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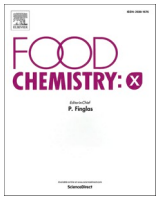

The manufacturing process provides green teas with differentiated nonvolatile profiles and influences the deterioration of flavor during storage at room temperature

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ABSTRACT

Hundreds of green tea products are available on the tea market and exhibit different characteristics. In the present study, seven types of green tea were processed, and their nonvolatile profiles were analyzed by liquid chromatography–mass spectrometry. Non-spreading green tea contained higher concentrations of catechins and flavonoid glycosides, but lower concentrations of amino acids, caffeine, and theaflavins. Non-rolling green teas with a straight appearance contained higher concentrations of flavonoid glycosides and theaflavins. In contrast, leaf-rolling green teas contained much lower concentrations of flavonoid glycosides and catechins. These seven green tea qualities all decreased following prolonged storage, concurrent with increasing concentrations of proanthocyanidins, catechins dimers, theaflavins, and organic acids. The leaf-rolling green teas exhibited reduced levels of deterioration during storage in terms of their nonvolatile profile and sensory quality. Findings show that moderate destruction on tea leaves during green tea processing is beneficial to both tea flavor and quality maintenance during storage.

1. Introduction

Green tea, as a non-fermented tea product, contains more astringent and bitter nonvolatile compounds than other tea products, including epigallocatechin gallate (EGCG), polyphenols, flavan-3-ols, and flavonol glycosides. These nonvolatiles provide a fresh, mellow, or sweet-after-taste to human sensation (Jiang et al., 2019). EGCG is the most abundant catechin in green teas and acts as an active oxygen scavenger due to its antioxidative activity (Wu et al., 2023). Green tea extracts have been applied as food additives due to their antimicrobial, anti-inflammatory, and anticancer properties (Silva et al., 2023). For example, previous research demonstrated that green tea extracts could retard the self-degradation of ready-to-eat sea cucumber (Qi et al., 2022).

Hundreds of green tea products are present in the tea market in China. Moreover, green tea accounts for 60% of the total tea production, followed by black tea and dark tea. Many Chinese green teas are well known worldwide, such as Longjing tea (Wang et al., 2020), Taiping Houkui tea (Zhou, Wang, Liu, & Lei, 2023), Lu'an guapian tea (Li et al., 2022), and Maofeng tea (Li et al., 2023). Japanese green teas also have a popular global reputation, including Sencha, Matcha, Gyokuro, and Kamairi-cha (Tan et al., 2019). These green tea products have their own characteristics and metabolite profiles (Yang et al., 2022; Zhang et al., 2024) due to differences in manufacturing processes (Wang et al., 2022), tea varieties (Yang et al., 2022), plucking season (Xu, Song, Li, & Wan, 2012), the maturity of fresh materials (Xu et al., 2021), geographic location, and garden cultivation.

The manufacturing process used to produce green tea generally includes spreading, enzymic inactivation, rolling/shaping/comminution, and drying. Previous research has demonstrated that the manufacturing process can exert significant influence on the metabolite profiles and sensory qualities of green tea products. When produced with a spreading step, green tea exhibits better quality, due to increased concentrations of

Abbreviations: AGC, automatic gain control; ANOVA, analysis of variance; C, catechin; CG, catechin gallate; DoT, dose over threshold; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GC, gallocatechin; GCG, gallocatechin gallate; GT, green tea; HPLC, high performance liquid chromatography; PCA, principal component analysis; UPLC–QTRAP–MS/MS, ultra–high performance liquid chromatography coupled with hybrid triple quadrupole–linear ion trap electrospray ionization mass spectrometry; VIP, variable importance for the projection.

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amino acids and aroma compounds (He et al., 2023). The enzymic inactivation stage (also known as 'fixation') is the most characteristic process for the production of green tea, including steaming and pan-frying methods. Steamed green tea contains more catechins compared to pan-fired green tea (Han et al., 2016). Recently, a new method, light-wave fixation has been shown to cause green tea product to accumulate more umami amino acids and catechins (Xue et al., 2023). A low fixation temperature in tea processing has been shown to result in more amino acids. In contrast, a high fixation temperature results in more polyphenols (Yu et al., 2023). The leaf-rolling procedure has been shown to influence the concentrations of caffeine, catechins, and theanine in green tea samples (Miyagishima et al., 2011).

Nonvolatile compounds are the main bioactive constituents and flavor contributors in green tea, including catechins, amino acids, procyanidins, alkaloids, flavonol/flavone glycosides, and organic acids. These compounds are all susceptible to high temperature, oxygen, and moisture during the storage of green tea, and can lead to quality deterioration and economic loss due to reduced marketability (Friedman, Levin, Lee, & Kozukue, 2009). Consensually, 'aged' green tea products are not commercially viable, largely due to the reduction of bioactive compounds and the resulting unpleasant flavor. In contrast, the adoption of an appropriate storage method can improve the quality of white tea, dark tea, or other tea products that undergo a fermented or post-fermented process (Dai et al., 2018).

In China, samples of green tea are stored in factories within a cold storage room before sale. This storage method can help to maintain the quality of tea and help to maximize sales. A previous study detected a 3% reduction in polyphenol content in green tea samples after a 150-day period of storage at −80 °C (Dai et al., 2019). In the present study, seven types of green tea (one non-spreading green tea, two non-rolling green teas, and four leaf-rolling green teas) were prepared by different manufacturing processes using a range of tea machinery (Fig. 1). Next, we comprehensively analyzed the nonvolatile profiles of the seven types of tea by applying an ultra-performance liquid chromatography and an ESI-triple quadrupole linear ion trap mass spectrometry (UPLC-QTAP-MS/MS) system. In addition, the seven types of tea underwent prolonged storage at room temperature to determine which green tea types can better maintain its flavor quality.

2. Materials and methods

2.1. Materials

2.1.1. Green tea samples

Fresh shoots of the 'Fuzao 2' tea variety (*Camellia sinensis* var. *sinensis* cv. Fuzao 2) were plucked in late April and processed into different types of green tea (Fig. 1), as described below.

Green tea sample 1 (GT1) was a non-spreading form of green tea. Fresh leaves were plucked and then fixed directly with a drum-type fixation machine (diameter: 40 cm; Zhejiang Sunyoung Machinery Co., Ltd., Quzhou, China) at 350 °C for 2–3 min (**Fig. S1**). A nonpressure rolling stage (with a distance of 3–5 cm between the barrel cover and the surface of the leaf) was conducted using a rolling machine (diameter: 35 cm; Zhejiang Sunyoung Machinery Co., Ltd., Quzhou, China) for 15 min. Drying was performed at 120 °C for 5–7 min, room temperature for 30 min, and 80 °C for 20–30 min. The same drying procedure was used to dry the other types of green tea.

An eight-hour indoor spreading step was performed before the other types of green tea were processed. Green tea sample 2 (GT2) was a non-rolling form of green tea that was fixed with a reciprocating fixation machine (Zhejiang Sunyoung Machinery Co., Ltd., Quzhou, China). Fixation was performed at 200 °C for 3–4 min, and the shaping stage was conducted in the same machine at 150 °C for 10–15 min prior to the drying procedure. Green tea sample 3 (GT3) was a non-rolling form of green tea; the fixation machinery for GT3 was the same drum-type fixation machine as used for GT1. The remaining processes were the same as for GT2. Green tea sample 4 (GT4) was a leaf-rolling form of green tea; the manufacturing process was the same as GT1 and included a spreading stage. Green tea sample 5 (GT5), 6 (GT6), and 7 (GT7) were the leaf-rolling types of green tea. An additional shaping procedure was conducted during the processing of GT5 which involved a drum-type drying machine (diameter: 60 cm; Zhejiang Sunyoung Machinery Co., Ltd., Quzhou, China), which operated at 130 °C for 10 min and 150 °C for 5 min. A light pressure rolling stage (with no space between the

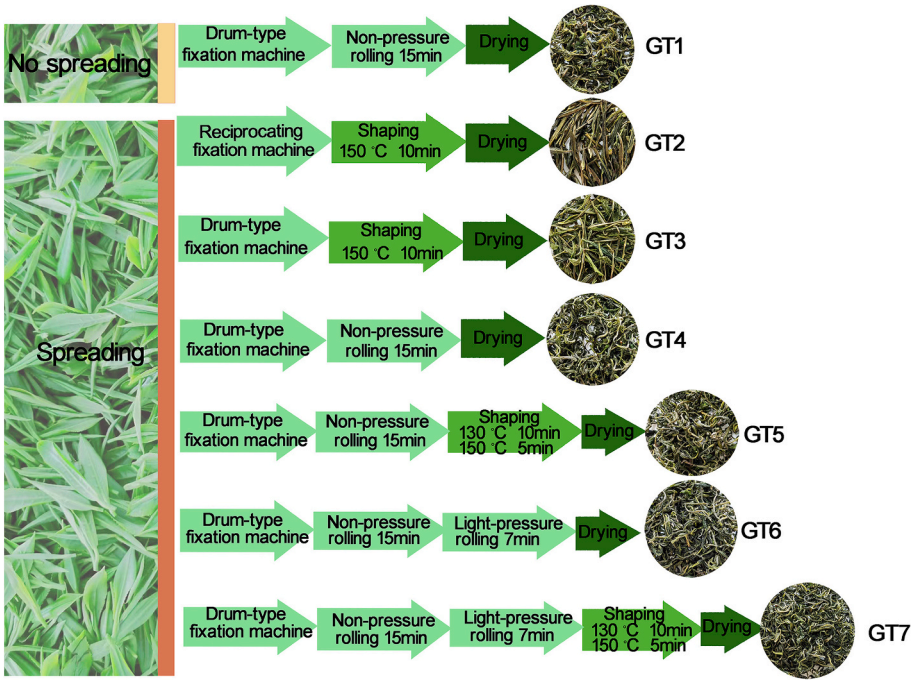

Fig. 1. Outlines of manufacturing processes for seven types of green tea. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

barrel cover and the leaf surface) was applied for 7 min when processing GT6 and GT7. The processing of GT7 incorporated an additional shaping procedure that involved a drum-type drying machine. The apparatus used in this study is shown in **Fig. S1**.

2.1.2. Chemicals

The following chemicals were purchased from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China): catechin (C), epicatechin (EC), epigallocatechin (EGC), gallocatechin (GC), gallocatechin gallate (GCG), epigallocatechin gallate (EGCG), epicatechin gallate (ECG), catechin gallate (CG), theaflavin, theaflavin-3-gallate, theaflavin-3′-gallate, and theaflavin-3,3′-di-*O*-gallate. Flavonoids and glycosides standards were purchased from Shanghai Macklin Biochemical Technology Co., Ltd. (Shanghai, China), including rutin, quercetin, vitexin, myricetin, myricetin 3-*O*-galatoside, kaempferol-3-*O*-glucoside quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, quercetin-3-*O*-glucosylrutinoside, proanthocyanidin B1, proanthocyanidin B2, proanthocyanidin B3, proanthocyanidin B4, *β*-glucogallin, and quinic acid. We also purchased a mixed amino acids standard from Sykam GmbH (Munich, Germany).

2.2. Methods

2.2.1. Determination of key parameters (L*, a*, b*, ΔE, and BI)

The color of the seven tea infusions was determined by a colorimeter (YS6060, 3nh Technology CO., Ltd., Shenzhen, China). Tea infusions (3 g of tea sample; 150 mL of boiling water) were prepared; after cooling to room temperature, tea infusions were placed on glass plates for measurements; values of *L*, *a*, and *b* were recorded for each sample at three different points. According to a previous study (El-Baset & Almoselhy, 2023), the total color variation index (Δ*E*) and browning index (BI) were also calculated in this study using the equations as follows:

$$\Delta E = \left[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2\right]^{1/2}$$

$$\mathrm{BI} = \frac{100}{0.17}\left(\frac{a^* + 1.75L^*}{5.645\,L^* + a^* - 0.012b^*} - 0.31\right)$$

2.2.2. HPLC analysis of catechins and caffeine

A HITACHI Chromaster HPLC system (HITACHI, Japan) was used to analyze the content of catechins and caffeine. Tea infusions (0.25 g of tea powder; 7 mL of boiling water) were prepared in a 90 °C water bath for 7 min. The residues were then re-extracted, as described above. The mixture of tea infusions was diluted four-fold using an initial mobile phase (10% B and 90% A), and then filtered through a 0.22 μm filter before LC analysis.

The HPLC column, column temperature, detection wavelength and mobile phases were as follows: HITACHI LaChrom C18 (150 mm × 4.6 mm, 5 μm), 40 °C, 280 nm, 0.04% (*v*/v) phosphoric acid in distilled water (A), and 100% acetonitrile (B), respectively. The flow rate and elution program were the same as those used in a previous study (Yang et al., 2022). Catechins and caffeine were identified and quantified according to authentic standards and calibration curves established from standards; each measurement was acquired in triplicate.

2.2.3. Analysis of free amino acids

Amino acid extracts were prepared as in Section 2.5. An S—433D amino acid analyzer (SYKAM, Munich, Germany), coupled to an LCA K07/Li column (SYKAM, Munich, Germany), was used to detect the amino acids in each tea sample. The elution and derivation flow rates were 0.45 and 0.25 mL/min, respectively. The mobile phases were purchased from SYKAM, including buffer A (0.12 N, pH 2.9), B (0.3 N, pH 4.2), and C (0.3 N, pH 8.0). Buffer D (500 mM NaOH, 0.68 mM EDTA) was prepared inhouse. The detection wavelengths were 440 nm for proline and 570 nm for all other amino acids. The gradient method used for elution was the same as described previously (Yang et al., 2022). The sixteen amino acids were identified and quantified according to authentic standards and by comparing peak areas for the analytical standards and tea samples; each sample was analyzed in triplicate

2.2.4. UPLC-QTRAP-MS/MS

The extraction of nonvolatile compounds was performed using 80% methanol solution. Tea powder (60 mg) was extracted twice with 1.8 mL of precooled solution. The aqueous solution was filtered through a 0.22 μm membrane prior to LC–MS analysis. Triple extractions for each sample were performed.

A Q-Exactive Focus Orbitrap LC–MS/MS System (Thermo Fisher Scientific, USA), coupled with a C18 column (3 mm × 150 mm × 2.7 μm; Poroshell 120 SB-C18, Agilent, California, USA) was used to analyze nonvolatile compounds. Firstly, we prepared the mobile phases: 0.4% acetic acid (*V*/V, acetic acid: distilled water, A) and 100% acetonitrile (B). The initial elution was 0.1% B and 99.9% A; this was then ramped to 7% B in 10 min (and held for 12 min). The mobile phase B was subsequently increased to 11% in 3 min, followed by a ramp to 12% in 5 min, a ramp to 14% in 1 min, a ramp to 35% in 12 min, and a ramp to 80% in 1 min (and held for 4 min). The column oven temperature, flow rate, and injection volume were set to 30 °C, 0.5 mL/min, and 10 μL, respectively.

The drying gas nitrogen and sheath gas flow were 8 L/min at 325 °C and 11 L/min at 350 °C, respectively. The nebulizer pressure and capillary voltage were 45 psi, and 3500 V. Analysis was performed in negative ionization mode. The full MS parameters were as follows: 70000 resolution, 5e6 AGC target, 200 ms maximum IT, and 80 to 1200 *m/z* scan range. The dd-MS^2 parameters were as follows: 17500 resolution, 1e5 AGC target, 60 ms maximum IT, 5 loop count, and 0.4 *m/z* isolation. The collision energy was set to15–40 V. The acquired MS and MS/MS data files were analyzed by Thermo Xcalibur 4.1 and TraceFinder 4.1 software packages.

2.2.5. Identification and quantification of nonvolatile compounds

The precursor ion and MS/MS fragment message of nonvolatiles were obtained from previous studies (Huang et al., 2021; Liu, Chen, Sun, & Ni, 2022; Zhuang et al., 2020) and from our current study. The retention time of the nonvolatiles with their authentic standards was compared with analytical standards. Other nonvolatiles were confirmed previously (Zhuang et al., 2020).

Thermo TraceFinder 4.1 software was used to establish a local database featuring the compound name, chemical formula, precursor *m/z*, polarity, and retention time (**Fig. S2**). The integration of nonvolatiles was then based on this newly established database. Compounds were quantified based on the peak areas of myricetin (10 mg/L).

2.2.6. Storage treatment

The seven green tea samples were placed separately into a sealed fresco bag and stored in a constant temperature (37 °C) incubator (Shanghai Yiheng Co., Ltd., Shanghai, China). As a control, tea samples were stored at −80 °C. Tea samples at different storage points (15, 30, 45, 60, 75, 90, 105, and 120 days) were stored at −80 °C for further analysis.

2.2.7. Sensory evaluation

Six tea experts evaluated the seven green teas by applying specific criteria: The National Standard of the People's Republic of China (GB/T 23776–2018). According to the standard, boiled water (98 °C, 150 mL) was poured onto the tea samples (3 g), and the infusions were poured into a bowl after 5 min of brewing. The overall taste quality was evaluated as follows: 7–8 (barely mellow, green and astringency), 8–9 (fresh, mellow and thick), and 9–10 (umami, sweet, fresh, and mellow).

The intensity of umami, astringency, and bitterness of the tea infusions were evaluated based on a 10-point scale: 0–2 (none or extremely weak), 2–4 (weak), 4–6 (middle), 6–8 (strong), and 8–10 (extremely strong). Five specific concentrations of glutamic acid

(umami), EGCG (astringency), and caffeine (bitterness) were prepared to train the tea evaluators.

2.2.8. Statistical analysis

Significant differences ($P < 0.05$) between tea samples were identified by one–way analysis of variance (ANOVA) and Duncan's multiple range tests in SPSS version 20.0 software (SPSS Inc., USA). The main taste compounds, nonvolatiles, and color were carried out in three replicates. Principal component analysis (PCA) and variable importance for the projection (VIP) plots were generated by SIMCA version 14.1 (Umetrics Corporation, Sweden).

3. Results and discussion

3.1. Nonvolatile profiles of seven types of green tea

3.1.1. Main taste compounds determined by HPLC

The seven green teas exhibited a distinguished appearance and flavor quality when processed by different manufacturing processes using a series of tea machinery (Fig. 1 and **Fig. S1**). Following reciprocating shaping, GT2 and GT3 exhibited a straight appearance. In contrast, the other green teas exhibited a curly appearance following rolling or drum-type shaping.

The color of the seven tea infusions was measured by determining *L* (brightness), *a* (red/green), and *b* (yellow/blue) values. The brightness of the seven tea infusions did not differ apparently; this was also the case for the *a* value. GT2 and GT3 tea infusions both had a high *b* value, thus indicating that these two green teas contained more nonvolatile compounds with a yellow coloration (Fig. 2). In addition, the ΔE and *BI* values of seven green tea infusions were shown in **Fig. S3**.

GT1, as a non-spreading green tea, contained higher concentrations of catechins compared to the other green teas, while GT4 and GT6 teas had lower concentrations of catechins. These data indicated that spreading reduced the concentrations of catechins, as typified by the difference in the processing of GT1 and GT4. As shown in **Table S1**, EGCG was highly concentrated in GT1; however, the concentration of EGCG in GT4 was less than in GT1, GT3, and GT5 green teas; there was no significant difference detected with the other teas. GT4 contained approximately 85% of the amount of EGCG in GT1; this was attributed to the degradation or epimerization of EGCG during spreading. Compared to GT4, the additional shaping stage used to produce GT5 led to an increase in EGCG content.

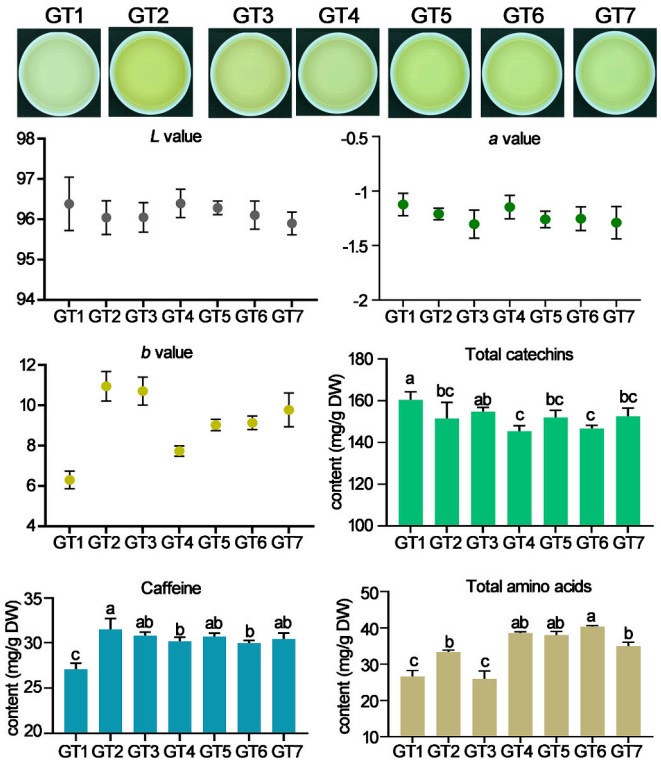

Fig. 2. Determination of color and main taste compounds in seven tea infusions.

Unexpectedly, GT1 contained the lowest concentration of caffeine compared to the other green teas (Fig. 2). Caffeine has a bitter characteristic and exhibits a high dose-over threshold (DOT) in tea infusions (Wen et al., 2022). This low concentration of caffeine in GT1 may exert a positive influence on sensory evaluation. Previous research suggested that caffeine was stored in the vacuoles of plants (Waldhauser & Baumann, 1996) while caffeine biosynthesis occurred in the chloroplasts of young leaves (Kato, Crozier, & Ashihara, 1998). Another study showed that shading increased the levels of caffeine in tea leaves (Ashihara, Sano, & Crozier, 2008). Our data indicate that eight-hour indoor spreading might enhance caffeine biosynthesis, most probably due to enzymic activity and substrates from intracellular degradation.

GT1, GT3, and GT7 teas contained low concentrations of amino acids. In contrast, GT4, GT5, and GT6 had high concentrations of free amino acids; this may exert a positive influence on sensory evaluation. As shown in **Table S1**, most amino acids, especially aspartic acid, glutamic acid, serine, and theanine, increased by 115%, 98%, 67%, and 20%, respectively, in GT4 when compared to GT1, thus suggesting that spreading improved the accumulation of amino acids, probably due to the degradation of intracellular protein; these results concurred with those of a previous study (He, Li, et al., 2023). The additional shaping procedure used in the production of GT7 dramatically reduced the levels of theanine compared to the GT6 green tea. Thermal degradation can reduce the levels of theanine during drying to produce nitrogen-containing heterocyclic compounds (Guo, Ho, Schwab, & Wan, 2021). Furthermore, reciprocating fixation had a low temperature response on tea leaves when compared to drum-type fixation. GT2 contained more amino acids compared to GT3 green tea; similar results were reported previously (Yu et al., 2023).

The color and taste of tea infusions are known to be closely related to flavonoids and glycosides, such as rutin, which provides a strongly astringent taste to the human tongue (Zhang, Cao, Granato, Xu, & Ho, 2020). These bioactive compounds, flavonoids, glycosides, and phenolic acids, require an application of mass spectrometry to identify accurately. Hence, in the present study, we used LC-MS/MS to analyze the nonvolatile profiles of the seven types of green tea.

3.1.2. Nonvolatile profiles of seven types of green tea determined by LC-MS/MS

In the present study, most of the nonvolatiles produced an intense $[M–H]^-$ ion signal in negative mode. In total, 79 nonvolatile compounds were identified by comparing their characteristic $[M–H]^-$ precursor ion, MS/MS fragment, and retention time (Huang et al., 2021; Liu et al., 2022; Zhuang et al., 2020). **Table S2** describes the identification of these compounds in detail, along with their relative concentration, which was calculated by comparing them with the peak area of myricetin (10 ppm). The GT3 tea had the highest total concentration of nonvolatiles (7098 mg/kg), followed by GT2 (6964 mg/kg), GT4 (6886 mg/kg), and GT1 (6819 mg/kg). GT5 had the lowest total concentration (6453 mg/kg) when compared to the other green teas. The total nonvolatile concentration did not differ significantly between GT1 to GT4, or between GT5 to GT7 ($P > 0.05$). These results implied that additional processing reduced the levels of the nonvolatile compounds, such as the thermal shaping procedure and leaf-rolling with pressure.

By applying homogenized calculation and the relative concentrations of the 79 nonvolatiles, we were able to produce a heatmap. Clustering analysis identified two subgroups: GT1–GT3 and GT4–GT7 (Fig. 3A). Most nonvolatiles were abundant in the former group, except for quinic acid and its derivatives, EC, C, and procyanidin C1. When

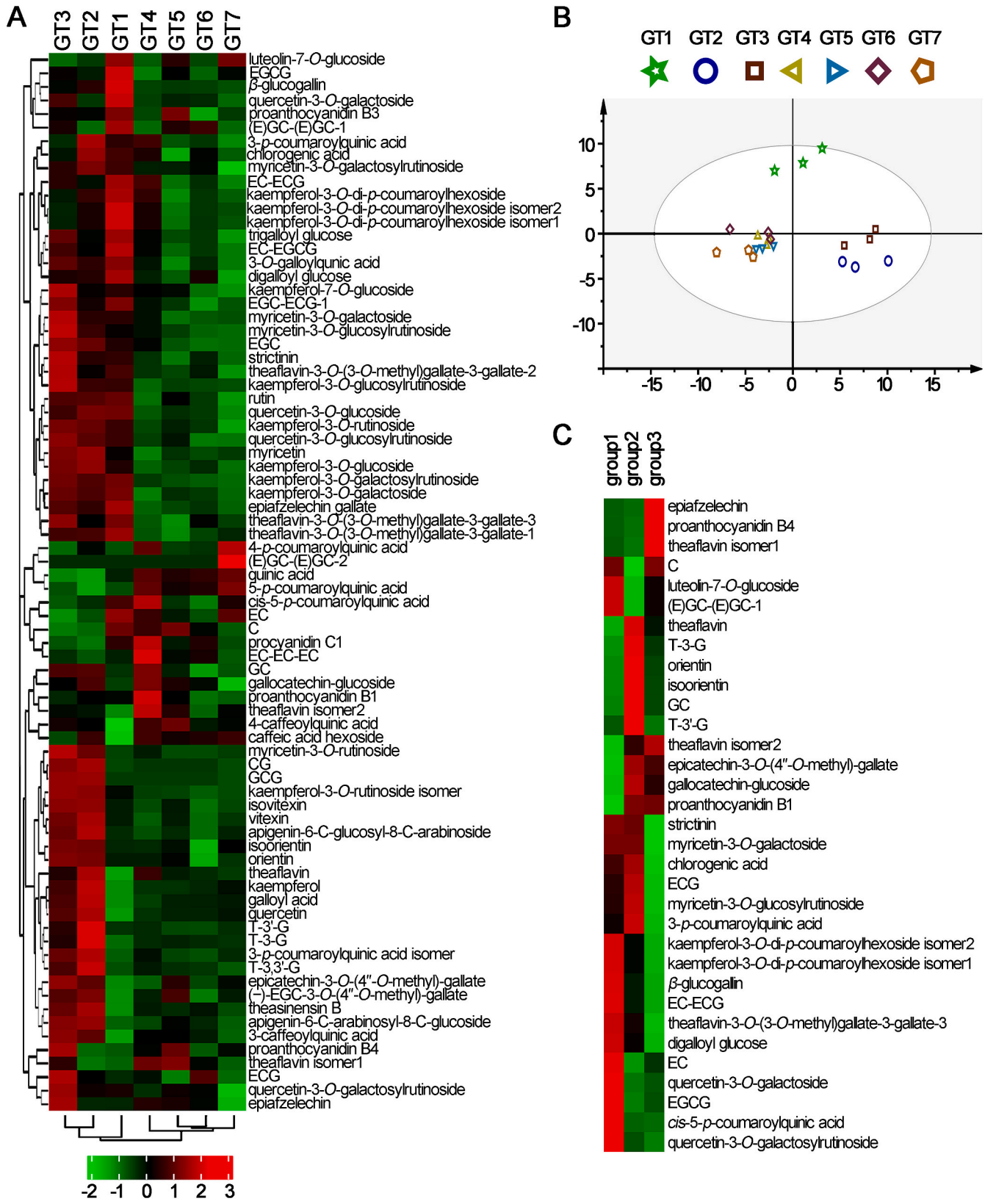

Fig. 3. Nonvolatile profiles of seven types of green tea identified by LC-MS/MS. (A) heatmap of 79 nonvolatiles; (B) principal component analysis (PCA); (C) 33 differential nonvolatiles between groups (VIP >1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

compared to GT2 and GT3, GT1 tea had lower concentrations of theaflavins, galloyl acid, kaempferol, non-*epi* catechins, quercetin, and catechin dimers. Principal component analysis (PCA) showed that GT1 tea was located distant from the other groups; GT2 and GT3 teas were located close to each other; and GT4, GT5, GT6, and GT7 teas were located close to each other (Fig. 3B). The nonvolatile profiles of non-spreading (GT1), non-rolling (GT2 and GT3), and leaf-rolling (GT4, GT5, GT6, and GT7) green teas differed apparently.

According to the PCA, we identified the seven green teas into group 1 (GT1), group 2 (GT2 and GT3), and group 3 (GT4– GT7). Next, we used a variable importance for the projection (VIP) plot to search for differential metabolites between groups (VIP >1). Fig. 3C shows thirty-three differential compounds. Group 1 was associated with higher concentrations of kaempferol-3-*O*-di-p-coumaroylhexosides, *β*-glucogallin, digalloyl glucose, EC, EGCG, quercetin glycosides, and cis-5-*p*-coumaroylquinic acid. These compounds mainly exhibited an astringency characteristic and strong bioactivity, especially cis-5-*p*-coumaroylquinic acid and EGCG (Wen et al., 2022). Plant flavonoids has been identified in the cytosol, vacuole, chloroplast, and endoplasmic reticulum, and most conjugated flavonoids are primarily found in the vacuole (Zhao & Dixon, 2010). Histochemical staining analysis previously showed that catechins were stored in the chloroplasts of mesophyll cells and vessel walls (Liu, Gao, Xia, & Zhao, 2009). GT1, which did not experience a spreading stage, contained more flavonoid glycosides and catechins than the other teas; this may be related to the subcellular location of polyphenol oxidases and their substrates (Olmedo et al., 2018). After a long period of spreading, membrane structures begin to loosen, thus accelerating transportation and transformation between enzymes and their substrates (Liu et al., 2022). It is possible that polyphenol oxidase catalyzed catechins to produce theaflavins or other polymers, while flavonoid glycosides and their catalytical enzymes (e.g., glycosidase) reacted to release the aglycones.

Group 2 had higher concentrations of theaflavins, such as theaflavin, theaflavin-3-gallate, and theaflavin-3′-gallate than the other groups. The high *b* value of GT2 and GT3 tea infusions might be associated with the high concentrations of theaflavins (Fig. 2). As the tea industry has developed, Chinese consumers appear to show preference for green tea products with a tidy appearance, such as Taiping Houkui green tea (Zhou et al., 2023) and albino green tea products (Yang et al., 2022). The reciprocating fixation machine is used to shape tea leaves in China so that these leaves have a straight appearance (**Fig. S1**). This machine is set at a high temperature which can cause a rapid loss of water from tea leaves; in addition, the edges of the leaves can easily be scorched, a characteristic that is not beneficial for shaping. Low temperature fixation and long-term shaping might enhance the oxidation of catechins, thus producing more theaflavins. We found that flavonoids and their glycosides were also more abundant in the GT2 and GT3 teas when compared to the third group (Fig. 3C). These two types of tea (which were produced without a rolling stage) featured tea leaves with a relatively complete internal structure. The leaf-rolling process is known to destroy the intracellular structure, thus promoting the outflow of water inside and the transfer of metabolites from deep leaf regions to the surface (Miyagishima et al., 2011).

In group 3 (GT4–GT7), most nonvolatiles were detected at relatively low levels, except for epiafzelechin, proanthocyanidin B4, and theaflavin isomers. The leaf-rolling and additional shaping procedure destroyed the leaf structure and forced more internal metabolites to be transferred to the surface. On the one hand, deep transformation occurred between substrates and residual enzymes after fixation, thus leading to lower levels of catechins and flavonoid glycosides. On the other hand, metabolites from the deep leaf regions were easily influenced by the thermal reaction arising from the drying procedure.

The seven types of green tea, produced by different manufacturing processes, exhibited different flavor qualities and nonvolatile profiles. At present, the storage of green tea remains a significant challenge for the tea industry that needs to be addressed urgently. The change and deterioration of green tea flavor and its bioactive compounds are irreversible during storage. Low temperature, oxygen isolation, and low moisture levels have all been attempted in an effort to store green tea effectively. In the present study, we aimed to determine which green tea product exhibited less variation in terms of metabolite profile and tea flavor after storage at room temperature.

3.1.3. Post-storage umami characteristics

When investigating the accelerated aging of green tea beverage, a previous study demonstrated that the storage of green tea beverage at 55 °C for 14 days led to the same magnitude of change as storage at 25 °C for one year (He et al., 2023). In the present study, we selected a constant storage environment (37 °C for 120 days) to determine which tea types exhibited relatively stable quality.

The umami intensity of the seven tea infusions continued to decrease with storage (Fig. 4A **and Table S3**). Amino acids are recognized as the main contributors to umami, particularly glutamic acid, theanine, and aspartic acid (Poojary, Orlien, Passamonti, & Olsen, 2017). The amino acid profiles of the seven types of green tea are measured over different storage durations. The total amino acid concentrations of the seven types of green tea fluctuated less after storage, except for GT1, GT3 and GT7. The total amino acid levels in the GT1 and GT3 teas increased after storage. In contrast, the GT7 tea exhibited a temporary decline in total amino acid concentration after 15 days of storage (Fig. 4B). PCA results further showed that the amino acid profiles of GT1 and GT2 teas were different from the other teas (Fig. 4C).

Next, we generated a heatmap that showed that most amino acids increased in concentration with prolonged storage **(**Fig. 4D**).** In particular, we noted that the concentrations of theanine, glycine and alanine increased in tea samples when stored for 15 days, except for the GT6 and GT7 teas. Previous research showed that the concentrations of aspartic acid, glutamic acid, and glutamine in a green tea beverage all decreased with aging at 55 °C, while the levels of pyroglutamic acid and theanine increased with storage (He, Jiang, et al., 2023). The degradation of theanine adducts may be contribute to an increasing level of theanine (Han et al., 2022). In the present study, 1-deocy-1-L-theanino-D-fructose ($C_{13}H_{24}N_2O_8$), a theanine-glucose adduct, was identified from MS data using its $[M–H]^-$theoretical *m/z* 335.1459 in negative mode. A peak of 335.1461 *m/z* was extracted, and its response showed a tiny decline with storage (**Fig. S4**). It is possible that the increased level of theanine may be attributed to the degradation of other theanine conjugates.

The levels of amino acids responsible for umami were relatively stable after storage; this finding was inconsistent with the reduction of umami intensity that was observed in the seven tea infusions. It is possible that an increase in the other taste senses (e.g., bitterness, stale, and astringency) may exert a negative influence on umami sensation. Some fatty acids are also known to be positively correlated with the umami taste of food (Min, Lee, Bae, & Moon, 2023). A previous study showed that the levels of lipid metabolites change rapidly during the storage of green tea, especially octadeca-dien-6-ynoic acid (an umami contributor), the levels of which were reduced to 10% after a 40-day period of storage (Zhou et al., 2023).

3.1.4. Nonvolatile profiles of seven types of green tea during storage

As expected, the intensities of astringency and bitterness for the seven tea infusions all showed a tendency to increase with storage, especially the astringent characteristic (Fig. 5A and B). The nonvolatile profiles of the seven types of green tea were analyzed at different storage points by LC-MS/MS. The total nonvolatile concentrations of the seven types of green tea (GT1– GT7) increased to 125%, 112%, 115%, 114%, 110%, 110%, and 108% of the original, respectively, within 120 days of storage (**Table S4**). For example, the total nonvolatile content of GT1 increased from 6819 mg/kg to 7227 mg/kg after a 30-day storage period, and then to 8556 mg/kg after a 105-day period of storage.

PCA showed that the tea samples stored for 0, 15, and 30 days clustered together. In contrast, tea samples stored for 45, 60, and 75

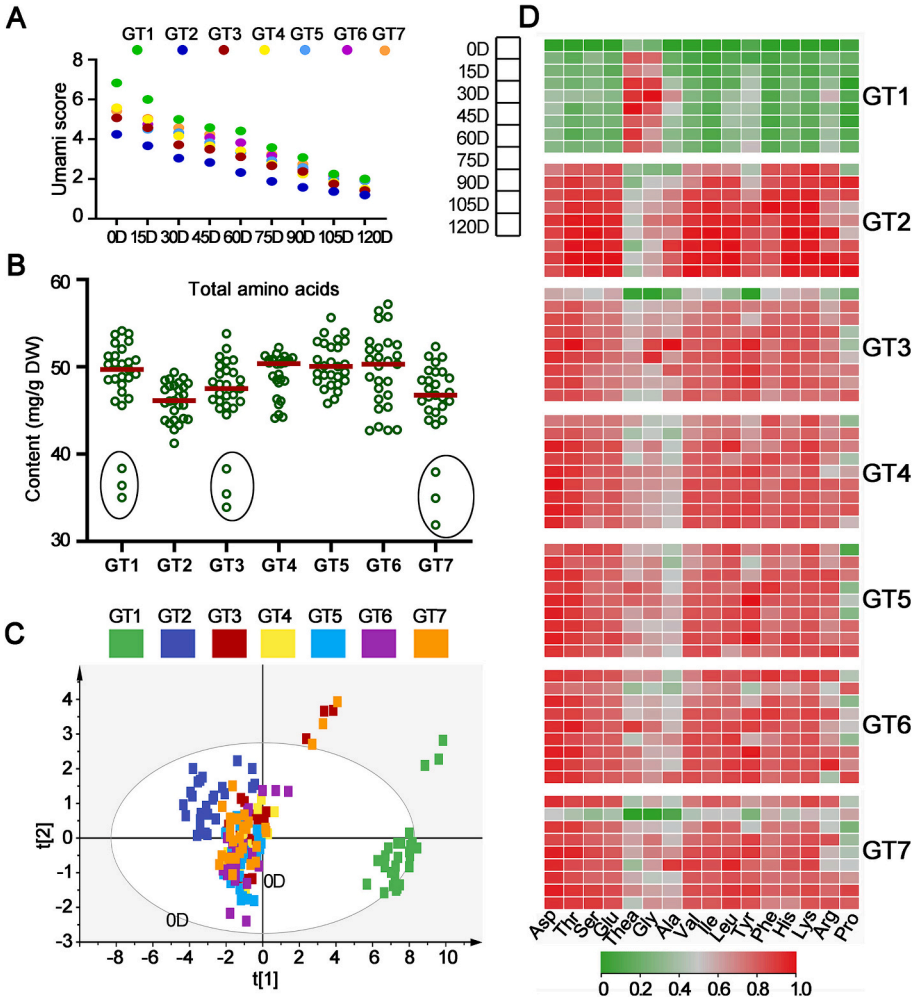

Fig. 4. Amino acids profiles of seven types of green tea during storage. (A) the intensity of umami; (B) total amino acid concentrations; (C) PCA result; (D) heatmap of 16 amino acids. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

days were clustered together, far away from the tea samples stored for 90 and 105 days (Fig. 5C). Fig. 5D shows twenty-six differential compounds with a VIP >1, including six proanthocyanidins (PAs), five organic acids, four theaflavins, eight flavonoid glycosides, two monomeric hydrolyzable tannins, and EGCG. The proportional differences in the concentrations of these compounds between stored samples and the control (stored at −80 °C) were calculated for the GT1, GT2, and GT5 teas, representing non-spreading, non-rolling, and leaf-rolling green teas, respectively.

Further analysis showed that the levels of proanthocyanidins (PAs) and catechin dimers increased significantly after storage. The concentrations of proanthocyanidins increased by 1.8–2.4-fold after a 15-day storage period in the non-spreading tea GT1; catechin dimers exhibited a more rapid increase in stored samples of the non-rolling and leaf-rolling tea types. PAs or catechin dimers are oligo- or polymers of monomeric flavan-3-ols, with a strong astringent characteristic (Zhuang et al., 2020), thus contributing to the increasing astringency intensities of the seven tea infusions with storage. The concentrations of isoorientin, orientin, quinic acid, 4-caffeoylquinic acid, and 4-*p*-coumaroylquinic acid increased in the GT1 sample after only a short period of storage. In contrast, the concentrations of these compounds showed a delayed increase in stored samples of the GT2 and GT5 teas.

The concentrations of theaflavins exhibited a dynamic change during storage and increased apparently after a 75-day storage period; the *b* values for the GT1, GT2, and GT5 tea infusions showed a tendency to increase with storage (**Fig. S5**); this was attributed to the dynamic changes in the concentrations of theaflavins. The concentrations of monomeric hydrolyzable tannins, digalloyl and trigalloyl glucoses, increased in the GT1 and GT5 samples over a 90-day storage period, but exhibited a delayed increase in the stored samples of GT2. The concentrations of myricetin- and quercetin- glycosides, which are associated with a velvety-like taste characteristic (Zhang et al., 2020), declined dramatically during storage.

The levels of EGCG increased by 5–30% after a short period of storage but began to decrease after 60 days of storage. Previous work showed that the concentrations of n-ethyl-2-pyrrolidinone-substituted flavan-3-ols (EPSFs), formed from theanine and flavan-3-ols, increased significantly in samples of green tea over a 19-month storage period (Dai et al., 2020). Epigallocatechin gallate-8-*C*-*N*-ethyl-2-pyrrolidinone ($C_{28}H_{27}NO_{12}$) is the main form of EPSFs and its $[M–H]^-$ precursor ion *m/z* 568.1460 was identified from MS data. A very low response (*m/z* 568.1445) was identified and the response in samples that had been stored for 105 days seemed to increase at the same retention time when compared to the control (**Fig. S6**). It is suggested that the temporary increase of EGCG during a short period of storage may be attributed to its epimerization because of the reduced levels of GCG in stored samples of green tea. The reduced concentrations of EGCG may be attributed to its degradation in response to the increase in gallic acid.

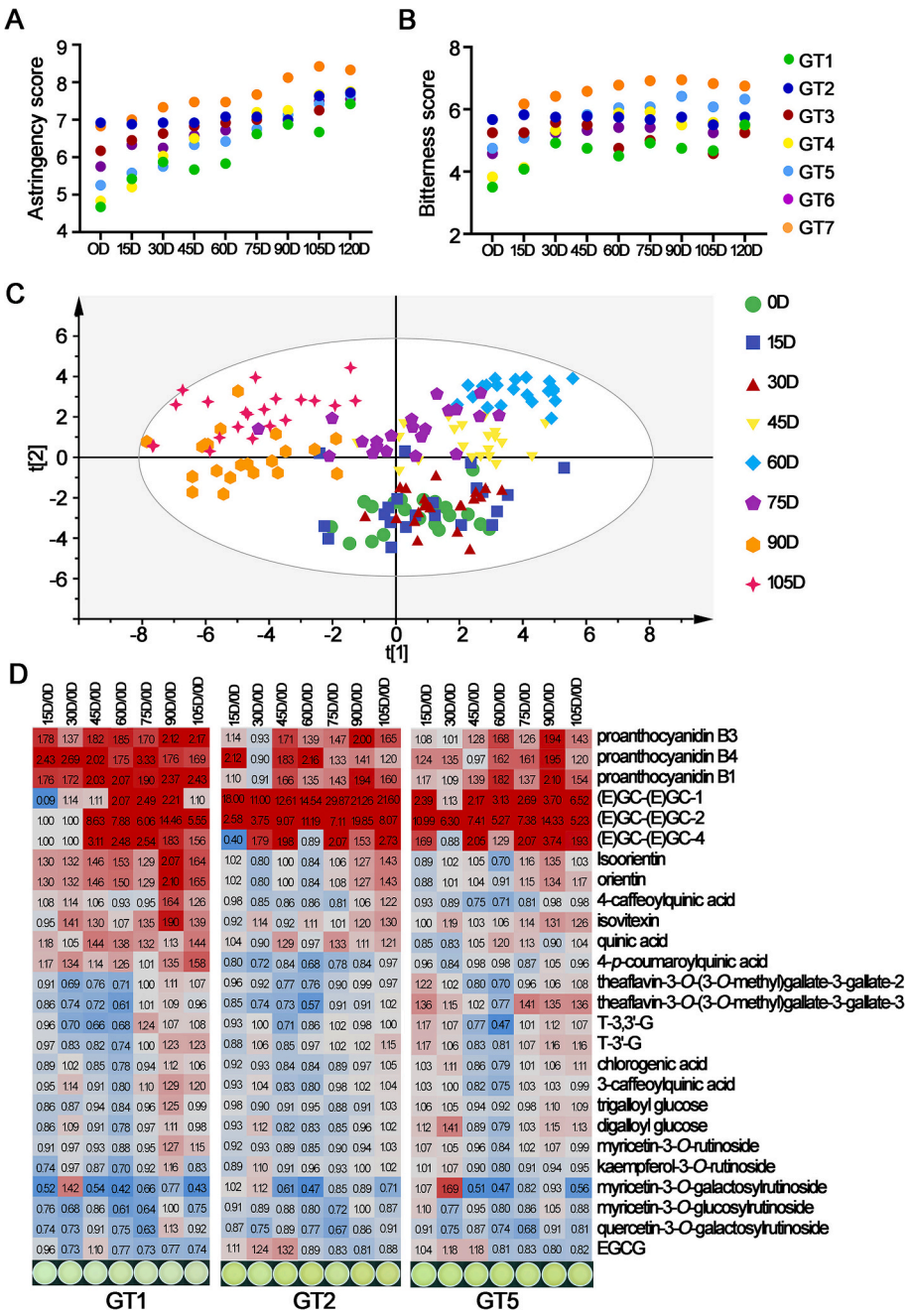

Fig. 5. Nonvolatile profiles of seven types of green tea during storage. (A) the intensity of astringency; (B) the intensity of bitterness; (C) PCA result; (D) 26 differential nonvolatiles between fresh samples and stored samples (VIP >1). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Sensory evaluation and flavor deterioration during storage

3.2.1. Sensory evaluation

GT1, GT4, GT5, and GT6 teas had a higher sensory score than the other teas and exhibited umami and mellow characteristics (**Table S5**). GT2, GT3, and GT7 exhibited stronger astringency than the other green teas and obtained a low sensory score. After storage, all seven types of green tea exhibited a reduction in flavor quality, especially with regard to a reduction in the umami characteristic (**Table S3**). It was clear that the deterioration of flavor in the seven types of green tea was unavoidable following storage at room temperature. Nevertheless, the variation of nonvolatile profiles in the seven types of green tea differed with storage. These data were useful in determining which types of green tea exhibited the lowest levels of deterioration during storage.

3.2.2. The rate of flavor deterioration in the seven types of green tea after storage

In this study, we established two linear regression equations to investigate the rate of flavor deterioration. One equation related to nonvolatiles and utilized data relating to total nonvolatile concentration during storage. The other equation related to flavor and utilized sensory evaluation data from the seven types of green tea during storage. The coefficients of these equations represented the rate of deterioration. The coefficients of the seven types of green tea (GT1– GT7) in terms of variation of nonvolatile profiles during storage were 238, 120, 132, 160, 102, 99, and 64, respectively (Fig. 6). GT1, a non-spreading green tea, exhibited the highest variable coefficient in terms of nonvolatile profile in relation to extended storage. In contrast, GT7 exhibited the least variation in the terms of nonvolatile profile; during processing, this tea

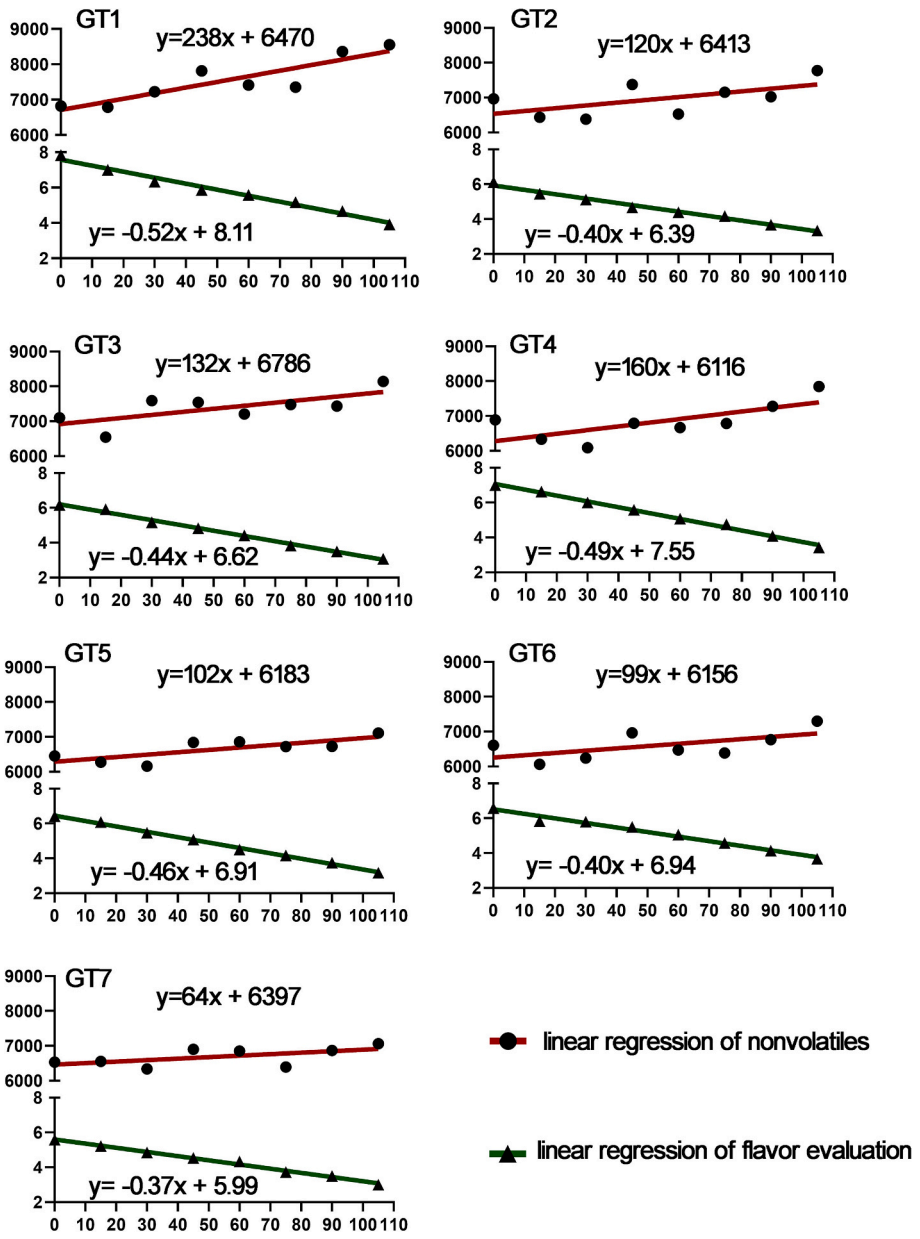

Fig. 6. Linear regression equations.

underwent leaf-rolling with light pressure and an additional shaping procedure.

The coefficients of the linear regression equations for the seven types of green tea in relation to sensory quality were 0.52, 0.40, 0.44, 0.49, 0.46, 0.40, and 0.37, respectively. GT7 also exhibited the least variation in terms of sensory quality during storage.

The non-spreading green tea (GT1) had a good flavor quality and contained an abundance of bioactive compounds; however, this type of tea was more vulnerable to storage. The non-rolling green teas (GT2 and GT3) exhibited less extensive variation than the GT1 and GT4 teas during storage. In contrast, their flavor quality was slightly inferior to the other teas; this was because they contained higher concentrations of theaflavins and caffeine, but lower concentrations of amino acids. The leaf-rolling green teas (GT4– GT7) experienced more extensive destruction of the tea leaves during processing but exhibited better stability in terms of metabolite profile and tea quality during storage. However, the GT5 and GT6 teas had a pleasant quality and were more stable during storage. We believe that the additional thermal shaping led to the GT7 tea losing more glycosides (with a velvety-like characteristic) but gaining more organic acids, such as *p*-coumaroylquinic acids (with a strong astringency) (Fig. 3A).

4. Conclusion

In the present study, seven types of green tea were comprehensively analyzed in terms of flavor quality and nonvolatile profile following storage. Non-spreading green tea (GT1), non-rolling green teas (GT2 and GT3), and leaf-rolling green teas (GT4–7) exhibited differences in tea flavor and nonvolatile profile. High concentrations of catechins and low concentrations of caffeine and amino acids were detected in GT1. In total, 79 nonvolatile compounds were identified by UPLC-QTRAP-MS/MS. The total relative concentrations of these nonvolatile compounds were higher in GT1– GT4 than in GT5– GT7. The flavor quality of the seven types of green tea infusions all decreased dramatically during storage at room temperature. The concentrations of proanthocyanidins, catechins dimers, theaflavins, and organic acids all increased with extended storage. In contrast, the concentrations of flavonoid glycosides and EGCG decreased. The non-spreading green tea exhibited greater

variation in nonvolatile profile and flavor quality during storage, while the leaf-rolling green teas exhibited a lower rate of deterioration. We suggest that the moderate destruction of tea leaves is beneficial in relation to good tea quality and flavor maintenance during storage.

Ethical statement

The authors declare that all experimental work involving humans was conducted in accordance with The International Code of Ethics of the World Medical Association (the Declaration of Helsinki).

The ethical approval of sensory evaluation is not required by national laws. No human ethics committee or formal documentation process is available for sensory evaluation.

All the authors ensure that the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research, including no coercion to participate, full disclosure of study requirements and risks, verbal consent of participants, no release of participant data without their knowledge, and the ability to withdraw from the study at any time.

CRediT authorship contribution statement

Hanchen Zhou: Data curation, Formal analysis, Funding acquisition, Investigation, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Yaqin Liu:** Data curation, Formal analysis, Methodology, Resources. **Qiong Wu:** Software, Methodology. **Xiaolei Zhang:** Validation, Resources, Methodology. **Hui Wang:** Resources, Methodology, Data curation. **Pandeng Lei:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

All the authors declare no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101371.

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