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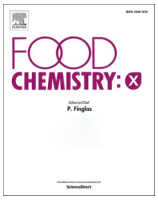

Study on taste quality formation and leaf conducting tissue changes in six types of tea during their manufacturing processes

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ABSTRACT

This study fristly investigated the taste quality formation and leaf conducting tissue changes in six types of Chinese tea (green, black, oolong, yellow, white, and dark) made from Mingke No.1 variety. Non-targeted metabolomics showed the vital manufacturing processes (green tea-de-enzyming, black tea-fermenting, oolong tea-turning-over, yellow tea-yellowing, white tea-withering, and dark tea-pile-fermenting) were highly related to their unique taste formation, due to different fermentation degree in these processes. After drying, the retained phenolics, theanine, caffeine, and other substances significantly impacted each tea taste quality formation. Meanwhile, the tea leaf conducting tissue structure was significantly influenced by high processing temperature, and the change of its inner diameter was related to moisture loss during tea processing, as indicated by its significant different Raman characteristic peaks (mainly cellulose and lignin) in each key process. This study provides a reference for process optimization to improve tea quality.

Introduction

Tea, one of the most popular beverages worldwide, has six major types (green, black, oolong, yellow, white, and dark) in China (Liu et al., 2019). The taste is refreshing for green tea (Zhuang et al., 2020), mellow for yellow tea (Wei et al., 2020; Wang, Yue, & Tong, 2021), mellowish for dark tea (Cheng et al., 2020; Hu et al., 2021), mellow and aromatic for oolong tea (Zeng, Zhou, Su, & Yang, 2020), sweet mellow for black tea (Li et al., 2021), and umami and sweet for white tea (Chen et al., 2020). Their traditional taste quality can be attributed to their respective unique processing methods, fresh leaf raw materials, processing environment, etc., and their taste difference is related to the changes in the content and proportion of amino acids, catechins, flavonoids, and other metabolites after processing (Liu et al., 2019; Chen et al., 2021). Currently, most studies focus on the changes of metabolites during single tea processing (white tea, oolong tea, etc.) (Hu et al., 2018; Chen et al., 2020), or directly compare the difference among tea products (Wang et al., 2019). However, tea product varies greatly in the source of raw materials (Chen et al., 2021), and the different chemical compositions of tea tree varieties may mask the impact of certain specific processes on tea taste. So using the same tea tree variety for different tea processing (Wang et al., 2019) is helpful for us to understand the effect of processes on the quality of different types of tea.

During processing, tea metabolite changes are reported to be closely related to leaf structure (Liu, Chen, Sun, & Ni, 2022), mainly including protective, vegetative, and conducting tissues. The conducting tissue not only transports water, inorganic salts, and photosynthetic products for the leaves, but also supports their spatial extension to ensure physiological function (Gamalei, 1989). Thus far, few studies have been performed on leaf conducting tissue changes during tea processing. Studies have shown that the conducting tissue plays a vital role in the synthesis and transport of carbohydrates, proteins, polyphenols, theanine, alkaloids, etc. in fresh leaves (Pratt & Jacobsen, 2016). In the postharvest stage, the leaves will continue the metabolism and synthesis reaction *in*

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vitro, and the conducting tissue also performs an important function (Li et al., 2021).

The aim of this paper was to investigate the taste quality formation and leaf conducting tissue changes in six types of tea during their manufacturing processes. Specifically, we used non-targeted metabolomics to analyze the change rule of nonvolatile components during six tea model manufacturing processes. Meanwhile, scanning electron microscopy and laser confocal Raman spectroscopy were used to preliminarily observe the changes in the structure and components of the leaf-conducting tissue during tea processing. This study provides a reference for process optimization to improve tea quality.

Materials and methods

Main chemicals

From Shanghai Yuanye Biotechnology Co., Ltd., we purchased the following standards of LC-MS grade: catechin standards ((+)-catechin/C, (-)-gallocatechin/GC, catechin gallate/CG, (-)-gallocatechin gallate/GCG, epicatechin/EC, (-)-epicatechin gallate/ECG, (-)-epigallocatechin/EGC, and epigallocatechin gallate/EGCG), amino acid standards (histidine, tryptophan, lysine, serine, glutamic acid, glutamine, aspartic acid, asparagine, arginine, tyrosine, proline, valine, isoleucine, leucine, threonine, phenylalanine, glycine, alanine, methionine, theanine, and γ-aminobutyric acid), phenolic acid standards (quinic acid, gallic acid, chlorogenic acid, caffeic acid, and p-coumaric acid), flavone standards (vitexin glucoside, vitexin, vitexin rhamnoside, quercetin, quercetin-7-O-glucoside, quercetin-3-O-rutin, quercetin, quercetin-3-O-glucosylrhamnoside, quercetin-7-O-α-L-rhamnoside, quercetin-3-galactoside, quercetin-3-O-β-D-galactopyranoside, myricetin, myricetin-3-O-galactoside, kaempferol, kaempferol, kaempferol, dihydrokaempferol-7-O-rhamnoside, kaempferol-3-O-glucoside, isovitexin, and isovitexin-2′′-O-arabinoside), theaflavin standards (theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-diggallate), alkaloid standards (theobromine, theophylline, and caffeine), (-)-epiafzelechin, procyanidin B1, procyanidin B1, and internal standard (ethophylline). Glycosidically bound volatiles were synthesized by Shandong University (Shandong, PRC).

Processing of tea samples

In August 2020, fresh tea leaves (Mingke No.1) were picked at Danding Tea Industry Co., Ltd., Danjiangkou city, Hubei province, PRC.

Green tea, yellow tea, dark tea, oolong tea, black tea and white tea were processed with 3 repetitions according to the basic processing technology. The specific process is as follows.

Green tea: fresh leaves → withering (spreading for 6 h) → fixation (280 °C, 4 ~ 5 min) → rolling for 40 min → first drying (110 °C, 20 min) → final drying (80 °C,1h). Samples were taken at fresh leaves (FL), withering leaves (W), de-enzyming leaves (D), rolling leaves (R) and green tea (GT).

Yellow tea: Yellow tea and green tea were processed with the same fresh leaves, de-enzyming leaves and rolling leaves. Put rolling leaves into the basket, cover them with wet cloth (temperature at 45 ℃, humidity 70%, 10 h). Then dry them at 110 ℃ for 20 min. Finally 80 ℃ dry them enough at 1 h to obtain the finished yellow tea. Samples were taken at yellowing leaves (Y) and yellow tea (YT).

Dark tea: Take about 500 kg of the above finished green tea (GT), sprinkle water to increase the moisture content of the tea to about 29%, then build a about 110 cm pile, and pile-ferment for 30 days. Then dry them at 110 ℃ for 30 min. Finally dry them enough to obtain the finished dark tea (80 °C, 1.5 h). Samples were taken at tea dhool (TD), pile-fermentation leaves(PF) and dark tea (DT).

White tea: withering (spreading at 2 ~ 3 cm thickness, temperature 25 ~ 28 ℃, humidity 67 ~ 80%, 72 h) → drying (60 °C, 1.5 h). Samples were taken at 24 h (W24), 48 h (W48), 72 h (W72) of withering leaves and white tea (WT).

Oolong tea: indoor withering (temperature 22 ℃, humidity 70%, 6 h) → first turning over (speed 20 r/min, 1 min) → withering 1 h → second turning over (speed 20 r/min, 4 min) → withering 2 h → third turning over (speed 20 r/min, 8 min) → withering 8 h → fixation (280 °C, 4 ~ 5 min) → rolling for 20 min → first drying (110 °C, 20 min) → final drying (80 °C, 1 h). Samples were taken at withering leaves (OW), turning-over leaves(TO), de-enzyming leaves(OD), rolling leaves (OR) and oolong tea (OT).

Black tea: ventilation withering 12 h → rolling for 60 min → fermentation (temperature 28℃, humidity 90%, 4 h) → first drying (110 °C, 20 min) → final drying (80 °C, 1 h). Samples were taken at withering leaves (BW), rolling leaves (BR), fermentation leaves (BF) and black tea (BT).

Sensory evaluation

Taste, aroma, appearance, brew color, and infused leaf of 6 finished tea products were evaluated independently by 5 professional tea tasters and evaluated according to the National Standard of China (GB/T23776-2018). The pictures of dry tea and tea soup are in Fig. S2.

Extraction and analysis of novolatile compounds in different tea samples

Each vacuum-ground freeze-dried sample (150 mg) was weighed into a 20 mL ground volumetric flask, followed by adding 7.5 mL of 75% (v/v) methanol solution (containing 5 μg/mL of theophylline as internal standard), extraction in a 70 ℃ water bath for 30 min, and then cooling to room temperature. Next, the extracted liquid was transferred into a 10 mL centrifuge tube, followed by centrifugation at 5000 g for 3 min, passing the supernatant through a 0.22 μm membrane into a brown sample bottle, sealing each bottle with parafilm, and storage at −20 ℃ for further analysis (Li et al., 2021).

Novolatile compounds in tea samples were analyzed using UHPLC-Q-TOF/MS as an analysis instrument (Agilent, California, USA) with the C18 chromatographic column (100 × 2.1 mm, 1.8 μm. Agilent, California, USA). Chromatographic conditions were as follows: mobile phase A, 0.1% (v/v) formic acid aqueous solution; mobile phase B, methanol; elution procedures:90–85% A (0–4 min), 85–75% A (4–7 min), 75–68% A(7–9 min), 68–60% A (9–16 min), 60–45% A (16–22 min), 45–5% A (22–28 min), 5% A (28–30 min), 5–90% A (30–31 min), and 90% A (31–35 min); injection volume: 3 μL; column temperature: 35 ℃. Mass spectrum conditions: ESI^+ mode, gas drying temperature (300 °C), capillary voltage (3.5 kV), flowing (8.0 L/min), spray pressure (3.5 psi), sheath temperature (350 °C), sheath washing (11.0 L/min), Auto MS/MS-scanning range (100–1200 Da), and collision energy (10, 20, and 30 V).

Analysis of conducting tissue

Analysis of conducting tissue structure. Except for dry tea (which was shrunk and curled after high-temperature treatment, thus difficult to slice for observation), all the samples in the processing of six types of tea were collected and fixed on 2.5% glutaraldehyde, followed by dehydration with gradient ethanol, incubation with isoamyl acetate for 20 min, and transfering each sample into the sample basket for critical point drying. After drying, the piece was transversely cut, pasted on the sample table, sprayed with gold, and finally placed in the scanning electron microscope chamber (JSM-6390LV) for observation. The length and width of the conducting tissue were measured by X2500 (2000 μm^2), and the electron microscope images were analyzed using Image J1.8.0 software.

Analysis of the conducting tissue components. Based on our previous research method (Yu et al., 2020), briefly, randomly select 5 leaves and slowly tear them apart along the main vein direction, the case in point is **Fig. S1.** Then their spectrum data were acquired using a laser confocal

Raman spectrometer (LabRAM HR Evolution, 50x objective lens, 100 μm, and 532 nm excitation wavelength) under the following conditions: test grating, 300 mm^{-1}; spectrum acquisition time, 12 s; scanning range, 200–1800 cm^{-1}. Post-processing and analysis of Raman spectral data were performed using the LabSpec6 software and ST-Japan database.

Statistical analysis

Data were analyzed using SPSS Statistics 20.00 ($P < 0.05$) and excel. Results were expressed as means ± standard deviation (mean ± SD, n = 3), and statistical significance was considered at $P < 0.05$. PCA (principal components analysis) and OPLS-DA (orthogonal partial least squares-discriminant analysis) were performed using SIMCA 14.1 software. Illustrations were drawn by Adobe Illustrator CC 2019 and OriginPro 2018.

Results and discussion

Metabolite profiles in the processing of six types of tea

Non-targeted metabolomics based on UPLC-Q-TOF/MS was used to analyze the nonvolatile components of all samples in the processing of six types of tea. After peak extraction and matching of the original document, the contaminated ions generated by column loss were deleted, and a total of 2109 characteristic ions were obtained. According to the traditional processing of the six teas, the nonvolatile components of samples at each process are systematically divided by PCA analysis (Fig. 1).

Green tea processing includes the procedures of spreading, de-enzyming, rolling, and drying (Li et al., 2022). During green tea processing (Fig. 1**A**), the nonvolatile components showd gradual changes. Specifically, the fresh leaves are located in the first quadrant, and after de-enzyming and rolling, nonvolatile components exhibit relatively small changes in the de-enzymed and rolled leaves, with both of them in the third quadrant, and a relative close distance between green tea and the de-enzymed leaves (in the arrow direction), suggesting that de-enzyming is a critical step in the formation of green tea taste quality. De-enzyming refers to the process of inactivating the activity of various enzymes at high temperatures to achieve the formation and transformation of certain substances, thus promoting the formation of quality characteristics, such as clear soup, and green leaves of green tea (Zhang et al., 2021). In green tea processing, rolling can produce some damp heat effect, but rolling is performed after cooling the de-enzymed leaves, and the rolling time is relatively short, so the nonvolatile components

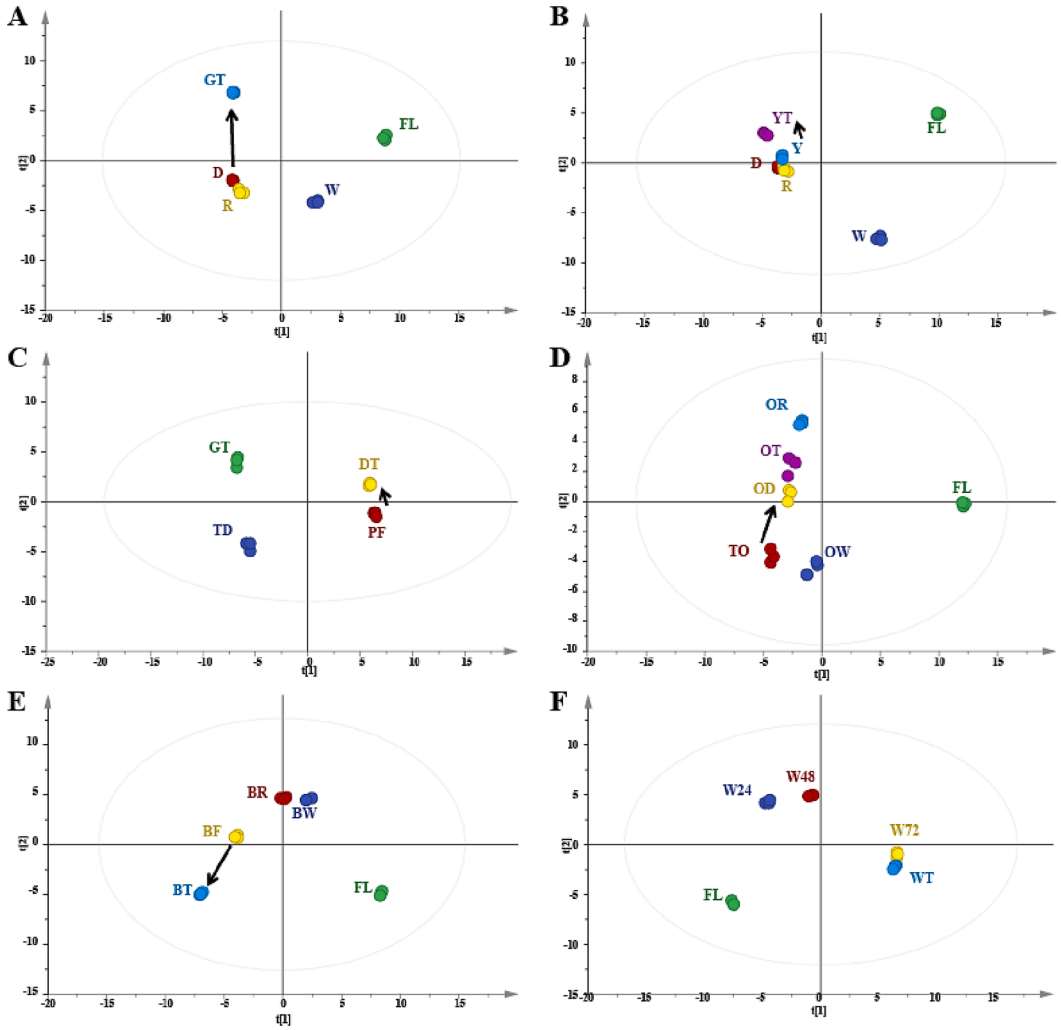

Fig. 1. PCA statistical analysis of no-volatile compounds in the processing of green tea (A), yellow tea (B), dark tea (C), oolong tea (D), black tea (E), and white tea (F). FL: fresh leaves, W: leaf withering, D: leaf de-enzyming, R: leaf rolling, Y: leaf yellowing, GT: green tea, YT: yellow tea; TD: tea dhool, PF: leaf pile-fermentation, DT: dark tea; WT: white tea, W24: withering 24 h, W48: withering 48 h, W72: withering 72 h; BT: black tea, BW: leaf withering, BR: leaf rolling, BF: leaf fermentation; OT: oolong tea, OW: leaf withering, TO: leaf turning-over, OD: leaf de-enzyming, OR: leaf rolling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

are similar in both de-enzymed and rolled leaves (Li et al., 2022).

Yellow tea has three "yellow" characteristics (yellow dry tea, yellow infusion, and yellow infused leaf), whose manufacturing procedures mainly include de-enzyming, rolling, yellowing, and drying (Guo, Ho, Schwab, & Wan, 2021). In Fig. 1**B**, the endogenous nonvolatile components of fresh leaves were seen to gradually change after those processes. It is worth noting that the main components overlap much between de-enzymed leaves and yellowed leaves. After de-enzyming, most biological enzymes of fresh leaves are inactivated, and the enzymatic reaction is significantly reduced, so the material transformation is relatively slow (Wang et al., 2020). However, the deliberate yellowing treatment caused more intense changes in the sample than rolling treatment, allowing the continuation of a series of chemical reactions dominated by non-enzymatic-hygrothermal reaction, thus promoting the quality characteristics of yellow tea (Wang, Yue, & Tong, 2021; Li et al., 2022). This study also found that the yellowed leaves were the closest to finished tea (Fig. 1**B**), confirming the importance of yellowing technology to yellow tea taste quality formation.

In this experiment, the processing of dark tea sample had the procedure of wet-piling, leading to obvious changes in its metabolites during processing (Fig. 1**C**). Specificlly, raw tea is first humidified to wet green tea, where water, as an important medium and reactant to transform dark tea quality components, can provide an environmental place for the oxidation, hydrolysis and isomerization of substances (Cao et al., 2018). Therefore, the distance is far between raw tea and tea dhool (TD), indicating the onset of material reaction at this time. It is worth noting that the distance is very close between the pile-fermented leaves and the finished tea, suggesting that dark tea's quality formation mainly occurs in the pile-fermentation process. Under the conditons of damp heat effect and microbial growth, a series of reactions take place in the pile-fermentation process, such as oxidation, hydrolysis, polymerization, and secondary metabolic transformation, which promote changes in composition and quality formation of dark tea (Hu et al., 2021).

The unique flavor quality formation of oolong tea is closely related to its processing technology, including picking → withering → turning-over → de-enzyming → rolling → drying (Hu et al., 2018). Based on most studies, turning-over is defined as the transformation process of inclusions through appropriate mechanical damage and water stress, which is also a critical processing technology to form the unique taste quality of oolong tea (Zeng, Zhou, Su,&Yang, 2020). In this study, we also found that during oolong tea processing, the component separation effect is obvious between the samples prepared in each process, and the main components of the turned-over leaves gradually moved closer to the finished tea (Fig. 1**D**, arrow direction), with a realtively close distance between the de-enzymed leaves and the finished tea, similar to PCA analysis of green and yellow teas.

Black tea processing includes the procedures of fresh leaf picking, withering, rolling, fermentation, and drying (Li et al., 2022). The fresh tea leaves are fermented under specific temperatures, humidity, pH, oxygen, and other environmental conditions, where a series of biochemical reactions occur, with polyphenol enzymatic oxidation as the core, gradually forming the quality characteristics of black tea (Li et al., 2021). In this study, the nonvolatile components of fresh leaves were far from those of other procedures (Fig. 1**E**), with significant changes in different manufacturing processes. The distance is the closest between fermented leaves and finished tea, and both of them had similar nonvolatile components, indicating that fermentation is a crucial process for the final taste quality formation of black tea.

In white tea processing, the nonvolatile components gradually changed (PCA in Fig. 1**F**). The distance was far between fresh leaves and 72 h-withered leaves, but close between 72 h-withered leaves and final tea, indicating that changes in nonvolatile components mainly occur in the withering process, thus a critical step in the quality formation of white tea. During withering, a series of chemical reactions (hydrolysis as the main body) take place, especially for long-term withering, resulting in most water loss and weight loss, as well as more dramatic quality changes (Chen et al., 2020).

In this study, we speculate that the unique taste quality formation of the six major tea types was found highly related to their respective vital processes, i.e., green tea-de-enzyming, yellow tea-yellowing, oolong tea-turning-over, black tea-fermenting, dark tea-pile-fermenting, and white tea-long-term withering. Samples in these stages were closest to the final taste quality of the finished tea. Experimental results indicated that the main components in the withered leaves of green tea, yellow tea and oolong tea were close to those of their finished products, respectively. This is probably related to the oxidation fermentation of their nonvolatile components. Specifically, green tea belongs to non-fermented tea; yellow tea mainly undergoes a thermal reaction in the yellowing stage, thus a low fermentation degree; oolong tea belongs to semi-fermented tea, with fermentation reaction primarily occurring in the turning-over stage (Liang et al., 2021). The de-enzyming process will inactivate biological enzymes (PPO, etc.) involved in fermentation, thus terminating or hindering this reaction (Zhang et al., 2021). This indicated that de-enzyming is equally essential for the taste quality of non-fermented or little-fermented tea. However, dark tea is different, whose nonvolatile components are more significantly influenced by damp heat and microorganisms.

Differential metabolite analysis

Currently, the metabolomics technology has been widely used in identification of tea flavor components, and metabolic data have massive and high-dimensional characteristics, so multivariate statistical analysis methods are usually used to simplify and reduce data dimensions and then screen differential metabolites (Vladimir, 2006). Based on the previous work of Yu et al. (2020), as well as retention time and standard comparison (**Table S1**), 66 nonvolatile components were identified in the original data of this study, including 15 amino acids, 8 catechins, 3 alkaloids, 4 theaflavins, 19 flavonoid glycosides, 7 phenolic acids, 8 glycosidically bound volatiles and 2 proanthocyanidins (**Supplemental materials-2**). As shown in the PCA score plot (Fig. 2**A**), the separation effect between samples was noticeable, indicating that the content of nonvolatile components in the one tea tree variety changed gradually after different processings (Wang et al., 2019).

Combined with the six critical manufacturing processes obtained above, OPLS-DA was used to screen the differential metabolites. As shown in Fig. 2**B**, after processing by different key processes, the treated leaves showed significant changes in nonvolatile components, with the distance being similar between the sampling points of de-enzymined and yellowed leaves, which was also observed in the above experiment (Fig. 2B). Based on OPLS-DA analysis, substance was first screened by VIP value > 1. Twenty-two differential metabolites were finally determined by Kruskal Wallis ($P < 0.05$), including 9 flavonoids, 5 phenolic acids, 4 glycosidically bound volatiles, 2 alkaloids, and 2 catechins (Fig. 2**D**, **Table S2**). These differential metabolites are probably the key components to form their respective characteristic taste quality.

Among the 22 different metabolites, the content of EGCG is relatively high, showing obvious changes, with the greatest reduction in fresh leaves after fermentation, followed by pile-fermentation, turning-over, long-term-withering, yellowing, and de-enzyming. The change in EGCG content is highly related to the fermentation degree of their respective tea types (Chen et al., 2020; Wei et al., 2020; Li et al., 2020; Hu et al., 2021), with its change in the order of full-fermentation (black tea) > post-fermentation (dark tea) > semi-fermentation (oolong tea) > micro-fermentation (white tea). The EGCG content of yellow tea decreased in the yellowing stage due to its heating effect, and in green tea, an unfermented tea, the EGCG content did not change significantly. In fermentation, tea polyphenols mainly undergo a series of complex and violent chemical changes with EGCG as the main body. For instance, catechins undergo enzymatic oxidation to produce tea pigment, and ester catechins experience a hydrolysis reaction to produce gallic acid and simple catechins under damp heat conditions (Qin, Li, Tu, Ma, &

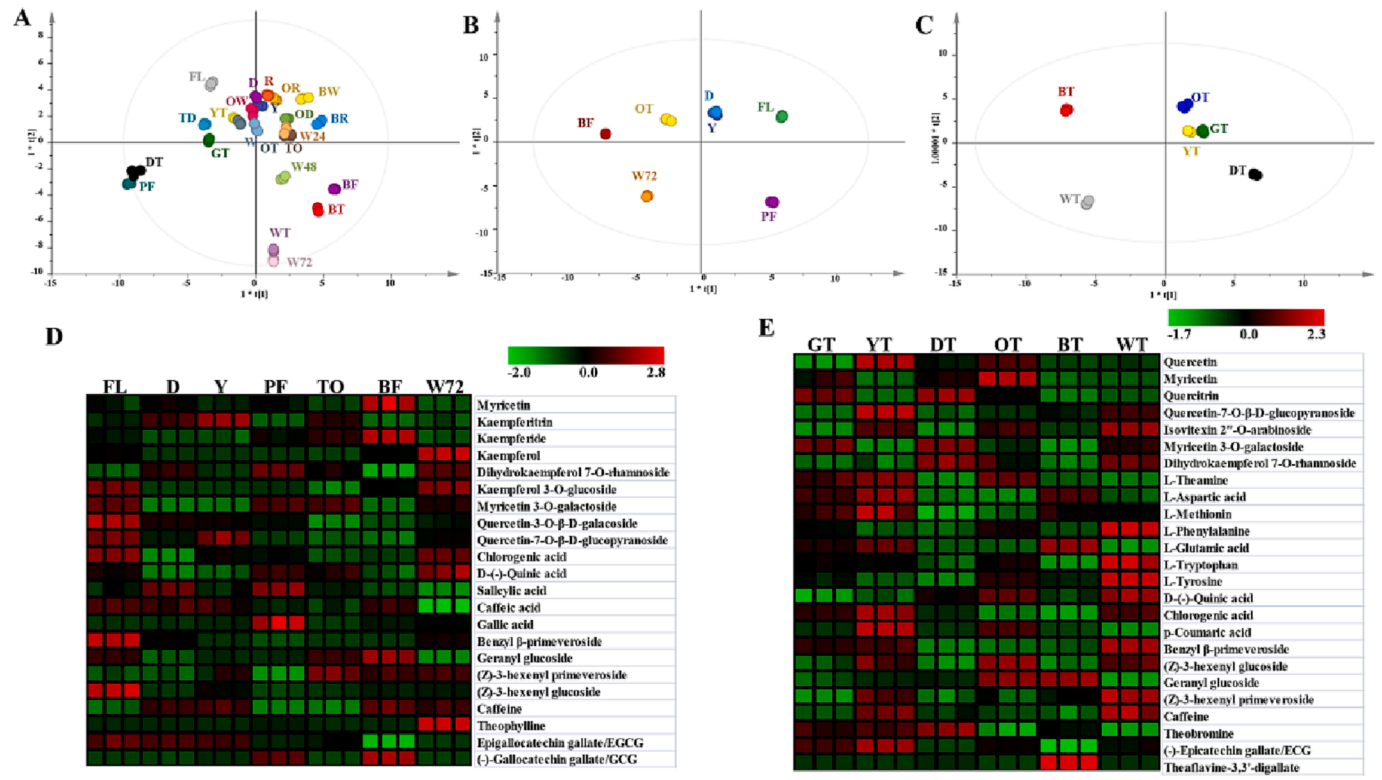

Fig. 2. Differential metabolite analysis. (A) PCA statistical analysis; (B-C) OPLS-DA statistical analysis; (D-E) heatmap; FL: fresh leaves, W: leaf withering, D: leaf de-enzyming, R: leaf rolling, Y: leaf yellowing, GT: green tea, YT: yellow tea; TD: tea dhool, PF: leaf pile-fermentation, DT: dark tea; WT: white tea, W24: withering 24 h, W48: withering 48 h, W72: withering 72 h; BT: black tea, BW: leaf withering, BR: leaf rolling, BF: leaf fermentation; OT: oolong tea, OW: leaf withering, TO: leaf turning-over, OD: leaf de-enzyming, OR: leaf rolling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Zhang, 2012). Therefore, the content of simple catechins (GCG, etc.) increased to a varying degree (**Table S2**) during fermentation, pile-fermentation, and yellowing, which could generate a damp heat environment.

The content and quantity of flavonoids are vibrant in tea, mainly including kaempferol, quercetin, myricetin, vitexin, etc., which are mostly combined with glucose, rhamnose, galactose, rutose, primrose, etc. to form flavonoid glycosides (Okello, Leylabi, & Mcdougall, 2012). In this study, the flavonoid content of the samples prepared with the same tea raw materials through different key processes had an irregular change rule, implying the changes of flavonoids during tea processing are relatively complex (Chen et al., 2020; Li et al., 2021). Among the five phenolic acids, gallic acid showed most obvious changes, with an increase in fermented types of tea, and the most significant increase in pile-fermented dark tea, which may be related to the hydrolysis of ester catechins to produce gallic acid under the damp heat effect (Qin, Li, Tu, Ma,&Zhang, 2012). In the withering process, the biosynthesis of caffeine, the alkaloid with the highest content, tended to continue, and after different processings, it showed an uptrend or did not change significantly, mainly because of its stable structure, less metabolic consumption, and main metabolism to produce theophylline (Ashihara, Kato, & Ye, 1998). As a purine base, theophylline is an intermediate product of caffeine metabolism, which increased in the withering process of white tea, consistent with previous studies (Chen et al., 2020). Glycosidically bound volatiles are mainly responsible for aroma formation, and compared with other nonvolatile components, they contribute less to tea taste (Yang, 2013), so they are less discussed in this study.

The metabonomic analysis method was used to screen the differential metabolites in the six types of tea, and 25 substances were obtained, including 7 flavonoids, 7 amino acids, 3 phenolic acids, 4 glycosidically bound volatiles, 2 alkaloids, 1 catechin and 1 theaflavin (Fig. 2**E**, **Table S3**), which will cause the difference in the taste quality of the 6 tea types. Note that the content of amino acids varied significantly in the six types of tea after drying treatment, thus becoming the primary differential metabolites.

Drying is the stage for tea quality formation and fixation, and changes in the content of nonvolatile components will make a difference in taste quality (Fu et al., 2020). The content of water-soluble substances in tea is 30%~48%, including polyphenols, alkaloids, amino acids, etc., and the composition, content, and interaction of each component will endow the tea infusion with a different taste style (Li et al., 2022).

Among the amino acids of tea, theanine accounts for over 40% of total free amino acids, and theanine itself has a fresh and mellow taste, thus able to alleviate bitterness and enhance the sweet note (Syu, Lin, Huang, & Lin, 2008). As shown in **Table S3**, the theanine content is relatively high in green tea and yellow tea, which could promote their fresh and mellow perception, but its content is relatively low in white tea. Meanwhile, as the main bitter substance, the content of caffeine is relatively high in white tea, which may be the main reason for its poor taste in this study. As a fully fermented tea, black tea is significantly lower than the other types of tea in catechin content, and its content of flavonoid glycosides (astringent substances) was also relatively low, thus endowing its infusion with a mellow taste. For oolong tea, the content of theanine and other free amino acids is relatively high, while caffeine is relatively low, which can promote a fresh and mellow perception (Li et al., 2022).

The differential metabolites also include theaflavine-3,3′-digitate and some phenolic acids. Theaflavins-3,3′-digitate is mainly produced by EGCG oxidation, which was significantly higher than other groups in black tea, followed by dark tea. The content of the three phenolic acids was relatively high in yellow tea and oolong tea (**Table S3**). Studies have

shown that theaflavine-3,3′-digitate and phenolic acid are also characterized by astringency, which can enhance bitterness and sweetness, thus increasing the sense of tea flavor layers (Okinda et al., 2006). Further sensory evaluation indicated that unlike traditional tea products, the green tea and yellow tea prepared in this study were slightly stimulating, with a mellow taste; black and dark tea were mellow and natural; oolong tea was heavy and fresh (Table 1). Additionally, all the six tea samples had flower-fruit fragrance, which might be related to the variety advantage of the fresh leaf raw material (Mingke No.1).

Collectively, under the same tea raw material condition, the different metabolites produced in the key manufacturing processes were mainly concentrated in phenols (catechins, flavonoids, and phenolic acids), with EGCG as the main body for reactions with a different degree of fermentation. The substances accumulated and transformed in these processes play a crucial role in taste quality formation. Finally, after drying, the type and content of nonvolatile components formed in the critical processes would be retained to the greatest extent and determine the amount of the final taste components.

Analysis of conducting tissue

During tea processing, the changes in leaf tissue structure and composition will affect tea quality formation (Yu, Liu, et al., 2020; Liu, Chen, Sun, & Ni, 2022). Before the tea leaves are completely dried, the leaf-conducting tissue theoretically play a role in redistributing water and soluble substances. However, it remains unclear whether conducting tissue changes contribute to the characteristic quality formation of tea. In this study, changes in the conducting tissue during tea processing were explored by scanning electron microscopy (Pathan et al. (2010)).

In Fig. 3**A**, it was shown that during green tea processing, the inner diameter decreased significantly after de-enzyming, followed by a plateau. High-temperature de-enzyming made the blade heated up rapidly in a short time, coupled with evaporation of a large amount of water (Zhang et al., 2021), edge shrinkage (Fig. 3**G-D**), and significant reduction ($P < 0.05$) in inner diameter. After yellowing, the inner diameter of yellowed leaves (Fig. 3**G-Y**) showed an uptrend, significantly larger than that of de-enzymed leaves and rolled leaves, but still smaller than that of the fresh leaves (Fig. 3**G-FL**). Yellowing refers to the hot-piling step of tea leaves after rolling by covering the tea dhool with a wet cloth, allowing the inner diameter to absorb water water, thus causing thermochemical reactions under damp heat conditions (Wei et al., 2020). After drying, the inner diameter decreased significantly ($P < 0.05$).

Table 1
Sensory evaluation of the six types of tea samples.

	Appearance	Brew color	Aroma	Taste	Infused leaf
Green tea	approach yellowish green	yellowish green	chest-nutty, slightly floral and fruity	mellowish, freshness, slightly stimulating	yellowish green
Yellow tea	auburnish yellow	auburnish yellow	chest-nutty, slightly sweet, slightly floral and fruity	mellowish, slightly stimulating	auburnish yellow
Dark tea	brownish yellow	brownish yellow	stale, slightly floral and fruity	mellowish	brownish
White tea	brownish auburn	greenish yellow	fresh, floral and fruity	clean and sweet	brownish red
Oolong tea	auburn bloom	brownish auburn	floral and fruit	mellow and thick	brownish auburn
Black tea	auburnish black	brownish red	pure, floral and fruity	sweet mellow	brownish red

Note: the appearance and infused leaf of six samples were only evaluated for color index.

Dark tea is made from raw green tea by humidifying, piling, and drying (Hu et al., 2021). The water content of raw tea is 6.17%, and 29.87% after humidifying. Due to water absorption, the inner diameter was detectable (Fig. 3**C**), and after pile-fermentation, the inner diameter was further enlarged and significantly higher ($P < 0.05$) than the previous stage.

During oolong tea processing, the conducting tissue structure changed significantly (Fig. 3**D**). In the turning-over process, the blade lost water and decreased in inner diameter (Fig. 3**G-TO**). When de-enzyming oolong tea, the temperature increased rapidly, causing the shrinkage of leaf tissue because of water loss, and further significant reducing ($P < 0.05$) the inner diameter.

During black tea processing, the conducting tissue structure changed significantly (Fig. 3**E**), and in the withering process, the leaves lost water due to transpiration (Speckman, Ewers, & Beverly, 2020), leading to significantly decrease ($P < 0.05$) in the inner diameter of withered leaves.

With the increase of withering degree in white tea, the inner diameter of withered leaves decreased significantly (Fig. 3**F**). After 24 h of withering, the leaves are dehydrated, causing the migration of water in prominent veins to branches and mesophyll cells (Pratt & Jacobsen, 2016), leading to a significant decrease ($P < 0.05$) in inner diameter, followed by a plateau. In Fig. 3**G-W72**, the inner diameter of the withered leaves was seen to be markedly lower ($P < 0.05$) than that of fresh leaves.

Taken together, the inner diameter changed regularly during tea processing. In the withering process, the leaves lost water, causing the migration of water in the stems to the main veins' xylem (Pratt & Jacobsen, 2016). With withering degree deepening, the fresh leaves lost water through stomata (Warrit, Landsberg, & Thorpe, 2010), thereby decreasing the inner diameter. Under the high-temperature conditions of de-enzyming and drying, the free and semi-combined water in the conducting tissue would gradually vaporize, leading to cell shrinkage and a sharp reduction in inner diameter. Therefore, the decrease in the conducting tissue's inner diameter can be concluded to be positively related to the water loss during tea processing. Interestingly, the inner diameter of samples was significantly increased in the processes of yellowing, pile-fermentation, and fermentation, indicating that the conducting tissue could absorb water to partially restore the inner diameter. The high humidity environment might favor the reaction and transformation of substances in the leaves (Zeng, Zhou, Su, & Yang, 2020). Note that depite the high humidity treatment (fermentation and pile-fermentation) of samples during processing, the inner diameter of their conducting tissue is still significantly lower ($P < 0.05$) than that of fresh leaves.

In addition, as shown in Fig. 3**G,** after processing the fresh leaves in different ways, some mass or filamentous substances appeared in the conducting tissue. Based on the changes in inner diameter and the observed substances, the conducting tissue can also be assumed to decompose to some metabolic substances during different manufacturing processes. To test this hypothesis, we used Raman spectroscopy to analyze the conducting tissue components of tea samples collected in the critical manufacturing processes (Fig. 4**A**). Raman spectrum is a characteristic molecular spectrum, and its band frequency, intensity, and shape are closely related to the molecular structure of the tested material, thus able to reflect the category and content change of characteristic substances (Dresselhaus, Jorio, Hofmann, Dresselhaus, & Saito, 2010).

Through database comparison and analysis, four prominent Raman characteristic peaks were identified in the conducting tissue, i.e., the

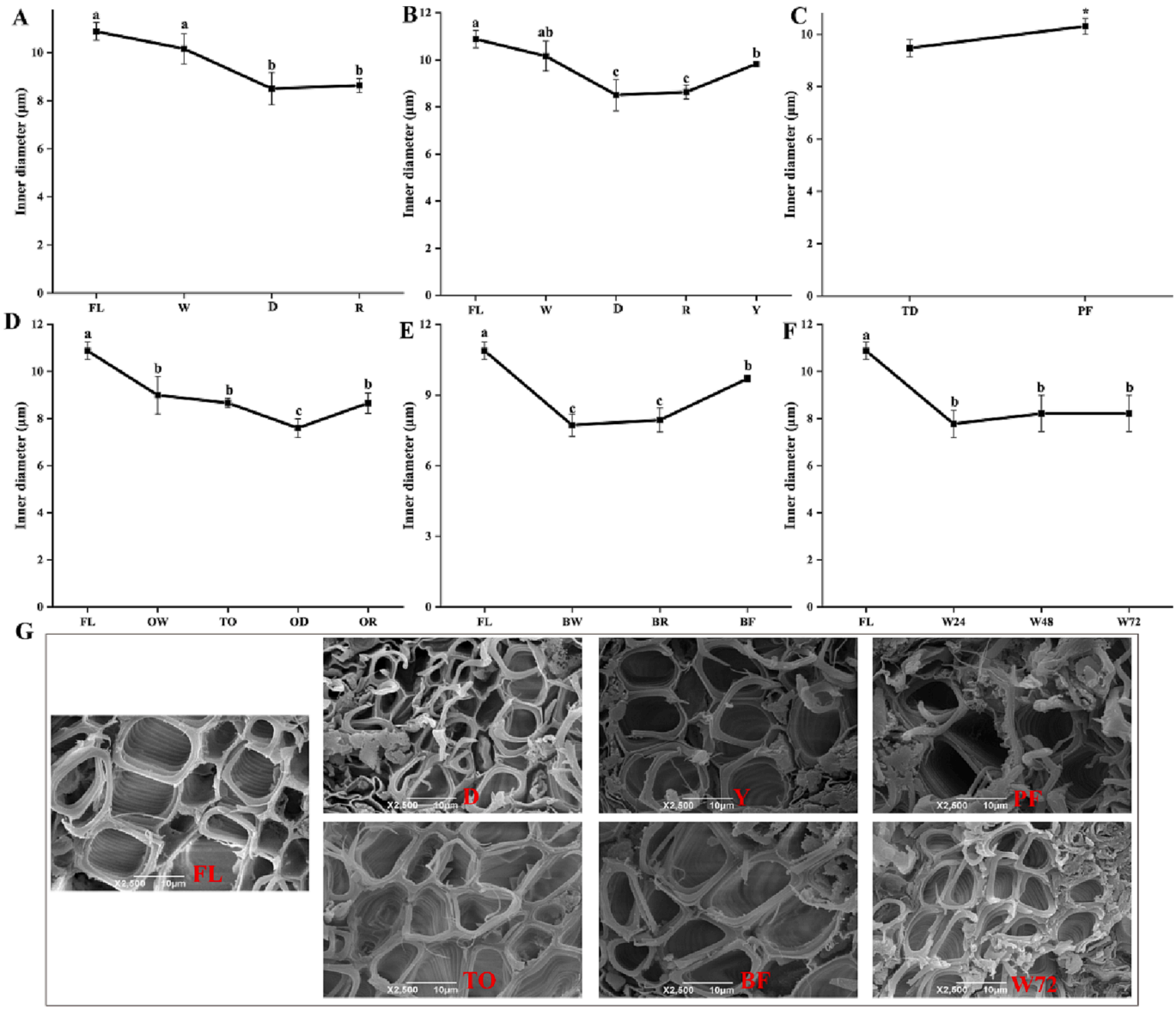

Fig. 3. Changes in inner diameter of conducting tissue (A-F) and SEM pictures (G). FL: fresh leaves, W: green tea leaf withering, D: green tea leaf de-enzyming, R: green tea leaf rolling, Y: leaf yellowing, TD: tea dhool, PF: leaf pile-fermentation, W24: withering 24 h, W48: withering 48 h, W72: withering 72 h, BW: black tea leaf withering, BR: black tea leaf rolling, BF: leaf fermentation, OW: oolong tea leaf withering, TO: leaf turning-over, OD: oolong tea leaf de-enzyming, OR: oolong tea leaf rolling. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

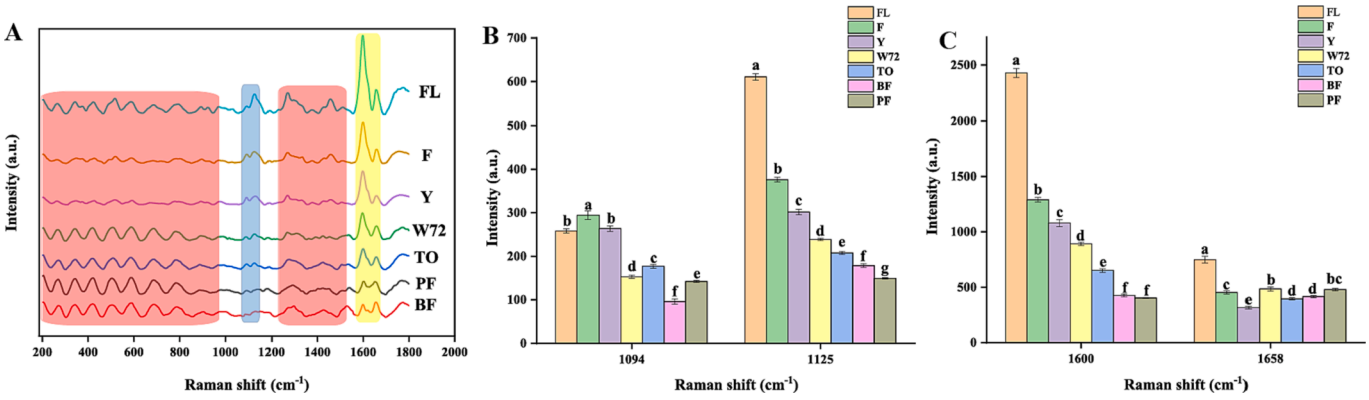

Fig. 4. Raman map (A) and changes in characteristic peak intensity (B-C). FL: fresh leaves, D: green tea leaf de-enzyming, Y: leaf yellowing, TD: tea dhool, PF: leaf pile-fermentation, W72: withering 72 h, BF: leaf fermentation, TO: leaf turning-over. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

characteristic cellulose peaks at 1094 cm^{-1} and 1125 cm^{-1}, and the lignin characteristic peaks at 1600 cm^{-1} and 1658 cm^{-1}, which are the main components of the conducting tissue (Pierantoni, Brumfield, Addadi, & Weiner, 2019). As shown in Fig. 4, after the same fresh tea leaves went through different manufacturing processes, the cellulose peak at 1094 cm-1 showed a slight increase in the de-enzyming and yellowing processes, and the intensity of the other peaks was significantly lower ($P < 0.05$) than that of fresh tea leaves. Note that the change law of the characteristic peak intensities (cellulose and lignin) was positively related to the fermentation degree of various teas in the manufacturing processes, which was in the order of de-enzyming > yellowing > withering 72 h > turning-over > pile-fermentation > fermentation, with a slight fluctuation for pile-fermentation, withering 72 h, and turning-over (Fig. 4**B-C**). Previous studies have shown that in the late stage of dark tea pile-fermentation, microorganisms can produce rich hydrolases (Wang, Peng, & Gong, 2011), which may hydrolyze the conducting tissue and change its cellulose and lignin content. During turning-over and long-term withering, the substances might be transferred and redistributed (Zeng, Zhou, Su, & Yang, 2020), thus causing fluctuations in the position and strength of Raman spectrum characteristic peaks. Fresh tea leaves were suggested to undergo the transfer of substances in the postharvest stage (Chen et al., 2020; Li et al., 2021).

Apart from the distinct peaks of identified lignin and cellulose, there are many other different characteristic peaks for the conducting tissue (marked red in Fig. 4**A**). Although the components could not be identified accurately, the difference in characteristic spectra could prove that the structural components of the conducting tissue itself or its contents had changed significantly in the critical manufacturing processes, and the products might also contribute to the quality formation of the corresponding type of tea.

Conclusions

In this study, non-target metabonomics, scanning electron microscopy, and laser confocal Raman microscopy were used to systematically analyze the taste quality formation and leaf conducting tissue changes in six types of tea during their manufacturing processes, and the following conclusions can be drawn.

Nonvolatile components and conducting tissue structure are significantly affected by the key manufacturing processes. The unique taste quality formation of the six teas is highly related to their respective vital manufacturing processes, i.e., green tea-de-enzyming, yellow tea-yellowing, dark tea-pile-fermenting, oolong tea-turning-over, black tea-fermenting, and white tea-withering, with the closeset distance between the leaves treated with these processes to their final tea taste quality. OPLS-DA analysis identified 22 different metabolites, with obvious changes in EGCG and other phenolic substances, and the fermentation reaction with EGCG as the main body was speculated to have a more significant impact on the characteristic quality formation of each tea. After drying, the content of converted and retained phenols, caffeine, theanine, and other amino acids has a more significant impact on the taste quality of the final tea products. Additionally, the inner diameter and structural components of the leaf conducting tissue would change during processing, and in some manufacturing processes, whose changes might contribute to the tea quality formation.

CRediT authorship contribution statement

Yuchuan Li: Conceptualization, Software, Formal analysis, Investigation, Writing – original draft. **Songhui Yu:** Conceptualization, Software, Formal analysis, Investigation. **Shuya Yang:** Supervision, Investigation. **Dejiang Ni:** Supervision. **Xinfeng Jiang:** Supervision. **De Zhang:** Supervision. **Jirong Zhou:** Supervision. **Chunlei Li:** Supervision. **Zhi Yu:** Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

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.2023.100731.

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