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A targeted and untargeted metabolomics analysis of 'Oriental Beauty' oolong tea during processing

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Abstract

Oriental Beauty, a deeply fermented variety of oolong tea, is famous for its fruity aroma and sweet taste. A targeted and untargeted metabolomics was used to comprehensively analyze the dynamic changes of taste and aroma metabolites during the processing stage. During the enzyme reaction stage, the catechin components were oxidized and degraded into theaflavins and oolongtheanins. The total abundance of aroma increased from 259.24 to 564.52 μg/L, and mainly monoterpenoids formed. During the nonenzymatic reaction stage, the total abundance of aroma decreased from 564.52 to 274.74 μg/L, and linalool was thermally converted to hotrienol. In this study, metabolomics changes were conducive to better control of tea quality.

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INTRODUCTION

Tea is a non-alcoholic beverage produced from fresh leaves of *Camellia sinensis*, and it has gained a variety of secondary metabolites such as theanine, catechins and terpenes, compounds that make up the sensory qualities of tea and also have certain health benefits[1]. Tea has been classified into six types depending on processing procedure and fermentation degree, among which oolong tea belongs to semi-fermented tea. Oolong tea retains longer biological activity in the tea leaves during processing, and the tea leaves respond to stresses in processing, giving it a unique floral and fruity aroma[2]. Oriental Beauty, a deeply fermented variety of oolong tea (60%–80% fermentation), is prized by experts for its fruity aroma and sweet taste. In Oriental Beauty manufacture, there are three differences from other oolong teas. The use of fresh tea leaves infested with the tea green leafhopper (*Empoasca onukii* Matsuda) is the most distinctive factor of Oriental Beauty tea, and the plucking standard is one bud with two leaves. The last factor provides a deep degree of fermentation during processing[3]. Current studies mostly focus on the effects of tea green leafhoppers on tea, but the impact of processing technology on the flavor quality of tea leaves is crucial.

The processing steps of oolong tea includes plucking, withering, shaking and rocking, stacking, fixing, rolling, and drying, and which has been classified into an enzymatic reaction (before fixing) and nonenzymatic reaction (after fixing). In the enzyme reaction stage, tea leaves can undergo stress reaction to some exogenous stimulation. For example, solar withering can upregulate phenylalanine and tryptophan-related synthetic genes to accelerate chemical conversion of flavonoids[4], and the transcriptional changes induced by dehydration stress can induce the transformations of catechins and amino acids[5]. Ultraviolet-B solar radiation (UV-B) induces tea leaves to release volatiles[6], and also induces the production of monoterpenes from the grape barrier, which protects the tissues from UV-B itself, and affects the wine flavor[7]. Continuous wounding during the shaking and rocking procedure is the main stress affecting tea quality. Wounding stress can activate some metabolite-related synthetic genes, such as indole, jasmine lactone, and (*E*)-nerolidol[8,9]. During the enzymatic reaction stage, the processing steps including withering, shaking and rocking, tea leaves are constantly being fermented. Fermentation (oxidation) in tea is a series of chemical reactions induced by endogenous enzymes that leads to the bitter and astringent flavonoid compounds, which are catalyzed by polyphenol oxidase (PPO) and peroxidase (POD) to produce catechin oligomers or polymerized products[10,11]. In the nonenzymatic reaction stage, the processing steps include fixing, rolling, and drying. The aim is to prevent the oxidation of enzymes at high temperature and to fix the quality of tea. The fixation step can convert amino acids into heterocyclic compounds to improve the flavor quality of tea[12], and reduction of compounds with green grassy notes[13].

In order to explore the dynamics and quality changes of Oriental Beauty during processing, in this study, liquid nitrogen was used to take tea samples. On the basis of the solid-phase microextraction (SPME) extraction method and gas chromatography–mass spectrometry (GC/MS) analysis method, untargeted analysis was conducted on aroma-related compounds in tea samples. Taste-related compounds of the tea sample were analyzed by liquid chromatography-tandem mass spectrometry (LC/MS) combined with statistical analysis. Dynamic variation in taste-related and odor-related metabolites in Oriental Beauty during processing were found.

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MATERIALS AND METHODS

Chemicals

(−)-Epigallocatechin gallate (EGCG), (−)-gallocatechin gallate (GCG), (−)-epigallocatachin (EGC), (−)-gallocatechin (GC), (−)-epicatechin gallate (ECG), (−)-catechin gallate (CG), (−)-epicatechin (EC), (−)-catechin (C), gallic acid (GA), caffeine, vitexin-2-*O*-rhamnoside, quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, cynaroside, quercetin-3-*O*-rhamnoside, kaempferol-7-*O*-rhamnoside, luteolin, quercetin, kaempferol, vitexin, theaflavin, theaflavin-3-gallate, theaflavin-3'-gallate, and theaflavin-3,3'-gallate (all with ≥ 95% purity) were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Acetonitrile (HPLC grade), methanol (HPLC grade), formic acid, and glacial acetic acid (HPLC, ≥ 99.9%) were purchased from Merck (Darmstadt, Germany). Ethyl caprate (99%) and ethanol (99%) were obtained from Sinopharm Group Chemical Reagent Co., Ltd. (Shanghai, China). Pure water used in this experiment was purchased from HangZhou Wahaha Group Co., Ltd. (Hangzhou, China).

Preparation of tea samples

The fresh tea leaves (cultivar 'Jinxuan' (JX) and 'Tieguanyin' (TGY)) were plucked in Dadian Country (Fujian Province, China). Oriental Beauty manufacture uses traditional processing, starting with fresh tea leaves (FTL), then withering (Wi-OB), followed by the first shaking and rocking (1S-OB), second shaking and rocking (2S-OB), third shaking and rocking (3S-OB), fourth shaking and rocking (4S-OB), fifth shaking and rocking (5S-OB), stacking (ST-OB), fixing (FX-OB), and final tea product (OB). Sampling methods include liquid nitrogen sampling, then maintainance at −80 °C for the subsequent assays.

Untargeted metabolomics analysis of samples based on LC/MS

Untargeted analysis of the tea samples was performed by ultrahigh-performance LC/MS. A Q Exactive LC/MS system (Thermo Fisher Scientific, Rockford, IL, USA) fitted with an ACQUITY UPLC HSS T3 column (1.8 μm, 2.1 × 100 mm, Waters, Milford, MA, USA) was used. The mobile phases A and B were H_2O/0.1% v/v formic acid and Acetonitrile, respectively. The elution gradient was as follow: 0–1.0 min, 5% B; 2.0 min, 10% B; 6.0 min, 35% B; 8.5–9.5 min, 100% B; and 10.0–12.0 min, 5% B. The total analysis time was 12 min. Sample injection volume was 5 μL, and the flow rate was 0.3 mL/min. The column and sampler manager were set at 40 and 10 °C, respectively. Before injection, tea infusions were filtered through a 0.22 μm Millipore filter.

Mass spectrometry (Orbitrap mass analyzer) parameters were set as follows. Data acquisition of mass spectrometry was performed in the negative ionization mode, and the spray voltage was 3.1 kV. The flow rates of sheath gas and auxiliary gas were 45 and 10 (in arbitrary units), respectively. The capillary and auxiliary gas heater temperature were 320 and 300 °C, respectively. The S-lens rf level was 50. Full-scan MS/data-dependent MS/MS (ddMS^2) was used as the scan mode. The resolutions of full-scan MS and ddMS^2 were set at 70,000 and 35,000, respectively. The normalized collision energy was 30%, and the mass scan range was mass-to-charge ratio (*m/z*) 66.7–1,000[14].

Identification of differential compounds was performed with reference to the Human Metabolome Database (www.hmdb.ca), Cloud Database (www.mzcloud.org), published literature and, where available, authentic reference standards.

Analysis of the volatile compounds in tea samples

Volatile compound extraction by SPME

Before extraction, we performed 5 min pretreatment of the SPME fiber in the gas chromatograph injection port at 230 °C, to remove remaining volatiles from the fiber. Dry tea samples (0.1 g) were weighed and placed in 20 mL headspace vials, and then boiling distilled water (5 mL) and decanoic acid ethyl ester (20 μL, 5 μg/L, internal standard) were added, the glass vial was then sealed. The vials were maintained at 60 °C in a water bath for 5 min, and then the SPME fiber was added and the vial was left in the water bath for a further 60 min. Subsequently, the volatiles were desorbed from the fiber in the injector of the GC/MS system for 5 min at 230 °C[15].

GC/MS analysis of volatile compounds

An Agilent 6890 gas chromatograph interfaced with an Agilent HP 5973 MSD ion trap mass spectrometer (Wilmington, DE, USA) was used for the analysis of volatiles. The separation was performed on a DB-5MS capillary column (30 m × 250 μm × 0.25 μm). The detailed detection method was referenced from a previously published article[16].

Identification of volatile compounds

The volatile compounds were identified by using retention indices, authentic standards, or comparison with mass spectra in the National Institute of Standards and Technology Library (NIST14.L). The linear retention indices were determined via sample injection with a homologous series of alkanes (C_5–C_{30}; Sigma-Aldrich, Shanghai, China).

Statistical analysis

Results are presented as mean ± standard deviation. Significance analysis and correlation analysis were performed by SPSS software (version 20.0). The statistical figure was employed for Origin 2022. Partial-least-squares discriminant analysis (PLS-DA) was performed using SIMCA-P 13.0 software (Umetric, Umea, Sweden). MultiExperiment Viewer software (version 4.7.4) was employed for heat-map analysis.

RESULTS AND DISCUSSION

Endogenous enzyme reaction stage

Alteration of non-volatile constituents during the endogenous enzyme reaction stage

To assess the variation in non-volatile metabolites during the enzyme reaction stage of Oriental Beauty, untargeted analysis based on LC/MS was employed. A total of 2394 mass/retention time figures were detected in the ESI$^-$ modes, which were reduced to 1201 single molecular features after filtering. PLS-DA was applied to distinguish different processing stage (Supplemental Fig. S1a, b). The key compounds with variable importance in projection (VIP) > 1 in SIMCA P were screened out. The importance of the ten key substances was evaluated by factoring in information about corresponding authentic standards, the Human Metabolome Database, and published literature (Supplemental Table S1).

The result is shown in Fig. 1a, b. There were a total of ten substances, which were assigned to two groups. One group (Fig. 1a) of compounds increased during enzymatic reaction, including GA, oolongtheanin, (*2'E,4'Z,8E*)-colneleic acid, and theaflavin-3,3′-gallate. Theaflavins and oolongtheanin were products of oxidation of catechins during enzymatic reactions.

Theaflavins have an effect on the astringency, brightness, color, and briskness of the black tea, and also have some health functions, such as anti-cancer activity[17], improvement of memory impairment and depression-like behavior[18]. The content of theaflavin-3,3′-gallate decreased in ST-OB stage, which may be caused by the oxidation and degradation of theaflavin to thearubigins[19]. Oolongtheanin was the characteristic dimer detected in oolong tea, and it was expected to have varieties of bioactivities[20]. Treatment of EGCG with $CuCl_2$ produced its related polymer oolongtheanin-3-*O*-gallate[21]. The content of

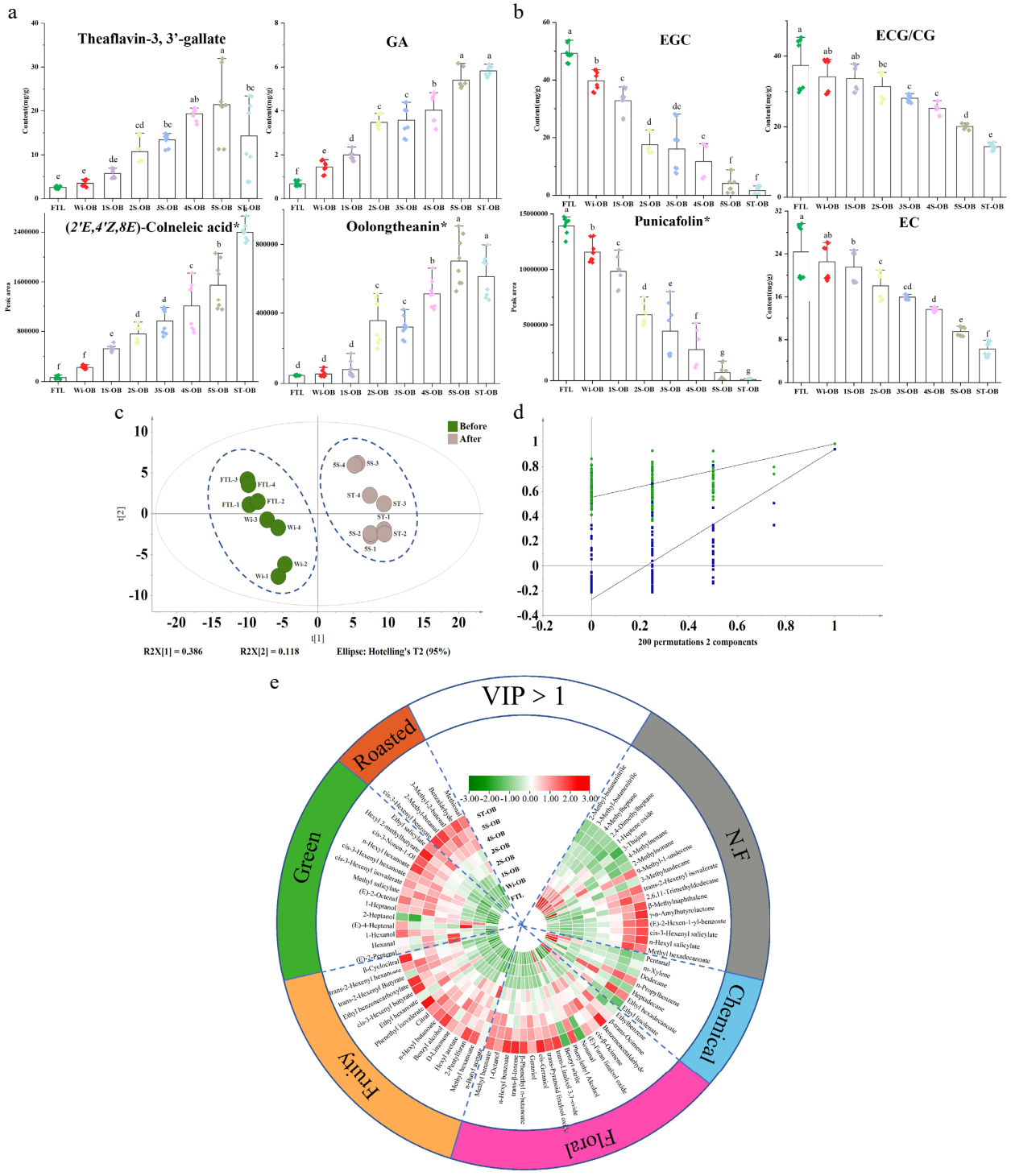

Fig. 1 Metabolite (compounds with variable importance in projection > 1) variations during enzyme reaction stage analyzed by LC/MS and GC/MS. (a) These non-volatile metabolites accumulate gradually in the enzymatic reaction stage. (b) These non-volatile metabolites decrease gradually in the enzymatic reaction stage. (c) The score scatter plots of PLS-DA of volatile metabolites. (d) Validation of the PLS-DA model. (e) Heatmap of differential volatile substances during enzyme reaction stage. VIP: variable importance projection, GA: gallic acid, EGC:(–)-epigallocatachin, ECG: (–)-epicatechin gallate, CG: (–)-catechin gallate, EC: (–)-epicatechin. Different lower case letters following the number indicate significant differences during the processing ($p < 0.05$). * Represents compounds that have not been validated by available standards.

GA showed a marked increase during the enzyme reaction. GA is a precursor of catechin and has strong antifungal activity against tea plant disease[22].

The other group showed a decrease during the enzyme reaction stage, including EGC, ECG/CG, EC, and punicafolin. The decrease in EGC, ECG/CG, and EC were due to the oxidative condensation of catechins into theaflavins and thearubigins. In the enzymatic reaction stage, catechins in tea leaves were oxidized and condensed under the action of enzymes to form theaflavins and oolongtheanin. As a result, the catechin content was greatly reduced during processing, which leads to an improvement in the taste quality of the tea leaves.

Alteration of volatile compounds during the endogenous enzyme reaction stage

Comparative analysis of the initial stage (FTL, Wi-OB) and the end stage (5S-OB, ST-OB) of the enzyme stage reaction showed that the two types of tea samples could be well distinguished (Fig. 1c). The vector value from 200 permutations suggested that this PLS–DA model was not outfitted (Fig. 1d). Subsequently, 76 volatile substances of variable importance projection (VIP) > 1 were filtered out and classified by aroma type to perform a heat-map analysis (Fig. 1e). It can be seen that the aroma was characterized by roasted, green, floral, and fruity notes, which increased with the enzyme reaction stage, and the abundance of some chemical notes compounds and unknown aroma compounds decreased. Therefore, the aroma quality of Oriental Beauty was improved.

During the enzyme reaction stage, the abundance of geraniol increased the most (103 fold), followed by 3-methyl-2-butenal (34 fold). Geraniol, an acyclic isoprenoid monoterpene with sweet rose notes, was shown to possess various pharmacological functions, including antioxidant, anti-inflammatory, and antitumor activities[23,24]. In industry, geraniol and nerol were obtained by selective hydrogenation of citral. Notably, the abundance of citral increased 25 fold during the enzymatic reaction stage. 3-Methyl-2-butenal is a natural product with almond and mild-buttery notes. In conclusion, the content of aromatic compounds of green, roasted, fruity, and floral showed an increasing trend in the enzymatic reaction stage, especially citral with strong lemon notes and geraniol with rose-like notes. The changes of these volatile compounds laid a formation of aroma quality of Oriental Beauty.

Nonenzymatic reactions stage

Alteration of non-volatile constituents during the nonenzymatic reaction stage

The nonenzymatic reaction stage is mainly the fixing process, which uses high temperature to stop the enzyme activity and fermentation, fix the quality of tea, and facilitate storage. Untargeted analysis (Supplemental Fig. S1c, d) results show that the abundance of EGCG, punicafolin, and EGC increased in the process of FX-OB (Fig. 2a), and these compounds showed no significant difference before and after non-enzymatic reaction. The content of theaflavin, theaflavin-3-gallate, and theaflavin-3, 3′-gallate significantly increase during the nonenzymatic reaction, and these compounds increase after FX-OB was perhaps due to polyphenol oxidase, which was also active and oxidized to form theaflavins[25]. The abundance of quinic acid and oolongtheanin decreased significantly during the nonenzymatic reaction stage. In past research, the abundance of quinic acid has been correlated with the grade and quality of tea[26,27].

Alteration of volatile constituents during the nonenzymatic reaction stage

High temperature also removed inferior odor, creating caramel and floral notes. During the nonenzymatic reaction stage, the total volatile-compound content was reduced from 564.52 μg/L to 274.74 μg/L. Application of PLS-DA was used to differentiate metabolite content differences between tea samples of different processing (Fig. 2c, d). The key compounds with VIP > 1 were screened out. Fifty-five different compounds were screened out, their contents were made into heat maps, and cluster analysis was conducted (Fig. 2e). The differentiated compounds between the different processes were divided into two groups. The content of compounds in group-a increased after fixing, while that of group-b decreased. Pentanal, 3-methylfuran, 3-octen-2-one, *cis*-2-penten-1-ol, and hotrienol were the top five metabolites contributing to the difference. 3-Methylfuran and 3-octen-2-one increased by 5–6 fold after fixing, and it had a roasted odor. This was mainly because the Maillard reaction occurs at high temperature to produce heterocyclic compounds, such as pyrrole and furan[28]. The content of *cis*-2-penten-1-ol and pentanal increased by 4.44 and 7.05 fold, respectively. The abundance of hotrienol increased by 3.89 fold after fixing. Dehydration of the 8-hydroxy linalool isomer afforded 3,7-dimethylocta-1,7-dien-3,6-diol. The allylic rearrangement and dehydration of this diol yielded hotrienol[29]. In this experiment, linalool content was reduced by 0.32 fold after fixing.

Dynamic changes of the main metabolites during processing

Using targeted analysis with LC/MS, we performed a quantitative test for flavor metabolites, including GC, EGC, C, EC, EGCG, GCG, ECG, GA, and caffeine (Table 1). The results indicated that the content of catechins significantly decreased ($p < 0.05$) before ST-OB and did not significantly change after FX-OB. The procedure before ST-OB was withering, shaking and rocking, and stacking. The decrease in catechin content during withering was caused by the change in flavonoid transcription induced by tea dehydration[30]. At the shaking and rocking stage, catechins were catalyzed by PPO-POD, resulting in theaflavins, theasinensins, thearubigins, and theabrownines[31]. Catechins also had some biological functions, such as anti-fungal and anti-insect activities, and induced plant defense[32,33]. Caffeine content fluctuated little while the GA content increased significantly during the whole process. Methyl gallate can be produced by GA, except for galloylated catechins[22]. The accumulation of GA content may be due to the lower conversion rate than the synthesis rate. GA add to the astringency of red wine and had antioxidant activity[34]. The flavonol *O*-glycosides of myricetin, quercetin and kaempferol were perceived to be astringent at very low levels[35]; the dynamic changes in the contents of these compounds are shown in Table 1. There were compounds with different trends; the content of vitexin-2-*O*-rhamnoside, luteolin, and kaempferol increased with the processing. Meanwhile, the content of quercetin-3-*O*-rutinoside, quercetin-3-*O*-galactoside, cynaroside, and quercetin 3-*O*-rhamnoside decreased with the processing. These metabolites were the main substances of bitterness and astringent taste in tea, and the reduction of these metabolites was beneficial to the improvement of tea quality.

Three main trends in metabolites (Fig. 3) were observed: (1) content decreases during processing (catechins, quercetin-3-*O*-rutinoside, and vitexin); (2) during the process, the content

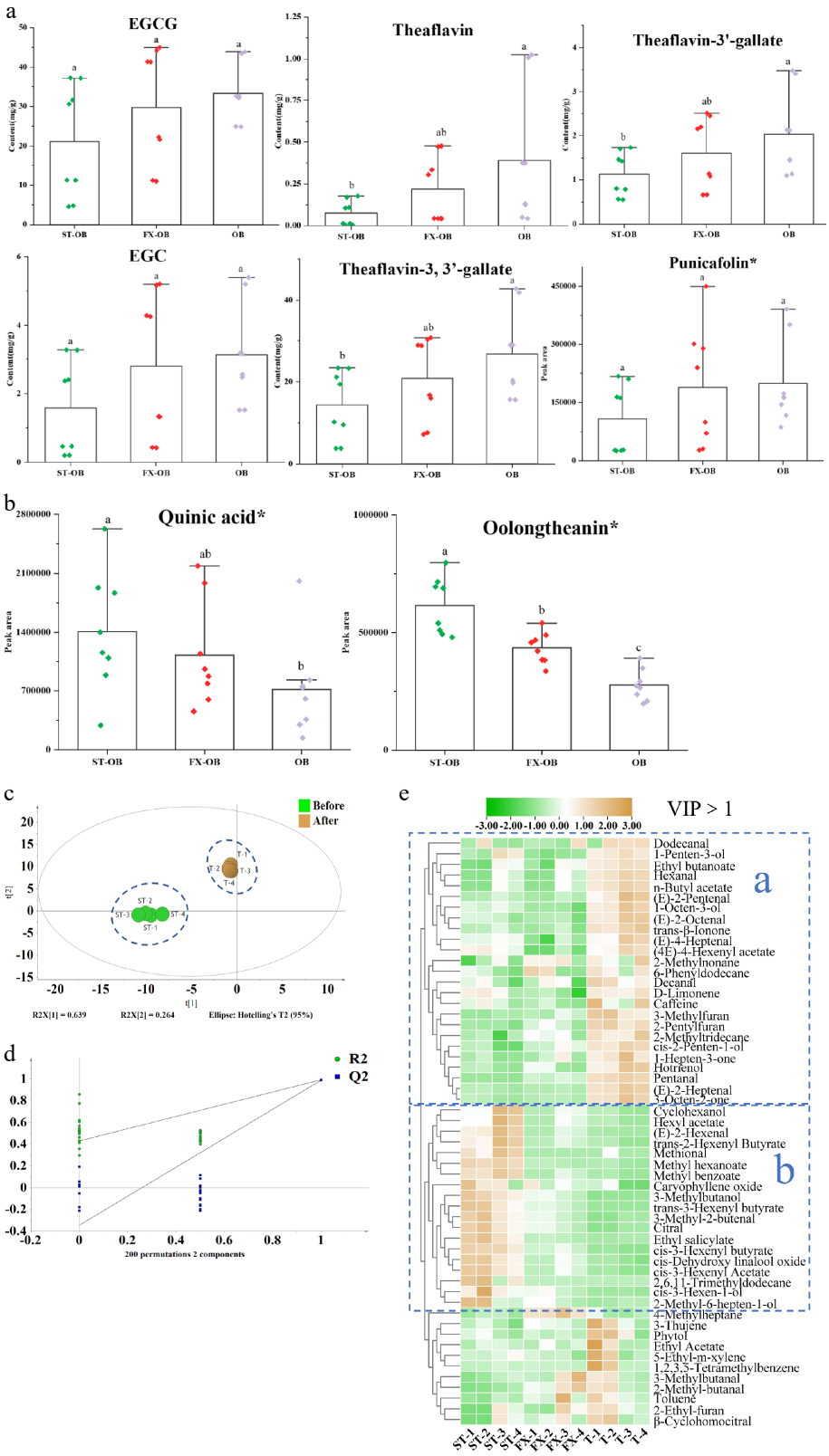

Fig. 2 Metabolite (compounds with variable importance in projection > 1) variations during nonenzymatic reaction stage analyzed by LC/MS and GC/MS. (a) These non-volatile metabolites accumulate gradually in the nonenzymatic reaction stage. (b) These non-volatile metabolites decrease gradually in the nonenzymatic reaction stage. (c) The score scatter plots of PLS-DA of volatile metabolites. (d) Validation of the PLS-DA model. (e) Heatmap of differential volatile substances during nonenzymatic reaction stage. VIP: variable importance projection, EGCG: (–)-epigallocatechin gallate, EGC: (–)-epigallocatechin. Different lower case letters following the number indicate significant differences during the processing ($p < 0.05$). * Represents compounds that have not been validated by available standards.

Table 1. Dynamic variation of major taste compounds.

Compounds (mg/g)	FTL	Wi-OB	1S-OB	2S-OB	3S-OB	4S-OB	5S-OB	ST-OB	FX-OB	OB
GC	8.730 ± 1.539^{a}	6.874 ± 0.341^{b}	6.394 ± 0.386^{b}	4.147 ± 0.363^{c}	3.829 ± 1.028^{c}	2.941 ± 0.754^{d}	1.445 ± 0.677^{e}	0.670 ± 0.514^{f}	0.927 ± 0.453^{ef}	1.126 ± 0.341^{ef}
EGC	49.248 ± 2.943^{a}	39.766 ± 3.377^{b}	32.811 ± 4.212^{c}	17.511 ± 3.132^{d}	16.029 ± 8.675^{d}	11.673 ± 6.057^{e}	4.117 ± 3.220^{f}	1.577 ± 1.380^{f}	2.800 ± 2.116^{f}	3.122 ± 1.482^{f}
C	34.730 ± 12.072^{a}	25.859 ± 4.542^{b}	25.784 ± 4.899^{b}	21.133 ± 3.301^{c}	20.225 ± 1.656^{c}	17.996 ± 1.792^{ce}	14.164 ± 0.725^{ef}	10.011 ± 1.040^{f}	11.188 ± 2.066^{f}	10.487 ± 1.299^{f}
EC	24.382 ± 5.110^{a}	22.508 ± 3.291^{ab}	21.541 ± 2.957^{b}	18.028 ± 2.620^{c}	15.915 ± 0.365^{cd}	13.624 ± 0.336^{d}	9.521 ± 0.851^{e}	6.231 ± 1.347^{f}	7.345 ± 0.583^{ef}	6.689 ± 0.306^{f}
EGCG	250.087 ± 3.465^{a}	230.995 ± 7.142^{ab}	214..127 ± 12.789^{b}	166.138 ± 12.478^{c}	143.142 ± 37.211^{d}	113.768 ± 33.531^{e}	55.881 ± 29.028^{f}	21.082 ± 14.399^{g}	29.749 ± 14.769^{g}	33.393 ± 7.166^{g}
GCG	0.901 ± 0.082^{a}	0.928 ± 0.082^{a}	0.858 ± 0.096^{a}	0.658 ± 0.059^{b}	0.581 ± 0.160^{b}	0.407 ± 0.131^{c}	0.211 ± 0.084^{d}	0.081 ± 0.037^{e}	0.135 ± 0.055^{de}	0.214 ± 0.0254^{d}
ECG	37.392 ± 7.412^{a}	34.177 ± 4.864^{b}	33.653 ± 3.649^{b}	31.376 ± 3.937^{bc}	28.058 ± 1.001^{cd}	25.238 ± 1.621^{d}	20.064 ± 0.703^{e}	14.346 ± 0.896^{f}	14.381 ± 1.357^{f}	13.458 ± 0.861^{f}
Total catechins	405.470 ± 30.134^{a}	361.106 ± 15.296^{b}	335.169 ± 13.269^{b}	258.990 ± 17.723^{c}	227.780 ± 44.836^{e}	185.647 ± 37.863^{f}	105.401 ± 33.025^{e}	53.940 ± 17.880^{g}	66.526 ± 14.339^{g}	68.489 ± 10.870^{g}
GA	0.680 ± 0.108^{f}	1.440 ± 0.277^{e}	1.988 ± 0.254^{e}	3.475 ± 0.271^{d}	3.584 ± 0.707^{d}	4.027 ± .0.730^{d}	5.398 ± 0.437^{c}	5.821 ± 0.218^{c}	6.755 ± 0.891^{b}	8.187 ± 1.247^{a}
Caffeine	68.601 ± 4.509^{ac}	71.635 ± 6.686^{abc}	71.447 ± 4.596^{abc}	73.500 ± 5.649^{c}	71.140 ± 4.084^{abc}	71.147 ± 5.983^{abc}	70.789 ± 5.863^{abc}	73.469 ± 6.177^{ce}	74.056 ± 4.884^{bc}	67.746 ± 2.673^{a}
Vitexin-2-*O*-rhamnoside	0.205 ± 0.077^{bc}	0.338 ± 0.205^{b}	0.347 ± 0.210^{b}	0.354 ± 0.205^{b}	0.336 ± 0.198^{b}	0.320 ± 0.190^{b}	0.317 ± 0.196^{b}	0.325 ± 0.212^{b}	0.360 ± 0.320^{b}	0.418 ± 0.247^{ab}
Quercetin-3-*O*-rutinoside	1.873 ± 0.339^{ab}	1.776 ± 0.427^{b}	1.746 ± 0.418^{abc}	1.783 ± 0.426^{ab}	1.688 ± 0.509^{abc}	1.524 ± 0.419^{abc}	1.508 ± 0.349^{abc}	1.366 ± 0.246^{c}	1.417 ± 0.367^{bc}	1.362 ± 0.345^{c}
Quercetin-3-*O*-galactoside	0.853 ± 0.684^{a}	0.808 ± 0.659^{a}	0.798 ± 0.656^{a}	0.808 ± 0.661^{a}	0.784 ± 0.636^{a}	0.743 ± 0.582^{a}	0.722 ± 0.514^{a}	0.709 ± 0.485^{a}	0.735 ± 0.508^{a}	0.696 ± 0.472^{a}
Cynaroside	0.046 ± 0.007^{acd}	0.052 ± 0.011^{cd}	0.050 ± 0.013^{acd}	0.052 ± 0.010^{acd}	0.048 ± 0.012^{acde}	0.044 ± 0.009^{acd}	0.040 ± 0.009^{abe}	0.036 ± 0.007^{b}	0.034 ± 0.006^{b}	0.034 ± 0.006^{b}
Quercetin 3-*O*-rhamnoside	0.022 ± 0.010^{acd}	0.022 ± 0.006^{acd}	0.021 ± 0.005^{cde}	0.025 ± 0.008^{d}	0.019 ± 0.004^{abce}	0.019 ± 0.003^{abce}	0.016 ± 0.002^{e}	0.016 ± 0.001^{be}	0.015 ± 0.003^{be}	0.015 ± 0.002^{be}
Luteolin	0.001 ± 0.000^{ac}	0.001 ± 0.000^{ac}	0.001 ± 0.001^{ac}	0.002 ± 0.001^{cde}	0.002 ± 0.001^{de}	0.002 ± 0.001^{e}	0.004 ± 0.001^{f}	0.006 ± 0.001^{h}	0.005 ± 0.001^{g}	0.009 ± 0.001^{b}
Quercetin	0.053 ± 0.029^{a}	0.047 ± 0.027^{a}	0.052 ± 0.032^{a}	0.051 ± 0.031^{a}	0.050 ± 0.029^{a}	0.048 ± 0.027^{a}	0.044 ± 0.024^{a}	0.045 ± 0.025^{a}	0.069 ± 0.048^{a}	0.068 ± 0.041^{a}
Kaempferol	0.046 ± 0.010^{a}	0.042 ± 0.014^{a}	0.046 ± 0.018^{a}	0.047 ± 0.019^{a}	0.045 ± 0.016^{a}	0.43 ± .015^{a}	0.045 ± 0.016^{a}	0.050 ± 0.021^{a}	0.066 ± 0.035^{a}	0.077 ± 0.040^{b}
Vitexin	0.037 ± 0.008^{a}	0.043 ± 0.006^{bcd}	0.046 ± 0.006^{cd}	0.046 ± 0.008^{d}	0.043 ± 0.004^{bcd}	0.041 ± 0.006^{abcd}	0.040 ± 0.006^{ab}	0.039 ± 0.006^{ab}	0.041 ± 0.005^{abcd}	0.046 ± 0.004^{bcd}

Different lowercase letters following the number indicate significant differences during the processing ($p < 0.05$). The results were presented in the form of mean values followed by the standard deviation.

initially decreases and subsequently increases (nerolidol, α-farnesene, and indole); and (3) an initial increase and then decrease in content during processing (methyl salicylate, β-trans-ocimene, benzaldehyde, benzyl alcohol, linalool, and (*E*)-2-hexenal). The total catechin content decreased by 0.15–0.18 fold, quercetin content decreased by 0.5–0.82 fold, vitexin increased by 0.8–1.6 fold.

The oxidation of tea flavanols by catechol oxidase remarkably affects the degradation of carotenoids[28]. The volatile carotenoid derivative β-trans-ocimene increased by 1.12–2.47 fold during the whole process. The content of benzyl alcohol was 3.5–53.7 fold that as was in FTL, and the content of benzaldehyde was 2.9–5 fold more in OB than in FTL. This was mainly caused by the protein degradation in the fermentation process that formed phenylalanine. Phenylalanine underwent further degradation to benzaldehyde, phenylacetaldehyde, benzyl alcohol, and phenylethyl alcohol[36]. Benzaldehyde and benzyl alcohol respectively contribute floral and almond notes in black tea[37]. The indole content decreased by 0.05–0.17 fold, and (*E*)-nerolidol decreased by 0.49–0.87 fold. Most noteworthy was that the characteristic volatiles of oolong tea are thought to be indole, jasmine lactone, and (*E*)-nerolidol[8], which had a low relative content in this experiment. This may be because the tea leaves of Oriental Beauty had high tenderness and easy fermentation, resulting in formation of monoterpene compounds such as linalool and geraniol[16].

Volatile secondary metabolites of tea were divided into three major groups according to their biosynthetic source: terpenoids (monoterpenes, homoterpenes, and sesquiterpenes), phenylpropanoid/benzenoid (indole), and fatty acid derivatives (green leaf volatiles)[38]. The dynamic changes of these compounds during processing are shown in Fig. 3. The total aroma relative content reached the highest value in 4S-OB–ST-OB, which was 530–584 μg/L. The abundance of all compounds decreased significantly during the processing steps of FX-OB. It may be that the high temperatures (200 °C) during the fixation process inactivated the enzymatic activity, and thus evaporated/degraded the volatile compounds. Before the FX-OB procedure, the variation trends of green leaf volatiles (GLVs) and monoterpenes were the highest in 2S-OB and 3S-OB, and then decreased. GLVs were mainly C6 aldehydes and alcohols in tea, and they had major inferior components of the flavor of tea (green grassy notes)[39]. Tea leaves could release a large amount of GLVs in a short time when stimulated by external stress, which was consistent with our results. Before ST-OB, monoterpene content increased by 2.93 fold, sesquiterpene content basically remained unchanged, and homoterpene increased by 5.61 fold.

Differences of main metabolites between JX and TGY varieties during processing

Tea varieties have a greater impact on tea quality, such as the composition, content and enzyme activity of biochemical components, which depend on the variety. The excellent characteristics of tea varieties can be further exerted under appropriate processing technology[40]. The changes of the main metabolites in JX and TGY during processing were compared and analyzed, and the results are shown in Fig. 4. The main nonvolatile compounds of the two varieties showed the same trend of change (except for caffeine and quercetin-3-*O*-galactoside) (Fig. 4a, b). The total catechin content of JX was lower than that of TGY, but the content of theaflavins in JX was higher, which may be the reason why the Oriental beauty tea processed by TGY has a strong taste. Comparison of the content of terpenoids, phenylpropanoid/benzenoid, and fatty acid derivatives

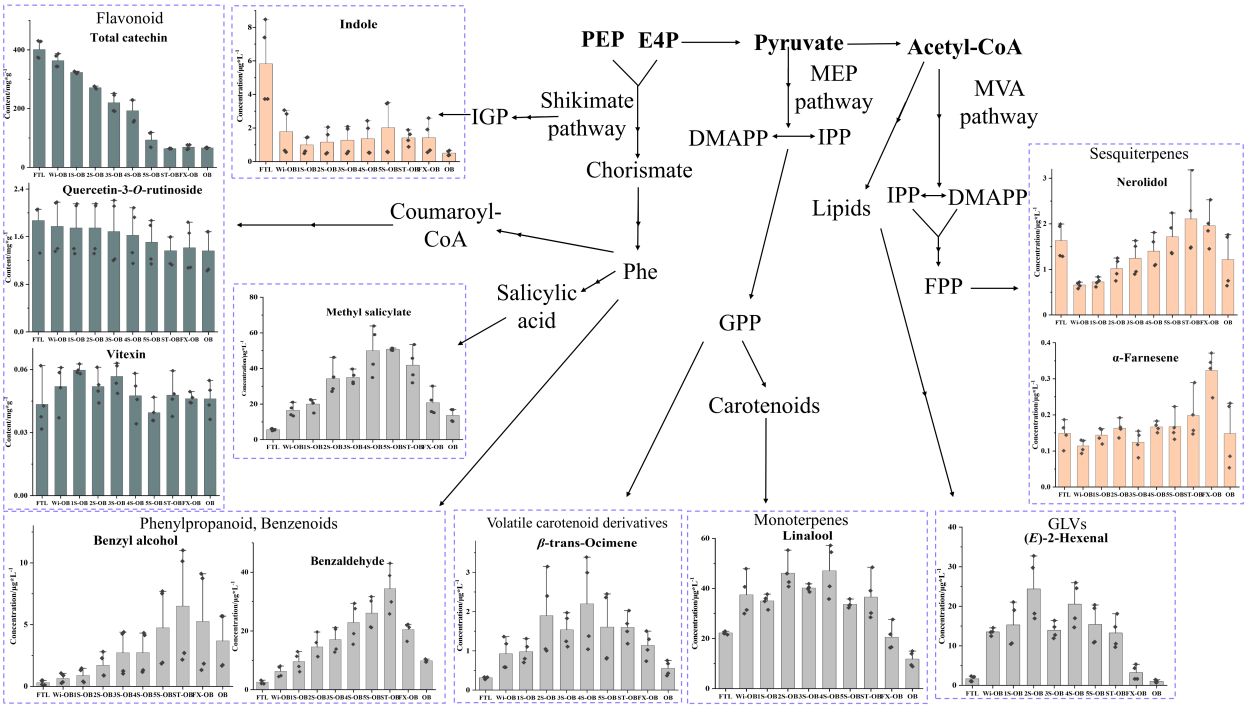

Fig. 3 Dynamic variation of major metabolites. DMAPP: dimethylallyl pyrophosphate, PEP: phosphoenolpyuvate, E4P: erythrose 4-phosphate, FPP: farnesyl pyrophosphate, GPP: geranyl pyrophosphate, IPP: isopentenyl pyrophosphate, Phe: phenylalanine, IGP: indole-3-glycerol phosphate, GLVs: green leaf volatiles, the methylerythritol phosphate (MEP) pathway, the mevalonic (MVA) acid pathway, lipoxgenase (LOX) pathway.

during processing of two varieties. Figure 4c and d shows that the indole and sesquiterpenes contents of the two varieties have the same trend, and the GLVs and monoterpenes have relatively large changes in JX. In TGY varieties, the content of homoterpenes increased sharply in 3S-OB and decreased after ST-OB, and this phenomenon did not appear in JX varieties. This may be due to differences between varieties.

CONCLUSIONS

In this study, untargeted metabolomics based on LC/MS and GC/MS was used to comprehensively compare the characteristics of taste and aroma metabolites in Oriental Beauty during the whole production process (Fig. 5). During the enzyme reaction stage, the content of GA significantly increased, and catechin was oxidized and degraded into oolongtheanins and theaflavins, leading to an increase in GA content. The total relative content of aroma increased and reached the maximum value at the ST-OB stage. Different from other oolong teas, monoterpenes such as linalool and geraniol were dominantly synthesized through the MEP pathway during the processing of Oriental Beauty. During the nonenzymatic stage, the content of theaflavin, theaflavin-3-gallate, and theaflavin-3,3′-gallate significantly increased. The increase after FX-OB was perhaps because polyphenol oxidase was also active and oxidized to form theaflavins. The total content of aroma decreased after FX-OB, and linalool heat treatment converted to hotrienol. These finding will provide an important theoretical basis for the quality changes in the processing of Oriental beauty.

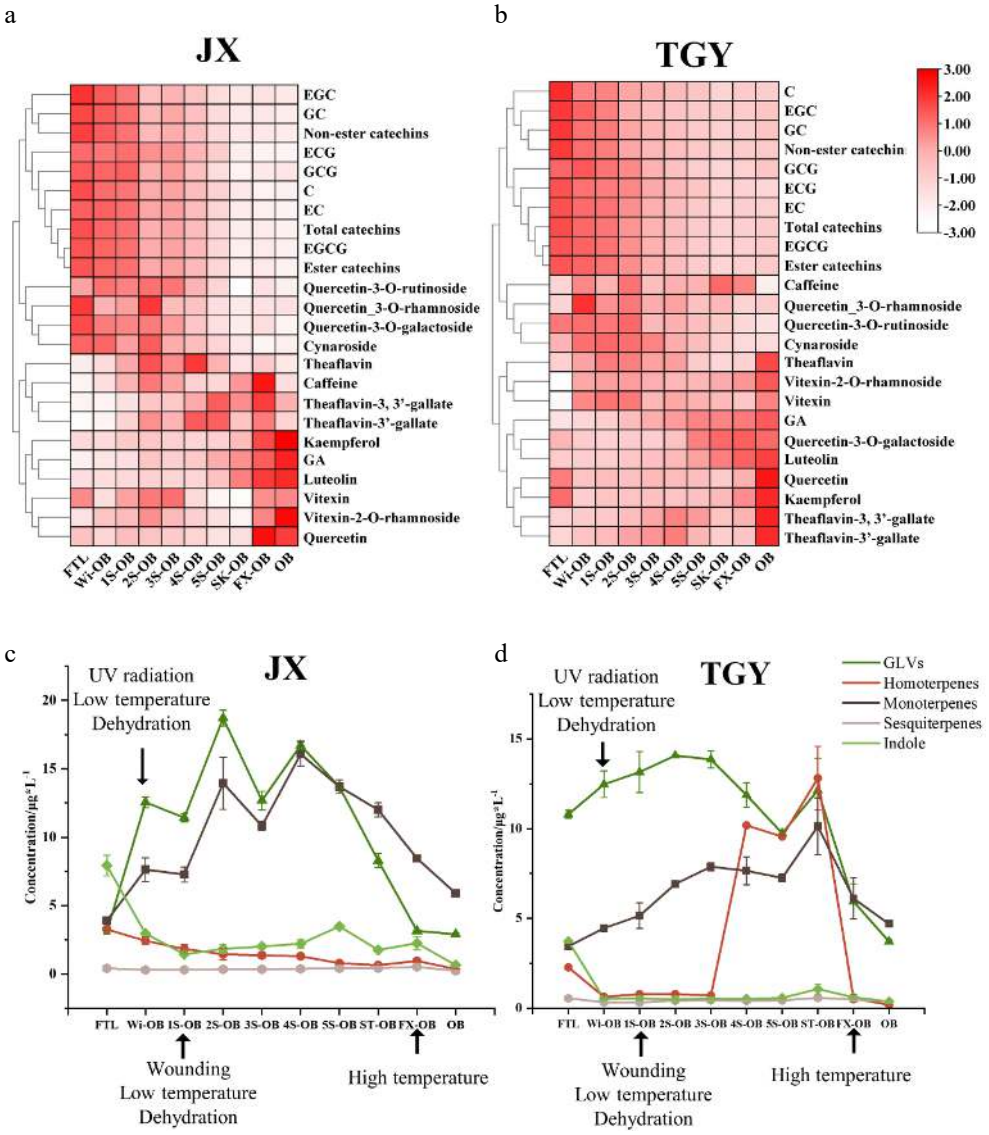

Fig. 4 Dynamic variation of major compounds between JX and TGY during processing. (a) Dynamic changes in the content of major non-volatile metabolites in JX varieties. (b) Dynamic changes in the content of major non-volatile metabolites in TGY varieties. (c) Dynamic changes in the content of major volatile metabolites in JX varieties. (d) Dynamic changes in the content of major volatile metabolites in TGY varieties. GLVs: green leaf volatiles (hexanal, (*E*)-2-hexenal, *cis*-3-hexen-1-ol,1-hexanol, *cis*-3-hexenyl acetate); homoterpenes ((3*E*)-4,8-dimethyl-1,3,7-nonatriene); monoterpenes (*β*-myecene, D-limonene, *β*-trans-ocimene, *cis*-*β*-ocimene, linalool, hotrienol, *trans*-linalool 3,7-oxide, *β*-cyclocitral, *cis*-geraniol, and citral); sesquiterpenes (*α*-cubebene, *β*-bourbenene, *β*-cubebene, caryophyllene, *α*-farnesene, *δ*-Cadinene, nerolidol, cubenol); JX: *Jinxuan*, TGY: *Tieguanyin*.

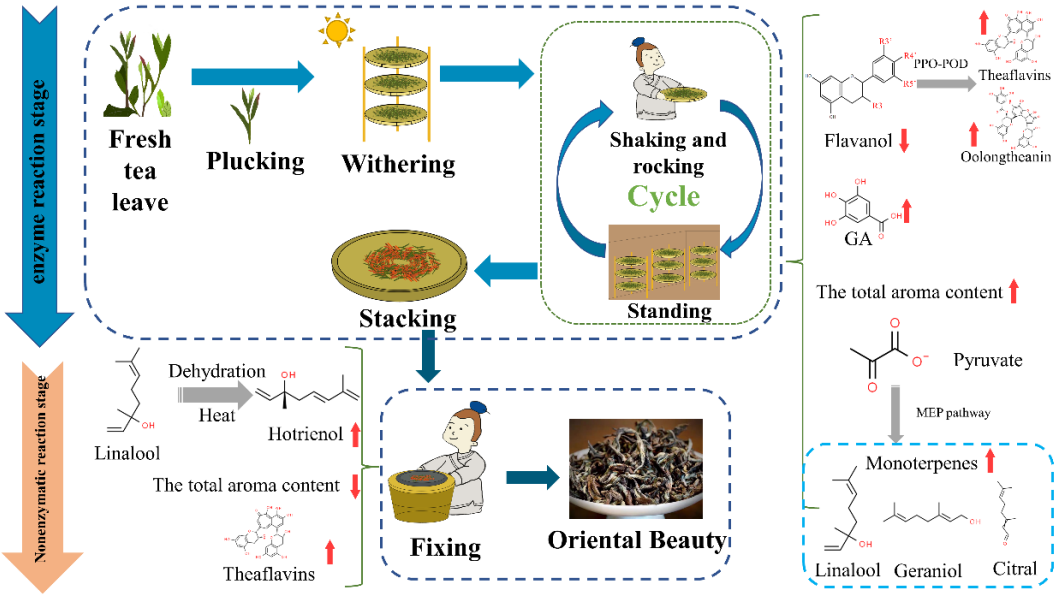

Fig. 5 The changes of taste and aroma metabolites in Oriental Beauty during the whole production process. PPO: polyphenol oxidase, POD: peroxidase, GA: gallic acid, MEP pathway: the methylerythritol phosphate pathway.

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Conflict of interest

The authors declare that they have no conflict of interest.

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