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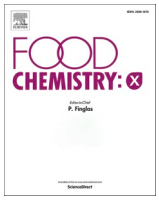

Evaluation of taste quality of Keemun congou black tea during ripening and the effect of this quality on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase

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ABSTRACT

Ripening is a key process driving the transformation of non-volatiles in Keemun congou black tea (KCBT), affecting its flavour profile and health functions. In this study, taste was quantitatively evaluated by using sensory and biomimetic electrodes and by employing metabolomic techniques. The results revealed that the content of polyphenols was greatly affected by ripening, catechins and flavonoids reduced by 63.5 % and 9.2 %, respectively, and theaflavins increased by 14.6 %, thereby attenuating the bitterness and astringency of the tea infusion while enhancing its sweetness and mellowness. Experiments regarding the inhibition of α-amylase and α-glucosidase activity and scavenging of 2,2-diphenyl-1-picrylhydrazyl radical revealed that ripening triggered the cascade reaction of polyphenols to form catechin polymers and flavonoid glycosides, thereby changing the dual biological functions of hypoglycaemia and free radical scavenging in vitro. Our study confirms the key role of ripening in enhancing the taste quality and potential health functional activities of KCBT.

1. Introduction

Tea, with its unique flavour profile and health benefits, is among the world's most widely consumed beverages (Sarma et al., 2023). Keemun congou black tea (KCBT) is the only black tea among China's top 10 most famous teas and is one of the world's three highly aromatic black teas (Xiao et al., 2022). The elaborate processing technique employed to produce KCBT is the key to its unique flavour characteristics and health benefits; KCBT processing comprises two stages, namely the primary production of the crude tea and the refined production of the congou tea (sifting, cutting, grading, refiring, blending, and packing) (Xu et al., 2023). The cyclic process of naturally aging and subsequently refiring crude tea is termed "ripening", which usually requires 4–5 months to complete (Huang et al., 2024). The ripening process for KCBT is a necessary step in the transition from primary to refined processing, and consists of five key stages. The degree of completion of each stage determines the progress of ripening, and has a direct impact on the unique flavour profile and health benefits of KCBT. Although studies have explored the formation of the flavour quality and health functions of KCBT (Guo et al., 2018; Ma, Wang, Xu, et al., 2022), few have examined the effects of ripening on the taste quality and potential health functions of KCBT.

The abundance of primary and secondary metabolites in tea play a key role in tea flavour contributing to the diversity of flavors present in tea infusion. Tea polyphenols, particularly catechins and flavonoid glycosides, are a group of substances that tend to contribute to bitterness and astringency in tea infusions. Polyphenols oxidise under enzymatic action to form theaflavins (TFs), thearubigins (TRs), and theabrownin (TB), and these oxidation products act as colouring agents that determine the colour of finished black tea and black tea infusion (Long et al., 2023). By contrast, free amino acids and soluble sugars enhance the umami and sweetness quality of tea infusions by antagonising their bitterness and astringency (Zhang et al., 2019); at the same time, sugars react with amino acids during the drying stage of the Maillard reaction to produce pigmented substances (e.g., melanoidins) and volatile compounds (e.g., heterocyclic compounds), which give tea its unique flavour profile (Yin et al., 2023). Changes in nonvolatile substances in tea during the enzymatic (withering and fermentation) and thermal (drying) stages

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have been widely reported (Chen et al., 2023; Huang et al., 2023; Qu et al., 2019). KCBT is made from crude Keemun black tea (KBT) through natural aging and refiring, which constitutes a ripening process that differs from the conventional drying process; because our previous study demonstrated that ripening enhanced the formation of the composite aroma qualities of KCBT (Huang et al., 2024), we hypothesised that ripening also improves the taste quality of KCBT infusion.

Substances such as epigallocatechin gallate (EGCG), flavonoids, and TFs have substantial effects on antioxidant activity, blood sugar regulation, and the prevention of cardiovascular diseases (Zhang et al., 2013). Notably, a growing body of evidence indicates that tea exhibits biological activities that facilitate the regulation of blood sugar and the scavenging of free radical; in addition, polyphenols in tea are associated with various biological activities and play key roles in the benefits of tea with respect to regulating blood sugar and providing antioxidant functions (Mi et al., 2024). The cyclic process of KCBT ripening, which involves natural aging and refiring, may accelerate the conversion of the aforementioned nonvolatile substances, particularly the oxidation and isomerisation of ester catechins, the consumption of free amino acids and sugars during the Maillard reaction, and the formation of soluble sugar and catechins complexes (Jiang et al., 2021), all of which may affect the taste quality and biological activity of KCBT.

Thus, the ripening process of KCBT, a typical representative of congou black tea, provides crude KBT with rich flavour characteristics and potential health functions that are noteworthy. In this study, sensory and bionic sensors were used to quantitatively evaluate the colour and taste quality of tea infusions of five samples during the ripening process of KCBT. In addition, metabolomics techniques were employed to analyze the changing patterns of the nonvolatile substances in the aforementioned samples as well as their influence on taste quality. Furthermore, in vitro bioactivity experiments were conducted to investigate the inhibitory effects of ripening on the activities of α-amylase and α-glucosidase and the scavenging ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The present study systematically investigated the effects of ripening on the formation of the flavour quality and potential health benefits of KCBT, thereby providing novel insights pertaining to the flavour quality and health functions of tea.

2. Materials and methods

2.1. Chemicals

Amino acids were obtained from Wako Pure Chemical Industries, Ltd. (Kanagawa, Japan). Standard sugars were purchased from IsoReag, TCI and CNW, (Shanghai, China). Caffeine, catechins, 3,5-dinitrosalicylic acid (DNS), α-glucosidase and α-amylase (porcine pancreas) were purchased from Shanghai Yuanye Biotechnology Company (Shanghai, China). Acarbose, DPPH, soluble starch, oxalic acid, and 4-nitrophenyl-α-D-glucopyranoside were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China); the purity levels of all these substances were at least 98 %. In addition, Methanol and acetonitrile were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Ethyl acetate and n-butanol were obtained from Xilong Scientific Co., Ltd. (Guangdong, China). Sulfosalicylic acid was obtained from Tianjin City Guang Fu Tech. Development Co., Ltd. (Tianjin, China). Hydrochloric acid (HCl), potassium chloride (KCl), and tartaric acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China); the purity levels of all these substances were at least 99 %.

2.2. Tea samples

The samples of crude KBT (*Camellia sinensis* var. *sinensis* cv. Zhuye) were processed in April 2021 in Qimen County (Anhui Province, China) using traditional primary production process and placed in a natural indoor environment with stable conditions, where ripening was conducted in accordance with the process depicted in Fig. 1(A). In total, five samples of crude KBT, first aging (FA), first refiring (FD), second aging (SA), and second refiring (SD) were collected; the temperature and humidity of the environment and the moisture of the samples during the ripening process are presented in Fig. 1(B, C). The collected samples were stored in a refrigerator at −80 °C until samples were collected, and freeze-dried and stored in a 4 °C refrigerator.

2.2.1. Quality of tea samples

The five samples collected during ripening were subjected to an initial sensory evaluation by three masters with tea-making experience, who used a common language to conduct a traditional sensory evaluation of KBT. All five samples conformed to the typical quality characteristics of KBT, i.e., red and bright infusion colour, sweet aroma and sweet taste, and some differences in the intensity of the characteristics (Table S1).

2.3. Quantitative evaluation of the taste profile of Keemun black tea infusion during ripening using sensory and electronic tongues

The quantitative evaluation of tea infusions through the use of sensory and electronic tongues. In brief, each tea infusion was prepared by combining 3 g of tea leaves and 150 mL of boiling water ensuring

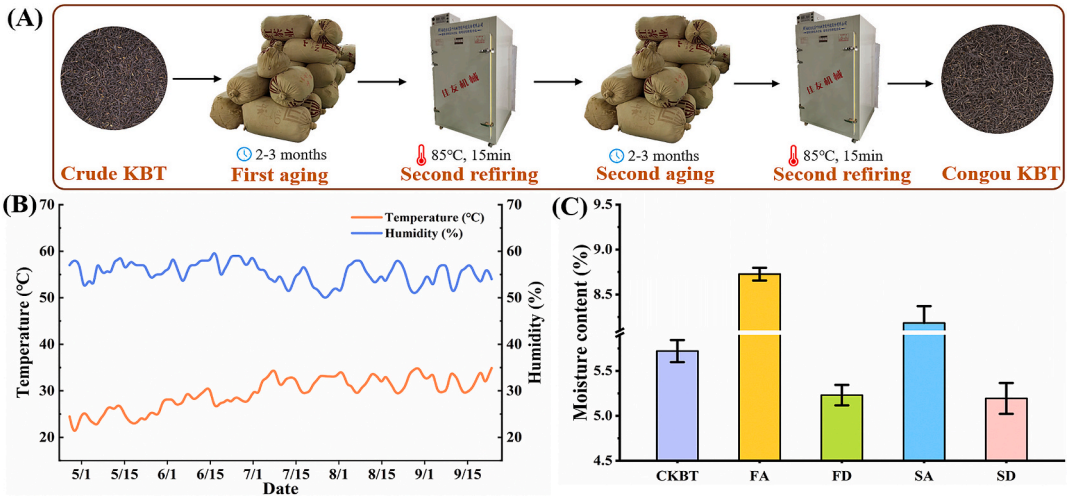

Fig. 1. Processing flow chart and main parameters during ripening of Keemun black tea; (B) Temperature and humidity variations in the natural indoor environment during the ripening; (C) Changes in moisture contents of Keemun black tea samples during ripening process.

thorough mixing for a duration of 5 min, followed by filtration. Twelve trained individuals (aged 23–30 years; six male and six female individuals) participated as evaluators in our evaluation, and the protocols for taste training and evaluation were adhered to as detailed in our prior research publication (Huang et al., 2022). The evaluators rated the bitterness, umami, astringency, sweetness, and richness of the five tea infusion samples on a 5-point scale (1, very weak; 2, weak; 3, moderate; 4, strong; 5, very strong; precision level, 0.1) in 25 °C room temperature environment. The sensory evaluation protocol for Keemun black tea was scrutinized and endorsed by the Human Sensory Ethics Inspection Committee at Anhui Agricultural University. Participants were aware of the potential risks and objectives of the evaluation. They joined the study on a voluntary basis, granted consent for the publication of their sensory data, and were assured that their personal information would be kept confidential. The electronic tongue method follows what we described in detail in our preceding research (Huang et al., 2022).

2.4. Determination of tea infusion colour

Tea infusions were obtained for each sample by the method described in 2.3. The parameters of aperture (F9), shutter (100 s), ISO sensitivity (ISO 500), and white balance (5500 K) were fixed, and a Nikon D610 digital camera was utilized to photograph the tea infusions, from which the L value (brightness), a* value (redness-greenness), and b* value (yellowness-blueness) were derived from the captured RGB images.

2.5. Analysis of free amino acids, catechins, TFs, caffeine, and gallic acid in Keemun black tea samples during ripening

Compounds for target quantification included (free amino acids, catechins, TFs, caffeine, and gallic acid) which were using an High-Speed Amino Acid Analyser (L-8900, Hitachi) and High performance liquid chromatography (HPLC, 1260 system, Agilent Technologies, Palo Alto, CA, USA), all detected exactly as described in detail in our preceding research (Huang, Lu, et al., 2022).

2.6. Determination of sugars of Keemun black tea samples during ripening

Isopropanol:methanol:water (3:3:2, *v*/v/v, 500 μL) was added to 20 mg of lyophilised tea powder and performed vortexing for 3 min, ultrasonication for 30 min, and centrifugation at 4 °C (3 min, 12,000 r/min). The supernatant (50 μL) was combined with 20 μL of an internal standard solution containing 1000 μg/mL each of adonitol and D-ribose, and the mixture was evaporated under a stream of nitrogen gas. The evaporated sample was transferred to a lyophilizer for freeze-drying prior to further derivatization. For derivatization, methoxyamine hydrochloride was combined with 100 μL of a 15 mg/mL pyridine solution. After incubation at 37 °C for 2 h, 100 μL of *N*,*O*-Bis(trimethylsilyl)trifluoroacetamide was added, vortexed, and allowed to react at 37 °C for an additional 30 min. The resulting mixture was diluted to the required concentration and analysed by gas chromatography–mass spectrometry (GC–MS). Sugar analysis was performed an Agilent 8890GC gas chromatograph interfaced with a 5977B mass spectrometer, with sugar compounds being separated on a DB-5MS column, 30 m in length, 0.25 mm in diameter, and 0.25 μm in film thickness. Helium was used as the carrier gas at a flow rate of 1 mL/min, and injections were performed in split mode with a split ratio of 5:1. The detection volume was 1 μL. The column temperature program was as follows: an initial held at 40 °C for 1 min, to 200 °C at 6 °C/min, then to 270 °C at 10 °C/min, further to 300 °C at 5 °C/min, and finally to 320 °C at 20 °C/min, and held for 5.5 min. All the samples were analysed using selected ion monitoring (Sun et al., 2016).

2.7. Analysis of metabolites in Keemun black tea samples during ripening through LC–Orbitrap–MS

Extraction and detection of non-volatile polyphenolic compounds using LC–Orbitrap–MS (Q-Exactive Focus, Thermo Fisher Scientific, MA, USA) according to our previously reported methods (Huang et al., 2024).

2.8. Analysis of TRs and TB in Keemun black tea samples during ripening

The analysis of TRs and TB in this study was performed in accordance with the Chinese agricultural industry standard NY/T 3675–2020 (Liu et al., 2023). An accurately weighed lyophilised 0.400-g sample was placed in 20 mL of boiling water, macerated for 5 min (with shaking performed every 2.5 min), and then cooled and centrifuged (4500 r/min, 10 min, 25 °C). The precipitate was fully extracted with 20 mL and 10 mL of boiling water, respectively, and the combined supernatant from three extractions was fixed in a 50-mL mother solution. The colorimetric solutions are described as follows. Colorimetric solution A: 2 mL of the mother solution was mixed with 2 mL of saturated oxalic acid solution and 6 mL of pure water, and 95 % ethanol was added to bring the total volume to 25 mL. Colorimetric solution B: 10 mL of the mother solution was mixed with 10 mL of n-butanol, and centrifuged (4500 r/min, 10 min) after continuous vortexing (2500 r/min, 10 min). Subsequently, the mixture was transferred to a separatory funnel for stratification; 2 mL of the underlying liquid was taken, 2 mL of saturated oxalic acid solution and 6 mL of water were added, and 95 % ethanol was added to bring the volume to 25 mL. Colorimetric solution C: 15 mL of the mother solution was mixed with 15 mL of ethyl acetate and centrifuged (4500 r/min, 10 min) after continuous vortexing (2500 r/min, 10 min). Next, 6 mL of the supernatant was added to 6 mL of sodium bicarbonate solution (25 g/L); this combination was mixed thoroughly and centrifuged (4500 r/min, 5 min). Thereafter, 4 mL of the supernatant was transferred and fixed, with 95 % ethanol being added to bring the volume to 25 mL; 95 % ethanol was used as a reference, and colorimetric solutions A, B, and C were used to measure the absorbance at 380 nm for E_A, E_B, and E_C, respectively. TB (1) and TRs (2) content levels were calculated using the following equations:

$$C_{TB} = \frac{16.944 \times E_B}{10 \times m} \quad (1)$$

$$C_{TRs} = \frac{16.944 \times \left(E_A - \frac{E_C}{2} - E_B\right)}{10 \times m} \quad (2)$$

where C_{TB} is the TB content (mg/g), C_{TRs} is the TRs content (mg/g), m is 0.400 g, and the Robert's empirical coefficient is 16.944.

2.9. Analysis of inhibition rates of α-amylase and α-glucosidase activities and scavenging rate of DPPH radical

Boiling water (8 mL) was added to lyophilised tea powder (0.500 g), which was extracted (100 °C, 45 min) in a thermostatic water bath and then centrifuged (3500 r/min, 10 min). The supernatant was then adjusted to 10 mL to obtain the tea infusion extract and subsequently diluted to the appropriate concentration. The activity inhibition experiments for α-amylase and α-glucosidase were minorly modified base on Johnson et al. (2011) and Luo et al. (2023). For the α-amylase inhibition assay, tea infusion extract (500 μL, 50, 40, 30, 20, and 10 mg/mL) and positive control (500 μL, 1 mM acarbose) were added to α-amylase solution (500 μL, 13 U/mL, 0.02 M sodium phosphate buffer, pH 6.9); the mixture was incubated at 25 °C for 10 min, supplemented with soluble starch solution (500 μL, 10 mg/mL, dissolved in sodium phosphate buffer and boiled for 15 min), incubated for 25 min, supplemented with 1 mL of DNS, heated in a boiling water bath (5 min), and finally diluted with 100 mL of distilled water; absorbance values were obtained at 520

nm. For the α-glucosidase inhibition assay, 100 μL of α-glucosidase solution (1 U/mL, 0.1 M sodium phosphate buffer, pH 6.9) was added to tea infusion extract at various concentrations (50 μL, 0.5, 0.4, 0.3, 0.2, and 0.1 mg/mL) and positive control (50 μL, 1 mM acarbose) at 25 °C for 10 min. After incubation was performed for 10 min, 50 μL of *p*-nitrophenyl-α-D-glucopyranoside solution (6 mM, 0.1 M sodium phosphate buffer, pH 6.9) was added. The absorbance value was obtained at 405 nm. For the DPPH radical assay, tea infusion extract at various concentrations (100 μL, 0.4, 0.3, 0.2, 0.1, and 0.05 mg/mL) was mixed with DPPH (100 μL, 0.1 mM), and absorbance values were obtained at 517 nm after incubation for 30 min. The inhibition rates for α-amylase and α-glucosidase activities and the scavenging rate for DPPH radical were calculated as follows:

$$\text{Inhibition / scavenging rate } (\%) = \frac{A_{control} - (A_{test} - A_{blank})}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance value without the addition of tea infusion extract, A_{test} is the absorbance value with the addition of tea infusion extract enzyme or DPPH, and A_{blank} is the absorbance value with tea infusion extract but without the enzyme or DPPH.

2.10. Data analysis

LC-MS data were visualised using Xcalibur software to match them with standard peaks, and metabolite-dependent MS/MS was performed using MSFINDER version 3.04 to identify unknown metabolites. An analysis of variance (Duncan's test) was conducted using SPSS 24.0, and significance levels for compound concentrations were calculated ($p < 0.05$). The Mantel test was performed using chiplot (https://www.chiplot.online/). In addition, image data were visualised using TBtools-II v2.012, Origin 2023b and Cytoscape_v3.7.2. Each test was conducted three times, the results are presented as means ± standard deviations.

3. Results and discussion

3.1. Infusion colour and taste characteristics of Keemun black tea during ripening

Tea infusion colour has an intuitive effect on the quality of KCBT during the ripening process. This study obtain tea infusion images of the five KCBT samples during the ripening process and extracted data regarding their brightness (L) and their red–green (a*) and yellow–blue (b*) values. The colour of tea infusion changed considerably with an increase in the degree of ripening (Fig. 2A). This change manifested as a gradual decrease of the L value of the tea infusion colour, particularly after the first drying. The value of a*, which represents the degree of red colour, gradually increased but did not change significantly, and the value of b*, which represents the degree of yellow colour, gradually decreased.

Ripening enhanced the sweetness and richness of the KCBT infusion and weakened its bitterness and astringency. A systematically trained sensory evaluation panel conducted quantitative descriptive analysis to evaluate the umami, sweetness bitterness, astringency, and richness of the five KCBT samples during the ripening process (Fig. 2B). The results indicated that when the degree of ripening increased, the sweetness and richness of the tea infusion increased significantly relative to the previously treated samples. In addition, the degree of ripening was significantly higher among the SD samples than among the crude KBT. The opposite trends were observed for bitterness, umami and astringency; that is, after ripening, the umami, bitterness, and astringency of the tea infusion had significantly weakened, and the infusion's intensity was significantly reduced relative to that of the crude KBT samples. Notably, the intensity of the various taste attributes changed more significantly after refiring than after aging, suggesting that refiring accelerated changes in the flavour-presenting substances. The results of the sensory evaluation and the electronic tongue evaluation were basically consistent, which confirmed that ripening has an effect on the taste of CKBT.

(A)

	CKBT	FA	FD	SA	SD
Infusion					
L	49.60±0.37a	49.59±0.49a	47.47±0.65b	46.06±0.33c	44.48±0.30d
a*	49.65±1.35a	50.14±1.56a	50.87±1.66a	51.08±1.09a	51.42±1.68a
b*	59.94±0.57a	59.72±1.04a	57.95±1.22b	56.75±0.83bc	55.23±0.86c

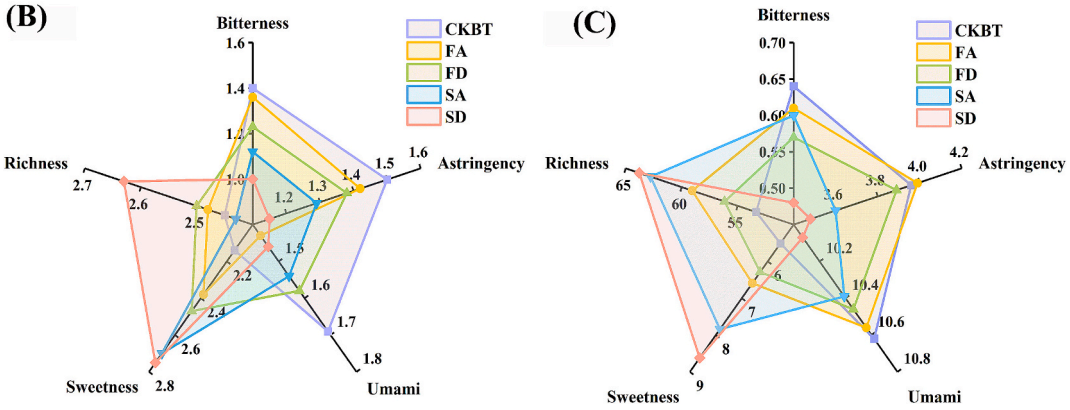

Fig. 2. (A) Changes of tea infusion colour with its brightness (L), redness (a*) and yellowness (b*) analysis during the ripening of Keemun black tea, different alphabets indicated to be significance at the level of $p < 0.05$. (B) Radar map of sensory quantitative evalution of tea infusion taste quality characteristics. (C) Radar map of electronic tongue quantitative evalution of tea infusion taste quality characteristics (Richness was obtained by calculating the area of closure of the radar map of bitterness, astringency, umami and sweetness taste).

However, we noticed that there were some minor deviations between the sensory evaluation and electronic tongue evaluation, which existed mainly in the values of the taste value of the two neighboring samples and the richness during the ripening. It may be that the differences in the taste characteristics of the neighboring samples were small; while the richness was mainly due to the different calculation of the two methods. The richness of the sensory evaluation is derived from the direct scoring by the sensory panelists based on the taste profile, which is a direct reflection of the flavour profile of the tea infusion and is affected by the interactions between the flavors, such as additive, synergistic, masking, etc. On the other hand, the richness value of electronic tongue evaluation is obtained by calculating the closed area of the radar chart consisting of bitterness, astringency, umami, and sweetness, which is affected by the values and differences of each taste and ignores the interactions among the taste, and thus there is a numerical difference between the two evaluation methods. We analyze the flavour changes during the ripening process from subjective and objective perspectives through the two methods to verify each other, and also provide the readers with method choices and references. Overall, these results indicated that ripening enhanced the sweetness and richness of KCBT but weakened its astringency and bitterness, thereby improving its overall taste characteristics.

3.2. *Characterisation of nonvolatile metabolite pattern during KCBT ripening*

The taste profile of tea infusions is significantly shaped by nonvolatile compounds such as free amino acids, sugars, caffeine, and polyphenols. These metabolites, in their various forms and concentrations, account for the wide array of flavors characteristic of different tea infusions (Zhang et al., 2020). Nonvolatile compounds were analysed in the five samples by employing both target and nontarget techniques and by taking advantage of the high-throughput and high-confidence results obtained through the LC–Orbitrap–MS technique and the accurate quantification achieved through HPLC. A total of 71 taste-presenting metabolites were identified in the five samples (Table 1, Table S2 and Fig. S1), of which 24 were relatively quantified using internal standards through LC-MS and the remaining 47 were accurately quantified for the reference compounds. TB and TRs were supplemented using UV spectrophotometry because they had not yet been identified. The identified taste-presenting substances were classified by chemical structure into seven groups, namely free amino acids (18), sugars (17), caffeine (1), catechins (11), flavonoids (16), phenolic acids (4), and soluble pigments (6); all these substances are collectively and significantly contributing to the taste and overall quality of the tea infusions.(Li et al., 2021).

A total of 18 free amino acids were detected in the five samples; among them, the concentrations of eight free amino acids (Ser, Gln, Thea, Ala, Thr, Val, Met, and Tyr) increased and subsequently decreased during the ripening process, and those of seven amino acids (Asp, Ile, Leu, Phe, Lys, His, and Arg) exhibited a decreasing trend; the remaining three (Glu, Gly, and Pro) did not experience significant changes during ripening. Compared with that of crude KBT, the total content of free amino acids in the SD samples decreased by 3.3 % during aging. Notably, the concentrations of almost all the free amino acids peaked after FA (22.880 mg/g); this increase could be due to the redistribution and rise in water content associated with short-term aging, which promote the hydrolysis of proteins in crude KBT and thus also promote the accumulation of amino acids. In the subsequent process that occurred because of the accelerated consumption of free amino acids, protein hydrolysis gradually weakened, the production–consumption equilibrium was broken, and the free amino acid content exhibited varying degrees of decrease. A considerable body of research results indicates that the degradation of free amino acids into Strecker aldehyde or their participation in the Maillard reaction during the drying leads to a reduction in amino acid content (Ho et al., 2015; Wang et al., 2022). In particular, high temperatures and long drying times exacerbate amino acid depletion, thereby considerably weakening the umami characteristics of tea infusions (Wang et al., 2023).

The ripening process altered the compositions of sugar fractions. Seventeen sugars (alditols) were detected in the five samples, namely five disaccharides and 12 monosaccharides (alditols). The content of the disaccharides during the ripening process accounted for 9.0 %–11.1 % of the total sugar content, and the content of the monosaccharides accounted for 88.9 %–91.0 % of the total sugar content; the dominant sugars were glucose, fructose, galactose, lactose, and sucrose, which collectively accounted for 93.4 %–93.9 % of the total sugar content. The total sugar content in the samples from the various ripening processes did not change significantly; however, the proportions of monosaccharides and disaccharides changed significantly. The content of disaccharides—namely lactose, maltose, and sucrose—increased significantly after ripening, particularly the content of sucrose, which exhibited a high sweetness intensity and may have enhanced the sweetness intensity of the tea infusions. Notably, the proportion of monosaccharides decrease slightly; a possible explanation for this phenomenon is that the thermal action of ripening helped to accelerate the nonenzymatic browning of monosaccharides through the caramelisation reaction (without amino acids) and the Maillard reaction (with amino acids) and thus contributed to the formation of the aroma of tea (Ho et al., 2015).

Catechins are key components that contribute to the astringency of tea, and their concentration is an essential element of tea flavour quality analyses (Zhuang et al., 2020). Six catechin monomers—namely (−) − epigallocatechin (EGC), (+) − catechin (C), (+) − gallocatechin (GC), (−) − EGCG (epigallocatechin gallate), (−) − epicatechin (EC), and (−) − epicatechin gallate (ECG)—were detected in the samples during the ripening process. In contrast to the concentrations of free amino acids and sugars, that of catechins significantly decreased during ripening from 9.992 to 6.261 mg/g. In particular, the concentrations of the four most abundant catechins—namely EGC, EGCG, ECG, and EC—decreased by 56.6 %, 71.9 %, 66.2 %, and 88.8 %, respectively, after ripening. By contrast, during the black tea rolling and fermentation phases, a large quantity of catechins was oxidized by polyphenol oxidase and peroxidase, and the resulting colourants included oxidatively polymerised substances such as TFs and TB, substantial amounts of which accumulated during ripening. The total content of TFs increased from 11.73 to 13.45 mg/g, representing an increase of 14.63 %. This outcome may be attributed to the conversion formation of high concentrations of catechins (Long et al., 2023). TRs are intermediate substances in the conversion of TFs to TB, and the content of TRs decreased significantly during SD. Our results suggest that secondary refiring accelerated the oxidative polymerisation of catechins and promoted the conversion of TRs to TB, resulting in significant increases in the contents of TFs and TB. These increases may be the main reason for the gradual change in the colour of the tea infusions from orange-red to red (Fig. 2A). The two cyclic processes of natural aging and refiring provided sufficient conversion time for mild natural oxidation to occur during the ripening process, which usually requires 2–3 months to complete (Fig. 1). In addition, variations in temperature and humidity in tea aging environments (Fig. 1B) gradually affect the oxidative polymerisation of polyphenols (e.g., catechins) and promote the formation of catechin polymers, such as epicatechin-(2beta- > 7,4beta- > 6)-catechin, epigallocatechin-(4beta- > 8)-catechin, theasinensin C, epicatechin-(4beta- > 8)-4'-*O*-methylgallocatechin, and 3,5-digalloylepicatechin. One study reported on the dynamic changes that catechins undergo only in the initial production process for Keemun black tea (Wen et al., 2023); our study revealed that the degree to which catechins and their oxidative polymerisation products transformed affected the formation of tea infusion taste and colour during the ripening process. The contents of colourants such as TFs, TRs, and TB substantially influence the colour of black tea infusions, and TFs are key components that influence the colour and taste intensity of tea infusions. Specifically, TFs exhibit lower levels of astringency and irritation compared with catechins (Zhang

Table 1
Concentrations changes of non-volatile compounds in KCBT during ripening.

No	Compounds	CKBT	FA	FD	SA	SD	Sourse	Identification
Amino acids								
1	Aspartic acid	0.857 ± 0.008^{ab}	0.878 ± 0.018^{a}	0.840 ± 0.017^{b}	0.801 ± 0.013^{c}	0.787 ± 0.005^{c}	Amino acid analyser	Std
2	Threonine	0.313 ± 0.003^{c}	0.343 ± 0.011^{a}	0.328 ± 0.008^{b}	0.320 ± 0.006^{bc}	0.314 ± 0.002^{c}	Amino acid analyser	Std
3	Serine	0.646 ± 0.007^{c}	0.705 ± 0.016^{a}	0.679 ± 0.016^{b}	0.662 ± 0.011^{bc}	0.653 ± 0.005^{c}	Amino acid analyser	Std
4	Glutamic acid	0.961 ± 0.013^{b}	1.027 ± 0.053^{a}	1.024 ± 0.018^{a}	0.994 ± 0.015^{ab}	0.994 ± 0.005^{ab}	Amino acid analyser	Std
5	Glutamine	1.328 ± 0.063^{b}	1.449 ± 0.036^{a}	1.251 ± 0.050^{b}	1.097 ± 0.035^{c}	1.026 ± 0.017^{c}	Amino acid analyser	Std
6	Theanine	10.987 ± 0.171^{c}	12.429 ± 0.224^{a}	11.66 ± 0.351^{b}	11.211 ± 0.164^{c}	10.870 ± 0.069^{c}	Amino acid analyser	Std
7	Glycine	0.050 ± 0.004^{a}	0.051 ± 0.001^{a}	0.050 ± 0.002^{a}	0.049 ± 0.002^{a}	0.050 ± 0.001^{a}	Amino acid analyser	Std
8	Alanine	0.361 ± 0.003^{c}	0.418 ± 0.013^{a}	0.406 ± 0.010^{ab}	0.403 ± 0.007^{ab}	0.393 ± 0.003^{b}	Amino acid analyser	Std
9	Valine	0.640 ± 0.007^{b}	0.717 ± 0.035^{a}	0.669 ± 0.065^{ab}	0.635 ± 0.027^{b}	0.635 ± 0.005^{b}	Amino acid analyser	Std
10	Methionine	0.011 ± 0.000^{b}	0.052 ± 0.001^{a}	0.012 ± 0.000^{b}	0.012 ± 0.001^{b}	0.014 ± 0.001^{b}	Amino acid analyser	Std
11	Isoleucine	0.326 ± 0.004^{ab}	0.333 ± 0.010^{a}	0.320 ± 0.008^{bc}	0.319 ± 0.005^{bc}	0.311 ± 0.002^{c}	Amino acid analyser	Std
12	Leucine	0.352 ± 0.005^{a}	0.356 ± 0.007^{a}	0.345 ± 0.015^{ab}	0.333 ± 0.006^{bc}	0.325 ± 0.002^{c}	Amino acid analyser	Std
13	Tyrosine	0.663 ± 0.007^{c}	0.710 ± 0.015^{a}	0.696 ± 0.018^{ab}	0.691 ± 0.012^{ab}	0.678 ± 0.005^{bc}	Amino acid analyser	Std
14	Phenylalanine	0.739 ± 0.009^{ab}	0.777 ± 0.050^{a}	0.752 ± 0.056^{ab}	0.717 ± 0.014^{ab}	0.694 ± 0.006^{b}	Amino acid analyser	Std
15	Lysine	0.414 ± 0.009^{a}	0.410 ± 0.008^{a}	0.392 ± 0.010^{b}	0.382 ± 0.007^{b}	0.377 ± 0.002^{b}	Amino acid analyser	Std
16	Histidine	0.087 ± 0.000^{ab}	0.088 ± 0.005^{a}	0.083 ± 0.004^{abc}	0.080 ± 0.002^{c}	0.081 ± 0.001^{bc}	Amino acid analyser	Std
17	Arginine	1.730 ± 0.026^{a}	1.723 ± 0.063^{a}	1.569 ± 0.051^{b}	1.614 ± 0.046^{b}	1.593 ± 0.009^{b}	Amino acid analyser	Std
18	Proline	0.431 ± 0.005^{a}	0.427 ± 0.025^{a}	0.432 ± 0.019^{a}	0.413 ± 0.014^{a}	0.405 ± 0.018^{a}	Amino acid analyser	Std
Sugars								
19	Lactose	0.248 ± 0.037^{b}	0.281 ± 0.004^{ab}	0.298 ± 0.011^{a}	0.308 ± 0.012^{a}	0.298 ± 0.022^{a}	GC–MS	Std
20	Cellobiose	0.012 ± 0.001^{a}	0.012 ± 0.000^{a}	0.012 ± 0.001^{a}	0.012 ± 0.000^{a}	0.012 ± 0.000^{a}	GC–MS	Std
21	Maltose	0.031 ± 0.002^{b}	0.034 ± 0.002^{ab}	0.034 ± 0.001^{ab}	0.033 ± 0.002^{ab}	0.035 ± 0.002^{a}	GC–MS	Std
22	Trehalose	0.010 ± 0.000^{a}	0.010 ± 0.001^{a}	0.009 ± 0.000^{a}	0.010 ± 0.000^{a}	0.009 ± 0.000^{a}	GC–MS	Std
23	Sucrose	0.119 ± 0.002^{d}	0.152 ± 0.009^{b}	0.174 ± 0.005^{a}	0.135 ± 0.005^{c}	0.155 ± 0.008^{b}	GC–MS	Std
24	D-Xylulose	0.007 ± 0.000^{a}	0.006 ± 0.000^{a}	0.007 ± 0.000^{a}	0.007 ± 0.000^{a}	0.007 ± 0.000^{a}	GC–MS	Std
25	D-Xylose	0.022 ± 0.000^{a}	0.021 ± 0.001^{a}	0.022 ± 0.000^{a}	0.021 ± 0.000^{a}	0.020 ± 0.002^{a}	GC–MS	Std
26	Xylitol	0.008 ± 0.000^{a}	0.008 ± 0.001^{a}	0.008 ± 0.000^{a}	0.008 ± 0.000^{a}	0.009 ± 0.000^{a}	GC–MS	Std
27	D-Sorbitol	0.007 ± 0.000^{b}	0.018 ± 0.001^{a}	0.017 ± 0.002^{a}	0.019 ± 0.000^{a}	0.017 ± 0.001^{a}	GC–MS	Std
28	D-Mannose	0.063 ± 0.001^{b}	0.068 ± 0.005^{ab}	0.067 ± 0.003^{ab}	0.071 ± 0.000^{a}	0.071 ± 0.003^{a}	GC–MS	Std
29	D-Glucuronic acid	0.011 ± 0.000^{b}	0.014 ± 0.000^{a}	0.013 ± 0.001^{ab}	0.013 ± 0.002^{ab}	0.013 ± 0.000^{ab}	GC–MS	Std
30	Glucose	2.042 ± 0.037^{a}	1.928 ± 0.126^{a}	1.946 ± 0.070^{a}	1.981 ± 0.007^{a}	2.059 ± 0.083^{a}	GC–MS	Std
31	D-Galactose	0.295 ± 0.010^{a}	0.242 ± 0.012^{b}	0.241 ± 0.010^{b}	0.236 ± 0.002^{b}	0.232 ± 0.011^{b}	GC–MS	Std
32	L-Fucose	0.032 ± 0.001^{a}	0.030 ± 0.002^{a}	0.031 ± 0.001^{a}	0.033 ± 0.002^{a}	0.030 ± 0.002^{a}	GC–MS	Std
33	D-Fructose	1.632 ± 0.039^{d}	1.753 ± 0.022^{c}	1.792 ± 0.047^{bc}	1.901 ± 0.029^{a}	1.873 ± 0.080^{ab}	GC–MS	Std
34	D-Arabinose	0.088 ± 0.002^{a}	0.067 ± 0.001^{b}	0.069 ± 0.002^{b}	0.062 ± 0.001^{c}	0.064 ± 0.001^{c}	GC–MS	Std
35	L-Rhamnose	0.014 ± 0.000^{a}	0.013 ± 0.001^{b}	0.012 ± 0.000^{b}	0.012 ± 0.000^{b}	0.012 ± 0.000^{b}	GC–MS	Std
Alkaloids								

(*continued on next page*)

Table 1 (*continued*)

No	Compounds	CKBT	FA	FD	SA	SD	Sourse	Identification
36	Caffeine	33.059 ± 2.347^{a}	32.243 ± 1.684^{a}	32.242 ± 0.174^{a}	32.919 ± 1.249^{a}	31.934 ± 2.557^{a}	HPLC	Std
Catechins								
37	(+)-Gallocatechin	0.314 ± 0.009^{ab}	0.243 ± 0.023^{c}	0.328 ± 0.018^{a}	0.307 ± 0.019^{ab}	0.282 ± 0.009^{b}	HPLC	Std
38	(−)-Epigallocatechin	1.420 ± 0.008^{a}	1.189 ± 0.013^{b}	0.750 ± 0.014^{c}	0.608 ± 0.021^{d}	0.480 ± 0.007^{e}	HPLC	Std
39	(+)-Catechin	0.212 ± 0.023^{a}	0.190 ± 0.016^{a}	0.198 ± 0.014^{a}	0.199 ± 0.007^{a}	0.194 ± 0.003^{a}	HPLC	Std
40	(−)-Epigallocatechin gallate	3.174 ± 0.095^{a}	1.784 ± 0.142^{b}	1.680 ± 0.065^{bc}	1.511 ± 0.088^{cd}	1.376 ± 0.066^{d}	HPLC	Std
41	(−)-Epicatechin	2.607 ± 0.019^{a}	1.685 ± 0.064^{b}	1.317 ± 0.030^{c}	0.487 ± 0.045^{d}	0.291 ± 0.018^{e}	HPLC	Std
42	(−)-Epicatechin gallate	2.265 ± 0.465^{a}	1.457 ± 0.209^{b}	1.231 ± 0.049^{b}	1.104 ± 0.135^{b}	0.637 ± 0.016^{c}	HPLC	Std
43	Epicatechin-(2beta- > 7,4beta- > 6)-catechin	0.035 ± 0.002^{c}	0.101 ± 0.004^{a}	0.089 ± 0.002^{b}	0.090 ± 0.005^{b}	0.100 ± 0.005^{a}	LC-MS	MS
44	3,5-Digalloylepicatechin	0.017 ± 0.001^{c}	0.053 ± 0.000^{a}	0.043 ± 0.006^{b}	0.047 ± 0.008^{ab}	0.046 ± 0.004^{ab}	LC-MS	MS
45	Epicatechin-(4beta- > 8)-4'-*O*-methylgallocatechin	0.008 ± 0.000^{b}	0.043 ± 0.000^{a}	0.039 ± 0.009^{a}	0.038 ± 0.008^{a}	0.039 ± 0.003^{a}	LC-MS	MS
46	Theasinensin C	0.040 ± 0.002^{c}	0.042 ± 0.001^{bc}	0.044 ± 0.003^{b}	0.049 ± 0.001^{a}	0.050 ± 0.002^{a}	LC-MS	MS
47	Epigallocatechin-(4beta- > 8)-catechin	0.291 ± 0.009^{ab}	0.236 ± 0.006^{c}	0.252 ± 0.014^{c}	0.277 ± 0.013^{b}	0.299 ± 0.004^{a}	LC-MS	MS
Flavonoids								
48	Kaempferol 3-rhamnoside 7-xyloside	0.192 ± 0.025^{b}	0.214 ± 0.004^{ab}	0.207 ± 0.011^{ab}	0.226 ± 0.006^{a}	0.227 ± 0.008^{a}	LC-MS	MS
49	Myricetin 7-(6″-galloylglucoside)	0.016 ± 0.001^{e}	0.040 ± 0.002^{a}	0.028 ± 0.002^{c}	0.022 ± 0.003^{d}	0.034 ± 0.003^{b}	LC-MS	MS
50	Myricetin 3-galactoside	0.115 ± 0.007^{a}	0.085 ± 0.004^{b}	0.079 ± 0.004^{b}	0.081 ± 0.007^{b}	0.079 ± 0.002^{b}	LC-MS	MS
51	Quercetin 3-glucoside-7-rutinoside	0.279 ± 0.004^{a}	0.251 ± 0.001^{b}	0.252 ± 0.009^{b}	0.261 ± 0.003^{b}	0.262 ± 0.011^{b}	LC-MS	MS
52	Kaempferol 3-rungioside	0.049 ± 0.001^{bc}	0.051 ± 0.001^{ab}	0.048 ± 0.002^{c}	0.052 ± 0.001^{a}	0.053 ± 0.002^{a}	LC-MS	MS
53	Kaempferol 7-galactoside 3-rutinoside	0.039 ± 0.001^{b}	0.038 ± 0.002^{b}	0.041 ± 0.002^{a}	0.041 ± 0.001^{a}	0.040 ± 0.001^{ab}	LC-MS	MS
54	Vitexin 2''-O-rhamnoside	0.054 ± 0.003^{c}	0.059 ± 0.003^{ab}	0.057 ± 0.003^{bc}	0.062 ± 0.002^{a}	0.061 ± 0.001^{ab}	LC-MS	MS
55	Kaempferol 3-rhamnoside	0.048 ± 0.003^{b}	0.051 ± 0.003^{b}	0.053 ± 0.004^{ab}	0.056 ± 0.002^{a}	0.058 ± 0.003^{a}	LC-MS	MS
56	Rutin	0.244 ± 0.004^{a}	0.227 ± 0.003^{bc}	0.219 ± 0.007^{c}	0.236 ± 0.010^{ab}	0.220 ± 0.009^{c}	LC-MS	Std
57	Luteolin 7-rhamnoside	0.050 ± 0.002^{c}	0.053 ± 0.001^{b}	0.055 ± 0.002^{b}	0.060 ± 0.001^{a}	0.060 ± 0.003^{a}	LC-MS	MS
58	Quercetin 3-galactoside	0.229 ± 0.005^{a}	0.189 ± 0.006^{c}	0.197 ± 0.007^{c}	0.212 ± 0.003^{b}	0.199 ± 0.008^{c}	LC-MS	MS
59	Quercetin 3-rhamnoside	1.223 ± 0.029^{a}	0.974 ± 0.012^{c}	0.933 ± 0.097^{c}	1.113 ± 0.008^{b}	0.985 ± 0.033^{c}	LC-MS	MS
60	Myricetin	0.092 ± 0.003^{c}	0.103 ± 0.003^{a}	0.094 ± 0.002^{bc}	0.098 ± 0.002^{b}	0.097 ± 0.003^{b}	LC-MS	Std
61	Kaempferol	0.021 ± 0.001^{b}	0.022 ± 0.001^{b}	0.022 ± 0.001^{b}	0.024 ± 0.001^{a}	0.024 ± 0.001^{a}	LC-MS	Std
62	Quercetin	0.021 ± 0.000^{b}	0.014 ± 0.001^{c}	0.018 ± 0.002^{bc}	0.034 ± 0.007^{a}	0.017 ± 0.004^{bc}	LC-MS	Std
63	3,7-Dimethylquercetin	0.027 ± 0.001c	0.031 ± 0.002ab	0.029 ± 0.002^{bc}	0.032 ± 0.001^{ab}	0.034 ± 0.002^{a}	LC-MS	MS
Phenolic acids								
64	Gallic acid	3.176 ± 0.129^{a}	2.893 ± 0.042^{bc}	2.960 ± 0.125^{b}	2.719 ± 0.108^{c}	2.854 ± 0.148^{bc}	HPLC	Std
65	Theogallin	2.907 ± 0.107^{a}	2.416 ± 0.036^{b}	2.070 ± 0.184^{c}	1.927 ± 0.044^{c}	1.885 ± 0.057^{c}	LC-MS	MS
66	Chlorogenic acid	0.065 ± 0.001^{a}	0.064 ± 0.002^{a}	0.052 ± 0.005^{b}	0.048 ± 0.001^{bc}	0.047 ± 0.002^{c}	LC-MS	MS
67	*p*-Coumaric acid	0.036 ± 0.001^{a}	0.031 ± 0.001^{b}	0.031 ± 0.002^{b}	0.032 ± 0.000^{b}	0.032 ± 0.002^{b}	LC-MS	Std
Soluble pigments								
68	TF	0.129 ± 0.011^{b}	0.133 ± 0.012^{ab}	0.145 ± 0.003^{ab}	0.150 ± 0.005^{a}	0.143 ± 0.010^{ab}	HPLC	Std
69	TF-3-G	4.078 ± 0.371^{b}	4.149 ± 0.538^{b}	4.712 ± 0.102^{ab}	4.935 ± 0.215^{a}	4.649 ± 0.406^{ab}	HPLC	Std
70	TF-3,3′-G	6.458 ± 0.442^{b}	6.578 ± 0.691^{b}	7.632 ± 0.242^{a}	7.031 ± 0.592^{ab}	7.579 ± 0.448^{a}	HPLC	Std
71	TF-3′-G	1.070 ± 0.000^{e}	1.071 ± 0.001^{d}	1.073 ± 0.001^{c}	1.076 ± 0.000^{b}	1.080 ± 0.000^{a}	HPLC	Std

(*continued on next page*)

Table 1 (*continued*)

No	Compounds	CKBT	FA	FD	SA	SD	Sourse	Identification
72	TRs	75.542 ± 8.436[a]	75.048 ± 5.939[a]	75.020 ± 15.450[a]	70.988 ± 1.676[a]	61.987 ± 4.671[b]	UV–Vis	OD
73	TB	52.244 ± 1.290[b]	51.114 ± 10.045[b]	53.585 ± 5.503[b]	59.304 ± 3.389[b]	76.460 ± 8.260[a]	UV–Vis	OD

Source of compounds: HPLC, Compound detection by HPLC; GC–MS, Compound detection by GC–MS; LC-MS, Compound detection by LC-Orbitrap-MS; UV–Vis: Compounds were identified by UV–Vis. Different letters indicate significance at the $p < 0.05$ level. Methods of identification: MS, compounds were identified by mass spectra; Std, reference compounds; OD, optical density of UV–Vis spectrophotometer.

et al., 2020), and they play a crucial role in the formation of Golden Circle tea infusions. Furthermore, TFs are believed to provide specific health benefits, and thus black tea products with higher TF content tend to be valued and priced more highly (He, 2017).

A study regarding black tea taste revealed that the main contributors to the taste quality of black tea are flavonoids; specifically, the low threshold and velvety flavour of flavonoids contribute substantially to the intense sweetness and richness of black tea (Scharbert et al., 2004). The 16 flavonoids that detected (e.g., kaempferol, myricetin, kaempferol 3-rhamnoside, kaempferol 3-rhamnoside 7-xyloside, and myricetin 7-(6″- galloylglucoside)) underwent significant changes during maturation, exhibiting gradual concentration increases. Ma et al. (2021) reported that the concentrations of most flavonoids found in Pu-erh tea increased during the aging process, possibly because of the oxidation of catechin monomers and the reduction of sugars or amino acids to form flavonoids (Zhang et al., 2014); this finding aligns with our results pertaining to the reduction of catechin monomers and amino acids. In conclusion, in the present study, the alternating of aging and refiring and the mild natural oxidation during the ripening stage provided suitable conditions (i.e., sufficient reaction time and the necessary temperature and humidity) for the transformation of free amino acids, sugars, and polyphenols. These processes ultimately influenced the taste profile of KCBT.

3.3. *Analysis of the correlation between changes in nonvolatile substances and taste attributes during KCBT ripening*

The quantitative analysis of flavour compounds, electronic tongue assessment, and sensory taste evaluation revealed a correlation between shifts in taste quality during ripening and changes in major taste-

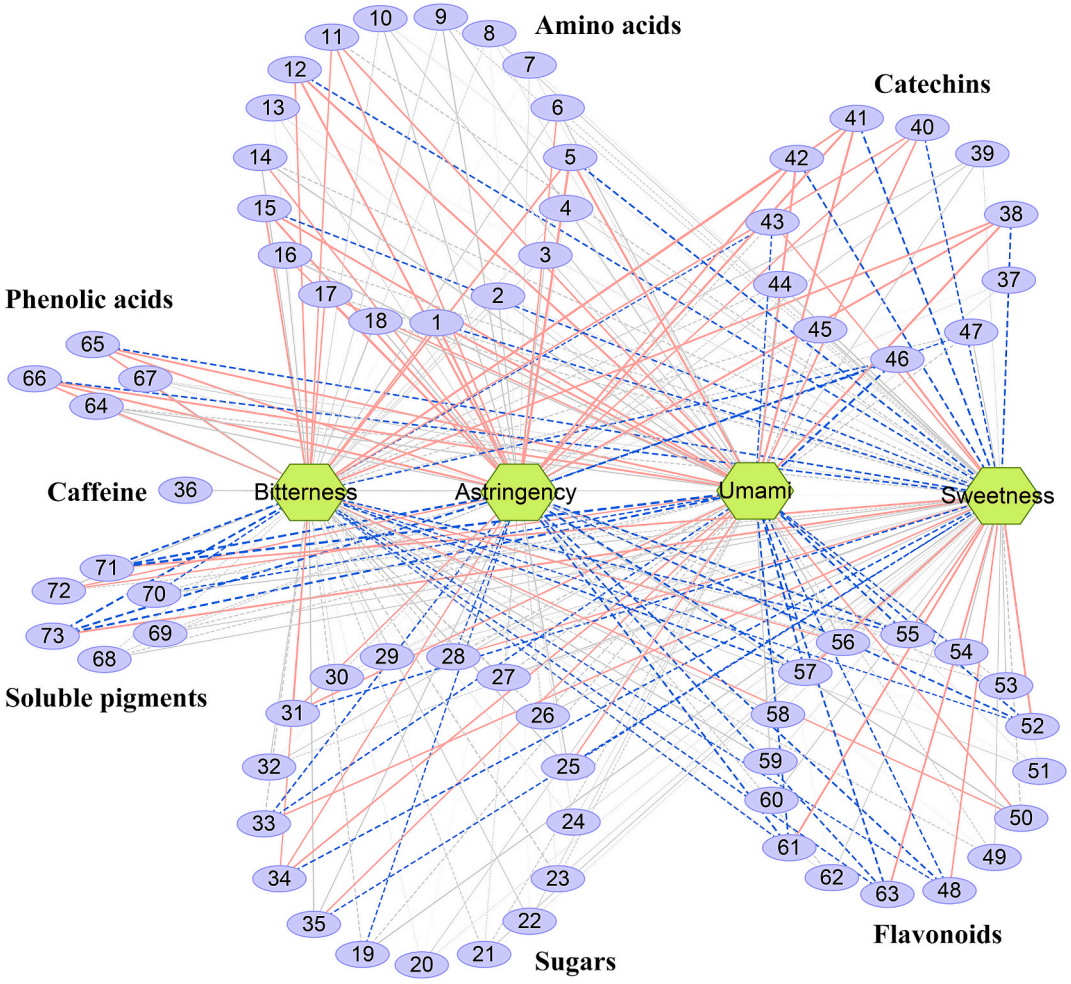

Fig. 3. Correlation analysis of taste quality and non-volatile compounds during ripening of Keemun black tea. Solid and dashed lines represent indicate and negative correlations, respectively (red indicate significant positive correlation and blue indicate significant negative correlation. The compounds corresponding to serial numbers 1–73 are listed in Table1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

presenting substances. To further investigate the effect of ripening on the taste quality of the tea infusions when the contents of major taste-presenting compounds underwent changes, Pearson correlation analysis was conducted to clarify the relationship between the major flavour-presenting substances and the various taste attributes.

Strong correlations between the main taste-presenting substances and taste quality were identified (Fig. 3 and Table S3), and these correlations were significant for most of these substances. Specifically, catechins, free amino acids, and phenolic acids were positively correlated with changes in bitterness, astringency, and umami during ripening. Among these substances, four catechins (EGC (38), EGCG (40), EC (41), and ECG (42)) and seven free amino acids (Lys (15), Arg (17), Asp (1), His (16), Gln (5), Ile (11), and Leu (12)) exhibited significant positive correlations with taste quality ($r > 0.5$). Specifically, the four catechins were the main contributors to astringency (EGC ($r = 0.789$), EGCG ($r = 0.608$), EC ($r = 0.822$), and ECG ($r = 0.756$)), and the seven amino acids were the main contributors to bitterness (Lys ($r = 0.683$), Arg ($r = 0.584$), His ($r = 0.402$), Ile ($r = 0.578$), and Leu ($r = 0.642$)) and umami (Asp ($r = 0.630$) and Gln ($r = 0.726$)) but were negatively correlated with sweetness ($r < -0.4$). These findings suggest that a decrease in the concentrations of catechins, free amino acids, and phenolic acids was the main reason for the increase in the sweetness of the tea infusions. By contrast, soluble pigments, except for TRs (72), were negatively correlated with bitterness and astringency and positively correlated with sweetness, with the correlations reaching significance ($p < 0.05$) for TB (73, $r = 0.770$) and TF-3′-G (71, $r = 0.733$). This result suggests that changes in pigmentation during ripening also influence the taste quality of tea infusions, primarily by reducing their bitterness and astringency and by enhancing their sweetness. The results for flavonoids (e.g., kaempferol 3-rhamnoside 7-xyloside (48), kaempferol 3-rungioside (52), kaempferol 7-galactoside 3-rutinoside (53), vitexin 2 “-O-rhamnoside (54), kaempferol 3-rhamnoside (55), luteolin 7-rhamnoside (57), myricetin (60), kaempferol (61), and 3,7-dimethylquercetin (63)) were similar to those for TFs. TFs and flavonoids are major components that contribute to the taste intensity of tea infusions, and ripening promotes both the conversion of catechins to TFs, TRs, and TB and the accumulation of flavonoids, thereby enhancing the sweetness and richness of tea infusions.

In addition, no significant correlations were identified between caffeine (36, $|r| < 0.3$, $p > 0.05$) or most of the sugars (19–35, $|r| < 0.5$, $p > 0.05$) and the changes in the umami, sweetness, bitterness, or astringency; this outcome may have been due to the nonsignificant effect of the ripening process on the concentrations of caffeine and soluble sugars and the weak association between the sweetness of the tea infusions and the concentrations of soluble sugars. This finding, which aligns with the findings of Scharbert and Hofmann (2005), when considered alongside the quantitative results presented in Table 1, indicates that a significant collective reduction in the concentrations of catechins, free amino acids, and phenolic acids had a positive effect on the weakening of the bitterness and astringency of the tea infusions during ripening. This finding also indicates that the ripening process drove the oxidative polymerisation of catechins and other substances; increased the generation of catechin polymers, TFs, and flavonoid glycosides; effectively weakened the bitterness and astringency of the tea infusions; and enhanced their sweetness and mellowness.

3.4. *Evaluation of biological and antioxidant activities of samples obtained during KCBT ripening*

To evaluate the effect of ripening on the biological and antioxidant activities of KCBT, we examined how various degrees of ripening influenced the inhibition of α-amylase and α-glucosidase activities and the scavenging ability of DPPH radical in the samples. Furthermore, we explored the effect of ripening on the potential health benefits of KCBT

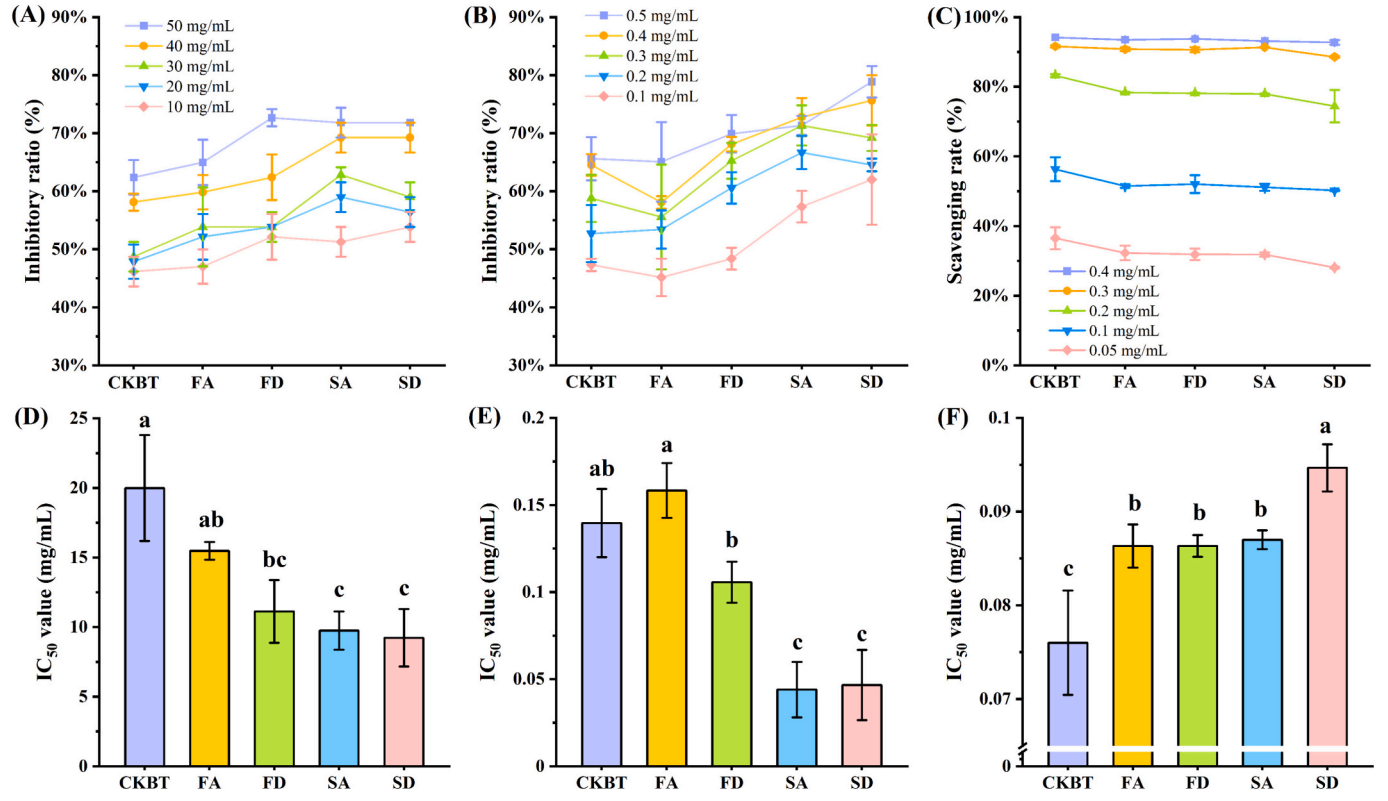

Fig. 4. Enzyme activity inhibition and antioxidant capacity of tea infusion extracts during ripening of Keemun black tea. (A) α-amylase activity inhibition rate; (B) α-glucosidase activity inhibition rate; (C) DPPH radical scavenging rate; (D) α-amylase activity IC_{50} value; (E) α-glucosidase activity IC_{50} value; (F) DPPH radical scavenging IC_{50} value.

(Table S4). As shown in Fig. 4(A, B), the inhibition of α-amylase and α-glucosidase activities in the tea infusions at a given concentration increased gradually with an increase in the degree of ripening, specifically increasing by 15.07 %–21.05 % and 17.22 %–31.06 %, respectively. The half-maximal inhibitory concentration (IC_{50}) values of α-amylase and α-glucosidase decreased by 53.82 % and 66.11 % (Fig. 4D,E), respectively, and the inhibitory effect of tea broth at high concentrations indicated a clear dose-dependent effect; that is, inhibitory efficiency increased synchronously with an increase in the tea infusion concentration. The two rounds of refiring were crucial for the inhibition of α-amylase and α-glucosidase activities because the IC_{50} values exhibited significant differences before and after refiring. Specifically, the ripened KCBT exhibited higher levels of in vitro α-amylase and α-glucosidase activity inhibition. These results suggest that the potential hypoglycaemic effect of ripened KCBT tea was enhanced, and thus the tea's value and health benefits were enhanced.

Proposed in 1958, the DPPH method has since been widely used to quantify the antioxidant capacity of biological specimens, phenolics, and foods (Luo et al., 2023; Sharma & Bhat, 2009; Zhang et al., 2013). The present results pertaining to the DPPH radical scavenging capacity of the samples obtained during varying degrees of ripening (Fig. 4C) indicated that similar to the results for α-amylase and α-glucosidase, the scavenging rates of the samples that underwent different ripening processes for DPPH radical exhibited a concentration-dependent property. That is, their scavenging rates increased synchronously with an increase in tea infusion concentration. The scavenging rate of DPPH radical through tea infusion gradually decreased during the ripening process, the scavenging rate decreased by 1.59 %–3.38 % when the tea infusion concentration was ≥0.3 mg/mL and decreased by >10 % when the concentration was <0.3 mg/mL. The IC_{50} value of the crude KBT sample was 0.076 mg/mL (Fig. 4F), which was significantly lower than that of the ripened SD sample (0.095 mg/mL). Catechins are the main antioxidants of polyphenols (Ma, Wang, Xu, et al., 2022), and therefore the decrease in the scavenging rate of DPPH radical during ripening may be due to the large amount of degradation of catechins.

3.5. *Mantel test analysis of tea polyphenols that exhibited in vitro biological and antioxidant activities during KCBT ripening*

Many related studies have demonstrated that metabolites (particularly polyphenolic compounds with many phenolic hydroxyl groups) influence the strength of the biological activity and antioxidant capacity of tea infusions (Ma, Wang, Zhou, et al., 2022; Mi et al., 2024). Similarly, our study revealed that ripening had a considerable effect on polyphenolic compounds and was significantly associated with the taste quality. Accordingly, we analysed the rates of α-amylase and α-glucosidase activity inhibition and the DPPH radical scavenging rate in relation to the concentration of polyphenolic compounds by conducting the Mantel test (Fig. 5); our results indicated that the concentrations of EGC, theasinensin C, and kaempferol 3-rhamnoside were significantly

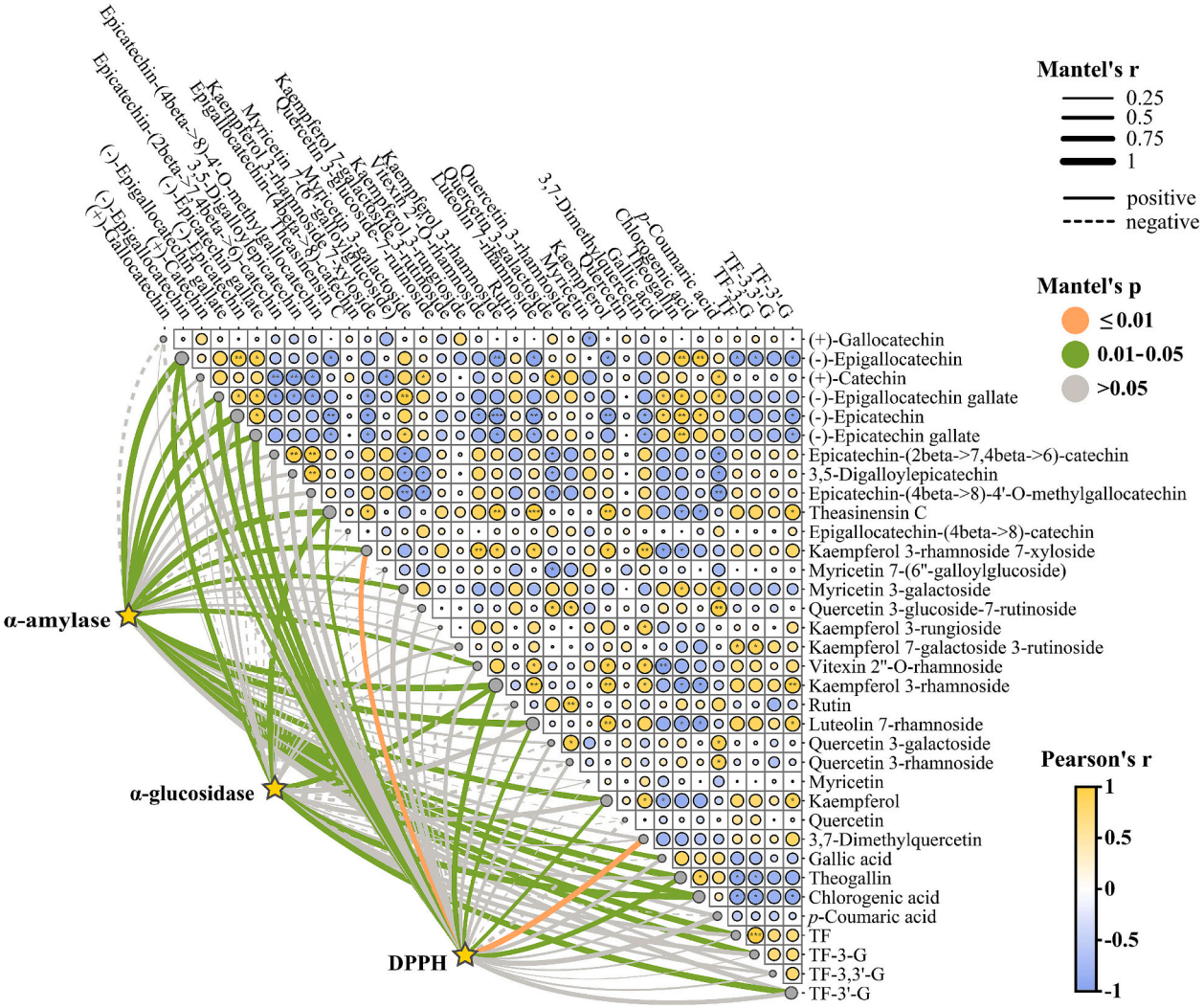

Fig. 5. Mantel test analysis of changes in tea polyphenols and DPPH, α-amylase and α-glucosidase during ripening of Keemun black tea.

correlated with the inhibition of α-amylase and α-glucosidase activities ($r > 0.5, p < 0.05$). The content of compounds such as TFs and flavonoid glycosides increased during the ripening process of KCBT, while the inhibition of α-amylase and α-glucosidase by the tea infusion gradually increased, which may be attributed to the fact that ripening promotes the oxidation of catechins and flavonols, and the inhibition of the enzyme activities showed a higher capacity, especially EGC, theasinensin C, and kaempferol 3-rhamnoside, which may form complexes with α-amylase or α-glucosidase through hydrogen bonding and hydrophobic interactions, thereby inhibiting the catalytic activities and regulating the contents of various sugars.

The phenolic hydroxyl groups in the molecular structure of tea polyphenols exhibit antioxidant properties that enable them to scavenge radical (Li et al., 2013; Ma, Wang, Zhou, et al., 2022). Our Mantel test analysis revealed that two flavonoids, namely kaempferol 3-rhamnoside 7-xyloside and 3.7-dimethylquercetin, were the most significantly effective at scavenging DPPH radical ($r > 0.5, p < 0.01$), followed by the catechin monomers (EGCG, ECG, EGC, and EC; $r > 0.5$, $p < 0.05$). Ripening weakened the ability of tea infusion to scavenge DPPH free radicals during the ripening process of KCBT (Fig. 4). The contents of catechin monomers (EGCG, ECG, EGC, and EC) decreased significantly with increasing degree of ripening (Table 1), which could be attributed to the auto-oxidation and thermal degradation of catechin monomers by the twice aging and refiring during the ripening, where one part of catechins oxidized to polymers and the other part was thermally degraded or isomerized. There is a significant positive correlation between the content of catechin monomers and the DPPH free radical scavenging capacity, so ripening drove the oxidative polymerisation of catechins and other substances to be consumed, which in turn leads to a series of cascade reactions that produce numerous catechin polymers and tea colourants and weak the DPPH free radical capacity. On the other hand, the content of theaflavins increased significantly (Table 1), but did not enhance the scavenging capacity of DPPH radicals, although it has been shown that the enzymatic oxidation of black tea to form polyphenol oxidation products such as TFs, TRs, and TB also possess in vitro antioxidant activity, which may be related to the complex tea infusion system, flavonoid glycosides and catechin monomers showed stronger scavenging DPPH radicals and competitiveness when catechins and their oxides were present simultaneously in the tea infusion. This finding suggests that the flavonoid glycosides and catechins monomers exhibit stronger antioxidant capacity compared with TFs. Thus, this study revealed that EGC and kaempferol 3-rhamnoside 7-xyloside exhibited enzyme activity inhibition and DPPH radical scavenging capacity ($r > 0.5, p < 0.05$), which are major health functions. This finding suggests that these two compounds are key metabolites that enhance the health benefits of KCBT during ripening.

4. Conclusion

In summary, ripening is a key step in the formation of the unique flavour quality and health benefits of KCBT. The quantitative evaluations of taste quality and metabolites in this study revealed that ripening promoted the conversion of catechins, flavonoids, and TFs in crude KBT, which in turn significantly weakened the bitterness and astringency of crude KBT infusions while enhancing their sweetness and mellowness, thereby substantially improving the value of KCBT. Furthermore, ripening drives the cascade reaction of polyphenols to form catechin polymers and flavonoid glycosides, which exhibit positive biological functions, namely enzyme activity inhibition and free radical scavenging, and thus enhance the health benefits of KCBT. In particular, EGC, Theasinensin C and kaempferol 3-rhamnoside contribute considerably to these effects. In conclusion, ripening improved the flavour profile and enhanced the potential biological activities of KCBT. The findings of the present study collectively provide novel insights into the alteration of flavour substances and the formation of health benefits in KCBT during the ripening process.

CRediT authorship contribution statement

Wenjing Huang: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Zhibin Ye:** Formal analysis, Data curation. **Yida Wu:** Data curation. **Tianzi Yu:** Software. **Wei Zhao:** Supervision. **Zihao Qi:** Validation, Methodology, Data curation, Supervision. **Yanqun Jiang:** Supervision. **Qiuyan Liu:** Supervision, Methodology, Formal analysis. **Guofu Lu:** Resources, Conceptualization. **Jingming Ning:** Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they possess no competing financial interests or personal affiliations that might be perceived as influencing the research presented in this study.

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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.2025.102264.

Data availability

Data will be made available on request.

References

Chen, L., Wang, H., Ye, Y., Wang, Y., & Xu, P. (2023). Structural insight into polyphenol oxidation during black tea fermentation. *Food Chemistry: X, 17*. https://doi.org/10.1016/j.fochx.2023.100615

Guo, X., Long, P., Meng, Q., Ho, C. T., & Zhang, L. (2018). An emerging strategy for evaluating the grades of Keemun black tea by combinatory liquid chromatography-Orbitrap mass spectrometry-based untargeted metabolomics and inhibition effects on alpha-glucosidase and alpha-amylase. *Food Chemistry, 246*, 74–81. https://doi.org/10.1016/j.foodchem.2017.10.148

He, H.-F. (2017). Research progress on theaflavins: Efficacy, formation, and preparation. *Food & Nutrition Research, 61*(1). https://doi.org/10.1080/16546628.2017.1344521

Ho, C.-T., Zheng, X., & Li, S. (2015). Tea aroma formation. *Food Science and Human Wellness, 4*(1), 9–27. https://doi.org/10.1016/j.fshw.2015.04.001

Huang, W., Fang, S., Su, Y., Xia, D., Wu, Y., Liu, Q., Zou, L., Lu, C., Deng, W.-W., & Ning, J. (2023). Insights into the mechanism of different withering methods on flavor formation of black tea based on target metabolomics and transcriptomics. *LWT - Food Science and Technology, 189*. https://doi.org/10.1016/j.lwt.2023.115537

Huang, W., Liu, Q., Fu, X., Wu, Y., Qi, Z., Lu, G., & Ning, J. (2024). Fatty acid degradation driven by heat during ripening contributes to the formation of the "Keemun aroma". *Food Chemistry, 451*. https://doi.org/10.1016/j.foodchem.2024.139458

Huang, W., Lu, G., Deng, W.-W., & Ning, J. (2022). Effects of different withering methods on the taste of Keemun black tea. *LWT - Food Science and Technology, 166*. https://doi.org/10.1016/j.lwt.2022.113791

Jiang, Z., Han, Z., Qin, C., Lai, G., Wen, M., Ho, C. T., … Wan, X. (2021). Model studies on the reaction products formed at roasting temperatures from either Catechin or tea powder in the presence of glucose. *Journal of Agricultural and Food Chemistry, 69*(38), 11417–11426. https://doi.org/10.1021/acs.jafc.1c03771

Johnson, M. H., Lucius, A., Meyer, T., & Gonzalez de Mejia, E. (2011). Cultivar evaluation and effect of fermentation on antioxidant capacity and *in vitro* inhibition of α-amylase and α-glucosidase by highbush blueberry (*Vaccinium corombosum*). *Journal of Agricultural and Food Chemistry, 59*(16), 8923–8930. https://doi.org/10.1021/jf201720z

Li, F., Li, S., Li, H. B., Deng, G. F., Ling, W. H., & Xu, X. R. (2013). Antiproliferative activities of tea and herbal infusions. *Food & Function, 4*, 530–538. https://doi.org/10.1039/c2fo30252g

Li, Q., Jin, Y., Jiang, R., Xu, Y., Zhang, Y., Luo, Y., Huang, J., Wang, K., & Liu, Z. (2021). Dynamic changes in the metabolite profile and taste characteristics of Fu brick tea during the manufacturing process. *Food Chemistry, 344*, Article 128576. https://doi.org/10.1016/j.foodchem.2020.128576

Liu, C., Li, J., Li, J., Xue, J., Wang, M., Jian, G., Zhu, C., & Zeng, L. (2023). Differences in the quality of black tea (Camellia sinensis var. Yinghong no. 9) in different seasons and the underlying factors. *Food Chemistry:X, 20*, Article 100998. https://doi.org/10.1016/j.fochx.2023.100998

Long, P., Rakariyatham, K., Ho, C.-T., & Zhang, L. (2023). Thearubigins: Formation, structure, health benefit and sensory property. *Trends in Food Science & Technology, 133*, 37–48. https://doi.org/10.1016/j.tifs.2023.01.013

Luo, J., Liang, L., Xie, Q., Qiu, Y., Jiang, S., Yang, Y., Zhu, L., Fu, Y., Chen, S., Wang, W., & Yuan, H. (2023). Differential analysis of phytochemistry and antioxidant activity in five citrus by-products based on chromatography, mass spectrometry, and spectrum-effect relationships. *Food Chemistry: X, 20*. https://doi.org/10.1016/j.fochx.2023.101010

Ma, B., Wang, J., Xu, C., Ren, X., Ma, C., & Zhou, B. (2021). Differences of phenolic components in Puer raw tea with various storage periods and their effects on the *in vitro* antioxidant capacities. *Journal of Tea Science, (in Chinese), 42*(1), 51–62.

Ma, B., Wang, J., Xu, C., Wang, Z., Yin, D., Zhou, B., & Ma, C. (2022). Interrelation analysis between phenolic compounds and *in vitro* antioxidant activities in Pu-erh tea. *LWT - Food Science and Technology, 158*. https://doi.org/10.1016/j.lwt.2022.113117

Ma, B., Wang, J., Zhou, B., Wang, Z., Huang, Y., Ma, C., & Li, X. (2022). Impact of harvest season on bioactive compounds, amino acids and *in vitro* antioxidant capacity of white tea through multivariate statistical analysis. *LWT - Food Science and Technology, 164*, Article 113655. https://doi.org/10.1016/j.lwt.2022.113655

Mi, S., Han, S., Wang, M., & Han, B. (2024). Comprehensive analysis of the effect of etiolated tea cultivars and harvest seasons on volatile compounds and *in vitro* antioxidant capacity in steamed green teas. *Food Chemistry: X, 22*. https://doi.org/10.1016/j.lwt.2022.113655

Qu, F., Zhu, X., Ai, Z., Ai, Y., Qiu, F., & Ni, D. (2019). Effect of different drying methods on the sensory quality and chemical components of black tea. *LWT - Food Science and Technology, 99*, 112–118. https://doi.org/10.1016/j.lwt.2018.09.036

Sarma, A., Bania, R., & Das, M. K. (2023). Green tea: Current trends and prospects in nutraceutical and pharmaceutical aspects. *Journal of Herbal Medicine, 41*. https://doi.org/10.1016/j.hermed.2023.100694

Scharbert, S., & Hofmann, T. (2005). Molecular definition of black tea taste by means of quantitative studies, taste reconstitution, and omission experiments. *Journal of Agricultural and Food Chemistry, 53*(13), 5377–5384. https://doi.org/10.1021/jf050294d

Scharbert, S., Holzmann, N., & Hofmann, T. (2004). Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse. *Journal of Agricultural and Food Chemistry, 52*(11), 3498–3508. https://doi.org/10.1021/jf049802u

Sharma, O. P., & Bhat, T. K. (2009). DPPH antioxidant assay revisited. *Food Chemistry, 113*(4), 1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008

Sun, S., Wang, H., Xie, J., & Su, Y. (2016). Simultaneous determination of rhamnose, xylitol, arabitol, fructose, glucose, inositol, sucrose, maltose in jujube (*Zizyphus jujube mill.*) extract: Comparison of HPLC–ELSD, LC–ESI–MS/MS and GC–MS. *Chemistry Central Journal, 10*(1). https://doi.org/10.1186/s13065-016-0171-2

Wang, J., Bi, H., Li, M., Wang, H., Xue, M., Yu, J., Ho, C.-T., Zhang, L., Zhuo, Q., Jiang, J., Wan, X., & Zhai, X. (2023). Contribution of theanine to the temperature-induced changes in aroma profile of Wuyi rock tea. *Food Research International, 169*. https://doi.org/10.1016/j.foodres.2023.112860

Wang, J., Li, M., Wang, H., Huang, W., Li, F., Wang, L., … Wan, X. (2022). Decoding the specific Roasty aroma Wuyi rock tea (*Camellia sinensis: Dahongpao*) by the Sensomics approach. *Journal of Agricultural and Food Chemistry, 70*(34), 10571–10583. https://doi.org/10.1021/acs.jafc.2c02249

Wen, M., Zhou, F., Zhu, M., Han, Z., Lai, G., Jiang, Z., Long, P., & Zhang, L. (2023). Monitoring of pickled tea during processing: From LC-MS based metabolomics analysis to inhibitory activities on α-amylase and α-glycosidase. *Journal of Food Composition and Analysis, 117*. https://doi.org/10.1016/j.jfca.2022.105108

Xiao, Z., Cao, X., Zhu, J., Chen, F., & Niu, Y. (2022). Characterization of the key aroma compounds in three world-famous black teas. *European Food Research and Technology, 248*(9), 2237–2252. https://doi.org/10.1007/s00217-022-04039-2

Xu, Y., Liu, Y., Yang, J., Wang, H., Zhou, H., & Lei, P. (2023). Manufacturing process differences give Keemun black teas their distinctive aromas. *Food Chemistry: X, 19*. https://doi.org/10.1016/j.fochx.2023.100865

Yin, X., Wei, Y., Li, T., Zhang, J., Zou, L., Cui, Q., Lu, C., & Ning, J. (2023). Heterocyclic compounds formation in large-leaf yellow tea induced by the Maillard reaction at different roasting temperatures. *LWT - Food Science and Technology, 182*. https://doi.org/10.1016/j.lwt.2023.114856

Zhang, J., Sun-Waterhouse, D., Su, G., & Zhao, M. (2019). New insight into umami receptor, umami/umami-enhancing peptides and their derivatives: A review. *Trends in Food Science & Technology, 88*, 429–438. https://doi.org/10.1016/j.tifs.2019.04.008

Zhang, L., Cao, Q.-Q., Granato, D., Xu, Y.-Q., & Ho, C.-T. (2020). Association between chemistry and taste of tea: A review. *Trends in Food Science & Technology, 101*, 139–149. https://doi.org/10.1016/j.tifs.2020.05.015

Zhang, L., Xia, Y., & Peterson, D. G. (2014). Identification of bitter modulating maillard-catechin reaction products. *Journal of Agricultural and Food Chemistry, 62*(33), 8470–8477. https://doi.org/10.1021/jf502040e

Zhang, Y., Li, Q., Xing, H., Lu, X., Zhao, L., Qu, K., & Bi, K. (2013). Evaluation of antioxidant activity of ten compounds in different tea samples by means of an on-line HPLC–DPPH assay. *Food Research International, 53*(2), 847–856. https://doi.org/10.1016/j.foodres.2013.03.026

Zhuang, J., Dai, X., Zhu, M., Zhang, S., Dai, Q., Jiang, X., Liu, Y., Gao, L., & Xia, T. (2020). Evaluation of astringent taste of green tea through mass spectrometry-based targeted metabolic profiling of polyphenols. *Food Chemistry, 305*. https://doi.org/10.1016/j.foodchem.2019.125507