated significant disparities in non-volatile metabolites subsequent to fixation. By implementing chemometrics

techniques to isolate essential metabolites, a PLS-DA model was established, with results depicted in Figure 2A and 2B. The findings indicate improved isolation of tea samples post-fixation, with variable importance in projection (VIP) values exceeding 2. Thirty metabolites were identified, as visualized in Figure 2C, 12 of which decreased post-fixation, while the rest increased. These key metabolites contained 10 lipid compounds, indicating that the lipids changed greatly during the process of fixation.

Lipids contribute not only to energy provision, texture, and mouthfeel but also significantly influence the odour and flavour formation of food (Shahidi & Hossain, 2022). Thermal treatment is a routine used to moderate lipid oxidation, thereby enhancing the palatability of food (Zhang et al., 2022). For instance, non-volatile lipids are converted to volatile metabolites such as aldehydes and alcohols, which contribute to the green and fresh notes in green tea (Matsui, Kurishita, Hisamitsu, & Kajiwara, 2000). Further examination of lipid metabolite alterations following fixation revealed that the majority of glycerol esters displayed a downward tendency, while lyso-phosphatidylcholine (LPC) and lyso-phosphatidylethanolamine (LPE) increased, as illustrated in Figure 2D. The presence of LPC and LPE in peanuts and green tea were also found to decrease post-thermal treatment (Zhang et al., 2023; Wang et al., 2021) and a previous study showed that LPC levels (16:0, 18:1, 18:2, and 18:3) notably increased after the rolling and fermentation stages in black tea processing (Liu et al., 2023). A previous study revealed that phosphatidic acids were the most significantly reduced lipids during green tea manufacturing (Li et al., 2021).

Additionally, carbonyl compounds from lipid oxidative products can interact with amino acids to form lipid-derivatived aroma compounds (Shahidi & Hossain, 2022). Combined with the lipid metabolic pathway (Figure 2D), thermally-induced processes potentially decreased the content of phosphatidic acids, forming LPC and LPE and releasing the esterified form of polyunsaturated fatty acids.

3.3. Differences in sensory and metabolites among three varieties of final tea

Tea plants from different varieties contain varying biochemical compositions, such as large-leaf varieties with a high level of polyphenols, and albino cultivars with a high content of amino acids (Zhao et al., 2022). In this experiment, Longjing teas made from three varieties were analysed and well discriminated, as shown in Figure 3A, B. Using a criterion of a VIP value greater than 2, key differential metabolites were identified and visualized in a heat map (Figure 3C), with a total of 35 metabolites identified. The BY variety exhibited higher levels of epigallocatechin, 3-methylellagic acid, and dihydromyricetin. It was found that the intensity of the sweet aftertaste increased with the molar concentration of epigallocatechin (Zhang et al., 2020). Ellagic acid is a polyphenol that results from the dimerization of gallic acid, and it is known to contribute to the characteristic astringent taste due to its stable cross-links with proteins (Bakkalbasi et al., 2009). Additionally, dihydromyricetin, the main bioactive component in vine tea, has been extensively studied for its potential health benefits (Carneiro et al., 2021).

In the LJ variety, isoorientin-7-*O*-glucoside, orientin-2''-*O*-galactoside, 3-isopropylmalic acid, 2-propylmalic acid, epicatechin gallate, cryptochlorogenic acid

(4-*O*-caffeoylquinic acid), morin-3-arabinoside, and quercetin-3-*O*-arabinoside were found to be more abundant compared to the other two varieties. The QTZ variety exhibited higher levels of naringenin-7-*O*-glucoside (flavanones), kaempferol-3-*O*(2''-galloyl) galactoside, and epicatechin-3-(3''-*O*-methyl) gallate. Previous studies have reported that the flavonol glycoside profiles of dry teas were able to discriminate tea plant cultivars, rather than the manufacturing procedure (Zhang et al., 2018). In this study, it was also observed that the key differential compounds were predominantly flavonol glycosides.

To quantify specific sensory factors attributes (i.e., bitterness, astringency, and umami), an organoleptic test was conducted. As shown in Figure 3D, the BY variety achieved the highest total and umami scores, while the LJ variety scored highest in bitterness. On the other hand, the QTZ variety exhibited a pronounced astringency. Combining these results with the metabolite analysis, it was inferred that the high content of epigallocatechin in the BY variety might contribute to its high total and umami scores. Additionally, previous studies have reported that the superior performance of the BY variety was attributed to its high level of amino acids and low levels of catechins and caffeine, which reduced astringency and bitterness while enhancing the umami taste (Feng et al., 2014). This observation was further supported by the dynamic changes in 12 amino acids (Figure 3E), where the content of L-glutamic acid and L-glutamate were highest in the BY variety. These compounds are known to be the primary contributors to the umami taste in green tea. Therefore, the L-glutamic acid and L-glutamate content may be the reason for the intense umami

taste of the BY variety. Additionally, previous studies have identified caffeoyl- or feruloyl-substituted quinides as contributors to the bitter taste in roasted coffee (Frank et al., 2006). Hence, it is possible that cryptochlorogenic acid (4-*O*-caffeoylquinic acid) contributes to the strong bitterness of the LJ variety. Similarly, compounds found enriched in the QTZ variety, including naringenin-7-*O*-glucoside (a flavanone), kaempferol-3-*O*(2''-galloyl) galactoside, and epicatechin-3-(3''-*O*-methyl) gallate (polyphenols), are known to present bitter flavours. However, these results were only tentative and based on previous studies. To further demonstrate these findings, correlation analyses between sensory results and these key metabolites, as well as sensory verification experiment, were conducted.

3.4. Correlation analysis and the sensory verification experiment

The data analysis workflow, illustrated in Figure 4A, involved the utilization of a PLS-DA model to identify 35 metabolites exhibiting differential expression (VIP > 2). Subsequently, these 35 metabolites were assessed for correlation with the sensory evaluation results. The significance was set as the absolute value of the correlation coefficients surpassing 0.8. The findings revealed significant correlations solely between these metabolites and the sensory attributes of umami and astringency, whereas no correlations were observed with bitterness. Astringency, a critical sensory characteristic of green tea, is primarily influenced by hydrolysable and condensed tannins (Granato et al., 2014). Among the 8 metabolites showing a strong correlation with astringency (Figure 4B), compounds such as N-(sulfonyl) phenylalanine, jaceosidin-7-*O*-galactoside, epicatechin-3-(3''-*O*-methyl) gallate, 4-hydroxy-3-

346 methoxyphenyl 1-*O*-*β*-D-(6'-*O*-galloyol)-glucopyranoside, and L-aspartic acid
347 displayed a significantly positive correlation with the astringency score. Conversely,
348 dihydromyricetin (ampelopsin), epigallocatechin, and L-phenylalanine exhibited a
349 notably negative correlation with the astringency score.

350 To further validate their actual taste contributions, epigallocatechin, L-
351 phenylalanine, L-aspartic acid, and dihydromyricetin were selected for additional
352 experiments (Figure 4C). The results indicated that L-aspartic acid and
353 dihydromyricetin augmented the astringency score, while L-phenylalanine reduced it.
354 Epigallocatechin displayed varying effects depending on its concentration. In green
355 tea, L-glutamic acid, L-theanine, and L-aspartic acid are considered as main
356 contributors to the umami flavour (Yu et al., 2014). Previous research demonstrated
357 that L-aspartic acid significantly inhibited the astringency of EGCG at low
358 concentrations (0.02 mg/mL), but significantly intensified the astringency at medium
359 to high concentration (0.17-1.39 mg/mL) (Liu et al., 2023). This finding suggests that
360 L-aspartic acid bidirectionally modulates astringency at different concentrations,
361 which possibly the reason for the positive correlation with astringency observed in
362 this study. Despite exhibiting a negative correlation in the correlation analysis,
363 dihydromyricetin was found to enhance the astringency score. On the other hand, the
364 bitterness of L-phenylalanine, which was proven to be a major contributor to
365 bitterness in bamboo shoots (Gao et al., 2019), reached an equivalent level to
366 berberine hydrochloride at a concentration of 0.5 mg/mL. However, the interaction
367 between L-phenylalanine and astringency remains unknown and requires further

investigation to better understand their relationship.

Shifting focus to the umami taste, although 8 metabolites exhibited strong correlations with umami taste, L-leucine demonstrated a negative correlation (Figure 4D). To validate this finding, we conducted a sensory verification experiment (Figure 4E), and the results indicated that the umami score decreased upon the addition of L-leucine. L-leucine itself exhibits a bitter taste, but previous research suggested that changes in L-leucine concentration were difficult for the human body to perceive. (Scharbert & Hofmann, 2005). Based on the results, L-aspartic acid, L-phenylalanine, and L-leucine were identified as essential taste metabolites in Longjin tea.

Furthermore, an analysis of the changes in L-aspartic acid, L-phenylalanine, and L-leucine during processing was conducted using a heat map (Figure 4F), revealing distinct variations in the levels of these metabolites among the three cultivars. Specifically, QTZ exhibited the highest content of L-aspartic acid and L-leucine, whereas BY had the highest level of L-phenylalanine. Notably, all three amino acids displayed an increasing trend across the three varieties during processing. The increase in amino acids was attributed to the high temperature employed during processing, which promoted protein hydrolysis. Similar observations have been reported in other studies on the processing of green tea (Wang et al., 2021).

3.5. Dynamic changes in the main taste metabolites of three different varieties of Longjing tea during processing

The primary taste constituents of tea are amino acids (umami), flavonoid

(bitterness and astringency), and alkaloids (bitterness) (Zhang et al., 2020). In order to gain a more comprehensive understanding of the transformations of the non-volatile compounds during Longjing tea processing, we focused on three salient metabolic pathways: the flavonoid pathway, the amino acid pathway, and the alkaloid pathway. The modifications in these metabolic pathways are discussed in detail blow.

3.5.1 Modifications in the flavonoid metabolic pathway

The major components of the flavonoid metabolic pathway in tea include flavones and flavone glycosides, flavonol glycosides, and flavanols, as depicted in Figure 5. Flavones and flavonols are predominantly present as *O*-glycosides, with a glycoside moiety attached to the C-3 position of the aglycones. These compounds play a crucial role in contributing to the bitter taste of tea (Fang et al., 2019). During processing, the concentrations of flavones (such as vitexin, apigenin, and isovitexin) and most apigenin glycosides generally exhibited an upward trend, particularly after the fixation process. However, exceptions were observed for apigenin-4'-*O*-glucoside, apigenin-7-*O*-glucoside (cosmosiin), and isovitexin-7-*O*-glucoside (saponarin). Although the abundance of flavones and flavone glycosides differed among the three tea varieties, the overall tendency during processing was similar.

The concentration of quercetin significantly increased after the fixation process. Quercetin is known to contribute to the green colour of tea infusions (Wang et al., 2004), which may explain intensified tea infusion colour observed after fixation. The detected flavonol glycosides were categorized as kaempferol glycosides, quercetin glycosides, and myricetin glycosides. The abundance of quercetin glycosides

primarily displayed a notable decrease during processing, particularly after fixation, whereas the trends for kaempferol glycosides and myricetin glycosides varied.

Flavanols, which are the most characteristic and abundant metabolites in tea, are considered the primary contributors to the astringency and bitterness taste (Zhang et al., 2020). In this study, different types of flavanols exhibited diverse changes. Epigallocatechin gallate and catechin gallate showed an increase during processing. Previous research has indicated that catechins undergo various transformations, including isomerization, optical isomerization, hydrolysis, thermal polymerization, and pyrolysis, during the processing of green tea (Huang et al., 2005).

3.5.2 Modifications in the amino acid metabolic pathway

Dynamic alterations in amino acids during processing are illustrated in Figure 6A. Throughout the tea manufacturing process, significant changes occurred in the abundance of various amino acids. Initially, fresh tea leaves contained high concentrations of L-theanine and L-glutamic acid, which gradually diminished during processing. L-theanine, a unique amino acid found in different tea types, plays a prominent role in contributing to the umami taste of tea (Zhang et al., 2020). It has been suggested that the decrease in L-theanine content is attributed to the Maillard reaction between theanine and glucose, resulting in the formation of Amadori rearrangement products, methylpyrazine and 2,5-dimethylpyrazine, which are found in various teas (Guo et al., 2018; Han et al., 2022).

Following withering, the concentrations of L-valine, L-aspartic acid, L-tyrosine,

and L-tryptophan significantly increased. The rise in proteinaceous amino acids is attributed to protein degradation facilitated by the hydrolytic activity of endogenous peptidases during withering, particularly in black tea (Chen et al., 2020). Notably, L-arginine contributes to sweetness, while tryptophan and phenylalanine contribute to the astringency and bitter taste of tea (Zhu et al., 2020). These changes in amino acid composition likely represent differentially expressed metabolites responsible for the distinctive taste profile of Longjing green tea.

The dynamic changes observed in L-valine, L-aspartic acid, L-theanine, L-glutamic acid, L-tyrosine, and L-tryptophan were similar across the three tea varieties studied. However, there were variations in the abundance of L-arginine during processing. In the case of QTZ, L-arginine content appeared to keep increasing, while BY and LJ exhibited a tendency to decrease followed by an increase. The variability in L-arginine levels could be attributed to varietal specificity.

3.5.3 Modifications in the alkaloid metabolic pathway.

Caffeine and theobromine are the primary alkaloids found in tea and are responsible for contributing to the characteristic bitter taste of tea infusions. As depicted in Figure 6B, the processing of tea leaves had a significant impact on the levels of caffeine and theobromine. Specifically, the content of theobromine consistently decreased throughout the processing stages, while caffeine initially increased, decreased after roasting, and then increased again. This observed phenomenon can be attributed to the conversion of theobromine, an intermediate compound in the biosynthesis of caffeine, into caffeine during the processing steps

(Xia et al., 2017). Furthermore, the fluctuation in caffeine content throughout the processing stages can potentially be attributed to sublimation of caffeine caused by exposure to high temperatures. It is worth noting that when comparing LJ and QTZ with BY, the content of both theobromine and caffeine was found to be the lowest in BY. These findings highlight the dynamic changes in caffeine and theobromine levels during tea processing and demonstrate the influence of processing on the composition and taste characteristics of tea.

4. Conclusion

This study combined widely targeted metabolomics and chemometrics to investigate the effects of cultivars and processing on Longjing green tea's metabolite profile. A total of 580 non-volatile metabolites were identified, highlighting alterations in flavonoid, amino acid, and alkaloid metabolic pathways. The fixation process potentially reduced phosphatidic acid levels, leading to the formation of LPC, LPE, and the release of esterified polyunsaturated fatty acids. Distinct metabolites and taste profiles were observed for the three cultivars, with L-glutamic acid and L-glutamate predominant in BY, and accumulate flavones predominant in LJ. Correlation analysis linked taste attributes with metabolites, revealing that a certain concentration of L-aspartic acid increased tea astringency. Changing trends of key metabolites during processing were similar among cultivars except for L-arginine in QTZ. These findings advance our understanding of Longjing green tea quality improvement and contribute to the development of high-quality tea varieties. Future research will focus on volatile metabolite dynamics in Longjing green tea.

Ethical statement

All the participants (healthy and nonsmokers from TRICAAS) were conducted in accordance with the principle set forth in the Declaration of Helsinki and informed written consent was obtained. This study was approved by the Zhejiang Gongshang University Human Ethics Committee.

CRediT authorship contribution statement

Lin Zeng: Writing – original draft, Methodology, Investigation, Data curation, Formal analysis, Visualization. **Yan-Qing Fu**: Writing – review & editing, Resources, Data curation, Methodology, Supervision. **Ying Gao**: Writing – review & editing, Resources, Software, Visualization. **Fang Wang**: Writing – review & editing. **Jun-Feng Yin**: Writing – review & editing. **Marie-Laure Fauconnier**: Writing – review & editing. **Lijing Ke**: Investigation, Data curation. **Yong-Quan Xu**: Conceptualization, Writing – review & editing, Data curation, Formal analysis, Investigation, Project administration.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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