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Non-volatile and volatile metabolite analyses and objective quantitative technique reveal the effect of fixation methods on the flavor quality and metabolites of green tea

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ABSTRACT

Fixation methodology serves as the critical determinant in shaping green tea's multi-dimensional quality attributes; however, the impact of different fixation methods and the corresponding combinations on the overall metabolites and quality of in green tea remains unclear. In this study, non-volatile metabolites (NVMs) and volatile metabolites (VMs) analyses and objective quantitative techniques were used to determine the effects of electromagnetic roller-hot air coupling fixation (ROHF), electromagnetic roller-steam coupling fixation (ROSF), electromagnetic roller-hot air-steam coupling fixation (RHSF), electromagnetic single roller fixation (ROLF), and carding fixation (CDF) on the quality of green tea. Forty-four NVMs, 99 VMs, and 15 flavor objective quantitative indexes were identified, with 12 differential NVMs and 9 differential VMs statistically screened. The green tea processed via ROLF was lustrous and emerald green with a bright and clear liquor; ROSF resulted in low caffeine and flavonoid glycosides contents and an umami (UMS) taste; and RHSF was associated with high *trans*-β-ionone, hexanal and 1-octen-3-ol levels, with a floral and fresh aroma. These findings provide a precise control scheme for the customized processing of green tea, and promote the upgrading of traditional fixation process to intelligent standardization.

1. Introduction

Tea is a botanical beverage consumed globally, with green tea being the preferred variety. With its exceptional flavor and aroma, its wide array of antimicrobial, antioxidant, anticancer, and anti-aging properties have contributed to its widespread appeal (Kochman et al., 2020; Yan et al., 2020). The processing steps for green tea include spreading, fixation, shaping, and drying, with fixation being the most important factor influencing green tea quality. Fixation is performed under short-term high-temperature conditions and important for moisture distribution within the leaves, enabling the subsequent shaping process. Fixation also terminates the activities of enzymes such as polyphenol oxidase, glycosidase, and lipoxygenase, interrupting the enzymatic reactions and promoting heat-induced chemical reactions within the leaves. Such reactions include the thermal decomposition of macromolecules, such as proteins and polysaccharides, and the loss of low-boiling-point volatile metabolites (VMs) responsible for grassy

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Abbreviations

ROHF	Electromagnetic roller-hot air coupling fixation
ROSF	Electromagnetic roller-steam coupling fixation
RHSF	Electromagnetic roller-hot air–steam coupling fixation
ROLF	Electromagnetic single roller fixation
CDF	Carding fixation
VMs	Volatile metabolites
NVMs	Non-volatile metabolites
HS-SPME	Headspace solid-phase microextraction
GC-MS	Gas chromatography-mass spectrometry
SRS	sourness
SWS	sweetness
BRS	bitterness
STS	saltiness
UMS	umami
GPS	general perceived taste
SPS	specific perceived taste
L	Brightness
a	red-green degree
b	yellow-blue degree
C	color saturation
h	hue angle
LL	Liquid brightness
La	Liquid red-green degree
Lb	Liquid yellow-blue degree
VIP	Variable importance in projection
OAV	Odor activity value
OPLS-DA	Orthogonal partial least squares discriminant analysis
EGCG	Epi-gallocatechin-3-gallate
ECG	Epicatechin gallate
EGC	Epigallocatechin
EC	Epicatechin
GCG	Gallocatechin gallate
CG	Catechin gallate
GC	Gallocatechin
C	Catechin
TAC	total catechins
TSC	total simple catechins
TETC	total ester catechins
TPs	Tea polyphenols
Que-glu	Quercetin-3-O-glucoside;
Kae-rut	Kaempferol-3-O-rutinoside;
Kae-glu	Kaempferol-3-O-glucoside;
Vit-glu	Glucosyl-vitexin
Que-glu-gen	Quercetin 3-O-β-d-glucose-7-O-β-d-gentiobioside;
Myr-gal	Myricetin 3-O-galactoside;
Vit-rha	Vitexin-2-O-rhamnoside;
TAFGs	The total amount of flavonoid glycosides
UAAs	Umami amino acids
SAAs	Sweet amino acids
BAAs	Bitter amino acids

flavors. Therefore, the fixation process lays the foundation for forming green tea's distinctive color and aroma (Ho et al., 2015).

The most common fixation methods include roller, hot air, steam, and pan-frying fixation techniques (Hua et al., 2017; Ye et al., 2022; Yu et al., 2023; Zhu et al., 2009), with different fixation methods resulting in different colors, aromas, and tastes. The roller and pan-frying fixation methods result in a strong aroma with a mellow taste, but suboptimal color and liquor (Yu et al., 2023). To overcome the limitations of the individual fixation methods, the use of combined or coupled fixation techniques has been extensively investigated, such as roller and hot air fixation steam, which reduced tea polyphenols (TPs) and catechin contents, increased amino acid and soluble sugar (SSs) levels, and enhanced freshness (Hua et al., 2015). However, previous studies on the effects of fixation methods on green tea quality remain limited in scope, lacking comprehensive and systematic investigation into how fixation methods influence objective quantitative quality parameters, volatile metabolites (VMs), and non-volatile metabolites (NVMs). To address these research gaps, we aim to further explore multiple heat-source fixation methods and identify key differential compounds among various fixation methods through comprehensive analysis.

In this study, the most commonly-used electromagnetic single roller fixation (ROLF) and carding fixation (CDF) methods were compared to three new fixation methods: electromagnetic roller-hot air coupling fixation (ROHF), electromagnetic roller-steam coupling fixation (ROSF), and electromagnetic roller-hot air–steam coupling fixation (RHSF), which were developed previously by our team (Hua et al., 2015). Objective features (i.e., appearance color, liquor color, and taste attributes) were assessed via quantitative indexes, important NVMs (catechins, flavonoid glycosides, free amino acids, SSs, caffeine (CAF), etc.) were investigated via absolute quantitative analysis, and VMs were subjected to semi-quantitative and quantitative analysis via headspace solid-phase microextraction (HS-SPME) and gas chromatography-mass spectrometry (GC-MS) to analyze the effect of fixation methods on green tea flavor quality and related metabolites. Orthogonal partial least-squares discriminant analysis (OPLS-DA), variable importance in projection (VIP), significant difference analysis, and odor activity value (OAV) were used to identify the key differential metabolites responsible for the differences in the color, aroma, and taste of green tea samples obtained by the different fixation methods, and obtain the optimal fixation methods for different flavor attributes of high-quality green tea. This study is expected to enrich the theoretical foundation of green tea processing chemistry and provide technical guidance for the targeted and precise production of characteristically flavored green tea.

2. Material and methods

2.1. Experimental materials

Fresh Zhongcha 108 tea samples (*Camellia sinensis* L.) comprising one bud and one leaf were collected in May 2021 at Yuyao City (Ningbo, China). Anthraquinone, phloroglucinol, and xanthone were sourced from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Formic acid, acetic acid, and acetonitrile (Chromatography grade) were obtained from Merck Group (Darmstadt, Germany). The catechin, free amino acids, and flavonoid glycoside component standards were procured from Sigma-Aldrich (Burlington, MA, USA). Authentic VMs standards (purity >95 %) were supplied by TCI Chemical Industry Development Co., Ltd. (Shanghai, China), Yuanye Biological Technology Co. Ltd. (Shanghai, China), and Sigma-Aldrich (Shanghai, China).

2.2. Green tea processing

The process and parameter treatment for green tea processing were as follows.

(1) Spreading: leaves with a thickness of 2.0 cm were incubated in a continuous green tea spreading machine (YJY-20M, Yuyao Yaojiangyuan Tea Machinery Co., Ltd., Ningbo, China) set to 25.0 ± 1.0 °C and 65 ± 3.0 % relative humidity for 12 h until the moisture content of tea leaves reached 70 %.

(2) Fixation: spread leaves were treated using the five investigated fixation methods under specific parameters (Fig. 1). ROLF, ROHF,

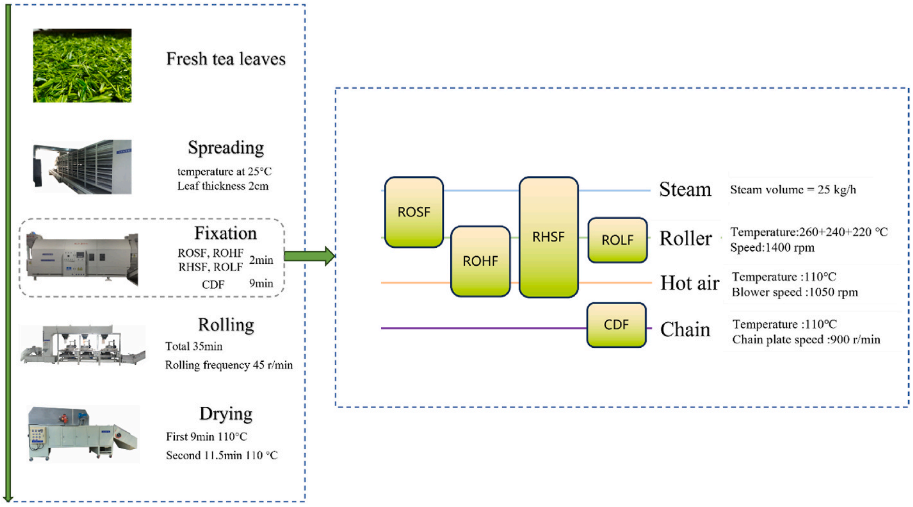

Fig. 1. Processing procedure for green tea and the key processing parameters involved. Note: ROHF, electromagnetic roller-hot air coupling fixation; ROSF, electromagnetic roller-steam coupling fixation; RHSF, electromagnetic roller-hot air–steam coupling fixation; ROLF, electromagnetic single roller fixation; CDF, carding fixation.

ROSF, and RHSF were performed over 2.0 min using an electromagnetic roller-hot air coupling fixation machine (YJY-GH4550-80B, Yuyao Yaojiangyuan Tea Machinery Co., Ltd., Ningbo, China), with the roller speed set to 22 r/min. CDF was performed using a carding drying machine (6CMD-6018, Zhejiang Lvfeng Machinery Co., Ltd., Quzhou, China) over 9.0 min. Fixation was conducted until the moisture content of the fixated leaves reached 40–45 %.

(3) Rolling: treated leaves were spread out and cooled for 1.0 h before rolling (45-type, Yuyao Yaojiangyuan Tea Machinery Co., Ltd., Ningbo, China) with the following process: no pressure rolling for 10 min, light rolling for 10 min, medium rolling for 10 min, and light rolling for 5 min.

(4) First drying: a fuel-powered hot air-drying machine (YJY-RY-25, Yuyao Yaojiangyuan Tea Machinery Co., Ltd., Ningbo, China) was used to supply a hot air temperature of 110 °C for 9.0 min until the moisture content reached 20–25 %.

(5) Second drying: leaves were then cooled for 30 min, and a fuel-powered hot air-drying machine (YJY-RY-25, Yuyao Yaojiangyuan Tea Machinery Co., Ltd., Ningbo, China) used to produce a hot air temperature of 110 °C for 11.5 min until the moisture content was below 7.0 %.

2.3. Analysis of tea quality objective quantitative indexes

2.3.1. Appearance and liquor color evaluation

Objective color indexes were assessed using a Konica Minolta portable spectrophotometer (CM-600d, Shanghai, China). Brightness (*L*), red-green degree (*a*), yellow-blue degree (*b*), color saturation (*C*), and hue angle (*h*) were obtained.

Tea was weighed to obtain 3 g samples (to 0.001g) that were infused in 150 mL boiling water in porcelain cups over 4 min. The obtained solutions were then filtered through 400-mesh filter cloth and cooled to 25 °C ± 2 °C. Objective liquor color indexes were evaluated using a Konica Minolta tabletop spectrophotometer (CM-5 type, Shanghai, China) via the liquid brightness (*LL*), liquid red-green degree (*La*) and liquid yellow-blue degree (*Lb*) results.

2.3.2. Electronic tongue analysis of taste

An a-Astree electronic tongue system (α-Astree II type, Alpha MOS, Toulouse, France) was used to fingerprint the taste characteristics of the obtained green tea liquor. Experiments were repeated thrice for repeatability. Characteristics encompassed five taste sub-attributes: sourness (SRS), sweetness (SWS), bitterness (BRS), saltiness (STS), and umami (UMS), as well as two comprehensive attributes: general perceived taste (GPS) and specific perceived taste (SPS).

2.4. NVMs analysis

2.4.1. TPs, catechin component, and CAF determination

NVMs detection was also conducted on the green tea liquor described in Section 2.3.1. TPs were analyzed using the Folin-Phenol reagent colorimetric method following the Chinese standard GB/T 8313–2018. Catechin components and CAF were determined using the Waters 2469 series high-performance liquid chromatography system (HPLC, Milford, MA, USA) (Wang et al., 2022).

2.4.2. Determination of total free amino acids, amino acid components, and SSs

Total free amino acids were detected based on the Chinese standard GB/T 8314–2013 using the ninhydrin colorimetric method. Free amino acid components were detected using an automatic amino acid component analyzer (Hua et al., 2021). Total SSs was determined using the sulfuric acid-ninhydrin colorimetric method (Wang et al., 2020a).

2.4.3. Detection of flavonoid glycoside components

The flavonoid glycoside components were determined using an HPLC system (Waters, Milford, MA, USA) (Wang et al., 2022) equipped with a chromatographic C18 column (4.6 mm × 250 mm, 5 μm, Waters, Milford, MA, USA), using a 0.15 % formic acid aqueous solution for mobile phase A and pure acetonitrile for phase B and the following mobile phase elution gradient: 0–2 min, 6–17 % B; 2–22 min, 17–19 % B; 22–23 min, 19–30 %; 23–25 min, 30 % B; 25–26 min, 30~6 % B; and 26–30 min, 6 % B. The flow rate was 1 mL/min, the column temperature was 35 °C, the injection volume was 20 μL, and the detection wavelength was 360 nm.

2.5. Analysis of VMs

2.5.1. Sample pre-treatment

A 0.5 g tea sample, 1 mL distilled water, and 10 μL ethyl decanoate (20 mg/L) were added to a 20 mL headspace vial and a manual handle with a DVB/CAR/PDMS fiber head (Agilent Technologies Inc., Supelco, PA, USA) inserted immediately. VMs absorbance was then continued over 60 min in a metal heating vessel at a constant temperature of 60 °C before inserting the fiber into the GC-MS for 5 min.

2.5.2. GC-MS analysis

An Agilent 7890B - 7000C (Agilent Technologies, Palo Alto, CA, USA) with an HP-5ms Ultra Inert capillary column (30 m × 0.25 mm × 0.25 μm) was utilized for VMs detection, with 99.999 % pure helium at a flow rate of 1.0 mL/min serving as the carrier gas. The injection port temperature was maintained at a constant 250 °C. The temperature program for the column was set to follow a specific sequence—initially holding at 50 °C for 5 min, increasing to 150 °C at 4 °C/min, maintaining for 2 min, and finally increasing to 270 °C at 10 °C/min, where it was maintained for 6 min.

Qualitative analysis was performed using an Agilent Mass Hunter, with VMs results compared to compounds in the NIST library (https://webbook.nist.gov/chemistry/). Compounds with a similarity score >80 % were selected. The Kovats' retention index for each compound was calculated using a linear equation based on normal alkanes (C7 ~ C40) and compared to the theoretical retention indices using a criterion of <30.

All screened VMs were subjected to relative quantitative analysis based on the concentration and peak area of decanoic acid ethyl ester (an internal standard). The formula used is as follows:

$$Ci=(Cis*Ai)/Ais$$

Where Ci represents the concentration (μg/L) of the unknown VMs, Ai denotes the peak area of the unknown VMs, Cis the concentration (μg/L) of decanoic acid ethyl ester (internal standard), and Ais the peak area of decanoic acid ethyl ester.

The standard curve was established using the standard to identify the absolute concentration of the key difference VMs (Table S4). All standard substances (including decanoic acid ethyl ester and n-alkanes) were provided by Shanghai Yuanye Biotechnology Co., Ltd.and Shanghai Aladdin Biochemical Technology Co., Ltd., and the purity was higher than 98 %.

2.5.3. OAV analysis

The OAV was used to calculate the ratio of the concentration (μg/L) of VMs in a tea sample to the odor threshold (OT) in water. The formula used is as follows:

$$OAV=C/OT$$

where C (μg/L) indicates the concentration of each VMs and OT (μg/L) refers to the odor threshold of VMs in water.

2.6. Statistical analysis

Detection was repeated thrice, and data from each trial were presented as the average. Statistical significance was analyzed using SPSS (version 22.0, IBM, Armonk, NY, USA). Origin 2018 (Origin Lab Co., Northampton, MA, USA) was used to create bar charts depicting the metabolite contents under different fixation methods. Principal component analysis (PCA) and OPLS-DA were performed using the SIMCA-P14 (Umetrics, Umea, Sweden) to identify the key differential NVMs and VMs among different fixation methods.

3. Results and discussion

3.1. Effects of fixation methods on objective quantitative indicators of green tea

3.1.1. Appearance and liquor color properties

The fixation methods had a significant differential impact on the green tea's appearance and liquor color attributes. The highest *L* value was observed under the ROHF treatment, followed by RHSF and ROLF, with the lowest observed under ROSF and CDF. The highest *a* and *h* values for green tea appearance were observed under ROLF, followed by ROHF and ROSF, and were significantly lower under RHSF and CDF ($P < 0.05$). The *b* and *C* values were significantly higher under RHSF treatment ($P < 0.05$), followed by ROLF, ROHF, ROSF, and CDF (Fig. 2A). Of the fixation methods employed, the most favorable *L* and *a* values were obtained for green tea processed using ROLF, which was also vibrant green (Fig. 2C), consistent with previous reports (Wu et al., 2022). This because the primary components responsible for color are chlorophyll and its derivatives (Liang et al., 2008) and the lower frequency of mechanical movement in the ROLF treatment results in relatively low cellular fragmentation and lower leaching of water-soluble metabolites, limiting the production of yellowish-brown pheophytin (Lanfer-Marquez et al., 2005). Furthermore, the absence of hot blast and hot steam under ROLF ensured a certain degree of humidity, preventing degradation of chlorophyll, and generating darker-colored pyropheophytin or pyropheophorbide (Dai et al., 2017; Donlao and Ogawa, 2019; Yu et al., 2019).

The liquid brightness (*LL* value) was higher under ROSF, ROLF, and CDF, while the liquid red-green degree (*La* value) was significantly lower under ROSF ($P < 0.05$). The liquid yellow-blue degree (Lb value) was significantly higher under RHSF ($P < 0.05$) (Fig. 2B). The best liquor color was observed under ROLF, with higher *LL* and *La* values and lower Lb values consistent with the image of green tea liquor in Fig. 2D, which displayed a green and bright color quality. The color of green tea liquor was primarily composed of flavonoids and their glycosides, along with some water-soluble chlorophyllins (Wang et al., 2004). The multiple heat source and long mechanical motions under the RHSF and CDF treatments enhanced the isomerization, hydrolysis, and oxidative polymerization of flavonoids, resulting in the polymerization of the flavonoids to create larger molecular pigments (Lai and Guo, 2012), and the flavonoids were released from the hydrolysis of flavonoids glycosides complex with catechins to produce brown compounds, further deepening the color of the tea (Cui et al., 2022; Dai et al., 2017).

3.1.2. Electronic tongue taste analysis

The high sensitivity and analytical capacity of electronic tongues enabled the precise evaluation of various taste attributes of tea, thereby facilitating the assessment of discerning indicators such as quality, region, and grade (Wu et al., 2022). The taste attributes radar plot of green tea with different fixation methods showed significant differences, especially for CDF treatment ($P < 0.05$; Fig. 2E). The highest sweetness attribute was observed under ROHF (7.53), whereas CDF resulted in significantly low sweetness (2.99, $P < 0.05$). The umami attribute was higher under RHSF, ROHF, and ROLF as compared to the other treatments, with values of 6.52, 6.38, and 6.28, respectively, while the lowest umami attribute was observed for CDF (5.03). CDF also resulted in significantly higher bitterness levels compared to the other treatments (8.52, $P < 0.05$), whereas ROHF resulted in lower bitterness levels (5.12). The sourness attribute was higher under ROHF and ROSF and significantly lower under CDF treatment ($P < 0.05$).

Overall, the green tea with ROSF exhibited the best taste flavor, with a refreshing taste and low bitterness and astringency. This was consistent with the tea sensory evaluation, with umami mellow taste (Table S1). In contrast, green tea with CDF and RHSF showed the poorest flavor profile, with the highest bitterness and lowest sweetness and umami.

3.2. Effect of fixation methods on NVMs in green tea

3.2.1. TPs, catechin components, gallic acid (GA), CAF, and SSs contents

TPs were the most important flavor and functional components of tea, with catechins being the most abundant. Catechins, GA, and CAF were the key NVMs responsible for the bitterness and concentration of the green tea taste (Rossetti et al., 2009). We found significant differences in the TPs, catechin, GA, and CAF components among the green teas under different fixation methods (Fig. 3A). The TPs, total catechins (TAC), total simple catechins (TSC), and total ester catechins (TETC) contents laid in the order RHSF > CDF > ROLF > ROHF > ROSF, with

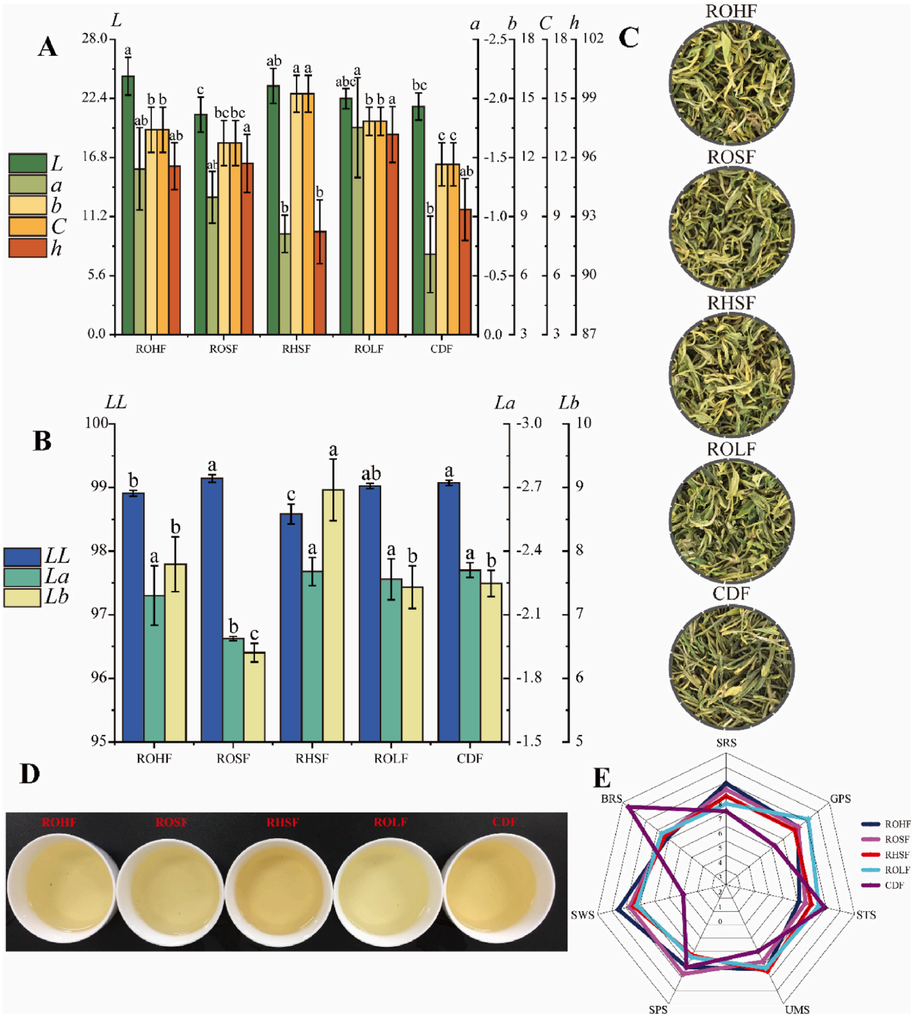

Fig. 2. Effects of different fixation methods on the objective quantitative indexes of green tea. (A) appearance color indexes, (B) liquor color index properties, (C) appearance photos, (D) liquor photos, and (E) electric tongue taste indexes. Note: *L*, brightness; *a*, red-green degree; *b*, yellow-blue degree; *C*, color saturation; *h*, hue angle; *LL*, brightness of liquor color; *La*, red-green degree of liquor color; *Lb*, yellow-blue degree of liquor color; SRS, sourness; SWS, sweetness; BRS, bitterness; STS, saltiness; UMS, umami; GPS, general perceived taste; SPS, specific perceived taste; different lower-case letters represent significant differences between groups ($P < 0.05$).

GC, EGC, EGCG, GCG, and ECG being highest under RHSF treatment, followed by CDF, ROLF, ROHF, and ROSF. The highest C content was observed under CDF treatment. Overall, the TSC content was lower than the TETC content, with the highest TSC/TETC ratio observed under ROSF and ROHF and the lowest under RHSF. Meanwhile, the GA and SSs contents were significantly higher under RHSF ($P < 0.05$), while CAF was significantly higher under ROLF treatment ($P < 0.05$).

Heat and time also significantly impacted the conversion of phenolic substances. Multiple thermal energy effects under RHSF treatment and long-term thermal effects under CDF treatment promoted continuous and rapid contact between tea and metal drums under high temperature, resulting in a higher degree of cell breakage and increasing the leaching of TPs, catechin components, and GA (Tu et al., 2023), which led to the bitterness under RHSF and CDF treatments (Fig. 2E). The TEC/TETC ratio was widely used to characterize the bitterness and astringency of tea liquor, with a higher TEC/TETC value implying less bitterness and a smoother taste (S. J. Deng et al., 2022; Zhang et al., 2015). The highest TEC/TETC ratio (0.500 and 0.497) was observed under ROHF and ROSF, respectively, in line with the lower bitterness values obtained using the e-tongue (Fig. 2E).

3.2.2. Flavonoid glycoside components

Flavonoid glycosides, with relatively low astringency thresholds (0.00115–19.80 μmol/L), were primary indicators for tea liquor greenness and played a crucial role in the bitterness of tea (X. M. Deng et al., 2022; Zhu et al., 2021). Eight flavonoid glycosides were detected, mainly kaempferol, quercetin, vitexin, and myricetin combined with the corresponding O or C glycoside (Fig. 3B). The total flavonoid glycosides (TAFGs) followed the trend CDF (16.446 ‱) > RHSF (16.049 ‱) > ROLF (14.783 ‱) > ROHF (14.491 ‱) > ROSF (13.448 ‱). The different flavonoid glycosides contents varied under different fixation methods, with quercetin-3-O-glucoside (Que-glu), kaempferol-3-O-rutinoside (Kae-rut), kaempferol-3-O-glucoside (Kae-glu), glucosyl-vitexin (Vit-glu), and rutin higher under CDF treatment, and quercetin 3-O-β-d-glucose-7-O-β-d-gentiobioside (Que-glu-gen), myricetin-3-O-galactoside (Myr-gal), and vitexin-2-O-rhamnoside (Vit-rha) higher under RHSF. All flavonoid glycoside components were significantly lower under ROSF ($P < 0.05$).

The intense friction associated with CDF treatment damaged the cellular tissue, releasing the bound flavonoid glycosides from the broken cytosol vesicles, resulting in the highest TAFGs content and the strongest bitterness and astringency (Shi et al., 2023). In contrast, the high

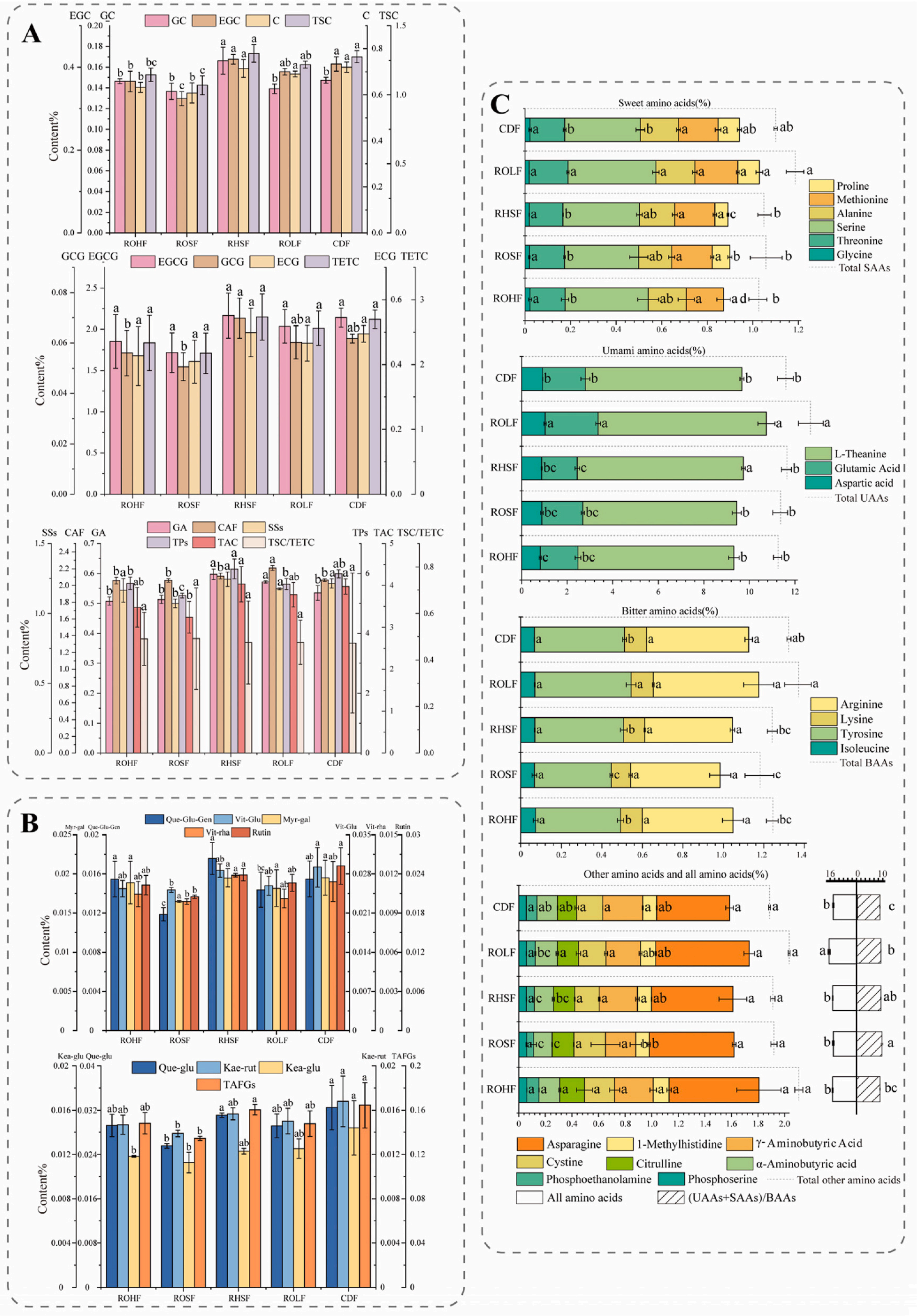

Fig. 3. Effects of different fixation methods on 44 non-volatile metabolites of green tea. (A) tea polyphenols, catechins components, gallic acid, soluble sugars, and caffeine, (B) flavonoid glycosides components, and (C) free amino acid components. Note: EGCG, epi-gallocatechin-3-gallate; ECG, epicatechin gallate; EGC, epi-gallocatechin; EC, epicatechin; GCG, gallocatechin gallate; CG, catechin gallate; GC, gallocatechin; C, catechin; TAC, total catechins; TSC, total simple catechins; TETC, total ester catechins; TPs, tea polyphenols; Que-glu, quercetin-3-O-glucoside; Kae-rut, kaempferol-3-O-rutinoside; Kae-glu, kaempferol-3-O-glucoside; Vit-glu, glucosyl-vitexin; Que-glu-gen, quercetin 3-O-β-d-glucose-7-O-β-d-gentiobioside; Myr-gal, myricetin 3-O-galactoside; Vit-rha, vitexin-2-O-rhamnoside; TAFGs, the total amount of flavonoid glycosides; UAAs, umami amino acids; SAAs, sweet amino acids; BAAs, bitter amino acids; different lower-case letters represent significant differences between groups ($P < 0.05$).

sensitivity of flavonoid glycosides to high temperature and humidity under ROSF promoted the hydrolysis of flavonoid glycosides to form flavonoid compounds and glycosides (Chaaban et al., 2017), resulting in lower bitterness and astringency. Quercetin, kaempferol, and their related glycosides were also the primary components influencing green color of green tea liquor (Long et al., 2023; Wang et al., 2021). Under ROLF treatment, the relatively high contents of que-glu and que-glu-gen resulted in a bright green liquor color (Fig. 2D), and higher *LL* and La values (Fig. 2B).

3.2.3. *Amino acid components*

Free amino acids contributed substantially to green tea's fresh and brisk taste. They were also essential substrates for forming VMs, such as pyrazines, furans, alcohols, and aldehydes, which played a significant role in shaping the aromatic flavor of green tea (Ho et al., 2015; Feng et al., 2019). As shown in Fig. 3C, theanine was the highest among the 21 amino acid components, accounting for 52 %, followed by glutamic and aspartic acid. The total amount of free amino acids followed the same trend: ROLF > CDF > ROHF > ROSF > RHSF. The fixation method led to

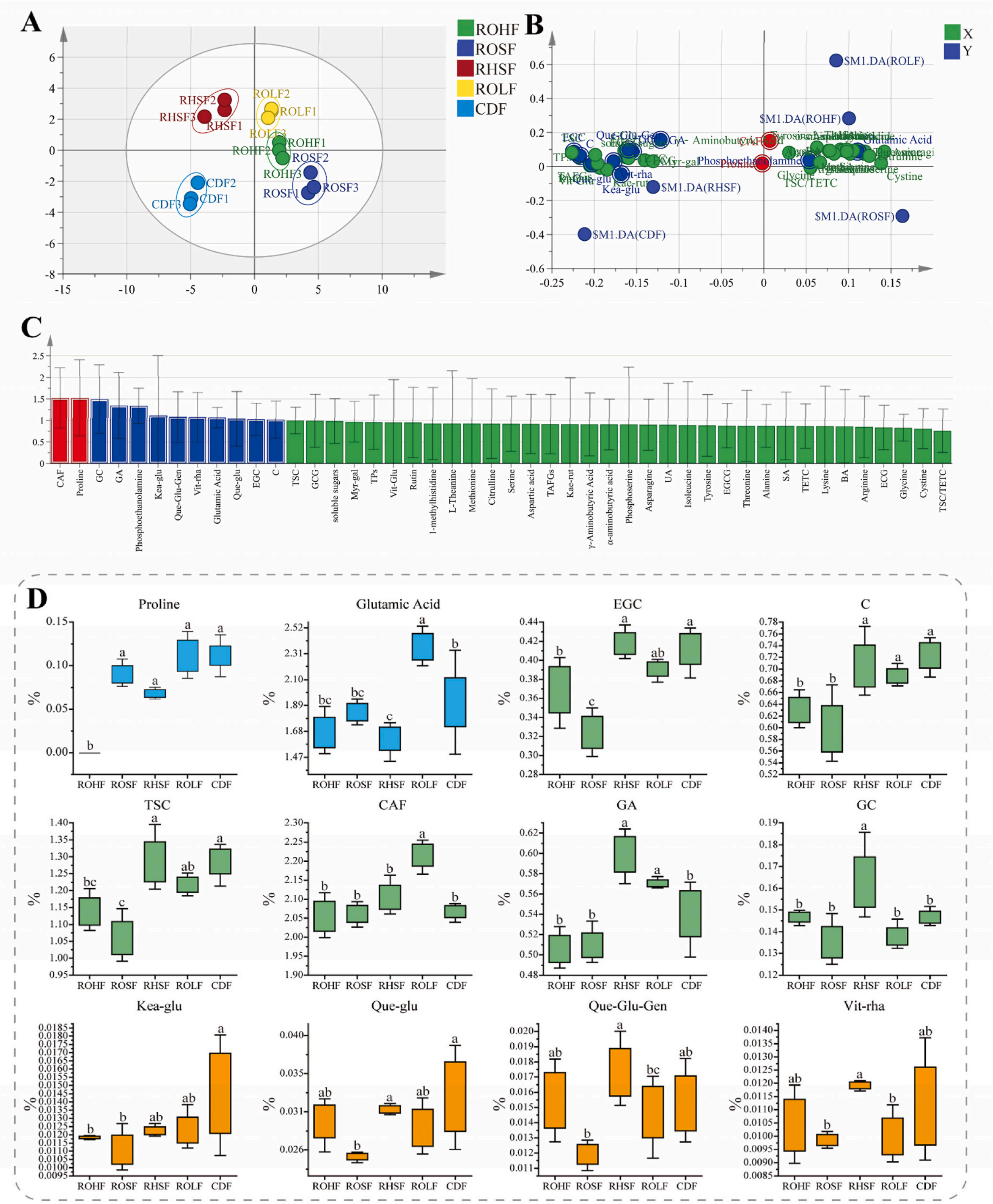

Fig. 4. Screening and comparison of key differential non-volatile metabolites (NVMs) among green tea samples with different fixation methods. (A) Orthogonal partial least-squares discriminant analysis score plots, (B) loading plots, (C) variables important in the projection (VIP) plots of NVMs, and (D) histogram analysis of 12 differential NVMs with VIP >1 in tea samples with different fixation methods. Note: Different lower-case letters represent significant differences between groups ($P < 0.05$).

differential effects regarding the different free amino acid components. Based on the taste attribute, the 21 free amino acid components were divided into sweet (SAAs, e.g., serine, proline, glycine, alanine, and histidine), umami (UAAs, e.g., aspartic acid, glutamic acid, and theanine), bitter (BAAs, e.g., isoleucine, tyrosine, lysine, and arginine), and other free amino acids (Yu et al., 2022). The SAAs, UAAs, and BAAs were significantly higher under ROLF ($P < 0.05$), while the lowest SAAs and UAAs were observed under ROHF and the lowest BAAs under ROSF. The other 10 free amino acids, including asparagine and γ-aminobutyric acid, exhibited the highest content under ROHF treatment and the lowest content under CDF treatment. Overall, ROLF produced the highest SAAs, UAAs, and BAAs content. In contrast, ROSF treatment resulted in higher SAAs and UAAs and the lowest BAAs content, which was more conducive to excellent taste for green tea.

During fixation, proteins were hydrolyzed to peptides and ultimately free amino acids under the thermal environment and enzyme activity (Ouyang et al., 2024). The single thermal action involved in ROLF treatment was found to promote the maximum retention of free amino acid components ($P > 0.05$), which multiple or prolonged thermal effects promoted deamination, decarboxylation, and the Maillard reactions, and led to a sharp decrease in the content of free amino components under RHSF, ROSF, and ROHF as compared to ROLF ($P > 0.05$). Free amino acids, with different taste attributes, showed a complex influence on green tea taste, with excessive retention leading to a lack of the desired aroma and insufficient retention leading to a lack of freshness in terms of taste (Qu et al., 2024; Yu et al., 2024). For this reason, the (UAAs + SAAs)/BAAs ratio was used to indicate the role of free amino acids in the quality taste of tea. The (UAAs + SAAs)/BAAs ratio under ROSF treatment was the highest among different fixations treatments, with a higher umami and sweet attribute, lower bitterness attribute, and the highest SPS comprehensive taste (Fig. 2E).

3.2.4. Analysis of key differential NVMs for green tea treated with different fixation methods

OPLS-DA was used to conduct multivariate statistical analysis based on all 44 NVMs to explore the key differential NVMs among the green tea under different fixation methods. The various fixation methods are distinguishable on the OPLS-DA score plot (Fig. 4A), with ROLF, ROHF, and ROSF clustering on the right side, CDF on the lower-left side, and RHSF on the upper-left side. The model-related values of $R^2X = 0.981$, $R^2Y = 0.983$, and $Q^2 = 0.787$ indicated that the model was accurate and reliable. The loading plot (Fig. 4B) displayed the content distribution of NVMs in green tea produced by different fixation treatments, with ROHF treatment resulting in high levels of free amino acids, ROSF treatment resulting in high levels of TSC/TETC, and RHSF treatment in high levels of flavonoid glycosides and TETC.

The VIP was used to further screen the key differential NVMs that affected green tea taste and color flavor caused by different fixation methods. Based on the principle of VIP >1, 12 key NVMs: proline, CAF, GC, GA, kea-glu, que-glu, que-glu-gen, glutamic acid, vit-rha, EGC, C, and TSC were identified (Fig. 4C), with proline and CAF (VIP >1.5) were crucial to tea taste. Proline was associated with a sweet taste, glutamic acid was associated with an umami taste, C, GC, and EGC were associated with an astringent taste, and CAF and the four flavonoid glycosides were associated with a bitter taste (Han et al., 2016; Zhang et al., 2020).

As shown in Fig. 4D, the fixation methods showed differential effects on key differential NVMs. ROLF treatment was beneficial for the retention of proline, glutamic acid, and CAF, meaning that the SPS value of e-tongue was low (Fig. 2E). ROSF treatment, with the combined effect of dampness and heat (Yu et al., 2023), promoted a huge decrease in the flavonoid glycosides (kea-glu, que-glu, que-glu-gen, and vit-rha), CAF, and catechins, resulting in lower bitterness and astringent attributes and a higher SPS comprehensive taste value. The flavonoid glycosides also caused bright yellow-green color to the tea liquor, so the high flavonoid glycoside content under ROLF treatment resulted in the best liquor quality of green tea (Fig. 2B and D).

3.3. Effects of fixation methods on VMs in green tea

3.3.1. Screening of VMs

A total of 31 alkanes, 19 esters, 12 alcohols, 10 alkenes, nine aldehydes, four ketones, six aromatic hydrocarbons, and seven others were detected using GC-MS, 99 VMs (Table S2), with alcohols, esters, alkanes, and aldehydes being comparatively high in abundance (Fig. 5A). The results indicated that different fixation methods had significantly different effects on the VMs categories of green tea. Alcohols, esters, ketones, alkenes, and aldehydes were significantly higher under RHSF ($P < 0.05$), alkanes were significantly higher under ROSF ($P < 0.05$), and all VMs categories were lower under ROLF ($P < 0.05$). These results indicated that multiple thermal effects (roller, hot air, and steam) accelerated the reaction of VMs in tea leaves, such as amino acid decarboxylation and lipid thermolysis (Xue et al., 2023), which promoting the accumulation of VMs.

3.3.2. OPLS-DA

To investigate the impact of the fixation method on aroma quality and VMs of green tea, OPLS-DA was employed based on 99 VMs. Green tea samples under five fixation methods were clearly distinguished in the score plot (Fig. 5B), with CFD and RHSF distributed in the upper-right corner, ROHF in the lower-right corner, ROSF in the upper-left corner, and ROLF in the lower-left corner. Five principal components were selected using the OPLS-DA model, with the independent variable fit index $R^2X = 0.993$, dependent variable fit index $R^2Y = 0.999$, and prediction fit index $Q^2 = 0.623$, indicating robust explanatory and predictive capabilities, together with ensured stability and reliability for the model.

To further clarify the impact of the fixation method on VMs and green tea aroma, VIP was used to obtain the differential VMs based on the principle of VIP >1. Thirty-five differential VMs (Table S3), primarily esters, alcohols, and aldehydes, were screened (Figs. 5C), and 10 types of esters were observed, including methyl salicylate, butanoic acid ethyl ester, and formic acid octyl ester; together with five types of alcohols, such as geraniol, *trans*-linalool oxide (pyranoid), and 1-octen-3-ol; three types of aldehydes, such as heptanal, hexanal, and benzaldehyde; three types of alkenes, such as β-myrcene, D-limonene, and β-ocimene; and three types of ketones, such as 3-tridecanone and *trans*-β-ionone. Based on the 35 differential VMs identified above, standard curves were established using gradient concentrations of reference standards to achieve absolute quantification. Ultimately, absolute quantification was successfully completed for nine differential VMs (Fig. 5D). Methyl salicylate, geraniol, β-myrcene, and D-limonene were significantly highest under ROHF treatment, while 1-octen-3-ol, hexanal, benzaldehyde, and *trans*-β-ionone were significantly highest in the RHSF treatment, resulting in a refreshing aroma with strong floral or fruity in both treatments. Heptanal was significantly highest in the CDF treatment, which contributed to an overly pronounced grassy odor.

3.3.3. OAV analysis

OAV was an important indicator to objectively evaluate the contribution of VMs to aroma (Hua et al., 2024). The aroma thresholds and aromatic characteristics of 9 differential VMs were determined from a literature review into VIP analysis (Chen et al., 2022; Guo et al., 2021; Ouyang et al., 2022; Xie et al., 2023). It was observed that all 9 VMs OAV values were greater than 1 (Table 1). Among them, heptanal, *trans*-β-ionone, hexanal, geraniol, 1-octen-3-ol had an OAV value greater than 1000, indicating that they were the most critical VMs for green tea aroma caused by different fixation treatments; methyl salicylate and β-myrcene had OAV values between 100 and 1000, indicating that they were the key VMs; D-limonene and benzaldehyde had OAV values between 1 and 100, indicating that they were important VMs (Feng et al., 2019; Ouyang et al., 2022; Xie et al., 2023). Green tea under RHSF treatment had the highest *trans*-β-ionone, hexanal, and 1-octen-3-ol ($P < 0.05$), with the best aroma flavor. Meanwhile, the high content of

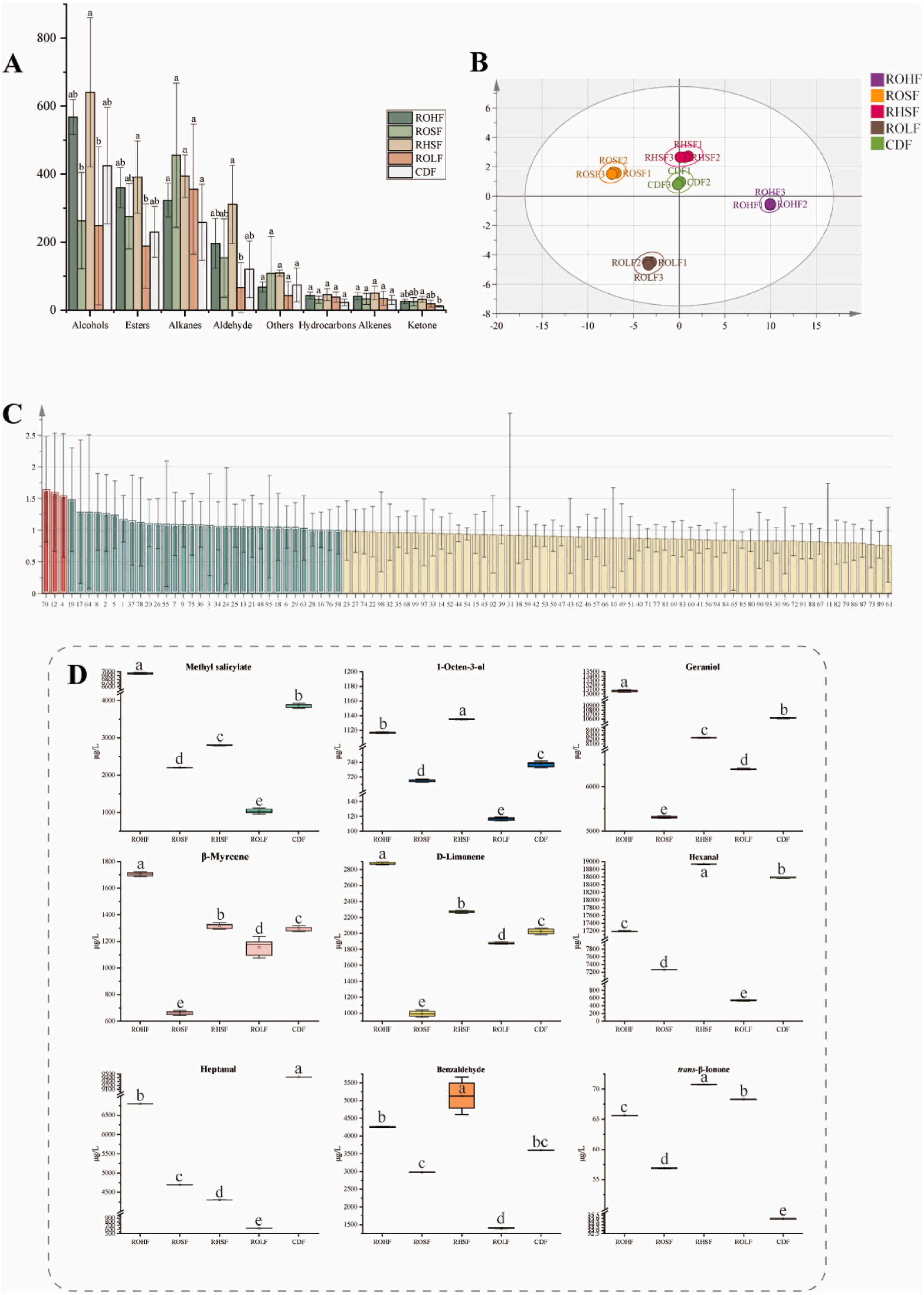

Fig. 5. Screening and comparison of key differential volatile metabolites (VMs) among green tea samples with different fixation methods. (A) histogram analysis of VMs categories, (B) orthogonal partial least-squares discriminant analysis score plots, (C) variables important in the projection plots of VMs, and (D) histogram analysis of nine key differential VMs with OAV >1 in tea samples with different fixation methods. Note: Different lower-case letters represent significant differences between groups ($P < 0.05$).

Table 1

Aroma characteristics and odor activity values (OAV) of 9 differential volatile metabolites (VMs) in green tea samples with different fixation methods.

VMs	OT (μg/L)	Odor	ROHF	ROSF	RHSF	ROLF	CDF
Heptanal	0.90	Grassy	7549.46 ± 1.79b	5215.24 ± 1.25c	4782.29 ± 1.19d	705.68 ± 0.37e	10467.78 ± 0.31a
***trans*-β-Ionone**	0.01	Violet, floral	6561.32 ± 0.96c	5687.19 ± 2.46d	7074.33 ± 1.28a	6828.67 ± 1.58b	3493.94 ± 1.89e
Hexanal	4.5	Green	3820.38 ± 1.72c	1614.21 ± 0.39d	4207.78 ± 1.44a	119.77 ± 2.24e	4129.20 ± 2.44b
Geraniol	7.5	Rose-like, sweet	1742.35 ± 1.90a	708.47 ± 1.87e	1098.75 ± 1.01c	852.64 ± 1.21d	1415.36 ± 1.00b
1-Octen-3-ol	1	Sweet, fucumber	1116.78 ± 0.39b	715.09 ± 1.17d	1135.29 ± 0.16a	116.70 ± 1.31e	737.45 ± 2.34c
Methyl salicylate	40	Mint-like	173.81 ± 0.35a	55.15 ± 0.13d	70.04 ± 0.24c	25.96 ± 1.14e	96.52 ± 0.90b
β-Myrcene	15	Geranium-like	113.67 ± 0.67a	44.14 ± 0.68d	87.78 ± 0.91b	77.08 ± 2.93c	86.40 ± 0.79b
D-Limonene	34	Citrus-like	84.61 ± 0.30a	29.24 ± 0.73e	66.79 ± 0.28b	55.22 ± 0.22d	59.57 ± 0.69c
Benzaldehyde	350	Almond-like	12.15 ± 0.03b	8.51 ± 0.01c	14.66 ± 0.82a	2.82 ± 1.71d	10.30 ± 0.00bc

Notes: Electromagnetic Roller-hot air coupling fixation (ROHF), electromagnetic roller-steam coupling fixation (ROSF), electromagnetic roller-hot air–steam coupling fixation (RHSF), electromagnetic single roller fixation (ROLF), and carding fixation (CDF). Different lowercase letters in the same row indicate significant differences between fixation types ($p < 0.05$) based on the least significant difference test.
A: OT, odor threshold in water based on the literature (Chen et al., 2022; Guo et al., 2021; Hua et al., 2024; Ouyang et al., 2022; Xie et al., 2023).

methyl salicylate, geraniol, β-myrcene under ROHF treatment contributed to a floral aroma to green tea. Under the CDF treatment, heptanal reached its maximum OAV value, which led to an overly pronounced grassy aroma to green tea.

Three heat sources and high temperatures were found to promote the conversion and volatilization of low-boiling-point aldehydes with a grassy flavor under RHSF treatment (Wang et al., 2021), accelerating the enzymatic and thermal cracking conversion reactions of carotenoids to generate *trans*-β-ionone with a floral aroma (Baldermann et al., 2010). Meanwhile, unsaturated fatty acids, such as α-linolenic and palmitoleic acid, oxidized to form C6 aldehydes, such as hexanal and 1-octen-3-ol with a green and fruity aroma (Ho et al., 2015; Shao et al., 2022). In addition, glycoside substances under ROHF treatment underwent significant hydrolysis under thermal action to produce methyl salicylate, geraniol, β-myrcene, and D-limonene, which producing a pleasant fruity and floral aroma to green tea (Hua et al., 2024; Yang et al., 2013). Overall, RHSF treatment promoted uniform and rapid thermal action from the inside out, which was beneficial for forming and enriching floral and fresh VMs, so that green tea under RHSF treatment exhibited the best aroma flavor with long persistence (Hua et al., 2018b; Qiu et al., 2022).

4. Conclusions

Different fixation methods had significant effects on objective quantitative indicators, NVMs, VMs, and the flavor quality of green tea, with different fixation methods producing green tea with distinctive flavor profiles. Green tea processed by ROLF exhibited the highest *L*, *a*, and *h* values for appearance, the highest *LL* and *La* values, and lower Lb for liquor color, characterizing the tea with a vibrant green appearance and bright liquor. A total of 44 NVMs were quantitatively measured, and 12 key differential NVMs were identified among five fixation methods. Green tea processed by ROSF exhibited lower levels of catechins, CAF, BAAs, and flavonoid glycosides, relatively high levels of SAAs and UAAs, and the highest ratio of (UAAs + SAAs)/BAAs, resulting in low bitterness and sourness attributes with e-tongue, and the taste characterized as refreshing and smooth. Ninety-nine VMs were detected, and nine key differential VMs were selected based on VIP and OAV. Green tea processed by RHSF exhibited higher levels of *trans*-β-ionone, hexanal, and 1-octen-3-ol, resulting in an optimal aroma characterized by a refreshing floral aroma. This results provided theoretical support and technical guidance for the targeted and precise production of characteristically flavored green tea. In our subsequent research, we intend to explore the evolution of VMs and NVMs during the different fixation methods, and analyze the regulatory mechanisms underlying the differences in the green tea flavor under various fixation methods.

CRediT authorship contribution statement

Xizhe Zhu: Methodology, Investigation, Conceptualization, Writing – original draft. **Liyue Yang:** Formal analysis, Investigation, Software. **Zhiwen Ge:** Investigation, Methodology. **Wen Ouyang:** Investigation, Methodology. **Jinjin Wang:** Data curation, Methodology. **Ming Chen:** Investigation, Data curation. **Yaya Yu:** Data curation. **Shunyin Wu:** Data curation. **Yanhua Qin:** Data curation. **Chunyong Huang:** Investigation. **Guofu Zhang:** Resources, Supervision, Project administration. **Yating Zhang:** Investigation. **Haibo Yuan:** Conceptualization, Investigation. **Yongwen Jiang:** Supervision, Conceptualization, Resources, Funding acquisition. **Jinjie Hua:** Supervision, Conceptualization, Writing – review & editing, Project administration.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2025.101037.

Data availability

Data will be made available on request.

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