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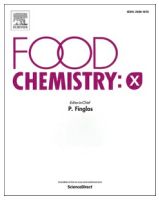

Study on flavor quality formation in green and yellow tea processing by means of UPLC-MS approach

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ABSTRACT

Yellow tea (YT) has an additional process of yellowing before or after rolling than green tea (GT), making YT sweeter. We analyzed the variations of composition and taste throughout the withering, fixing and rolling steps using UPLC-MS/MS and sensory evaluation, and investigated the influence of various yellowing times on flavor profile of YT. 532 non-volatile metabolites were identified. Withering and fixing were the important processes to form the taste quality of GT. Withering, fixing and yellowing were important processes to form flavor profile of YT. Withering mainly regulated bitterness and astringency, and fixing mainly regulated bitterness, astringency and sweetness of YT and GT. Yellowing mainly regulated sweetness of YT. Trans-4-hydroxy-L-proline and glutathione reduced form as the key characteristic components of YT, increased significantly during yellowing mainly through Arginine and proline metabolism and ABC transporters. The paper offers a systematic insight into intrinsic mechanisms of flavor formation in YT and GT.

1. Introduction

Tea, the most favorable soft drinks worldwide (Tang et al., 2019), which is responsible for numerous health activities, for example anti-diabetic, anticancer, antioxidation, antiinflammation, cardiovascular-protective effects (Gan, Li, Sui, & Corke, 2018; Guo, Sun, Yu, & Qi, 2017; Nam et al., 2018). In general, tea is categorized into green, yellow, white, oolong, dark and black tea, in accordance with the degree of fermentation (Leung et al., 2016; Liu et al., 2013; Zhu et al., 2015). The biotransformation of chemical constituents in leaves after various processing resulting in teas with distinct flavors and bioactivities. For example, total catechin content in the six tea types is different, which resulted in different capacities for antioxidant activity (Xie et al., 2021). The processing method is considered to be a significant factor that contributes to the variety of taste and aroma of tea (Feng et al., 2019). GT is made by withering, fixing, rolling and drying. The baking process remarkably increased the content of aromatic organic volatile compounds (AOVCs), especially the aromatic pyrrole substances (Fu et al., 2020). 1,2-Dihydro-1,1,6-trimethyl-naphthalene is a major contributor to the formation of chestnut-like aroma during the drying process (Wang et al., 2022). The key taste characteristics of GT are bitterness and astringency (Deng et al., 2022). Glycosylation, pyrolysis, and oxidative polymerization are critical reactions during roasting of GT, leading to significantly increasing in organic acids, catechins and their derivatives, and flavonoid glycosides, and significantly decreasing in some amino acids and their derivatives (Liu et al., 2023). The processing of YT includes withering, fixing, rolling, yellowing and drying. The content of some aromatic AOVCs including 1-octen-3-ol, phenylacetaldehyde, β-ketone heptanal and 1-octanol change significantly in the yellowing process, so as to increasing the mushroom and sweet aromas and decreasing the floral, grassy, and fruity aromas (Wei et al., 2022). Old fire roasting is an essential step to form strong nutty, roasted, and woody

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odors of YT (Guo, Ho, Schwab, & Wan, 2021). YT is milder as well as sweeter than GT, which is intimately linked to a decrease trend in astringent-bitter substances such as flavones, caffeine, and flavanol glycosides, and an increase trend in sweet amino acids and gallic acid (Fan et al., 2022). YT has an additional yellowing process than GT, resulting in a difference in flavor and aroma attributes between green and yellow teas. Therefore, the comparison of the flavor qualities of green and yellow teas can facilitate the elaboration of the effect of processing on the flavor qualities of green and yellow teas as well as the reasons for the differences in flavor qualities between YT and GT.

The chemical constituents undergo significant changes throughout tea processing, which can make a crucial impact on the quality of the tea, especially its aroma and flavor attributes. However, what chemicals have changed and how the taste qualities of GT and YT are formed during processing is not clear yet. It is essential to have an understanding of the dynamical transformations of the composition and taste quality of YT and GT. Therefore, we analyzed the changes in the material composition and the influence on the taste quality of GT and YT leaves during different processes by extensively targeted metabolomics techniques and sensory review. This paper initially demonstrated the mechanism of taste quality formation throughout the processing of GT and YT.

2. Chemical compositions and methods

2.1. Tea samples

The large-leaf *Camellia sinensis* var. Yinghong NO.9 tea samples with one bud and two leaves were picked as raw materials on Oct 9, 2019 to make into GT and YT respectively. The processing steps of both GT and YT referred to previous studies (Wen et al., 2023). The samples from the pre-processing of YT (fresh leaves, spreading, fixing and twisting) were the same as those from GT. Samples were taken and dried at the end of each process (picking, withering, fixing and rolling). 12 samples were taken from 4 different groups (Ya: Fresh tea leaves, Yb: After withering, Yc: After fixing, Yd: After rolling). Tea samples were collected at 4, 8, 16, 24 and 48 h from the yellowing procedure, they were labelled as Ye, Yf, Yg, Yh and Yi, respectively. Each group consists of three replicates.

2.2. Sensory evaluation

Informed consent was obtained from all tea tasters involved in the review before the sensory review began. In according with the national standard tea sensory evaluation procedure (GB/T 23776–2018), an evaluation team consisting of 10 tea experts (half men and half women) evaluated qualities of the finished GT and finished YT after 48 h of yellowing.

2.3. Sample preparation and extraction

A hybrid mill (MM 400, Retsch) with zirconia beads was used to crush the freeze-dried leaf for 1.5 min at 30 Hz. An overnight extraction at 4 °C in 0.6 mL 70% aqueous methanol was carried out with 100 mg milligrams of powder. Centrifugation at 10,000*g* for 10 min was followed by absorbing (CNWBOND Carbon-GCB SPE Cartridge, 250 mg, 3 mL; ANPEL, Shanghai, China) and filtrated (SCAA-104, 0.22 μm pore size; ANPEL, Shanghai, China) the extracts, followed by UPLC-MS/MS method.

2.4. Conditions of UPLC

UPLC- MS system (UPLC, Shim-pack UFLC SHIMADZU CBM30A system, MS, Applied Biosystems 4500 Q TRAP) were used to analyze. Condition of UPLC was set as follow, UPLC: column, Waters ACQUITY UPLC HSS T3 C18 (1.8 μm, 2.1 mm * 100 mm); Solvent A: purified water with 0.04% acetic acid; solvent B: acetonitrile plus 0.04% acetic acid consist of the mobile phase. We used a gradient program for sample measurements, which used the starting conditions of 5% B, 95% A. Reference is made to previously published papers regarding gradient elution (Sun et al., 2023).

2.5. ESI-Q TRAP-MS/MS

LIT and triple quadrupole (QQQ) scans used in this experiment were API 4500 Q TRAP UPLC/MS/MS triple quadrupole linear ion trap mass spectrometers equipped with an ESI Turbo IonSpray interface, both for positive and negative ion modes and regulated by Analyst 1.6.3 software (AB Sciex). The operating parameters of the ESI source were in accordance with published literature (Sun et al., 2023). Instrument tuning and mass calibration were respectively carried out using 10 and 100 μmol/L polypropylene glycol solutions in QQQ and LIT modes. QQQ scans were obtained as MRM experiments with the collision gas (nitrogen) set at 5 psi.

DP and CE were conducted on individual MRM transitions and further optimized for DP and CE. A specific set of MRM transitions was monitored during each time period based on the metabolites eluting within that time range. All raw MS data were analyzed using Analyst (v1.6.3) and MultiqQuant.

2.6. Multivariate statistical analysis

Unsupervised principal component analysis (PCA) was conducted to identify within-groups and between-group variations by using the prcomp statistical function in R (http://www.r-project.org/). 3D PCA plots were produced by the scatterplot3d R package (version 0.3.41) (Uwe Ligges, 2003). The hierarchical clustering of metabolites and samples was illustrated by a heatmap with dendrograms. Pearson's correlation coefficients were obtained using the cor function of the R package and displayed as a heatmap.

2.7. Orthogonal projection to latent structures-discriminant analysis

VI*P* values were acquired by the MetaboAnalystR R *package* (Dennis et al., 2003). VIP $\geq$ 1, fold change $\geq$2 and fold change $\leq$0.5 were used as indicators to screen for differentially expressed metabolites between groups.

2.8. KEGG analyses

To further investigate the underlying biological pathways of these dysregulated metabolites, we performed KEGG pathway annotation and enrichment analysis (Kanehisa, Sato, Furumichi, Morishima, & Tanabe, 2019). Metabolite set enrichment analysis (MSEA) was performed by utilizing the pathways with mapped differentially altered metabolites. The P value to indicate significance.

2.9. Statistical analysis

All continuous variables are expressed as the mean ± standard deviation (SD). Statistical analyses were performed using the R software (version 4.0.2, http://www.r-project.org/) and were considered significant at $P < 0.05$.

3. Results

3.1. Taste review of tea samples

Experts consistently believed that YT had a sweet, mellow flavor after 48 h of yellowing, and GT had bitter, astringent and sweet taste (Table S1), consistenting with previously published articles (Wen et al., 2023). The sweetness enhanced and the bitterness and astringency diminished during the yellowing process of YT. The colour of GT liquor

was orange and the colour of YT liquor was bright-yellow due to the breakdown of the oxidation of carotenoids, chlorophyll, and the formation of theaflavins under the high humidity and heat of yellowing (Yu et al., 2019) (Fig. S5).

3.2. Overview of non-volatile metabolites

The PCA of the withering, fixing and rolling process (the same process for GT and YT) and the yellowing process (a specific process for YT) revealed that samples were clustered with groups were clustered while samples between groups were discrete. This indicated that the metabolic profiles captured the variance of different groups and had good stability between biological replicates (Fig. 1A). Although the cumulative variable explainability of the principal component (< 0.63) and the variable explainability of the first principal component (< 0.37) were not high (Fig. S1), we found significant differences between Ya and Yb, Yb and Yc, Yd and Yg, Yd and Yh, Yd and Yi, respectively (Fig. 1A). Moreover, the hierarchical clustering heatmap displayed the similar results (Fig. 1B).

After peak extraction and alignment, 532 metabolic features (Table S2) were identified, including 144 (27.07%) flavonoids, 106 (19.92%) phenolic acids, 68 (19.74%) amino acids and their derivatives, 47 (8.83%) nucleotides and their derivatives, 38 (7.14%) lipids, 37 (6.95%) organic acids, 28 (5.26%), tannins, 17 (3.20%) alkaloids, 7 (1.32%) lignans and coumarins, 1 (0.19%) terpenoid, and 37 (7.33%) others (Fig. 1C). Strikingly, we discovered that the 532 metabolites were detected in each sample, however, the relative content of each metabolite was different in each sample (Table S2).

532 non-volatile metabolites were examined for dynamic changes during processing. The total content of non-volatile metabolites showed an increasing tendency and 11 metabolites (except tannins) changed significantly during processing ($p < 0.05$) (Fig. 1D). Amino acids and their derivatives, lipids and others showed an increasing tendency and increased significantly at the withering, fixing and 4 h of yellowing stages. Phenolic acids, nucleotides and their derivatives increased significantly during fixation and tended to increase during yellowing. Lignans and coumarins increased in rolling step. Flavonoids, terpenoids and organic acids decreased sharply during the withering process, and then increased significantly and reached a maximum peak during the fixing process. The alkaloids show a tendency to decrease and then increase, reaching a maximum peak during the rolling stage, decreasing significantly at the beginning of the yellowing stage and then stabilizing.

3.3. Influence of withering, fixing and rolling on the quality development of GT and YT

To identify the metabolic features of taste quality of GT and YT, we carried out analysis of variance and KEGG enrichment analysis for each

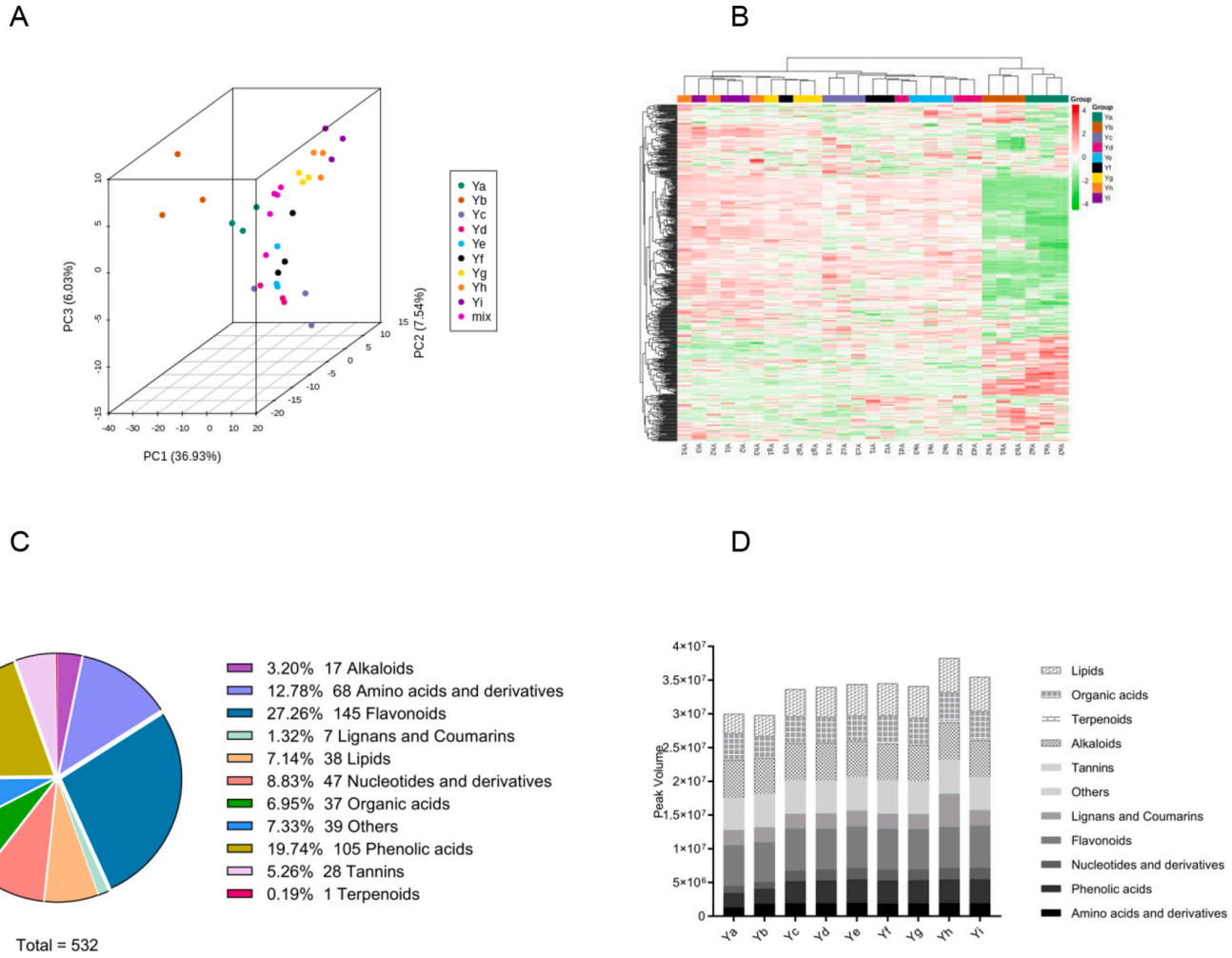

Fig. 1. The overall metabolic profile distribution among different treatment groups in Yinghong NO.9 Tea samples: (A) 3D PCA score plots of sample distribution in all 27 tea samples; (B) Heatmap and dendrogram of components from different treatment groups; (C) Pie chart of the categories of metabolites; (D) Plot of peak volume for the categories of metabolites.
Note: mix refers to mixed samples.

pair comparison. Firstly, we analyze the implications of withering, fixing and rolling on the content of taste compounds so as to reveal the mechanism of taste quality formation in GT. At the same time, these three processes are also the pre-processes of the taste quality formation in YT. Model validation was performed on the OPLS-DA model. The predictive parameters for evaluating the model are R^2X, R^2Y and Q^2, which were close to 1, indicating that the model was stable and usable (Fig. S3A—C).

The OPLS-DA score plot showed that the relative amounts of metabolites differed significantly between Ya and Yb (Fig. S2 A), with 27 up-regulated differentially expressed metabolites and 5 down-regulated differentially expressed metabolites (Fig. 2A). 16 differential metabolites with flavor profiles and 18 metabolites with taste thresholds were identified (Table 1). Of those, L-valine, L-isoleucine, alpha-hydroxyisobutyric acid, phe, *L*-phenylalanine, L-(+) -lysine, adenosine, 2-hydroxybutanoic acid had bitter flavor, with thresholds above 2000 ppm except for ph-phe (370 ppm) and L-(+) -lysine (434 ppm) (Du et al., 2023; Kohl, Behrens, Dunkel, Hofmann, & Meyerhof, 2013). Theaflavin, theaflavin 3′ -gallate, theaflavin 3-gallate, theaflavin 3,3′-digallate all showed a sense of fold convergence, with threshold about 10 ppm. 3′-aenylic acid had sweet flavor, and its threshold was not found. L-glutamine had salty flavor with a large threshold of 7300 ppm. Glutathione reduced form displayed a sense of oral fullness with a large threshold of 950 ppm. γ-Aminobutyric acid presents a sense of oral dryness with a low threshold of 2.1 ppm. Of note, glutathione reduced form was down-regulated differential metabolite, and the others were up-regulated differential metabolites. KEGG enrichment analysis was performed for the 32 differential metabolites mentioned above, and 30 metabolites pathways were significantly enriched ($p < 0.05$), and the top 20 pathways were shown in Fig. 2B. The bitter taste characteristic components are up-regulated mainly through Tropane, piperidine and pyridine alkaloid biosynthesis, Aminoacyl-tRNA biosynthesis, Metabolic pathways, Biosynthesis of secondary metabolites, 2-Oxocarboxylic acid metabolism, Biosynthesis of amino acids, ABC transporters, etc. γ-Aminobutyric acid as astringent characteristic components was up-regulated via Alanine, aspartate and glutamate metabolism, Arginine and proline metabolism, beta-Alanine metabolism, Butanoate metabolism, Nicotinate and nicotinamide metabolism and Metabolic pathways, and the remaining astringent differential metabolites were not significantly enriched in the metabolic pathway.

The relative amounts of metabolites differed significantly between Yb and Yc (Fig. S2B), among which 86 were up-regulated differentially expressed metabolites and 13 were down-regulated differentially expressed metabolites (Fig. 2C). 27 differential metabolites had taste characteristics and 31 differential metabolites had taste threshold, and 4 were taste down-regulated differentially expressed metabolites (Table 1). Of the up-regulated differential metabolites, nicotinamide, riboflavin, thymine, cytosine, phe, herbacetin, adenosine, guanine, guanosine, histamine both cytidine and cytidine displayed bitter flavor. The bitterness threshold for histamine was a minimum of 70 ppm, was significantly enriched to Histidine metabolism, Metabolic pathways, Biosynthesis of secondary metabolites. 3′-Aenylic acid, 2,4-dihydroxy benzoic acid, DL-alanyl-DL-phenylalanine presented sweet flavor, and the threshold of 2, 4-dihydroxy benzoic acid was 231 ppm. It was found that the production of bitter metabolites such as histamine and sweet metabolites such as DL-alanyl-DL-phenylalanine was promoted by the thermal hydrolstysis of the protein during fixing steps. Ferulic acid, 2-furanoic acid, protocatechuic acid, 4-hydroxybenzoic acid and pyrocatechol showed astringency flavor, with the threshold values all below 100 ppm. Ferulic acid had been found to be a possible precursor of vanillin and the main flavor component of vanilla extract (Sharma et al., 2021). Protocatechuic acid, 4-hydroxybenzoic acid and pyrocatechol, etc. are vanilla flavor metabolites (Tripathi, Ramachandra Rao, & Ravishankar, 2002). Cyclic AMP presented with freshly flavored, however its thresholds was above 30,000 ppm. γ-Aminobutyric acid presented a sense of oral dryness with a low threshold of 2.1 ppm, and KEGG analysis significantly enriched for six metabolic pathways contain Alanine, aspartate and glutamate metabolism, Arginine and proline metabolism, beta-Alanine metabolism, Butanoate metabolism, Nicotinate and nicotinamide metabolism and Metabolic pathways. Vanillin had a silky oral coating sensation with a low threshold of 38 ppm, and up-regulated by Biosynthesis of secondary metabolites and Metabolic pathways. Glutathione reduced form showed oral fullness flavor. Among the down-regulated differential metabolites, theaflavin and methyl gallate were astringent with thresholds of 9.024 ppm and 0.232 ppm, respectively. Both 2-aminoisobutyric acid and trans-4-hydroxy-L-proline had sweet flavor, with thresholds of 1030 ppm and 790 ppm respectively. Trans-4-hhydroxy-L-proline were down-regulated by Arginine and proline metabolism, Metabolic pathways and ABC transporters. These findings indicated that the fixing process may mainly regulate the bitterness, astringency and sweetness of tea. KEGG analysis of the above 99 differentially expressed metabolites identified 17 obviously enriched pathways ($p < 0.05$) (Fig. 2D). Most of the bitter taste characteristic components increased significantly by Pyrimidine metabolism, Metabolic pathways, Purine metabolism and ABC transporters. 3′-Aenylic acid as a sweetness differential metabolite, decreased significantly by Purine metabolism and Metabolic pathways, and the other 2 upregulated sweetness differential metabolites were not significantly enriched in the pathways. The astringent characteristic components were enriched to a total of 11 pathways.

Similarly, OPLS-DA score plot exposed distinct differences in the metabolites compared between Yc and Yd (Fig. S2C), with 1 up-regulated and 1 down-regulated, respectively (Table 1, Fig. 2E). The up-regulated trans-4-hydroxy-L-proline displayed sweet flavor with the threshold of 790 ppm, while the down-regulated luteolin 7-o-glucoside (cynaroside) also displayed the sweet flavor. The findings demonstrated that the rolling step may mainly regulate the sweetness of GT and YT by altering the relative content of flavonoids. KEGG enrichment analysis of the above two differentially expressed metabolites discovered three significantly enriched pathways ($p < 0.05$) (Fig. 2F). Trans-4-hydroxy-L-proline was significantly enriched to Arginine and proline metabolism, Metabolic pathways and ABC transporters, and luteolin 7-o-glucoside was significantly enriched to flavone and flavonol biosynthesis.

3.4. The taste profiles of the YT after yellowing for 48 h

532 taste substances were detected in YT after yellowing for 48 h, and 111 metabolites were found with taste characteristics. Of those, there were 43 metabolites with bitter flavor, 26 with astringent flavor, 26 with sweet flavor, 7 with sour flavor, and 4 with umami flavor (Fig. 3A). Meanwhile, 128 compounds were found with flavor thresholds, among which 6 compounds had flavor thresholds <1 ppm, 3 compounds had flavor thresholds between 1 and 10 ppm, and 31 compounds had flavor thresholds between 10 and 100 ppm. There were 42 compounds with the flavor thresholds between 100 and 1000 ppm, 36 compounds between 1000 and 10,000 ppm, and 10 compounds >10,000 ppm (Fig. 3B). Statistical analysis revealed that compounds with bitter flavor was significantly higher than that of other compounds, followed by astringent and sweet metabolites, and the least relative content of YT after yellowing for 48 h was umami metabolites (Fig. 3C). The radar charts of the flavors of YT and GT products indicated that the sweetness and freshness and other flavors of YT were more intense than those of GT (Fig. 3D). The flavor threshold of metabolites in the YT after yellowing 48 h was mainly between 100 and 1000 ppm, followed by 1000–10,000 ppm. This indicated that the threshold of bitter compounds in finished YT might be higher and thus the bitterness flavor was not obvious.

Moreover, we performed statistical analysis on the flavor thresholds of each class of taste metabolites retrieved. The flavor thresholds of 43 metabolites with bitter were mainly distributed in 1000–10,000 ppm (16/43, 37.21%), 100–1000 ppm (15/43, 34.88%), and > 10,000 ppm

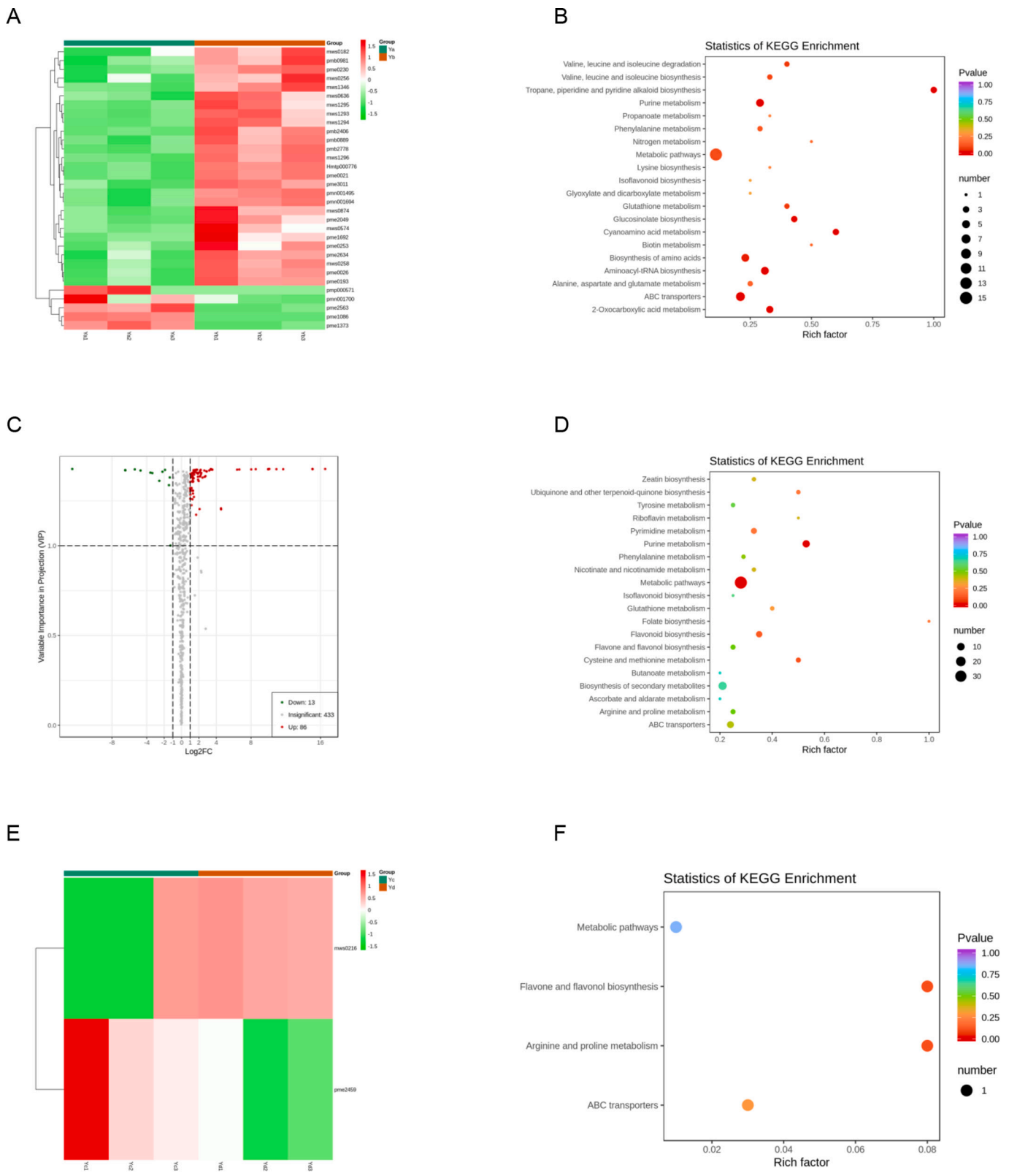

Fig. 2. The differential metabolites and associated KEGG pathways after withering, fixing and rolling: (A) Heatmap of differential metabolites between Ya and Yb; (B) KEGG enrichment of differential metabolites between Ya and Yb; (C) Heatmap of differential metabolites between Yb and Yc; (D) KEGG enrichment of differential metabolites between Yb and Yc; (E) Heatmap of differential metabolites between Yc and Yd; (F) KEGG enrichment of differential metabolites between Yc and Yd.

Table 1
Taste thresholds and characteristics of different metabolites in yellow tea processing.

Index	Group	Formula	Compounds	Class I	CAS	Flavor threshold	Flavor descriptor
Hmcn002875	9	C15H10O7	6-Hydroxyluteolin	Flavonoids	18,003–33-3	–	–
HmdP001587	5	C34H26O22	Tercatain	Tannins	103,744–87-2	–	–
Hmtn001120	1	C14H20O8	5-(2-Hydroxyethyl)-2-O-glucosylohenol	Phenolic acids	–	–	–
Hmtn001302	1	C13H16O8	Glucosyloxybenzoic acid	Phenolic acids	–	–	–
Hmtp000776	16	C8H9NO3	4,5,6-Trihydroxy-2-cyclohexen-1-ylideneacetonitrile	Alkaloids	–	–	–
Hmyp007396	12	C37H68NO9P	PC(oxo-11:0/18:2)	Lipids	–	–	–
Lmhn001773	15	C19H13NO10	Caffeoylnicotinoyltartaric acid	Phenolic acids	–	–	–
Lmhn003246	6	C24H22O13	Sinapoylcaffeoyltartaric acid	Phenolic acids	–	–	–
Lmhn202452	12	C13H12O8	Cis-*p*-Coumaroyltartaric acid(cis-Coutaric acid)	Phenolic acids	–	–	–
Lmmp003783	12	C21H18O13	Quercetin glucuronic acid	Flavonoids	–	–	–
Lmmp003903	1	C23H22O12	Kaempferol acetyl-glucoside	Flavonoids	–	–	–
Lmtn002565	14	C14H18O9	1'-O-Vanilloyl-β-D-glucoside	Phenolic acids	–	–	–
Lmzn001582	9	C18H28O9	5′-Glucopyranosyloxyjasmanic acid	Phenolic acids	–	–	–
Qingke_Rfmb089–2-3	9	C18H34O4	9,10-Dihydroxy-12-octadecenoic acid	Lipids	263,399–34-4	–	–
Zmhn001926	1	C13H16O8	Salicylic acid O-glycoside	Phenolic acids	–	–	–
Zmhn003099	14	C21H20O10	Kaempferol 3-O-α-L-rhamnoside(X)	Flavonoids	–	–	–
Zmxn001997	1	C13H16O8	Isosalicylic acid O-glycoside	Phenolic acids	–	–	–
mws0014	9	C10H10O4	Ferulic acid	Phenolic acids	1135–24-6	13 a	pleated astringency a
mws0024	3	C7H6O5	Gallic acid	Flavonoids	149–91–7	140 a	bitterness a
mws0032	9	C15H10O8	Myricetin	Flavonoids	529–44-2	10 a	–
mws0124	1	C13H14N2O3	N-(3-Indolylacetyl)-L-alanine	Amino acids and derivatives	57,105–39-2	–	–
mws0126	16	C26H54NO7P	1-Stearoyl-sn-glycero-3-phosphocholine	Lipids	19,420–57-6	–	–
mws0133	1	C6H6N2O	Nicotinamide	Others	98–92-0	855 a	bitterness a
mws0177	9	C5H4O3	2-Furanoic acid	Organic acids	88–14-2	18 a	astringency a
mws0180	15	C7H6O4	2,5-Dihydroxybenzoic acid	Phenolic acids	490–79-9	90 a	–
mws0182	15	C8H8O3	*p*-Hydroxyphenyl acetic acid	Phenolic acids	156–38-7	20 a	–
mws0183	15	C7H6O4	Protocatechuic acid	Flavonoids	99–50-3	31 a	pleated astringency a
mws0216	8	C5H9NO3	Trans-4-Hydroxy-L-proline	Amino acids and derivatives	51–35-4	790 a	sweet a
mws0227	4	C6H13NO2	L-Leucine	Amino acids and derivatives	61–90-5	5500 a	bitterness a
mws0232	1	C17H20N4O6	Riboflavin	Others	83–88-5	376 a	bitterness a
mws0237	9	C9H16O4	Anchoic Acid	Organic acids	123–99-9	188 a	sour a
mws0250	4	C9H11NO3	L-(−)-Tyrosine	Amino acids and derivatives	60–18-4	908 a	bitterness a
mws0251	7	C5H6N2O2	Thymine	Nucleotides and derivatives	65–71-4	525 a	bitterness a
mws0255	11	C4H5N3O	Cytosine	Nucleotides and derivatives	71–30-7	890 a	bitterness a
mws0256	4	C5H11NO2	L-Valine	Amino acids and derivatives	72–18-4	2460 a	bitterness a
mws0258	6	C6H13NO2	L-Isoleucine	Amino acids and derivatives	73–32-5	2097 a	bitterness a
mws0289	1	C23H46NO7P	LysoPE 18:1	Lipids	89,576–29-4	–	–
mws0345	9	C6H11NO2	Pipecolinic acid	Organic acids	535–75-1	–	–
mws0458	1	C8H8O3	Vanillin	Phenolic acids	121–33-5	38 a	silky smooth mouth coating feeling a
mws0467	6	C9H10O3	3-(4-Hydroxyphenyl)-propionic acid	Phenolic acids	501–97-3	–	–
mws0520	16	C11H13NO4	N-Acetyl-L-tyrosine	Amino acids and derivatives	537–55-3	–	–
mws0574	8	C4H8O3	α-Hydroxyisobutyric acid	Organic acids	594–61-6	>10,400 a	bitterness a
mws0609	1	C10H12N5O7P	Guanosine 3′,5′-cyclic monophosphate	Nucleotides and derivatives	7665-99-8	–	–
mws0628	1	C7H6O2	4-Hydroxybenzaldehyde	Phenolic acids	123–08-0	11 a	–
mws0636	16	C18H20N2O3	Phe-Phe	Amino acids and derivatives	2577-40-4	370 a	bitterness a
mws0639	15	C7H6O4	2,3-Dihydroxybenzoic Acid	Organic acids	303–38-8	20 a	–
mws0748	11	C16H18O9	1-Caffeoylquinic acid	Phenolic acids	1241-87-8	–	–
mws0749	9	C7H6O3	4-Hydroxybenzoic acid	Phenolic acids	99–96-7	92 a	astringency a
mws0874	1	C10H14N5O7P	3′-Aenylic acid	Nucleotides and derivatives	84–21-9	–	sweet †
mws0884	1	C10H12N5O6P	Cyclic AMP	Nucleotides and derivatives	60–92-4	32,900 a	umami a
mws0885	15	C7H6O4	2,4-Dihydroxy benzoic acid	Phenolic acids	89–86-1	231 a	sweet a
mws0914	9	C15H12O5	Pinobanksin	Flavonoids	548–82–3	–	–
mws1050	5	C5H9NO4	O-Acetylserine	Amino acids and derivatives	5147-00-2	–	–

(*continued on next page*)

Table 1 (*continued*)

Index	Group	Formula	Compounds	Class I	CAS	Flavor threshold	Flavor descriptor
mws1060	3	C10H12N4O5	9-(β-D-Arabinofuranosyl)hypoxanthine	Nucleotides and derivatives	7013-16-3	–	–
mws1140	1	C15H12O5	Naringenin chalcone(4,2′,4′,6’-Tetrahydroxychalcone)	Flavonoids	73,692–50-9	–	–
mws1167	2	C4H4O5	Oxaloacetic acid	Organic acids	328–42-7	500 a	–
mws1195	5	C10H10O3	Methyl p-coumarate	Phenolic acids	3943-97-3	–	–
mws1200	5	C10H10O3	Trans-4-Hydroxycinnamic Acid Methyl Ester	Phenolic acids	19,367–38-5	–	–
mws1212	5	C11H12O4	Methyl ferulate	Phenolic acids	2309-07-1	–	–
mws1293	10	C29H24O12	Theaflavin	Tannins	4670-05-7	9.024 a	pleated astringency a
mws1294	10	C36H28O16	Theaflavin-3-gallate	Tannins	30,462–34-1	10.74 a	pleated astringency a
mws1295	10	C36H28O16	Theaflavin-3’-Gallate	Tannins	28,543–07-9	10.74 a	pleated astringency a
mws1296	6	C43H32O20	Theaflavin 3,3’-Digallate	Tannins	30,462–35-2	11.284 a	pleated astringency a
mws1326	9	C15H10O7	Herbacetin	Flavonoids	527–95-7	–	bitterness †
mws1346	4	C6H11NO4	DL-2-Aminoadipic acid	Alkaloids	542–32-5	–	–
mws1354	1	C10H10O4	Trans-ferulic acid	Phenolic acids	537–98-4	–	–
mws1358	9	C6H6O2	Pyrocatechol	Phenolic acids	120–80-9	99 a	astringency a
mws1434	14	C21H20O10	Isovitexin	Flavonoids	29,702–25-8	–	–
mws1587	6	C6H13NO2	α-Aminocaproic acid	Amino acids and derivatives	327–57-1	2625 a	bitterness a
mws2125	5	C3H4KO6P	Phosphoenolpyruvic acid	Organic acids	4265-07-0	–	–
mws2213	1	C9H8O2	Cinnamic acid	Phenolic acids	140–10-3	–	–
mws2623	15	C18H34O2	11-Octadecanoic acid(Vaccenic acid)	Lipids	506–17-2	–	–
mws2627	14	C16H12O7	Tamarixetin	Flavonoids	603–61-2	–	–
mws4134	3	C20H32N6O12S2	Oxidized Glutathione	Amino acids and derivatives	121–24-4	400 a	–
mws4176	1	C12H16N2O3	DL-Alanyl-DL-phenylalanine	Amino acids and derivatives	1999-45-7	–	sweet †
mws5035	1	C15H22N2O3	Leucylphenylalanine	Amino acids and derivatives	56,217–82-4	–	–
mws5038	6	C12H22O11	Isomaltulose	Others	13,718–94-0	–	–
mws5042	16	C11H14N2O3	Glycylphenylalanine	Amino acids and derivatives	721–66-4	–	–
pmb0374	1	C5H5N5	Aminopurine	Alkaloids	452–06-2	–	–
pmb0423	1	C10H10O4	Hydroxy-methoxycinnamate	Phenolic acids	–	–	–
pmb0492	3	C34H37N3O6	N′,N″,N‴-p-Coumaroyl-cinnamoyl-caffeoyl spermidine	Alkaloids	–	–	–
pmb0653	15	C27H30O15	Di-C,C-hexosyl-apigenin	Flavonoids	–	–	–
pmb0681	14	C20H18O9	Apigenin 8-C-pentoside	Flavonoids	–	–	–
pmb0854	15	C26H48NO7P	LysoPC 18:3	Lipids	–	–	–
pmb0855	16	C24H50NO7P	LysoPC 16:0	Lipids	17,364–16-8	–	–
pmb0864	1	C19H40NO7P	LysoPE 14:0	Lipids	–	–	–
pmb0876	1	C21H44NO7P	LysoPE 16:0	Lipids	53,862–35-4	–	–
pmb0889	6	C18H30O2	Punicic acid	Lipids	544–72-9	–	–
pmb0981	14	C10H14N5O7P	Adenosine 5′-monophosphate	Nucleotides and derivatives	61–19-8	–	–
pmb2319	16	C23H48NO7P	LysoPC 15:0	Lipids	–	–	–
pmb2406	16	C25H52NO7P	LysoPC 17:0	Lipids	–	–	–
pmb2620	5	C11H12O4	3,4-Dimethoxycinnamic acid	Phenolic acids	14,737–89-4	–	–
pmb2778	6	C18H32O3	9,10-EODE	Lipids	65,167–83-1	–	–
pmb2940	1	C17H22O10	1-O-β-D-Glucopyranosyl sinapate	Phenolic acids	78,185–48-5	–	–
pmb3068	9	C16H18O8	1-O-p-Coumaroyl quinic acid	Phenolic acids	–	–	–
pmb3072	1	C22H26O12	3-O-p-coumaroyl shikimic acid O-hexoside	Phenolic acids	–	–	–
pmb3099	13	C4H11O4P	Diethyl phosphate	Organic acids	598–02-7	–	–
pmd0136	16	C26H54NO7P	LysoPC 18:0	Lipids	–	–	–
pme0021	16	C9H11NO2	L-Phenylalanine	Amino acids and derivatives	63–91-2	9600 a	bitterness a
pme0026	6	C6H14N2O2	L-(+)-Lysine	Amino acids and derivatives	56–87-1	434 a	bitterness a
pme0152	5	C5H8N2O2	5,6-Dihydro-5-methyluracil	Nucleotides and derivatives	696–04-8	–	–
pme0183	9	C5H5N5O	2-Hydroxy-6-aminopurine	Nucleotides and derivatives	3373-53-3	–	–
pme0193	6	C5H10N2O3	L-Glutamine	Amino acids and derivatives	56–85-9	7300 a	salty a
pme0230	1	C10H13N5O4	Adenosine	Nucleotides and derivatives	58–61–7	20,580 a	bitterness a
pme0253	16	C8H15NO3	N-Acetyl-L-leucine	Amino acids and derivatives	1188-21-2	81 a	sour a
pme0274	4	C6H13NO2	6-Aminocaproic acid	Organic acids	60–32-2	3935 a	bitterness a
pme0278	5	C7H14N2O4	2,6-Diaminooimelic acid	Amino acids and derivatives	583–93-7	–	–
pme0295	15	C6H11NO3	4-Acetamidobutyric acid	Organic acids	3025-96-5	–	–
pme0309	5	C8H8O5	Methyl gallate	Flavonoids	99–24-1	0.232 a	astringency a

(*continued on next page*)

Table 1 (*continued*)

Index	Group	Formula	Compounds	Class I	CAS	Flavor threshold	Flavor descriptor
pme1086	2	C10H17N3O6S	Glutathione reduced form	Amino acids and derivatives	70–18-8	950 a	fullness of mouth a
pme1109	15	C5H5N5O	Guanine	Nucleotides and derivatives	73–40-5	760 a	bitterness a
pme1178	1	C10H13N5O5	Guanosine	Nucleotides and derivatives	118–00-3	17,840 a	bitterness a
pme1286	5	C14H20N6O5S	S-(5′-Adenosy)-L-homocysteine	Amino acids and derivatives	979–92-0	–	–
pme1373	2	C9H14N3O7P	2'-Deoxycytidine-5′-monophosphate	Nucleotides and derivatives	1032-65-1	–	–
pme1419	5	C6H14ClNO2S	L-Methionine methyl ester	Amino acids and derivatives	2491-18-1	–	–
pme1474	1	C11H15N5O3S	5'-Deoxy-5′-(methylthio)adenosine	Nucleotides and derivatives	2457-80-9	215.4 a	–
pme1692	7	C10H13N4O8P	Inosine 5′-monophosphate	Nucleotides and derivatives	131–99-7	–	–
pme1786	1	C29H35O17+	Malvidin 3,5-O-diglucoside (Malvin)	Flavonoids	–	–	–
pme1816	9	C16H18O9	Neochlorogenic acid(5-O-Caffeoylquinic acid)	Phenolic acids	906–33–2	50 a	bitterness a
pme2049	8	C4H8O3	2-Hydroxybutanoic acid	Organic acids	600–15-7	10,400 a	bitterness a
pme2122	14	C5H9N3	Histamine	Amino acids and derivatives	51–45-6	70 a	bitterness a
pme2253	7	C6H10O6	L-Gulonic-γ-lactone	Others	1128-23-0	–	–
pme2459	13	C21H20O11	Luteolin 7-O-glucoside(Cynaroside)	Flavonoids	5373-11-5	–	sweet #
pme2563	2	C8H14N2O5S	γ-Glu-Cys	Amino acids and derivatives	636–58-8	–	–
pme2634	4	C5H11NO2	DL-Norvaline	Amino acids and derivatives	760–78-1	–	–
pme2776	1	C10H12N4O4	2'-Deoxyinosine	Nucleotides and derivatives	890–38-0	–	–
pme2954	14	C15H10O7	Quercetin	Flavonoids	117–39-5	10 a	–
pme3007	3	C9H14N2O12P2	Uridine 5′-diphosphate	Nucleotides and derivatives	27,821–45-0	–	–
pme3011	1	C4H9NO2	γ-Aminobutyric acid	Organic acids	56–12-2	2.1 a	dry mouth feeling a
pme3017	5	C4H9NO2	2-Aminoisobutyric acid	Amino acids and derivatives	62–57-7	1030 a	sweet a
pme3174	11	C9H14N3O8P	Cytidine 5′-monophosphate(Cytidylic acid)	Nucleotides and derivatives	63–37-6	12,930 a	umami a
pme3188	9	C9H13N2O9P	Uridine 5′-monophosphate	Nucleotides and derivatives	58–97-9	–	–
pme3227	15	C27H30O14	Vitexin 2''-O-β-L-rhamnoside	Flavonoids	64,820–99-1	–	–
pme3443	5	C11H12O4	Sinapinaldehyde	Phenolic acids	4206-58-0	80 a	–
pme3475	1	C15H12O5	Butin	Flavonoids	492–14-8	–	–
pme3732	9	C9H13N3O5	Cytidine	Nucleotides and derivatives	65–46-3	4255 a	bitterness a
pme3961	1	C10H13N5O3	Deoxyadenosine	Nucleotides and derivatives	958–09-8	–	–
pmf0284	5	C10H10O3	Riboprine	Phenolic acids	7724-76-7	–	–
pmn001494	16	C22H26O8	(+)-Syringaresinol	Lignans and Coumarins	21,453–69-0	–	–
pmn001495	16	C19H38O4	Hexadecanoic acid 2,3-dihydroxypropyl ester	Lipids	542–44-9	–	–
pmn001519	9	C15H12O9	Galloyl Methyl gallate	Phenolic acids	–	–	–
pmn001526	7	C20H20O14	1,6-Bis-O-galloyl-β-D-glucose	Phenolic acids	–	–	–
pmn001530	7	C27H24O18	2,4,6-Tri-O-Galloyl-D-Glucose	Phenolic acids	108,043–99-8	–	–
pmn001531	1	C27H24O18	2,4,6-Tri-O-galloyl-β-D-glucose	Phenolic acids	–	–	–
pmn001547	15	C27H26O15	3-Hydroxy-5-Methylphenol-1-Oxy-β-D-(6'-O-Digalloyl)Glucose	Phenolic acids	–	–	–
pmn001588	12	C24H42O21	Nystose	Others	13,133–07-8	–	–
pmn001628	5	C20H18O14	Hexahydroxydiphenoylglucose	Phenolic acids	–	–	–
pmn001632	7	C34H28O22	Tetragalloylglucose	Tannins	–	–	–
pmn001669	5	C12H14O5	Methyl sinapate	Phenolic acids	20,733–94-2	–	–
pmn001694	16	C18H34O5	9,10,13-Trihyroxy-11-octadecadienoic acid	Lipids	–	–	–
pmn001700	13	C30H48O3	24,30-Dihydroxy-12(13)-enolupinol	Terpenoids	–	–	–
pmn001713	14	C27H30O15	Luteolin-7-O-β-D-rutinoside	Flavonoids	–	–	–
pmp000001	14	C16H12O6	Hispidulin	Flavonoids	1447-88-7	–	–
pmp000116	14	C21H20O10	Apigenin-8-C-glucoside	Flavonoids	3681-93-4	–	–
pmp000232	9	C16H18O8	Cis-3-p-coumaric quinic acid	Phenolic acids	–	–	–
pmp000413	14	C21H20O10	Genistein 8-C-glucoside	Flavonoids	66,026–80-0	–	–
pmp000571	14	C15H10O5	Apigenin	Flavonoids	520–36-5	–	–
pmp000572	12	C15H10O6	Luteolin	Flavonoids	491–70-3	–	–
pmp001250	9	C26H51NO7P+	PC(18:2)	Lipids	–	–	–
pmp001270	9	C24H48NO7P	LysoPC(16:1)	Lipids	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Index	Group	Formula	Compounds	Class I	CAS	Flavor threshold	Flavor descriptor
pmp001273	9	C26H50NO7P	LysoPC(18:2)	Lipids	–	–	–
pmp001283	5	C21H38O4	Glyceryl linoleate	Lipids	26,545–74-4	–	–
pmp001286	16	C26H54NO7P	LysoPC(18:0)	Lipids	–	–	–

†: https://cosylab.iiitd.edu.in/flavordb/search; #: http://www.flavornet.org/flavornet.html;
a: Compilations of Flavor Threshold Values in Water and other Media – 2nd ed. / LJ van Gemert (2015).

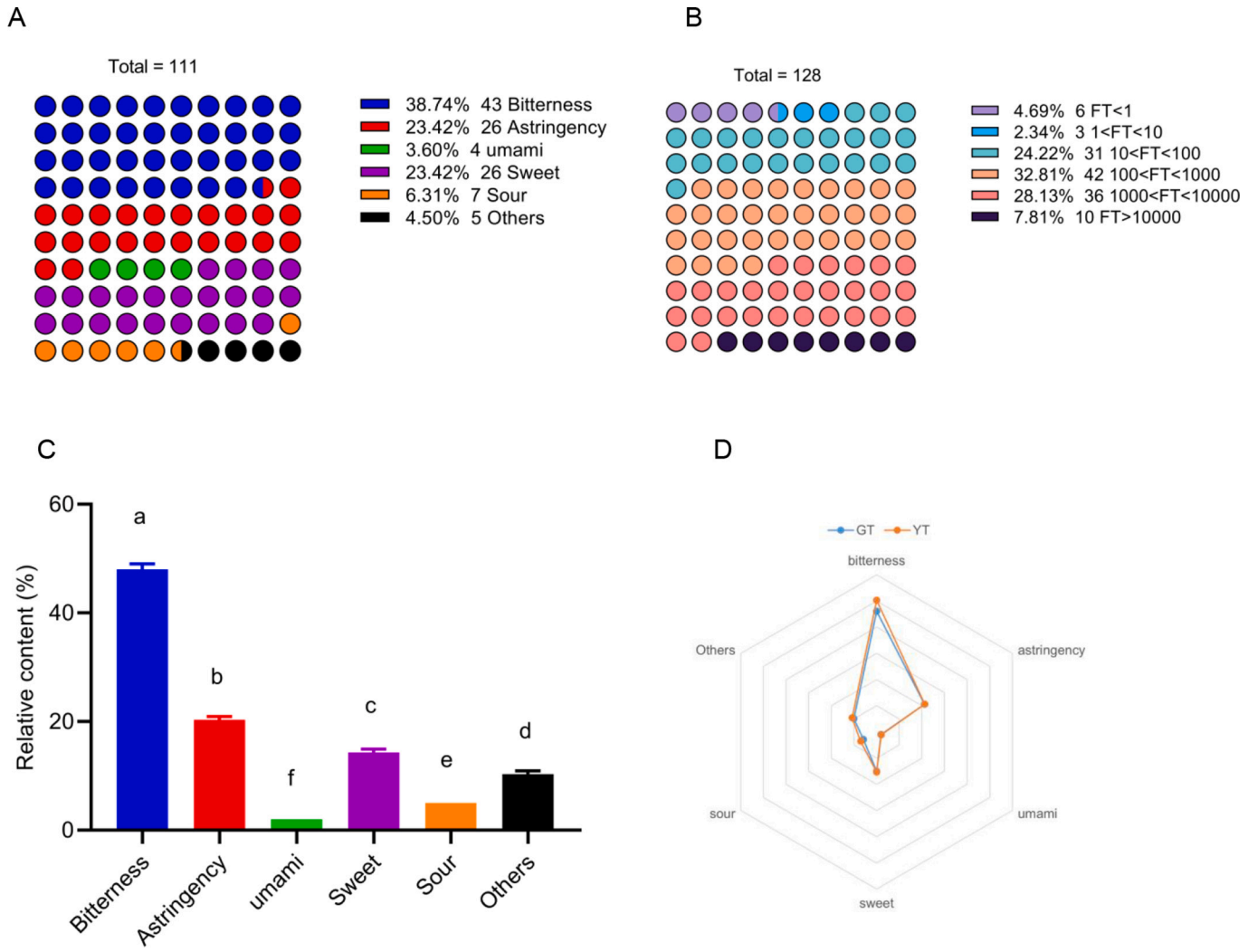

Fig. 3. The taste and quality characteristics of the yellow tea after yellowing for 48 h: (A) Flavor distribution of 111 metabolites with taste characteristics; (B) Flavor threshold distribution of 128 compounds with flavor thresholds; (C) Bar plot of the relative contents of different taste categories; (D) Radar chart of flavor profiles of all tea samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(6/43, 13.95%) (Fig. S3A). The flavor thresholds of the 26 metabolites with astringent were mainly distributed in 10–100 ppm (11/26, 42.31%) and 100–1000 ppm (9/43, 34.62%) (Fig. S3B). The flavor threshold of 26 sweet metabolites was mainly distributed in 1000–10,000 ppm (14/26, 53.85%), and there was 1 metabolite (3.85%) with the threshold >10,000 ppm (Fig. S3C). The flavor thresholds of 7 sour metabolites were mainly distributed in 100–1000 ppm (5/7, 71.43%) (Fig. S3D). The flavor thresholds of the 4 umami metabolites were distributed in 100–1000 ppm and > 10,000 ppm half by half (Fig. S3E). These results suggested that the taste of the YT after yellowing for 48 h might be mainly bitter and astringent with sweet taste.

3.5. *Influence of yellowing on the flavor profile development of YT*

Yellowing is a critical step that affects the flavor profile of YT. To investigated the influence of yellowing time on the flavor quality of YT, we set five treatment groups of yellowing for 4 h (Ye), 8 h (Yf), 16 h (Yg), 24 h (Yh) and 48 h (Yi).

The differential analysis revealed that there were 2 differential metabolites between Yd and Ye, 3 differential metabolites between Yd and Yf, 5 differential metabolites between Yd and Yg, 7 differential metabolites between Yd and Yh, and 12 differential metabolites between Yd and Yi, respectively (Fig. 4A-E). These findings suggested that the longer the yellowing treatment, the greater the number of differential metabolites. There were no differential metabolites with taste characteristics

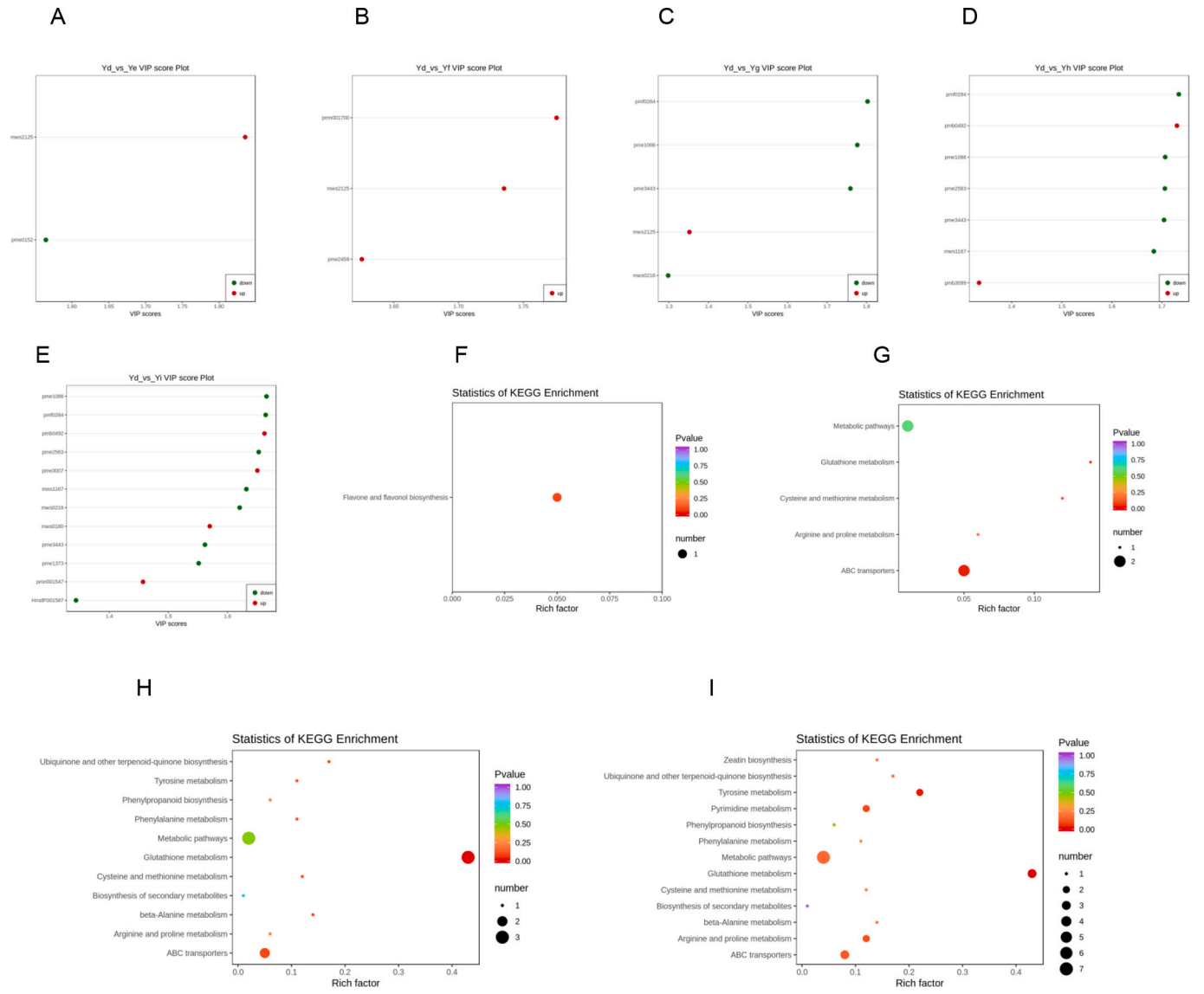

Fig. 4. The differential metabolites and associated KEGG pathways after yellowing: (A) VIP score plot of differential metabolites between Yd and Ye; (B) VIP score plot of differential metabolites between Yd and Yf; (C) VIP score plot of differential metabolites between Yd and Yg; (D) VIP score plot of differential metabolites between Yd and Yh; (E) VIP score plot of differential metabolites between Yd and Yi; (F) KEGG enrichment of differential metabolites between Yd and Yf; (G) KEGG enrichment of differential metabolites between Yd and Yg; (H) KEGG enrichment of differential metabolites between Yd and Yh; (I) KEGG enrichment of differential metabolites between Yd and Yi.

and taste thresholds found, and no enriched KEGG pathways were identified between Yd and Ye. Among the 3 differential metabolites between Yd and Yf, only 1 compound was found to have a specific taste, which is luteolin 7-o-glucoside (cynaroside) with sweet flavor and no threshold found. Flavone and flavonol biosynthesis ($p < 0.5$) were significantly enriched in those differential metabolites (Fig. 4F). While for the 5 differential metabolites between Yd and Yg, phosphoenolpyruvic acid was the same as that found in Yd versus Ye and Yd versus Yf comparisons. Two compounds were found to have taste characteristics and threshold values, including trans-4-hydroxy-L-proline with sweet flavor and 790 ppm and glutathione reduced form with a sense of oral fullness and 950 ppm. Glutathione metabolism, Cysteine and methionine metabolism, Arginine and proline metabolism and ABC transporters were significantly enriched for these 5 differential metabolites ($p < 0.5$) (Fig. 4G), which were intimately connected with the transport and metabolism of amino acids (Wu et al., 2022). For the 7 differential metabolites between Yd and Yh, there were 3 metabolites the same as those between Yd and Yg. Only 1 compound had taste characteristics and taste threshold, which was Glutathione reduced form. 9 markedly enriched metabolic pathways were recognized (Fig. 4H). Glutathione reduced form was significantly down-regulated via Cysteine and methionine metabolism, Glutathione metabolism, Metabolic pathways and ABC transporters. As for the 12 differential metabolites between Yd and Yi, there were 6 metabolites the same as those produced those between Yd and Yh, including oxaloacetic acid, N′,N″,N‴-p-coumaroyl-cinnamoyl-caffeoyl spermidine, glutathione reduced form, γ-glu-cys, sinapinaldehyde and riboprine, and only 2 compounds had taste characteristics and taste thresholds, which were trans-4-hydroxy-L-proline and glutathione reduced form. Only 3 compounds were identified with taste threshold values. 11 markedly enriched metabolic pathways were recognized (Fig. 4I). The above results indicated that the sweetness of yellow tea was enhanced and its bitterness and astringency were reduced with the extension of the yellowing time. Yellowing may have modulated the sweetness of yellow tea mainly by regulating the relative content of Luteolin 7-O-glucoside(Cynaroside) and Trans-4-Hydroxy-L-proline in the Arginine and proline metabolism, Metabolic pathways,

and ABC transporters metabolic pathway. Modulation of the ABC transporter protein metabolic pathway can alter some of the sugars, sugar alcohols and amino acid classes associated with sweetness, which in turn affects flavor quality.

4. Discussion

We analyzed the variations on the material composition and flavor quality of GT and YT during different steps by UPLC-MS/MS combined with sensory review, and further investigated the influences of different yellowing times on the development of the taste quality of YT. 532 non-volatile metabolites were examined for dynamic changes during processing. Most of non-volatile metabolites showed an increasing tendency and 11 metabolites (except tannins) changed significantly during processing. And amino acids and their derivatives, flavonoids, phenolic acids, organic acids and lipids undergo the most significant changes in the processes of withering, fixing and rolling steps. These findings indicated that the processing of GT and YT may promote the development of GT and YT flavors by altering the relative amounts of metabolites.

The above differential metabolite analyses suggest that withering and fixing might be the key processes that regulated the development of taste qualities in both GT and YT. The withering process mainly regulated the astringency and bitterness, while the fixing process mainly regulated the bitterness, astringency and sweetness. GT is prepared from freshly picked leaves by withering, fixing, rolling and drying. Withering and fixing play an essential part in the development of the taste quality of GT, while kneading has the least impact. Fixing not only brings a light, sweet and bitter taste characteristic to GT, but is also a key process in the development of the chestnut-like aroma of GT (Wang et al., 2020). Fixing involves a dramatic conversion of non-volatile metabolites of GT, because the high temperature treatment promotes hydrolysis, oxidation, isomerization, and other rapid thermochemical reactions of metabolites (Wang et al., 2021). The fixing process may mainly regulate the bitterness, astringency and sweetness of tea. In the early stages of smothering, amino acids and their derivatives, lipids and other classes increase significantly, while alkaloids decrease significantly. Free amino acids give a light and sweet taste to the tea broth and are also involved in the development of the chestnut aroma of GT (Cui et al., 2019). Withering may have increased the astringent-bitter flavor mainly by increasing markedly bitter metabolites such as phe-phe and L-(+)-lysine, and astringent metabolites such as catechin and γ-aminobutyric acid reduced the sense of oral fullness by significantly decreasing the content of glutathione reduced form. Bitterness and astringency are the key taste characteristics of GT, and the main contributors are catechins (Xu et al., 2018).

YT is prepared by withering, fixing, rolling, yellowing and drying. The procedures of withering, fixing and yellowing as the development of the flavors of YT in the early stages produces astringency and bitterness of YT. However, in comparison with GT, YT has a smoother, sweeter and less astringent and bitter taste due to the additional yellowing process than GT. Yellowing has been reported in the literature as an essential process for YT, where compounds and flavors are altered (Wei et al., 2021b). The yellowing time varies from a few hours to tens of hours, and the tea undergoes many thermochemical reactions such as non-enzymatic oxidation of polyphenols, chlorophyll decomposition and protein hydrolysis, which significantly change tea ingredients, forming YT's unique appearance “three yellows” (Wei et al., 2021a), and provides it with an attractive appearance and a unique flavor quality (Shi et al., 2021; Wang, Yue, & Tong, 2021). Flavonoids such as luteolin showed an upward trend during fixation, rolling and drying, and luteolin 7-o-glucoside (cynaroside) showed a downward trend and enriched to flavone and flavonol biosynthesis. This is because the leaves are damaged during rolling, allowing the enzyme to interact with the substrate and accelerate the hydrolysis of the flavonoid glycosides (Li et al., 2019). Trans-4-hydroxy-L-proline and glutathione reduced form increases significantly during the yellowing process mainly through Arginine and proline metabolism, Metabolic pathways and ABC transporters, resulting in an enhanced sweetness and reduced bitterness in YT. Literature reported that different rootstocks can modulate metabolic pathways such as ABC transporter proteins, which alter some of the sugars and sugar alcohols and amino acid classes associated with sweetness, thereby affecting the flavor quality of orange juice (Liu, Gmitter, Grosser, & Wang, 2023).

The above results show that the yellowing process may regulate the relative amount of luteolin 7-o-glucoside (cynaroside) and trans-4-hydroxy-L-proline via Arginine and proline metabolism, Metabolic pathways, ABC transporters, thereby modulating the sweetness in the taste quality of YT. Therefore, the sweetness of the YT enhanced and the bitterness decreased as the yellowing time was extended.

This paper uses the UPLC-MS/MS to monitor the dynamics of tea ingredients throughout the procedure of TT and GT, which is conducive to a deepen comprehensive and systematic insight into formation of mechanisms of the taste quality of GT and YT, and provides a theoretical foundation for tea processing and quality control.

5. Conclusion

Yellowing is the key factor that leads to flavor profiles of YT differing from GT. To understand the mechanism of taste quality formation throughout the process steps of GT and YT and the difference in flavor profiles between GT and YT resulting from the yellowing process. We analyzed the changes in the material composition and taste quality of GT and YT throughout the withering, fixing and rolling steps by UPLC-MS/MS combined with sensory review, and further investigated the influences of different yellowing times on the formation of the taste quality of YT. 532 non-volatile metabolites were detected, classified into 11 categories. The processing of GT and YT may have developed their respective taste quality by altering the relative content of non-volatile metabolites. PCA, HCA and differential metabolite analysis showed that withering and fixing may be the key processes to regulate flavor profiles formation of GT, while withering, fixing and yellowing may be the important processes to regulate the flavor profiles formation of YT. The withering procedure mainly regulates the bitterness and astringency, and the killing process mainly regulates the bitterness, astringency and sweetness, while the yellowing process mainly regulates the sweetness. Trans-4-hydroxy-L-proline and glutathione reduced form increases significantly during the yellowing process mainly by Arginine and proline metabolism and ABC transporters, resulting in an enhanced sweetness and reduced bitterness in YT. The overall taste profile of YT was mainly bitter and astringent with sweet taste, while GT might be dominated by bitterness and astringency. Traditional sensory review and e-tongue results illustrate that the YT with 48 h-yellowing showed sweet and mellow taste quality and the GT showed bitter, astringent and sweet taste quality. In this paper, UPLC-MS was used to monitor the dynamic changes of compounds during the processing of YT and GT, which is conducive to a more comprehensive and systematic understanding of the formation mechanism of the flavor quality of YT and GT, and provides a theoretical basis for future research on the processing and quality control of YT and GT.

Ethical statement

In order to ensure the morality and legitimacy of the scientific experiments, we made the following ethical statement. Ethics approval was not required by national law, and the establishment of a human ethics committee was not required under national regulations for sensory review. We confirmed that the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research, including no coercion to participate, full disclosure of study requirements and risks, written or verbal consent of participants, no release of participant data without their knowledge,

ability to withdraw from the study at any time.

CRediT authorship contribution statement

Lingli Sun: Visualization, Methodology, Formal analysis, Data curation, Writing – original draft. **Shuai Wen:** Data curation, Formal analysis, Methodology, Writing – original draft. **Suwan Zhang:** Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Junxi Cao:** Funding acquisition, Project administration, Resources. **Ruohong Chen:** Resources, Project administration, Funding acquisition. **Zhongzheng Chen:** Resources, Funding acquisition. **Zhenbiao Zhang:** Resources, Funding acquisition. **Zhigang Li:** Resources, Funding acquisition. **Qian Li:** Funding acquisition. **Zhaoxiang Lai:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision. **Shili Sun:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, “Study on flavor quality formation in green and yellow tea processing by means of UPLC-MS approach”.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101342.

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