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Zeng, L., Fu, Y.-Q., Gao, Y. et al. (6 more authors) (2024) Dynamic changes of key metabolites in Longjing green tea during processing revealed by widely targeted metabolomic profiling and sensory experiments. Food Chemistry, 450. 139373. ISSN 0308-8146

https://doi.org/10.1016/j.foodchem.2024.139373

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 compounds in Longjing green tea. A total of 580 non-volatile metabolites were identified by 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-glutamine, while Longjing 43 showed elevated levels of flavones. Correlation analysis and sensory verification indicated that the specific concentration of L-leucine could decrease the umami of the tea. These findings advance our understanding of Longjing green tea quality improvement and cultivar development.

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

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, anticancer effects and other functional activities (Schneider et al., 2009). Manufactured from the fresh new shoots of tea plant (*Camellia sinensis*), the typical green tea process involves picking, spreading, fixing, rolling, and drying. Sensory evaluations of green tea reveal a variety of aroma attributes, including faint, floral, chestnut-like, and other categories. Green tea infusion exhibits a bright green colour and the taste is usually bitter, astringent, sweet, 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, and alkaloids contribute significantly to its bitter taste (Ye et al., 2022). Amino acids constitute approximately 70% of the umami taste intensity of green tea, while soluble sugars are the primary source of sweetness (Yue et al., 2017).

Taste compounds in tea leaves could be influenced by the quality of fresh tea leaves (cultivars, growing conditions, and picking tenderness), processing technique, and storage condition. Firstly, storage time is one of the quality evaluation criteria for tea, especially for dark tea and white tea (Zhou et al., 2023). However, green tea is usually not recommended for long-term storage because its taste and aroma deteriorate quickly. Secondly, tea plant cultivar is closely associated with tea quality (Wang et al., 2021). For instance, Longjing 43 is considered to be the most suitable cultivar for

producing Longjing tea (Dragon well tea). The fresh and mellow taste of Anji bai tea processed from Baiye No.1 is profited from its high L-theanine content and low tea polyphenol levels (Zeng, Lin, Liu, & Liu, 2019). Finally, tea processing technologies affected the quality of final tea product through regulating metabolism. Many bioactive compounds vary significantly during the tea manufacturing process (Liao, Zhou, & Zeng, 2022). In the fixation or roasting process, amino acids could react with carbonyl compounds to form Strecker aldehydes that contributed to the formation of the tea aroma (Rizzi, 2008). The fixation stage was primarily associated with chlorophyll decomposition, phosphatidic acids reduction and glycolipids degradation (Li et al., 2021). However, previous studies have mostly focused on the changes within a single cultivar during the processing or the differences between different cultivars, with few investigating the differences in metabolite changes during the processing of different cultivars. The formation of tea flavour and quality is very complicated, and different cultivars may exhibit varying trends during processing. 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 metabolomics is mainly used for the accurate quantification of known metabolites, which has the characteristics of strong specificity and high sensitivity. This approach has been extensively utilized for several decades in the field of metabolism research. In contrast, untargeted metabolomics aims to detect and identify as many metabolites as possible in the samples, including known and unknown metabolites (Zeng et al., 2022). However, the identification of metabolites in untargeted metabolomics is complex and time-consuming (Hertzog et al., 2022). A recent advancement in metabolomics analysis 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 as the analysis tool.

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. The hypothesis was that different cultivars had varying metabolite profiles and exhibited different changes during the processing stage, which might be the primary cause of the differences in the quality of Longjing green teas. Hence, three mainly-planted tea cultivars were selected, including albino tea cultivar "Baiye No.1," current Longjing cultivars "Longjing 43" and the traditional cultivar

"Quntizhong." Fresh tea leaves from these cultivars were processed to Longjing green tea by using the same manufacturing technology. This study will investigate the differences in metabolite changes during the processing stage of different cultivars, and validate the results through sensory evaluation. The findings of this study are helpful in the understanding of quality improvements for Longjing green tea and high-quality tea cultivars development.

2. Materials and methods

2.1. Chemicals

The main chemicals used in this study included pure water (HangZhou Wahaha Group Co., Ltd., Hangzhou, China); methanol (MeOH, HPLC grade), and acetonitrile (HPLC, ⩾99.9%) (Merck, Darmstadt, Germany); formic acid (FA), (−)-Epigallocatechin (EGC), dihydromyricetin, and L-leucine (Aladdin, Shanghai, China).

2.2. Tea samples

In 2020, young shoots consisting of one bud and two leaves from *Camellia sinensis* cultivars 'Longjing 43 (LJ)', 'Quntizhong (QTZ)', and 'Baiye No.1 (BY)' were collected from Pan'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, the first drying (fixation) was carried out using an automatic flat tea stir-frying machine (6CCB-100ZD type, Zhejiang Hengfeng Technology Development Co., Ltd., Shaoxing, China) at temperatures between 180 and 195 °C for a period of 1 hour,

with a rotational speed of 395 xg and a leaf load of 80-100g. After the first drying, the shaping process was conducted for 30 min in the automatic, flat tea stir-frying machine for shaping at 180 °C for 60 min. Finally, the second drying was conducted for 30 min in a tea roasting machine (6CH-3.0 type, Zhejiang Hengfeng Technology Development Co., LTD, Shaoxing, China) with a temperature of 90 °C. Samples were collected and preserved for analysis during the processing stages, including fresh tea leaves (FTL), withering leaves (Wi), fixation/the first drying leaves (Fix), shaping leaves (Sha), and the second drying leaves/final tea products (Dry). 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

147 strong intensity). The mean values of the scores were calculated and reported (Zeng et
148 al., 2023).
149 *2.4. Sample preparation and extraction*
150 *2.4.1. Dry sample extraction*
151 Biological samples were subjected to vacuum freeze-drying using a Scientz-100F
152 lyophilizer (Ningbo Xinzhi Biotechnology Co., Ltd., Ningbo, China). Subsequently, the
153 dried samples were ground to a powder form using a MM-400 Retsch grinder (Verder
154 Shanghai Instruments and Equipment Co., Ltd., Shanghai, China) operating at a
155 frequency of 30 Hz for 1.5 min. For further analysis, 50 mg of the powdered sample
156 was weighed using an MS105DM electronic balance (Mettler Toledo Technology Co.,
157 Ltd., Shanghai, China). Subsequently, 1200 μL of a 70% methanolic aqueous internal
158 standard extract, pre-cooled to -20 °C, was added to the sample. The sample and extract
159 were vortexed once every 30 min for a duration of 30 seconds, repeating this process
160 six times. Following centrifugation at a rotation speed of 1152 xg for 3 minutes, the
161 supernatant was aspirated, and the sample was filtered using a microporous membrane
162 (ANPEL, Shanghai, China) with a pore size of 0.22 μm. The filtered sample was then
163 stored in an injection vial (Beijing Castemer Technology Development Co., Ltd.,
164 Beijing, China) for subsequent UPLC-MS/MS analysis.
165 *2.4.2. UPLC Conditions*
166 The sample extracts were subjected to analysis using a UPLC-ESI-MS/MS system
167 (UPLC, ExionLC™ AD) coupled with tandem mass spectrometry. The analytical
168 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 was set to 5500 V for positive ion mode and -4500 V for negative ion mode. The ion source gases I and II and the curtain gas were set at 50, 60, and 25 psi, respectively. The collision-activated dissociation was set to high. Pre-selection of qualitative ion pairs was conducted using the characteristic ion fragmentation information and quasi-molecular ion compositions of each metabolite for quantitative multiple reaction monitoring (MRM) analysis. MRM scans were acquired using a triple quadrupole mass spectrometer, with the collision gas (nitrogen) set to medium. The declustering potential and collision energy for each MRM transition were optimized through further declustering potential and

collision energy 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 (MetWare, Wuhan, China) for compounds 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 metabolites. 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 metabolites, 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-phenylalanine, EGC, dihydromyricetin, and L-leucine in the tea. The additive amount for each compound should be as follows:

L-phenylalanine: 8 mg and 24 mg

EGC: 8 mg and 24 mg

Dihydromyricetin: 200 mg and 400 mg

L-leucine: 12 mg and 36 mg

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 (Umetric, Umea, Sweden). Heat map analysis was performed using TBtools v2.003 software (Chen et al., 2020). Hierarchical cluster analysis and radar map visualization were conducted using Origin 2023b software (Origin Lab Corp., Northampton, MA). Pearson correlation analysis between taste attributes and chemical compounds was performed using SPSS software version 20.0 (SPSS, Chicago, IL, USA), and the resulting network diagram was generated using Cytoscape software version 3.9.1 (https: //cytoscape. Org). K-means clustering analysis was performed using R software version 3.5.1 (https://www.r-project.org/).

3. Results

3.1. Determination of non-volatile metabolites in three varying cultivars

A total of 2615 metabolites were obtained after peak-picking and alignment in the analysis. Subsequently, the metabolites were matched with the database based on their secondary mass spectra (containing all fragment ions of the substances) and retention time, resulting in 580 non-volatile metabolites with a matching degree of 70% or above. 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

other metabolites (Figure 1A).

To better understand the changes in non-volatile metabolites during Longjing tea processing, the hierarchical cluster analysis was performed on these three different cultivars (Figure 1B). The analysis revealed significant differences among three cultivars, indicating distinct metabolic profiles. When comparing variations in processing within the same cultivar, it was observed that samples taken before and after fixation process showed noticeable discrepancies. Furthermore, through a *K*-means plot analysis of the processing stages, four different change tendencies were identified for these metabolites (Figure 1C). Among them, a total of 271 non-volatile metabolites exhibited lower content in the Dry compared to the FTL, while 309 metabolites displayed higher content. This observation strongly indicated that the manufacturing process influenced the transformation and generation of metabolites.

3.2. Alteration of metabolites in fixation process

The hierarchical cluster analysis results demonstrated significant disparities in non-volatile metabolites subsequent to fixation. To find the essential difference metabolites, a PLS-DA model was established, which is shown in Figure 2A and 2B. This model effectively distinguished tea samples before and after fixation, and further screened 30 key metabolites with variable importance in projection (VIP) values exceeding 2, 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 fixation process. Further observation of lipid metabolite

alterations following fixation revealed that most glycerol esters displayed a downward tendency, while lyso-phosphatidylcholine (LPC) and lyso-phosphatidylethanolamine (LPE) increased, as illustrated in Figure 2D.

3.3. Differences in sensory and metabolites among three cultivars Longjing teas

Different tea plant cultivars contain varying biochemical compositions, such as large-leaf cultivars 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 cultivars were analyzed and well discriminated, as shown in Figure 3A, and 3B. Based on the criterion of 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 cultivar exhibited higher levels of (−)-Epigallocatechin (EGC), 3-methylellagic acid, and dihydromyricetin. In the LJ cultivar, isoorientin-7-*O*-glucoside, orientin-2''-*O*-galactoside, 3-isopropylmalic acid, 2-propylmalic acid, (−)-Epicatechin gallate (ECG), cryptochlorogenic acid (4-*O*-caffeoylquinic acid), morin-3-arabinoside, and quercetin-3-*O*-arabinoside were found to be more abundant than the other two cultivars. The QTZ cultivar exhibited higher levels of naringenin-7-*O*-glucoside (flavanones), kaempferol-3-*O*(2''-galloyl) galactoside, and epicatechin-3-(3''-*O*-methyl) gallate.

To quantify specific sensory factors attributes (i.e., bitterness, astringency, and umami) among three cultivars, an organoleptic test was conducted. As shown in Figure 3D, the BY cultivar achieved the highest total and umami scores, the LJ cultivar scored

293 highest in bitterness, and the QTZ cultivar exhibited a pronounced astringency.
294 Previous studies have reported that the superior performance of the BY cultivar was
295 attributed to its high levels of amino acids and low levels of catechins and caffeine,
296 which reduced astringency and bitterness while enhanced the umami taste (Feng et al.,
297 2014). This observation was further supported by the dynamic changes in 12 amino
298 acids (Figure 3E), where the content of L-glutamic acid and L-glutamine were highest
299 in the BY cultivar. These compounds are known to be the primary contributors to the
300 umami taste in green tea. Therefore, the L-glutamic acid and L-glutamine might be the
301 reason for the intense umami taste of the BY cultivar. Additionally, previous studies
302 have identified caffeoyl- or feruloyl-substituted quinides as contributors to the bitter
303 taste in roasted coffee (Frank et al., 2006). Hence, it was possible that cryptochlorogenic
304 acid (4-*O*-caffeoylquinic acid) contributed to the strong bitterness of the LJ cultivar.
305 Similarly, naringenin-7-*O*-glucoside (a flavanone), kaempferol-3-*O*(2''-galloyl)
306 galactoside, and epicatechin-3-(3''-*O*-methyl) gallate (polyphenols), which are known
307 to present bitter taste, were enriched in the QTZ cultivar. However, these results were
308 only tentative and based on previous studies. To further demonstrate these findings,
309 correlation analyses between sensory results and key metabolites, as well as sensory
310 verification experiment, were conducted.

311 *3.4. Correlation analysis and sensory verification*

312 The data analysis workflow, illustrated in Figure 4A, involved the utilization of a
313 PLS-DA model to identify 35 metabolites exhibiting differential expression (VIP > 2).
314 Subsequently, these 35 metabolites and 12 amino acids were assessed for correlation

315 with the sensory evaluation results. The significance was set as the absolute value of
316 the correlation coefficients surpassing 0.8. The findings revealed significant
317 correlations between these metabolites and the sensory attributes of umami and
318 astringency, whereas no correlations were observed with bitterness. Astringency, as the
319 characteristic taste of green tea, is primarily influenced by hydrolysable and condensed
320 tannins (Granato et al., 2014). Among the 7 metabolites showing strong correlations
321 with astringency, compounds such as N-(sulfonyl) phenylalanine, jaceosidin-7-*O*-
322 galactoside, epicatechin-3-(3''-*O*-methyl) gallate, and 4-hydroxy-3-methoxyphenyl 1-
323 *O*-*β*-D-(6'-*O*-galloyol)-glucopyranoside displayed the significantly positive
324 correlations with the astringency score. Conversely, dihydromyricetin (ampelopsin),
325 epigallocatechin, and L-phenylalanine exhibited notably negative correlations with the
326 astringency score.

327 To further validate their actual taste contributions, L-Phenylalanine, EGC, and
328 dihydromyricetin were selected to be added into tea infusion due to the difficulty in
329 obtaining other compounds (Figure 4B). The results showed that dihydromyricetin
330 enhanced the astringency, while L-phenylalanine reduced it. EGC displayed varying
331 effects depending on its concentration. Despite exhibiting a negative correlation in the
332 correlation analysis, dihydromyricetin was found to enhance the astringency score. On
333 the other hand, the bitterness of L-phenylalanine, which was proven to be a major
334 contributor to bitterness in bamboo shoots (Gao et al., 2019). However, the interaction
335 between L-phenylalanine and astringency remains unknown and required further
336 investigation to better understand their relationship.

Focused on the umami taste, although 8 metabolites exhibited strong correlations with umami taste. The majority of metabolites did not show a significant correlation with umami, except for L-leucine and N-(sulfonyl) phenylalanine, which presented a negative correlation. To validate this finding, we added L-leucine into tea infusion, and the results indicated that the umami score decreased upon the addition of L-leucine (Figure 4C), consistent with previous findings that L-leucine has a negative effect on umami taste. As a branched-chain amino acid, L-leucine itself has a bitter taste, which could explain the observed reduction in umami flavour. In conclusion, it was found that L-phenylalanine has a negative effect on astringency taste, while L-leucine can reduce the umami taste.

Based on the results, L-phenylalanine, epigallocatechin, dihydromyricetin, and L-leucine were identified as essential taste metabolites in Longjing tea. Furthermore, heat map was conducted to visualize the distinct variations in these compounds during processing in three cultivars (Figure 4D). Specifically, QTZ exhibited the highest content of L-leucine, whereas BY had the highest level of L-phenylalanine, epigallocatechin, and dihydromyricetin. These findings can help to explain the sensory evaluation results, with QTZ exhibiting the lowest umami flavour and BY having the lowest astringency taste. Notably, L-phenylalanine and L-leucine displayed an increasing trend across the three cultivars during processing. The increase in amino acids was attributed to the protein hydrolysis promoted by high temperature treatment during processing. 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 cultivars Longjing teas 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 comprehensively understand 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 were 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 played the 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 cultivars, the overall change tendency during processing was similar.

The concentration of quercetin significantly increased after the fixation process.

Quercetin was known to contribute to the green colour of tea infusion (Wang et al., 2004), which could explain the enhancement of 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 displayed a notable decrease trend during processing, particularly after fixation, whereas there were no significant changes observed in the trends for kaempferol glycosides and myricetin glycosides.

Flavanols, the most characteristic and abundant metabolites in tea, were considered the primary contributors to the astringency and bitterness taste of tea (Zhang et al., 2020). In this study, different types of flavanols exhibited diverse changes. (−)-Epigallocatechin gallate (EGCG) and (−)-catechin gallate (CG) decreased during the whole 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 (Wang et al., 2021), These transformations may be the reason for the observed decrease in EGCG and CG.

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, the abundance of amino acids varied significantly. Initially, fresh tea leaves contained high concentrations of L-theanine and L-glutamic acid, which gradually diminished during processing. Following withering, the concentrations of L-valine, L-aspartic acid, L-tyrosine, and L-tryptophan

401 significantly increased. These amino acids changes likely represented differentially
402 expression responsible for the distinctive taste profile of Longjing green tea.

403 The dynamic changes observed in L-valine, L-aspartic acid, L-theanine, L-
404 glutamic acid, L-tyrosine, and L-tryptophan were similar among the three tea cultivars.
405 However, the dynamic change trend of L-arginine during processing was different.
406 During processing L-arginine kept increasing in QTZ cultivar, but showed a trend of
407 first decrease and then increase in BY and LJ cultivars. The variability in L-arginine
408 levels could be attributed to varietal specificity.

409 *3.5.3 Modifications in the alkaloid metabolic pathway.*

410 Caffeine and theobromine are the primary alkaloids found in tea, and contributed
411 to the characteristic bitter taste of tea infusions. As depicted in Figure 6B, tea processing
412 had significant impact on the levels of caffeine and theobromine. Specifically, the
413 content of theobromine consistently decreased throughout the processing stages.
414 However, the content of caffeine showed an overall increasing trend and varied among
415 the three cultivars during the processing. This observed phenomenon could be
416 attributed to the conversion of theobromine, an intermediate compound in the
417 biosynthesis of caffeine, into caffeine during the processing steps (Xia et al., 2017).
418 Furthermore, the fluctuation in caffeine content throughout the processing stages could
419 potentially be attributed to sublimation of caffeine caused by exposure to high
420 temperature. It is worth noting that the content of both theobromine and caffeine was
421 found to be lower in BY than that in LJ and QTZ. These findings highlighted the
422 dynamic changes in caffeine and theobromine levels during tea processing and

demonstrated the influence of processing on the composition and taste characteristics of tea.

4. Discussion

4.1. Sensory and metabolomic profiling differences among three different tea cultivars

According to previous research, tea quality can be greatly influenced by various factors, such as cultivar, ecological environment, cultivation method, processing technology, and storage condition. Among these factors, cultivar plays a key role (Qin et al., 2023). The metabolite levels vary significantly among different cultivars, resulting in different sensory qualities of the final tea product. In this experiment, BY cultivar, also known as Anji Baicha, was chosen. It is a low-temperature-sensitive, periodic albino variant that produces albino young shoots in early spring when the environmental temperature is below 20-22 °C. Previous reports have indicated that albino tea contains high levels of amino acids and low levels of chlorophyll, catechins, and caffeine, which contribute to a favorable taste profile by reducing bitterness and astringency, and increasing umami. Similarly, in our study BY cultivar presented high umami characteristics and notably higher levels of L-glutamic acid and L-glutamine than the other two cultivars (Figure S1). L-glutamic acid could reduce the bitterness of ester catechins, and it was one of the main contributors to the umami taste in green tea (Liu et al., 2023). Additionally, the sensory experiment revealed that L-phenylalanine could reduce the astringency of tea leaves, and BY cultivar contained higher levels of

444 L-phenylalanine, which may contribute to its lowest astringency taste.

445 LJ and QTZ are typical cultivars used in the production of Longjing tea. QTZ is a
446 sexual cultivar reproduced from seeds under specific natural conditions. LJ is a superior
447 asexual cultivar bred from QTZ. In this experiment, LJ exhibited a pronounced
448 bitterness, while QTZ tea had a more noticeable astringency and lowest umami flavour.
449 Further correlation analysis and sensory validation experiment revealed that L-leucine
450 might be the main cause of the umami of QTZ tea, while the bitterness of LJ tea may
451 be attributed to cryptochlorogenic acid (4-*O*-caffeoylquinic acid). Currently, the
452 research on the sensory and metabolomic differences between sexual and asexual tea
453 cultivars is rarely reported, highlighting the need for further study in this area

454 *4.2. Metabolite changes during the processing of Longjing tea*

455 The metabolites' changes in tea processing mainly come from the different
456 chemical reactions that occur in the processing, including hydrolysis, substitution,
457 isomerization, oxidation-reduction, and other thermo-physical and chemical reactions.
458 The changes in metabolites have an important impact on the quality of tea. Different
459 kinds of tea have different processing technologies, and green tea processing includes
460 withering, fixation and drying. Withering is a crucial step in the formation of tea quality,
461 primarily aimed at reducing the moisture content of fresh tea leaves. This process
462 involves chemical transformations, increased activity of leaf enzymes, and the
463 oxidation of certain polyphenols, degradation of chlorophyll, hydrolysis of
464 polysaccharides and disaccharides into monosaccharides, and breakdown of proteins

into amino acids. These transformations play a key role in establishing the substance foundation for the formation of tea quality. In this experiment, significant increases were observed in the levels of L-valine, L-aspartic acid, L-tyrosine, phenylalanine, and L-tryptophan after the withering stage (Figure S1). This accumulation of amino acids during the withering stage might be due to the degradation of proteins by endogenous peptidases (Chen et al., 2020). Fixation is the most critical step in Longjing tea processing. The processing aims to inhibit the oxidation of polyphenols by inactivating the oxidase, and promote the volatilization of low-boiling-point aroma compounds, resulting in a unique aroma (Yu et al., 2023). In our study, it was found that the content of L-theanine decreased after fixation. Previous studies had reported that the decrease in L-theanine content was due to the Maillard reaction between theanine and glucose, leading to the formation of Amadori rearrangement products, such as methylpyrazine and 2,5-dimethylpyrazine, which are commonly found in various teas (Han et al., 2022).

In addition to amino acids, the levels of lipid metabolites also underwent significant changes after the fixation stage. Most glycerol esters showed a decrease trend, while LPC and LPE increased (Figure S1). Previous studies have demonstrated that LPC and LPE could induce lipid degradation under heat treatment to form carbonyl metabolites, such as hexanal, 2,4-dienal, and 1-octen-3-one (Lin & Blank, 2003). Therefore, it can be inferred that thermally-induced processes potentially decreased the content of phosphatidic acids, leading to the formation of LPC and LPE and the release of esterified forms of polyunsaturated fatty acids. Besides, in this study, flavonoids, important bitter compounds in tea, also showed some characteristic changes during

processing. The levels of apigenin, quercetin, and vitexin increased after fixation, while myricetin decreased. Previous studies have suggested that the increase in these flavonoids was related to the hydrolysis of flavonoid glycosides (Wang et al., 2021). However, in this experiment, there was no corresponding decrease in the metabolites of *O*-glycosides, which requires further investigation.

5. Conclusion

Based on the combination analysis of widely targeted metabolomics and chemometrics, this study investigated the effects of cultivars and processing on Longjing green tea's metabolite profile. A total of 580 non-volatile metabolites were identified, highlighting the alterations in flavonoids, amino acids, and alkaloids metabolic pathways. Fixation process potentially reduced phosphatidic acid levels, leading to the formation of LPC, LPE, and the release of esterified polyunsaturated fatty acids. Cultivar difference brought distinct metabolites and taste profiles, with more L-glutamic acid and L-glutamine in BY cultivar, and accumulated flavones in LJ. Correlation analysis between taste attributes and key metabolites revealed that certain concentration of L-leucine could decrease tea umami. Except for a few metabolites, the change trends of key metabolites during processing were similar among cultivars. These findings advanced our understanding of Longjing green tea quality improvement and were helpful in the development of high-quality tea cultivars. However, this study still had some limitations. For example, the fixation process might cause lipid degradation without specific measurement of the related degradation products,

Additionally, the correlation analysis between sensory evaluation and metabolites may be limited due to the small sample size. Further research will focus on investigating the degradation of lipids and their products during the fixation process.

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. **Shuang Liang**: Investigation, Data curation. **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.

Acknowledgments

This research was supported by the National Natural Science Foundation of China (32272368, 31872709, 32272771), the Key Research and Development Program of Zhejiang (2022C02033), the Science and Technology Cooperation Project of Zhejiang Province (2024SNJF028), and the Innovation Project for Chinese Academy of Agricultural Sciences. We would also like to thank the Instrumental Analysis Center in Tea Research Institute of the Chinese Academy of Agricultural Sciences.

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