Re-rolling treatment in the fermentation process improves the taste and liquor color qualities of black tea

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ABSTRACT

Fermentation is a vital process occurred under the premise of rolling affecting black tea quality. Theoretically, re-rolling during fermentation will remodel the biochemical conditions of tea leaves, and thus influence black tea quality. Herein, we studied the effect of re-rolling on black tea taste and liquor color. Sensory evaluation showed that re-rolling significantly weakened the astringency taste and improved the redness and luminance of liquor. With re-rolling, the color attributes of a* and L* and the contents of theaflavins and thearubigins were significantly improved. Metabolomics analysis showed that the contents of 110 non-volatile compounds were significantly different among black teas with different rolling treatments. In summary, re-rolling accelerated the oxidation of polyphenols into pigments, the hydrolysis of proteins into amino acids, and the metabolism of alkaloids, organic acids, glycosidically-bound volatiles, and lipids during the fermentation period. Our study provided a novel and simple way to improve black tea quality.

1. Introduction

Black tea is the most popular tea among six kinds of teas due to its fascinating flavor and accounts for approximately 75 % tea consumption in the world (Zhang et al., 2023; Zhang, Ho, Zhou, Santos, Armstrong & Granato, 2019). According to the manufacturing methods, there are two main classes of black teas: Congou and CTC (crush, tear, and curl) black teas. Congou black tea is a traditional Chinese black tea processed for about 400 years and present mellow or sweet-mellow taste, sweet and/ or floral aroma, and brightly red liquor color (Wu et al., 2022). Taste and liquor color are determined by non-volatile compounds. The main non-volatile compounds in black tea are catechins, theaflavins (TFs), thearubigins (TRs), theabrownins (TBs), caffeine, free amino acids, flavonoid glycosides, and phenolic acids (Zhang et al., 2019). TFs and TRs are the most characteristic substances conferring on black tea unique quality features differing from other kinds of teas.

The composition and concentration of flavor components in teas are largely affected by the manufacturing steps. The basic manufacturing steps of Congou black tea include withering, rolling, fermentation, and drying (Zhang et al., 2019). Of them, fermentation is the key processing step determining the flavor and quality of black tea. During this process, non-volatile substances undergo drastic changes, such as: catechins and other polyphenols are catalyzed into TFs by polyphenol oxidase (PPO), and further into TRs and TBs by peroxidase (POD) (Hua et al., 2022); proteins are hydrolyzed into free amino acids, which are further degraded into aroma components via Strecker degradation (Chen et al., 2020b); and RNAs are hydrolyzed into nucleotides, nucleosides, and alkaloids (Chen, Shi, Mu, Chen, Dai & Lin, 2020). These conversions confer on black tea its unique flavors. Owing to the importance of fermentation for black tea flavors, some studies have been made to explore the influences of fermentation conditions on black tea quality, such as fermentation time (Muthumani & Kumar, 2007), fermentation temperature (Hua et al., 2022), oxygen concentration in air (Chen et al., 2021), fermentation method (Hua et al., 2021), and cell fragmentation degree (Wu et al., 2022).

Various conversions occurred during the fermentation process take place under the premise of rolling. In fresh tea leaves, enzymes and corresponding substrates are generally distributed in the different subcellular regions. For instance, polyphenols are mainly stored in vacuole, while PPOs are mainly located in plastids (Xu et al., 2016). After rolling,

the cell structure is destroyed, which creates a chance for enzymes to adequately touch substrates and thus induces strong fermentation activities. However, as the fermentation progresses, biochemical conditions will develop to a direction that go against the accumulation of some important quality components, such as TFs and TRs (Muthumani and Kumar, 2007; Tan et al., 2016). TFs account for the formation of briskness and brightness, and TRs are responsible for the red color and taste strength of black tea infusion (Hua et al., 2021; Samanta, Cheeni, Das, Roy, Ghosh & Mitra, 2015). High-quality black tea requires a high content of both TFs and TRs, and that the ratio of TFs to TRs is about 1:10 (Jolvis Pou, 2016). It was reported that the contents of TFs and TRs greatly increased at first, and then decreased after reaching the maximum during the fermentation period (Muthumani and Kumar, 2007; Tan et al., 2016). This phenomenon may attribute to that the biochemical conditions of tea leaves, for instance the consumption of substrates (Hua et al., 2022), are not conducive to the accumulation of TFs and TRs as fermentation goes on. Thus, remodeling the biochemical conditions of tea leaves in the late fermentation period is beneficial to increase the contents of some quality components. Theoretically, re-rolling treatment will remodel the primary biochemical conditions in different parts of tea leaf cells during the late fermentation period and subsequently create a novel environment for various biochemical reactions, so as to influence black tea quality.

Therefore, the aims of this study were to evaluate the effect of re-rolling treatment on the qualities (especially the taste and liquor color) of black tea via sensory evaluation and chromatic aberration analysis, and to comprehensively investigate its influence on non-volatile compounds affecting taste and liquor color in black tea via a nontargeted metabolomic analysis.

2. Materials and methods

2.1. Chemicals and reagents

Deionized water was produced by a Milli-Q water purification system (Millipore, Billerica, Massachusetts). Liquid chromatography–mass spectrometry (LC–MS) grade methanol, formic acid, and acetonitrile were purchased from Merck Corporation (Darmstadt, Germany). Foline-phenol, ninhydrin, ethyl acetate, and *n*-butanol were purchased from Macklin Biochemical Co., Ltd (Shanghai, China). Na_2CO_3, $NaHCO_3$, and oxalic acid were purchased from J&K Scientific Corporation (Beijing, China). Authentic standards used for LC-MS based metabolomic analysis were obtained from Sigma Corporation (St. Louis, MO, USA) or Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

2.2. Manufacturing of black tea samples

Clonal Ningzhou No.2 (*Camellia sinensis* (L) O. Kuntze), a tea cultivar suitable to manufacturing black tea, was selected for our study. Fresh tea leaves (one bud and two leaves) were collected from the tea garden of Jiangxi Tonggu Tea Co., Ltd (Yichun, China) in July 5th, 2022. Approximately 25 kg fresh leaves were evenly spread on bamboo sieves

for indoor natural withering. Until the moisture content of tea leaves reached about 62 % after withering for about 19 h, tea leaves were then rolled using a specialized roller (Jiayou Machinery Co. Ltd., Quanzhou, China). Afterwards, rolled tea leaves were statically fermented at 30 °C with 90 % humidity in a specialized fermentation machine (JY-6CFJ-2B, Jiayou Machinery Co. Ltd., Quanzhou, China). The leaf thickness was about 8 cm. As the fermentation begins at the moment of rolling (Chen et al., 2022a; Jolvis Pou, 2016), the total time of rolling and fermenting were kept the same for three black tea samples. The rolling and fermenting durations for different samples were as follows: R1 underwent 1 h of rolling followed by 5 h of fermentation, R1.5 went through 1.5 h of rolling followed by 4.5 h of fermentation, and RR1.5 underwent a sequence of 1 h rolling, 2.5 h fermentation, 0.5 h re-rolling, and 2 h fermentation (Fig. 1). To ensure the inside and outside tea leaves can be evenly fermented, tea leaves were turned every 1.5 h. After ending fermentation, tea leaves were firstly dried at 110 °C for 20 min and then cooled for 30 min. Finally, tea leaves were secondly dried at 85 °C for 15 min to obtain black tea products. Black tea samples were stored at –20 °C and were ground into homogeneous powder using a mill (IKA Werke GmbH, Staufen, Germany) in prior to further analysis.

2.3. Sensory evaluation of black tea samples

Sensory evaluation was carried out according to the Chinese national standard of "Methodology of sensory evaluation of tea" (GB/T 23376–2018) and "Tea vocabulary for sensory evaluation" (GB/T 14487–2017). Non-powdered tea samples (3.0 g) were brewed with 150 mL boiled water for 5.0 min in a specialized cup. After brewing, tea infusions were transferred to a specialized bowl. Afterwards, the appearance, aroma, liquor color, taste, and brewed leaves were evaluated by three experienced assessors authenticated by professional organizations.

2.4. Chromatic aberration analysis of tea liquors

Tea liquors were prepared according to above sensory evaluation method, and the CIELAB parameters of tea liquors were determined by a CR-400 chroma meter (Konica Minolta, Inc. Japan). The lightness (L*), red/green coordinates (a*), and yellow/blue coordinates (b*) were recorded for 20 measurement points per sample. Each sample was determined for three times.

2.5. Determination of water extracts, total polyphenols, and total amino acids

Determination of water extracts was carried out according to the Chinese national standard of "Determination of water extracts content" (GB/T 8305–2013). Accurate 2.0 g of tea powders were extracted using 500 mL boiling water at 100 °C for 45 min, shaking once 10 min. Afterwards, tea liquor was filtered to obtain tea residues. Tea residues were dried at 120 °C for 2 h and subsequently weighed. The content of water extract is 100 % minus the percentage of residue, which represents
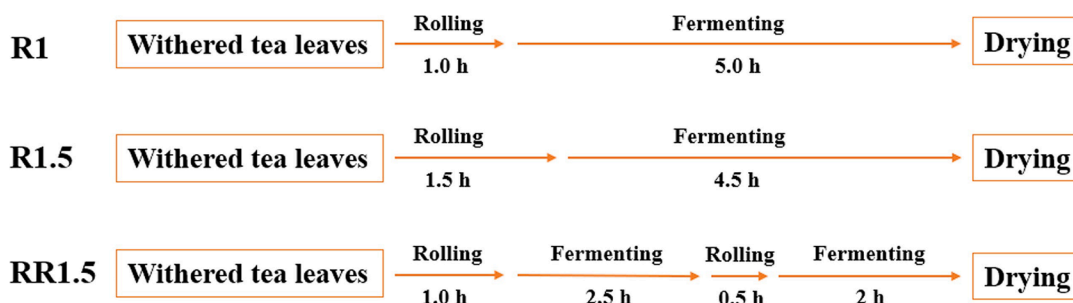

R1	Withered tea leaves	Rolling 1.0 h →	Fermenting 5.0 h →	Drying

R1.5	Withered tea leaves	Rolling 1.5 h →	Fermenting 4.5 h →	Drying

RR1.5	Withered tea leaves	Rolling 1.0 h →	Fermenting 2.5 h →	Rolling 0.5 h →	Fermenting 2 h →	Drying

Fig. 1. Processing schemes of three black teas with different rolling treatment (R1, R1.5, and RR1.5).

the weight proportion of water-soluble compounds in a tea.

Determination of total polyphenols was carried out according to the Chinese national standard of "Determination of total polyphenols and catechins content in tea" (GB/T 23376–2018). Accurate 0.2 g of tea powders were extracted using 5 mL 70 % (v/v) methanol at 70 °C for 10 min. The solutions were then centrifuged at 3500 r/min (Centrifuge 5810R, Eppendorf; Hamburg, Germany) for 10 min and the supernatants were collected. The sediments were extracted again, and the supernatants were collected again and combined with the first supernatants together. After mixing well, the supernatants were diluted 100 times using distilled water. Next, accurate 1 mL diluted liquor was mixed with 5 mL 10 % (v/v) foline-phenol. After reacting for 8 min, 4 mL 7.5 % (w/v) Na_2CO_3 were added and mixed well. The solutions were subsequently equilibrated for 60 min before measuring the absorbance. The absorbance was measured using a UV/Vis spectrophotometer (Shimadzu UV 2550, Kyoto, Japan). The wavelength was 765 nm and the cuvette was 10 mm. Distilled water and gallic acid were used as a black and to construct calibration curve, respectively.

Determination of total amino acids was carried out according to the Chinese national standard of "Tea-Determination of free amino acids content" (GB/T 8314–2013). Accurate 3.0 g of tea powders were extracted using 450 mL boiling water at 100 °C for 45 min (shaking once 10 min). The filter liquor was collected and the residue was washed with a little hot water. After cooling to room temperature, the filter liquor was filled to 500 mL with distilled water. The reacting solution consisted of 1 mL extraction liquor, 0.5 mL phosphate buffer (pH 8.0), and 0.5 mL 2 % (w/v) ninhydrin solution. After reacting for 15 min in boiling water, the solution was filled to 25 mL with distilled water and equilibrated for 10 min. The determination wavelength was 570 nm and the cuvette was 10 mm. Distilled water and theanine were used as a black and to construct calibration curve, respectively.

2.6. Determination of TFs, TRs, and TBs

TF, TR, and TB were measured using a spectrophotometric method referred to a previous study (Hua et al., 2021). Powdered tea samples (3.0 g) were brewed in 150 mL boiled water at 100 °C for 10 min. After filtering and cooling to room temperature, tea liquors were extracted to form four parts to severally measure absorbance values named as Ea, Eb, Ec, and Ed. Tea Liquor (30 mL) was firstly mixed with 30 mL of ethyl acetate in a funnel and shaken for 5 min. The ethyl acetate layer (2 mL) was diluted to 25 mL with 95 % (v/v) ethanol. The absorbance of this solution was named as Ea. Another 15 mL of ethyl acetate layer was mixed with15 mL of 2.5 % (w/v) NaHCO₃ solution and strongly shaken for 30 s. After standing layering, the upper ethyl acetate layer (4 mL) was diluted to 25 mL with 95 % (v/v) ethanol. The absorption of this solution was named as Ec. The first aqueous layer (2 mL) was mixed with 2 mL of saturated oxalic acid and 6 mL of water, and then diluted to 25 mL with 95 % (v/v) ethanol. The absorbance of this solution was named as Ed. Finally, tea liquor (15 mL) was mixed with 15 mL of *n*-butanol and shaken for 3 min in a separating funnel. The aqueous layer (2 mL) was mixed with 2 mL of saturated oxalic acid solution and 6 mL of water, and then diluted to 25 mL with 95 % (v/v) ethanol. The absorbance of this solution was named as Eb. The absorbance was measured with a UV/Vis spectrophotometer (Shimadzu UV 2550, Kyoto, Japan) at 380 nm with 95 % (v/v) of ethanol as a blank. The contents of total TFs, TRs, and TBs were obtained through the following formulates:

TF% = 2.25 × Ec/(1 − M).
TR% = 7.06×(2Ea + 2Ed − 2Eb − Ec)/(1 − M).
TB% = 7.06 × 2Eb/(1 − M).

In above formulates, M is the moisture content of tea sample.

2.7. Nontargeted metabolomics analysis

Extraction of non-volatile compounds was based on a previous work with slight modifications (Chen et al., 2020a). Accurate 0.1 g of tea powders were placed into a 15 mL centrifuge tube (Corning; NY, USA), and then 10 mL deionized water was added into the tubes. The tubes were incubated in boiling water (100 °C) for 10 min for extracting tea metabolites, with shaking once at 5 min. After cooling to room temperature, the solutions were centrifuged at 8 000 *g* (Centrifuge 5810R, Eppendorf; Hamburg, Germany) for 10 min at 4 °C, and the supernatants were filtered through a 0.22 μm membrane before metabolomics analysis.

An ultrahigh-performance liquid chromatography-quadrupole/orbitrap mass spectrometer (UHPLC-Q-Exactive/MS) system (Thermo Fisher Scientific; Waltham, MA, USA) was used for nontargeted metabolomics analysis. The chromatographic separation of metabolites was performed on an Acquity UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm, Waters, Manchester, UK), and the column temperature was set at 40 ℃. The mobile phase A and B was 0.1 % formic acid (v/v) solution and methanol (100 %), respectively. The gradient elution program was as follows: 0 min, 2 % B; 0.5 min, 2 % B; 8 min, 15 % B; 13 min, 35 % B; 15 min, 70 % B; 16 min, 85 % B; 16.5 min, 2 % B and 20 min, 2 % B. The injection volume was 3 μL. MS parameters were as follows: the source voltage was 3.5 kV; the capillary voltage for ESI + mode and temperature were set as 49 V and 300 °C, respectively; the flow rate and temperature of drying gas were maintained at 10 L/min and 325 °C, respectively; the mass scan range was *m/z* 80–1200; and the resolution of orbit-trap was 30000.

The raw data files were processed using Compound Discoverer 3.2 software (Thermo Fisher Scientific; Waltham, MA, USA) for peak picking and alignment with a mass width of 20 ppm and a retention time width of 0.2 min. After peak alignment, the individual mass intensities were normalized to the total mass intensity.

2.8. Data processing method

Principal component analysis (PCA) was performed using Simca-P 13.0 software (Umetrics AB, Umea, Sweden), and the data were auto-scaled (the data were centralized and then divided by the standard deviation). The difference significances were calculated by one-way analysis of variance (ANOVA) and Tukey s-b(K) test using a PASW Statistics software (Version 18.0, USA) with a threshold of $P < 0.05$. Heat-map of differential compounds was generated by MultiExperiment Viewer 4.8.1 (Oracle Corporation, Redwood, CA, USA) after the data were auto-scaled.

3. Results

3.1. Effect of re-rolling treatment on the sensory qualities of black tea

Sensory evaluation results of three black teas were shown in Table 1. In addition, the appearance, liquor, and brewed leaves of three black teas were photographed and shown in Fig. 2 to more intuitively display

Table 1
Sensory evaluation results of three black teas with different rolling treatment.

Factor	R1	R1.5	RR1.5
Appearance	Approach tight, auburn, have golden pekoe (85.0)	Tight, approach black, with little golden pekoe (86.0)	Tight, black (85.5)
Liquor color	Yellow, bright (88.5)	Orange, bright (90.0)	Orange red, brighter (91.0)
Aroma	Sweet, with little green scent (86.0)	Sweet, pure (89.0)	Sweet, with floral scent (91.5)
Taste	Approach mellow, with little astringency (86.0)	Mellow (88.0)	Mellow and thick (89.5)
Brewed leaves	Less even red (84.0)	Approach even red (86.0)	Approach even red (86.5)

Note: the number means the score.

Fig. 2. Appearance, liquor color, and brewed leaves of three black teas with different rolling treatment (R1, R1.5, and RR1.5). A: appearance; B: liquor color; C: brewed leaves.

the differences among three black teas. Compared to R1 and R1.5, RR1.5 presented tighter and darker appearance, redder liquor, thicker taste, sweeter aroma, and redder brewed leaves. The score of taste for R1, R1.5, and RR1.5 were 86.0, 88.0, and 89.5, respectively, and the score of liquor color for R1, R1.5, and RR1.5 were 88.5, 90.0, and 91.0, respectively (Table 1). These results demonstrated that re-rolling treatment was beneficial to improve the taste and liquor color qualities of black tea.

3.2. Effect of re-rolling treatment on the chromatic aberration of tea liquor

As shown in Fig. 3-A. The values of a* (representing red color) and L* (representing luminance) were significantly increased, while the value of b* (representing yellow color) was gradually decreased from R1 to RR1.5. Comparing with R1.5, the values of a* and L of RR1.5 were further increased (10.75 % and 11.37 %, respectively) and the value of b* was further decreased (9.37 %) (Fig. 3-A). These results further proved that re-rolling treatment was beneficial to improve the liquor color quality of black tea.
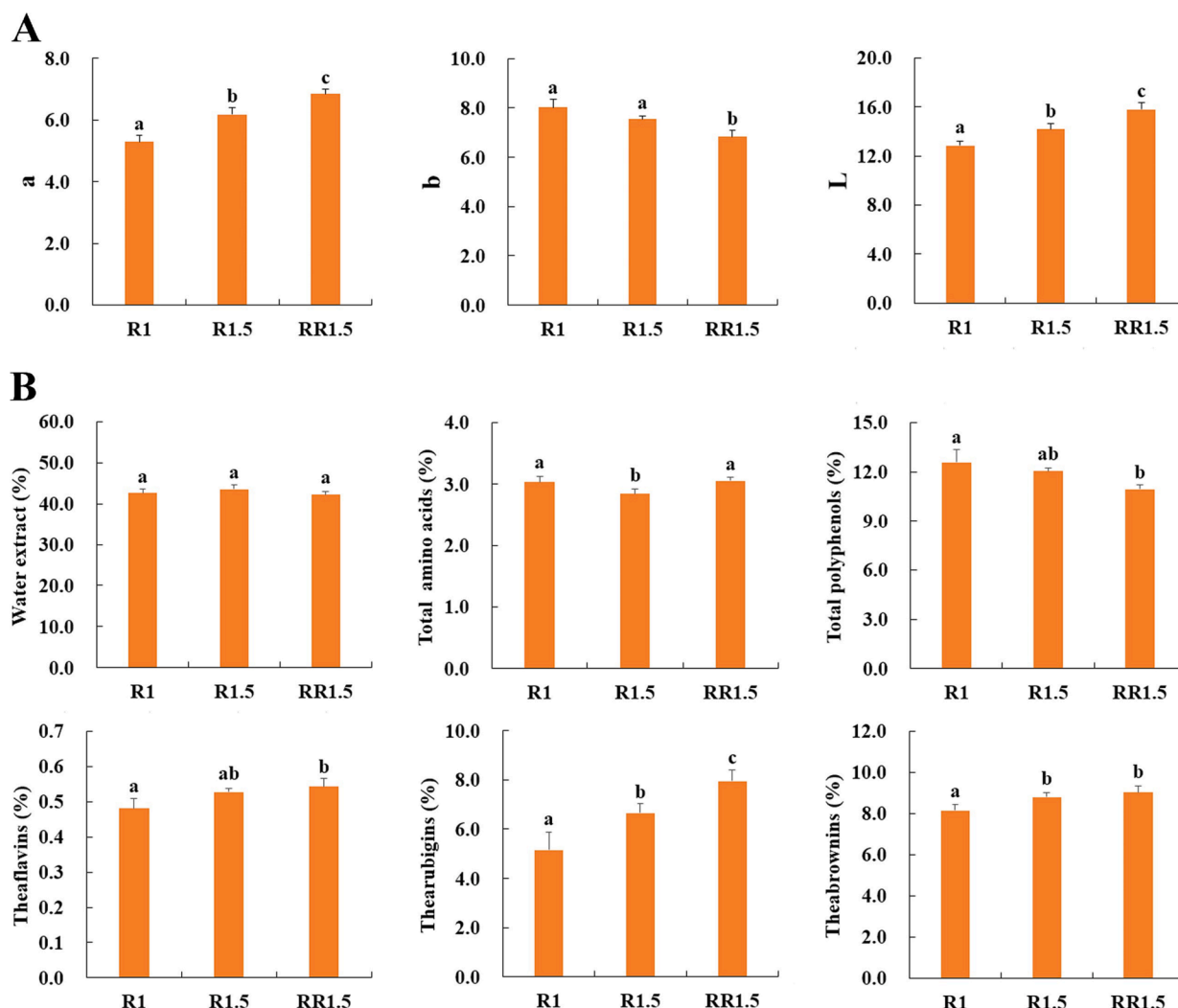
Fig. 3. Comparison of liquor color attributes and main biochemistry components among three black teas with different rolling treatment (R1, R1.5, and RR1.5). A: liquor color attributes; B: main biochemistry components.

3.3. Effect of re-rolling treatment on the general biochemistry components

Tea taste and liquor color are closely related to non-volatile compounds; thus, the general biochemistry components were determined. Water extracts is the total of all soluble components extracted by hot water and is positively correlated with taste thickness. The contents of water extracts in R1, R1.5, and RR1.5 were 42. 63 %, 43.52 %, and 42.29 %, respectively, and there was no significant difference in the content of water extracts among three black teas (Fig. 3-B). The content of total amino acids in R1, R1.5, and RR1.5 were 3.04 %, 2.85 %, and 3.06 %, respectively (Fig. 3-B). Compared to R1, R1.5 had significant lower content of total amino acids (Fig. 3-B), which is due to that the content of total amino acids is negatively correlated with the rolling time in black teas (Wu et al., 2022). Compared to R1.5, the content of total amino acids in RR1.5 was significantly increased and equal to that in R1 (Fig. 3-B). The content of total polyphenols in R1.5 (12.05 %) was significantly lower than that in R1 (12.58 %), and was further reduced in RR1.5 (10.94 %).

TFs, TRs, and TBs are the oxidative products of polyphenols catalyzed by PPO and POD (Zhang et al., 2019). The contents of TFs, TRs, and TBs were significantly increased from R1 to RR1.5, and the increase extent of TRs was larger than that of TFs and TBs (Fig. 3-C and Table S3). This means that the extension of rolling time and re-rolling treatment have the greatest effect on the formation of TRs, following by TFs and TBs.

3.4. Effect of re-rolling treatment on non-volatile compounds based on metabolomics analysis

To further deeply understand the effect of re-rolling treatment on black tea quality components, non-targeted metabolomics analysis was employed to compare the non-volatile compounds among three black tea samples. Based on authentic standards, MS^2 spectra, metabolomics database, and previous studies (Gao et al., 2022; Zhao et al., 2018), 140 non-volatile compounds were ultimately identified, covering 19 amino acids, 19 flavones/flavonols/flavanols, 15 dimeric flavanols, 30 flavone/flavonol glycosides, 10 phenolic acids, 20 alkaloids, 6 organic acids, 2 sugars, 6 glycosidically-bound volatiles (GBVs), 9 lipids, and 4 other compounds (Table S1).

The differential compounds between any two samples were screened by ANOVA ($P < 0.05$). There were 101, 85, and 60 differential compounds in the R1 vs R1.5, R1 vs RR1.5, and R1.5 vs RR1.5 groups, respectively; and 27 non-volatile compounds showed statistical differences in all three comparison groups (Table S2 and Fig. S1). Comparing with R1, the levels of most differential metabolites for R1.5 (5 up, 96 down) and RR1.5 (7 up, 78 down) were significantly down-regulated (Table S2). Enzymatic oxidation of polyphenols (such as catechins, flavone/flavonol glycosides, procyanidins, and phenolic acids), Strecker

degradation of amino acids, oxidative degradation of fatty acids and carotenoids, and hydrolysis of GBVs (Ho, Zheng & Li, 2015; Jolvis Pou, 2016) are occurred during the fermentation of black tea. Predictably, R1.5 and RR1.5 had higher fermentation degree comparing with R1 owing to the longer rolling time. This explained that most differential compounds showed lower levels in R1.5 and RR1.5 compared with R1 (Table S2). Comparing with R1.5, up- and down-regulated metabolites in RR1.5 were 45 and 15, respectively (Table S2). The number of decreased metabolites was dramatically less than that of increased metabolites, indicating that the fermentation degree of RR1.5 was inferior

to that of R1.5.

Further, we screened and compared the top differential compounds (TDCs) with high fold change (FC), and the results were shown in Fig. 4. Compared with R1, up-regulated TDCs in R1.5 were hydroxyhexadecanoic acid, sphinganine, palmitic acid, phytosphingosine, and theaflavin-3-gallate; down-regulated TDCs were EC-(4alpha->8)-ECG, linalool primeveroside, EGC 3,5-digallate, GMP, chlorogenic acid isomer, EC-(4beta->8)-EGCG, theasinensin F, GCG, theanine glucoside, GC, adenosine, and C amongst others (Fig. 4-A). Compared with R1, up-regulated TDCs in RR1.5 were quercetin 3-(3-p-coumaroylglucoside),
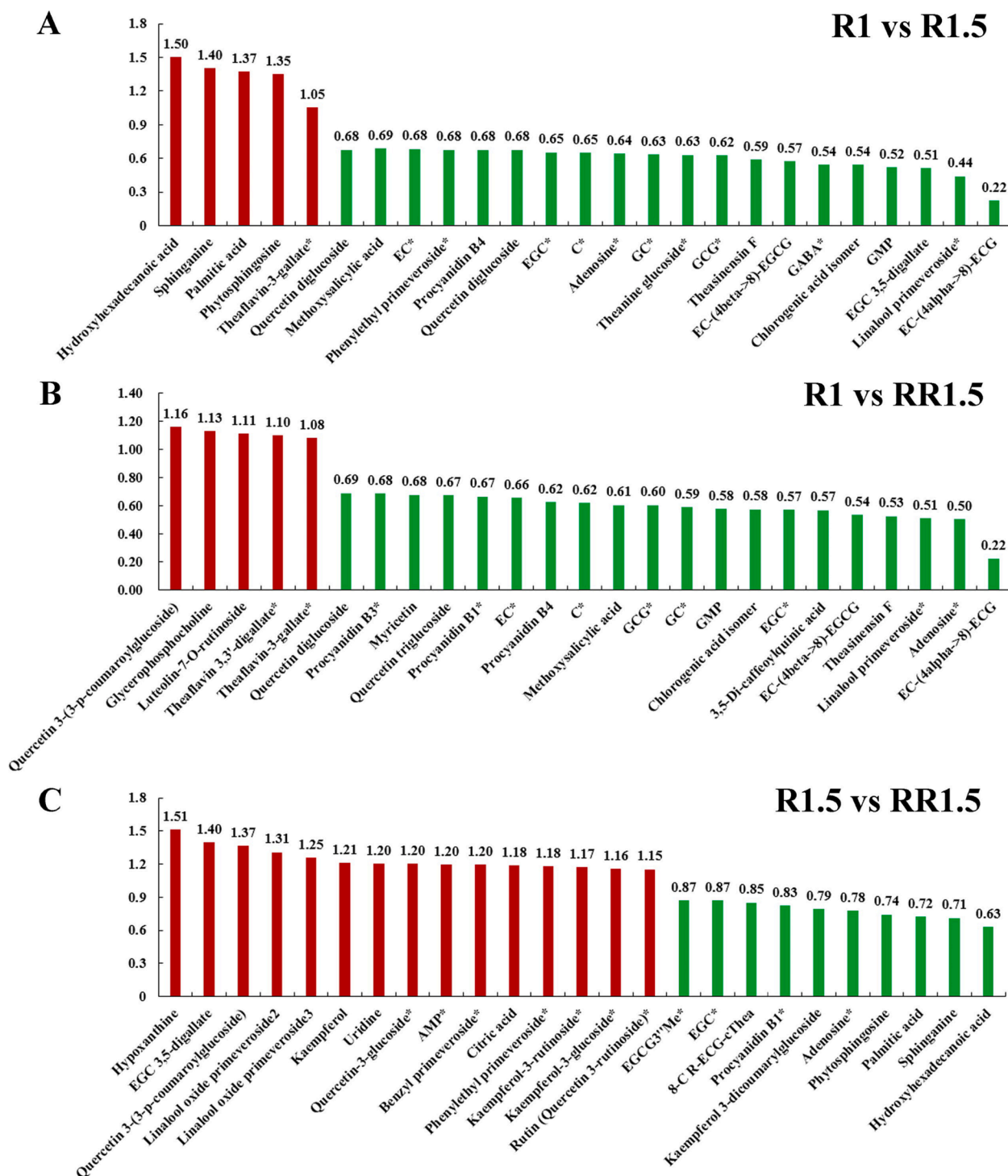

Fig. 4. Bar chart of top differential compounds. Red: up-regulated compounds; green: down-regulated compounds. The number above the column means fold change. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

glycerophosphocholine, luteolin-7-o-rutinoside, theaflavin 3,3′-digallate, and theaflavin-3-gallate; down-regulated TDCs were similar to these in R1.5 (Fig. 4-B). As down-regulated TDCs in both R1.5 and RR1.5 are mainly phenolic compounds, it supports that the oxidation of polyphenols is the dominant reaction during black tea fermentation (Jolvis Pou, 2016). Compared with R1.5, up-regulated TDCs in RR1.5 were mainly flavone/flavonol glycosides and GBVs, including hypoxanthine, EGC 3,5-digallate, quercetin 3-(3-p-coumaroylglucoside), linalool oxide primeveroside 2, linalool oxide primeveroside 3, kaempferol, uridine, quercetin-3-glucoside, AMP, benzyl primeveroside, citric acid, and phenylethyl primeveroside amongst others; down-regulated TDCs were hydroxyhexadecanoic acid, sphinganine, palmitic acid, phytosphingosine, adenosine, and kaempferol 3-dicoumarylglucoside amongst others (Fig. 4-C). GBVs and fatty acids are important aroma precursors in teas (Ho et al., 2015).

3.5. Overall comparisons of non-volatile compounds among the R1, R1.5, and RR1.5 black teas

Based on the peak areas of 140 non-volatile compounds, PCA was applied to overview the overall difference among the R1, R1.5, and RR1.5 black teas. As shown in Fig. 5-A, R1.5 and RR1.5 were separated from R1 by PC1 (explained 47.3 % total variance), and RR1.5 was separated from R1.5 and R1 by PC2 (explained 15.4 % total variance). This indicated that the levels of non-volatile compounds were obviously different among R1, R1.5, and RR1.5. In addition, RR1.5 located between R1 and R1.5, which once more supported that the fermentation degree of RR1.5 was inferior to that of R1.5. To better understand the effect of re-rolling treatment on quality components, significantly changed compounds were screened using a Tukey s-b(K) test with a criterion of $P < 0.05$. In total, 110 non-volatile compounds were screened (Fig. 5-B).

Amino acids are one of vital contributors to black tea taste and nutrition. In this study, the levels of 14 amino acids, including theanine, isoleucine, methionine, phenylalanine, GABA, and tryptophan etc., significantly differed among R1, R1.5, and RR1.5 (Fig. 5-B). Compared with R1, the levels of all differential amino acids in R1.5 were significantly decreased, implying that the extend of rolling time is not beneficial to the accumulation of free amino acids. This result is consistent with that in a previous study (Wu et al., 2022). The levels of most amino acids, including isoleucine, lysine, tryptophan, tyrosine, glutamic acid, proline, and valine, in RR1.5 were significantly higher than that in R1.5, and the levels of glutamic acid and valine were even higher than that in R1 (Fig. 5-B). The changes in the levels of individual amino acids were consistent with that of the total amino acids (Fig. 3-B).

Flavones, flavonols, and flavanols (catechins) derive from the flavonoid biosynthesis pathway in plants and all have a 2-phenyl-4H-chromene structure (Liu et al., 2021). As shown in Fig. 5-B, the levels of most flavones/flavonols/flavanols in R1.5 were significantly lower than that in R1 ($P < 0.05$). Compared with R1.5, the levels of most flavones/flavonols/flavanols, especially EGCG, EGC, and EGCG3″Me, were further decreased in RR1.5, while the levels of quercetin, kaempferol, epiafzelechin, and EGC 3,5-digallate were obviously increased (Fig. 5-B and Table S2).

Dimeric flavanols, such as TFs, procyanidins, and theasinensins, are polymerized from flavanols (catechins) under the action of a series of enzymes. TFs and theasinensins are simultaneous oxidized products of catechins, and they are competitively formed (Xu, Jiang, Zhang, Yang & Liu, 2015). Except for TFs, the contents of other dimeric flavanols in R1.5 and RR1.5 were significantly lower than that in R1 (Fig. 5-B). Unlike procyanidins and theasinensins, individual TFs showed diverse changes. Compared with R1, the levels of theaflavin in R1.5 was significantly decreased, and the level of theaflavin-3-gallate was opposite. Nevertheless, the levels of theaflavin, theaflavin-3-gallate, and theaflavin 3,3′-digallate in RR1.5 were significantly increased compared with R1 and R1.5 (Fig. 5-B and Table S1).

Flavone/flavonol glycosides are also important astringent compounds in teas (Scharbert & Hofmann, 2005). The glycosides of apigenin, kaempferol, quercetin, myricetin, and luteolin were detected in this study (Fig. 5-B and Table S1). Compared with R1, the levels of most flavone/flavonol glycosides in R1.5 and RR1.5 were significantly reduced. Compared with R1.5, flavone/flavonol glycosides in RR1.5 showed two alteration patterns: the levels of kaempferol-3-arabinoside, kaempferol-3-galactoside, and quercetin-3-glucoside etc. were significantly increased; while the levels of kaempferol-3-dicoumarylglucoside, quercetin-3-galactoside, and kaempferol-3-O-acetyl-glucoside etc. were slightly decreased (Fig. 5-B). These results indicated that re-rolling treatment was not beneficial to promote the oxidation of flavone and flavonol glycosides, which differed from the flavanols (catechins) and procyanidins.

Phenolic acids are a class of aromatic compounds with phenolic skeleton and carboxyl acid group. As shown in Fig. 5-B, the levels of most phenolic acids, including chlorogenic acid, theogallin, 4-coumaroylquinic acid, and 4-O-caffeoylquinic acid etc., in R1.5 and RR1.5 were significantly lower than that in R1, which was consistent with other phenolic compounds. Compared with R1.5, the levels of 4-coumaroylquinic acid, 4-O-caffeoylquinic acid, and 3-coumaroylquinic acid were significantly increased, and methoxysalicylic acid showed a reverse trend in RR1.5. The level of gallic acid, a well-known phenolic acid in teas, remained stable among three black teas ($P > 0.05$, Table S1).

Alkaloids are a class of alkaline nitrogen-containing organic compounds widely distributed in organisms (Zhang, Jin, Chen, Ercisli & Chen, 2022a). In tea plant, alkaloids are mainly purine alkaloids, such as caffeine, theobromine, theophylline, and adenine etc., followed by a small number of pyrimidine alkaloids (Zhang et al., 2022a). The levels of theobromine, cytosine, adenine, guanine, uracil, 5′-methylthioadenosine, and adenosine were gradually decreased from R1 to RR1.5; the levels of CMP, GMP, UMP, AMP, uridine, 5′-deoxy-5′-(methylsulfinyl)adenosine, and hypoxanthine were significantly decreased and then increased from R1 to RR1.5 (Fig. 5-B). In total, the levels of most alkaloids in R1.5 and RR1.5 were significantly lower than that in R1.

Organic acids are important contributors to the sourness taste and health promoting components in teas (Zhang et al., 2022b). As shown in Fig. 5-B, the levels of citric acid, pantothenic acid, and gluconic acid showed significant difference among R1, R1.5 and RR1.5.

GBVs are important precursors of aroma compounds (Song, Hartl, McGraphery, Hoffmann & Schwab, 2018). In this study, 6 aroma primeverosides were detected, and they were most hydrolyzed in R1.5, followed by RR1.5 and R1 (Fig. 5-B). Lipids are also important precursors of aroma compounds in teas (Li et al., 2017). The levels of palmitic acid, hydroxyhexadecanoic acid, sphinganine, and phytosphingosine in R1.5 were significantly higher than that in R1 and RR1.5, while the levels of LysoPC(18:3) and LysoPC(18:2) showed gradual decrease from R1 to RR1.5 (Fig. 5-B). The different alterations in the GBV and lipid contents would affect the aroma of R1, R1.5 and RR1.5 black teas (Table 1).

In addition, the levels of theanine glucoside, phaeophorbide b, and 8-C R-ECG-cThea were also significantly different among R1, R1.5 and RR1.5 (Fig. 5-B). Theanine glucoside is a newly discovered compound formed from theanine and glucose and our previous study showed that it increases with the prolong of black tea fermentation (Tan et al., 2016). 8-C R-ECG-cThea is also newly discovered compound formed from theanine and ECG in our previous study (Dai et al., 2018).

4. Discussion

4.1. Re-rolling treatment selectively accelerated the oxidation of catechins and procyanidins into pigments

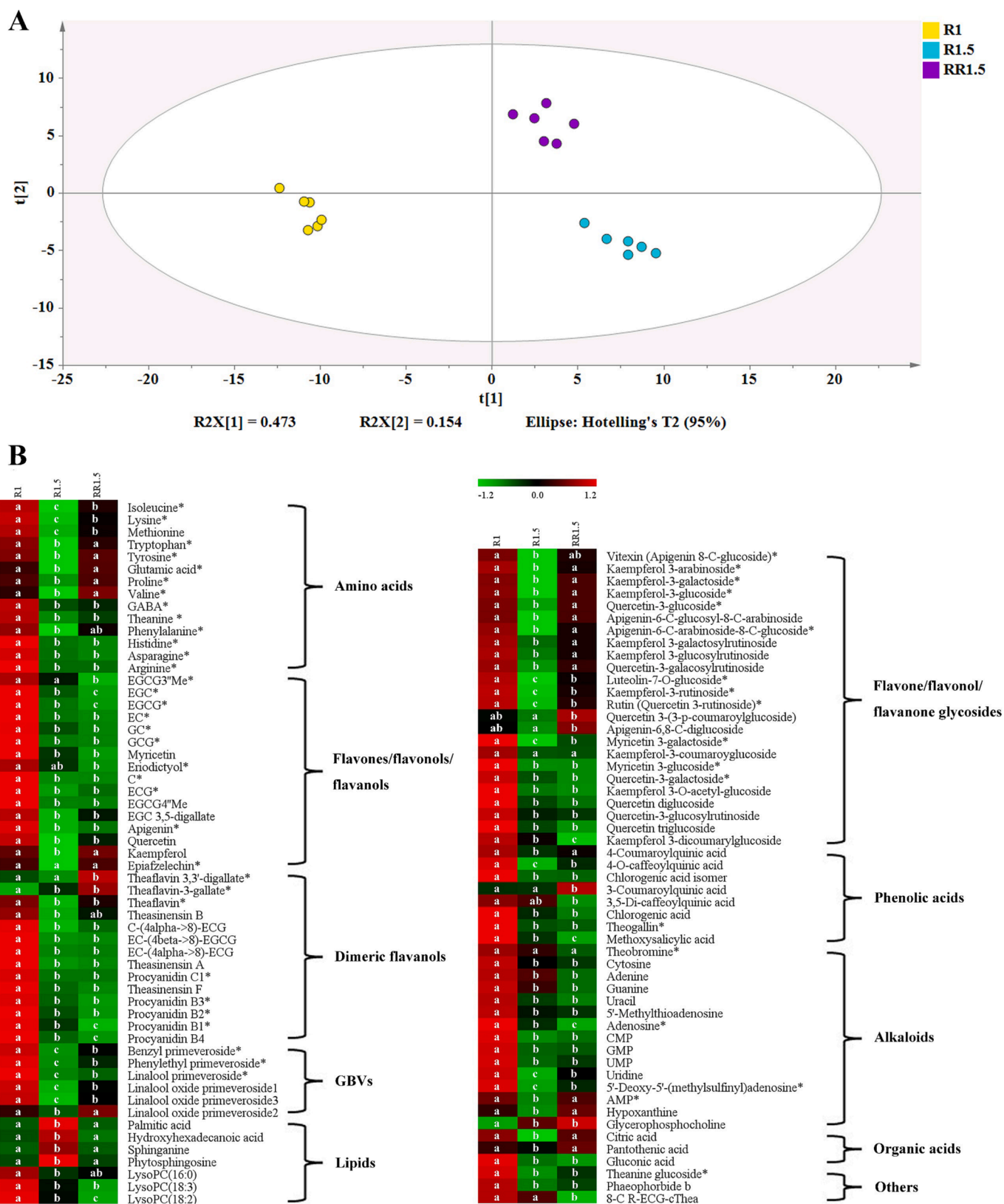Tea polyphenols are the dominant non-volatile compounds in teas

Fig. 5. Metabolomics analysis of the black teas with different rolling treatment (R1, R1.5, and RR1.5). A: PCA score plot ($R^2X = 0.627$, $Q^2 = 0.484$) of three black teas; B: Heat-map of the levels of differential nonvolatile compounds. The data for PCA and heat map were both auto-scaled. a, b, c: $P < 0.05$ for the changes with different letters (Tukey s-b (K) test).

and are also the precursors for synthesizing TFs, TRs, and TBs (Hua et al., 2022). In this study, the level of total polyphenols was significantly decreased, while the levels of TFs, TRs, and TBs were significantly increased from R1 to RR1.5 (Fig. 3-B). This implied that polyphenols were consumed to synthesize TFs, TRs, and TBs. In fresh tea leaves, polyphenols consist of flavanols (catechins), flavones and flavonols, flavone/flavonol glycosides, procyanidins, theasinensins, catechin dimers, and phenolic acids (Liu, Bruins, de Bruijn & Vincken, 2020). Compared with R1, the levels of almost all phenolic compounds in R1.5 were significantly decreased (Fig. 5-B and Table S2), which agreed with the contents of phenolic compounds in black tea are negatively correlated the rolling time (Wu et al., 2022). However, the levels of most flavones, flavonols, catechin dimers, theasinensins, and flavone/flavonol glycosides in RR1.5 were equal or significantly increased compared with R1.5, and only the levels of some catechins and procyanidins were significantly decreased (Fig. 5-B and Table S2). The above results jointly indicated that re-rolling treatment selectively accelerated the oxidation of catechins and procyanidins into pigments.

During the fermentation period, the contents of TFs and TRs generally increase at first, and then decrease after reaching the maximum (Muthumani and Kumar, 2007; Tan et al., 2016). This may owe to that the biochemical conditions in tea leaf cells are not conducive to the accumulation of TFs and TRs in the later fermentation stage, such as the consumption of substrates (Hua et al., 2022) and the effect of product inhibition (Wright, Mphangwe, Nyirenda & Apostolides, 2002). After re-rolling treatment, the spatial distributions of enzymes and substrates in tea leaf cells are reconstructed, which allows the enzymes, such as PPO and POD, to sufficiently contact with substrates again and reduce the product inhibition effect to enzymes. This reconstitution renews a relatively suitable circumstances for synthesizing TFs and TRs, which is similar to the initial rolling step. As a result, re-rolling treatment reduced the levels of flavanols (Fig. 5-B) and improved the levels of TFs and TRs (Fig. 3-B).

4.2. Re-rolling treatment accelerated the hydrolysis of proteins into free amino acids

In this study, the content of total amino acids in R1.5 (2.85 %) was significantly lower than that in R1 (3.04 %), while the content of total amino acids in RR1.5 (3.06 %) was significantly higher than that in R1.5 and was equal to that in R1 (Fig. 3-B). The difference in the contents of total amino acids among R1, R1.5, and RR1.5 is attributed to their different fermentation processes. During the fermentation period, free amino acids are degraded into volatile compounds or other small-molecule substance via Strecker and enzymatic degradation (Dudareva, Klempien, Muhlemann & Kaplan, 2013; Ho et al., 2015); on the other hand, proteins are enzymatically degraded to free amino acids (Chen et al., 2020b). Perhaps owing to the degradation rate is usually greater than the replenishment rate, the content of total free amino acids tends to decrease during the fermentation period (Chen et al., 2020b; Liu et al., 2023). However, some amino acids showed opposite change trends. Tan et al (2016) reported that glutamine, valine, proline, and tryptophan showed significant increasing during the fermenting process. In addition, the contents of glutamine, glycine, asparagine, and threonine were also showed be significantly increased after fermentation (Chen et al., 2020b; Yılmaz, Özdemir & Gökmen, 2020). It implied that re-rolling treatment influenced the degradation of proteins into free amino acids and/or the degradation of amino acids during the fermentation period.

Actually, fermentation activities also occur during the rolling step (Chen et al., 2022a; Jolvis Pou, 2016). It was found that the levels of glutamic acid, asparagine, aspartic acid, tryptophan, alanine, and serine showed significantly increasing after rolling (Chen et al., 2020b; Yılmaz et al., 2020); specially, the increase degree of glutamic acid, asparagine, and tryptophan after rolling was greater than that after withering (Yılmaz et al., 2020). Above results indicate that rolling is beneficial to

promote the hydrolysis of proteins into free amino acids. In our study, the levels of theanine and GABA, two non-proteinaceous amino acids, in RR1.5 were equal to that in R1.5 ($P > 0.05$; Fig. 5-B), indicating that re-rolling treatment have no significant effect on the degradation of amino acids. However, the levels of glutamic acid, tryptophan, isoleucine, lysine, tyrosine, proline, methionine, and valine in RR1.5 were significantly higher than that in R1.5 ($P < 0.05$; Fig. 5-B), which confirmed that re-rolling treatment facilitated the hydrolysis of proteins into free amino acids.

4.3. Re-rolling treatment improved the taste quality of black tea via enhancing the mellowness and thickness

A cup of good black tea infusion requires a mellow or sweet-mellow taste (Wang et al., 2014). The taste of R1 black tea was approach mellow with little astringency (Table 1). Astringency is an undesired taste for teas, and it was disappeared in the R1.5 and RR1.5 black teas. In addition, RR1.5 black tea had a thicker taste compared with the R1 and R1.5 black teas. Above results showed that re-rolling treatment improved the taste quality of black tea via reducing the astringency and promoting the mellowness and thickness.

In teas, astringency is caused by polyphenols, including flavanols (catechins) (Scharbert and Hofmann, 2005), flavone and flavonols (Zhao, Lin, Zhang, Lin, Yang & Ye, 2014), TFs (Samanta et al., 2015), flavone and flavonol glycosides (Scharbert and Hofmann, 2005), procyanidins (Zhang, Cao, Granato, Xu & Ho, 2020), theasinensins (Zhang et al., 2023), and phenolic acids (Chen et al., 2022b). Of them, flavanols (catechins), flavonols, procyanidins, and theasinensins are also contributors to the bitter taste (Preys et al., 2006; Scharbert and Hofmann, 2005; Zhang et al., 2023; Zhang et al., 2020). From R1 to RR1.5, the contents of total polyphenols were significantly decreased (Fig. 3-B), which agrees with the loss of astringency taste in the R1.5 and RR1.5 black teas (Table 1). In terms of various kinds of phenolic compounds, they displayed various change trends (Fig. 5-B). The levels of almost all phenolic compounds in R1.5 were significantly lower than that in R1. In RR1.5, the levels of most flavanols (catechins) and procyanidins, especially EGCG, EGC, EGCG3″Me, procyanidin B1, and procyanidin B4, were further decreased, while the levels of kaempferol, epiafzelechin, TFs, and most flavone and flavonol glycosides were significantly increased and equal to that in R1 (Fig. 5-B and Table S2). The decreasing intensity of flavanols (catechins) and procyanidins was larger than that of other phenolic compounds (Fig. 4). In addition, flavanols (catechins) account for 70–80 % of the total polyphenols (Chen et al., 2022b). These results indicated that the decreasing of flavanols (catechins), following by procyanidins, was the principal cause for the reduction of polyphenols and the loss of astringency in R1.5 and RR1.5.

TFs and TRs are the oxidation products of polyphenols. TFs contribute to the briskness taste of black tea infusion, and TRs contribute to the mouthfeel (thickness) and sweet taste (Hua et al., 2021; Samanta et al., 2015). The contents of TFs and TRs were significantly increased from R1 to RR1.5 (Fig. 3-B), which was consistent with that the mellowness and thickness of taste was gradually increased from R1 to RR1.5 (Table 1).

In addition to phenolic compounds, taste components also include free amino acids, alkaloids, nucleosides, and nucleotides etc. Free amino acids are important taste and nutrition components in tea (Wu et al., 2022). Various amino acids present different taste characteristics. Theanine, aspartic acid, and glutamic acid present umami taste; glycine, serine, alanine, proline, and threonine present sweet taste; and histidine, methionine, valine, arginine, leucine, isoleucine, phenylalanine, and tryptophan present bitter taste (Zhang et al., 2017). The various alterations in the levels of free amino acids have a complex effect on the taste of R1, R1.5, and RR1.5 black teas. Purine alkaloids are the important contributors to bitter taste (Zhang et al., 2020), while nucleosides and nucleotides have different taste characteristics. Adenosine was reported to enhance sweet taste (Dando, Dvoryanchikov, Pereira, Chaudhari &

Roper, 2012). GMP, IMP, XMP, and AMP are important contributors to the umami taste (Manninen, Rotola-Pukkila, Aisala, Hopia & Laaksonen, 2018). The alterations in the levels of alkaloids are one of the factors for the taste difference among R1, R1.5, and RR1.5.

4.4. Re-rolling treatment improved the liquor color quality of black tea via increasing the contents of TFs and TRs

Liquor color is an important quality factor for black teas. High-quality black tea requires a reddish and bright liquor color (Wang et al., 2014), especially with a golden-yellow rim on the surface of liquor (Hua et al., 2022). From R1 to RR1.5, the liquor color changed from yellow to red (Fig. 2-B), and the values of a* (representing red color) and L* (representing luminance) were significantly increased (Fig. 3-A). This means that the liquor of RR1.5 is redder and brighter than that of R1.5 and R1.

The well-known pigments in black tea are TFs, TRs, and TBs. TFs contribute to the brightness and yellowish color, and TRs contribute to the red and brown color of black tea infusion (Samanta et al., 2015). Thus, higher levels of TFs and TRs will result in redder and brighter black tea liquor. TBs are dark-brown pigments and generally considered to cause a side effect for black tea liquor color (Hua et al., 2021). The contents of TFs, TRs, and TBs were gradually increased from R1 to RR1.5, and the increase extent of TRs and TFs was larger than that of TBs (Fig. 3-C and Table S3), which is consist with the enhancement of red color and brightness of liquor from R1 to RR1.5 (Fig. 2-B, Fig. 3-A, and Table 1). This implies that re-rolling treatment mainly has a beneficial effect on the liquor color of black tea.

In addition to TFs, TRs, and TBs, flavone/flavonol glycosides and organic acids were also reported as the color contributors to black tea infusion (Long et al., 2024). It was reported that the addition of quercetin-3-O-glucoside, although presenting yellow hue, to liquor increased the red hue of black tea infusion, and quinic acid increased the brightness (L* value) but decreased the redness, yellowness and chroma of black tea infusion (Long et al., 2024). The levels of flavone/flavonol glycosides, including quercetin-3-O-glucoside, were the highest in R1, followed by RR1.5 and R1.5 (Fig. 5), and there was no significant difference in the quinic acid level (Table S1). This implied that flavone/flavonol glycosides and quinic acid played minor roles in the convert of liquor color and brightness from R1 to RR1.5. Therefore, re-rolling treatment is mainly to improve the liquor color quality of black tea by increasing the contents of TFs and TRs.

4.5. Re-rolling treatment is a novel and simple way to improve black tea quality

In order to improve black tea quality, especially the taste and aroma, many explorations have been made to date, such as oxygen-enriched fermentation (Chen et al., 2021), dynamic fermentation (Hua et al., 2021), exogenous enzyme addition (Chiang, Yang, Wang & Chen, 2022), and withering with LED light (Li et al., 2022) etc. However, these methods require either the specialized and expensive equipment or adding exogenous substance, which limits their potentiality to be widely used in the production practice. Comparing with the above methods, re-rolling treatment is easily to operate and not need expensive equipment. These advantages endow re-rolling an enormous practicability and utilization potentiality in tea manufacturing industry.

In addition, a problem that often appears is the insufficient fermentation in black tea production practice. Insufficiently fermented black tea usually retains high-abundance polyphenols, resulting in an astringency even a bitter taste, e.g., the R1 black tea in this study (Table 1). Re-rolling treatment could selectively accelerate the oxidation of catechins and procyanidins into pigments and the hydrolysis of proteins into free amino acids (Fig. 3 and Fig. 5), and thus improve the taste and liquor color quality (Table 1). This implies that re-rolling treatment can prevent insufficient fermentation during black tea processing.

Therefore, we regarded that re-rolling treatment is a novel and simple way to improve black tea quality.

5. Conclusion

In the present study, the effect of re-rolling treatment on the taste and liquor color qualities of black tea were comprehensively investigated. Re-rolling treatment weakened the astringency of black tea via promoting the oxidation of polyphenols (especially catechins and procyanidins) and the hydrolysis of proteins into free amino acids, and improved the redness and luminance of liquor via promoting the contents of TFs and TRs. Metabolomics analysis showed that re-rolling treatment also significantly influenced the conversion of flavone and flavonol glycosides, phenolic acids, alkaloids, organic acids, GBVs, and lipids. As re-rolling treatment obviously improved the black tea aroma, the effect of re-rolling treatment on aroma components is also worth study in future. In summary, this study provides a novel and simple way to improve black tea qualities and is beneficial to enrich the theory of black tea processing chemistry.

CRediT authorship contribution statement

Qincao Chen: Formal analysis, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Ying Fu:** Formal analysis, Investigation. **Wenting Heng:** Formal analysis, Investigation. **Shuai Yu:** Formal analysis, Investigation. **Feng Xie:** Resources. **Fang Dong:** Resources. **Zhi Lin:** Conceptualization, Funding acquisition, Writing – review & editing. **Weidong Dai:** Conceptualization, Formal analysis, Funding acquisition, Writing – review & editing. **Haihui Fu:** 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

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.2024.101143.

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