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Effects of different fixation methods on the aroma quality of Anjibai tea

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ABSTRACT

Fixation is a crucial step in the processing of Anjibai tea. In this study, gas chromatography tandem with mass spectrometry was used to analyze the aroma compositions of Anjibai tea across three types of fixation methods: roller-electrical fixation (REF), carding machine fixation (CMF), and roller-hot air fixation (RHF). Nine key volatile compounds were found responsible for Anjibai tea's aroma variations. Results from quantitative descriptive analyses, along with aroma recombination and omission tests, showed that linalool and geraniol significantly contribute to the formation of the medium flowery, fruity, and honey aromas in the REF samples; hexanal has a significant impact on the green aroma in the CMF samples; and 2-ethyl-3,6-dimethylpyrazine is an important aroma compound for the strong roasty aroma in the RHF samples. These findings provide an important theoretical foundation for improving the aroma quality of Anjibai tea and selecting the optimal fixation method.

1. Introduction

Anjibai tea, originating from Anji County, Zhejiang Province, is categorized within the green tea family. Its name, translating to "Anji's white tea," refers to the distinctive whiteness of its buds and leaves during a certain growth phase (Du, Shin, Wang, Lu, & Liang, 2009). The superior ecological environment of Anji area has led to the unique aroma quality of Anjibai tea, making it increasingly popular among tea lovers in recent years. With the gradual increase in market demand for Anjibai tea, the scale of Anjibai tea industry is rapidly growing, as of 2022, the area of tea plantations in Anji over 13,700 ha, with an output of 2100 tons of Anjibai tea and an output value of $44.11 million, and in 2023, production of Anjibai tea reached 2300 tons, with an output value of $49.46 million, which has become a veritable wealth industry. It is well known that the quality of tea aroma is affected by the season, growing environment and processing technology. Generally speaking, the aroma of green tea in spring is superior to that of green tea in summer, and the quality of tea produced at high altitude is superior to that at low altitude (Guo, Ho, Schwab, & Wan, 2021; Xiong et al., 2023). It's worth noting that although more than 700 volatile compounds have been identified in tea, only a small number of volatiles in fresh tea leaves are crucial to the overall aroma (Zhai, Zhang, Granvogl, Ho, & Wan, 2022). This suggests that in addition to an ideal cultivation environment, proficient processing is also important in achieving quality (Yang et al., 2021). The aroma quality of Anjibai tea is significantly influenced by its processing methods, namely fixation, carding, and drying, with fixation being the key process for quality formation, this study focus was on exploring the effects of different fixation methods on the aroma quality of Anjibai tea during its production.

Aroma, often regarded as the essence of tea, plays an important part in defining tea quality. Fixation, a pivotal step in green tea production, deactivates enzymes through high temperature, ceasing enzymatic oxidation, removing grassy flavors, and facilitating aroma release (Ouyang et al., 2022; Yin et al., 2022b). Research indicates that fixation has the most significant impact on green tea's volatile metabolites, following the trend of fixation > drying > rolling. The fixation process also significantly diminishes the quantity of aroma compounds in tea (Wang et al., 2021b; Wang et al., 2022b). Furthermore, the fixation method selected-distinguished by differences in temperature, duration, and heating technique-greatly influences the development of volatile compounds, resulting in unique aroma characteristics of teas (Han et al., 2017). Wang et al. (2020a) explored how different fixation methods affect the development of key volatile compounds in tea. By analyzing the impact of these methods on chestnut-scented green tea and the development of key volatile compounds, it was revealed that roller hot-air fixation is the most effective technique for creating a robust and long-lasting chestnut and floral aroma in tea.

Tea aroma has been a primary focus of tea research, emphasizing the

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importance of employing appropriate aroma extraction methods for the identification and analysis of aroma components in tea (Wang et al., 2022a; Zhai et al., 2022). Among them, solid-phase microextraction (SPME) is characterized by simplicity and effectiveness in the adsorption of volatile compounds. However, the selective adsorption nature of SPME (Table S1 shows a list of abbreviations) means it is less effective for compounds of weak volatility (Mu et al., 2018), indicating a need for complementary methods to achieve comprehensive aroma component analysis. Solvent-assisted flavor evaporation (SAFE), offering an efficient approach to pre-treatment for volatiles through solvent vaporization at low temperatures and high vacuum, complements SPME by facilitating the evaporation of aroma compounds and the removal of non-volatiles. This method ensures a more accurate representation of tea's aromatic profile in tea infusion (Corral, Leitner, Siegmund, & Flores, 2016). The synergy of SPME and SAFE, combined with gas chromatography-mass spectrometry (GC-MS) for the identification and analysis of volatiles, has been widely used in recent research on the aroma quality of tea (Baba & Kumazawa, 2014; Flaig, Qi, Wei, Yang, & Schieberle, 2020; Huang et al., 2022; Yu et al., 2023).

At present, there are three primary fixation methods for Anjibai tea: roller-electrical fixation (REF), carding machine fixation (CMF), and roller-hot air fixation (RHF). However, the mechanism by which these fixation methods affect the aroma quality of Anjibai tea remains unclear, so it is important to study the effect of different fixation methods on the aroma of Anjibai tea. In order to guide the producers for the choice of tea fixation method, to improve the production quality of tea and create more profit. In actual production, it has been found that CMF samples usually exhibit stronger green aroma. We speculate that this may be due to the lower temperature at which CMF is fixation, the restricted range of movement and uneven heating during fixation, which results in the retention of higher levels of aldehydes. And for the RHF samples usually exhibit stronger roasted aroma. This may be due to the fact that the fixation, the tea is subjected to the double heating of the inner wall of the drum and the steam, which results in a more even heating of the tea samples and facilitates the occurrence of the Maillard reaction to produce pyrazine compounds. Based on this, this study is the first to adopt a molecular sensory science approach to systematically investigate the volatile compounds in Anjibai tea. The objective is to investigate the effect of fixation methods on the aroma formation of Anjibai tea and to reveal the reasons behind the differences in aroma quality. This study has significant potential for improving the processing and production of Anjibai tea. Explaining practical problems through theoretical and scientific knowledge provides producers with confidence in their production, thus increasing investment and commercial scale and creating more profits.

2. Materials and methods

2.1. Processing and collection of tea samples

In March 2022, specimens comprising one bud and one leaf were collected from the 'White Leaf No.1' variety of Anjibai tea (Fig. S1) at the Tea Expo Garden of Zhejiang Anji Songming White Tea Co. Ltd. The collected tea leaves were evenly spread in a blower withering tank. Following this, the leaves were divided into three groups for fixation using three different methods (Fig. S2): REF (298 ± 5 °C, 1.6 min), CMF (185 ± 5 °C, 6 min), and RHF (300 ± 5 °C, 1.5 min). Each method aimed to achieve a consistent fixation level, maintaining the moisture content of 51 ± 1%. After the same carding and drying process, the tea samples were stored at 4 °C in a refrigerator to preserve the stability of their volatile compounds.

2.2. Chemical reagents and materials

Ethyl decanoate was obtained from Yuan Ye Biotechnology (Shanghai, China); methylene chloride was obtained from Tedia (Fairfield, OH, USA); C_6–C_{40} n-alkane samples were purchased from Sigma-Aldrich (Sigma-Aldrich, China); sodium chloride (NaCl) and anhydrous sodium sulfate (Na_2SO_4) were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China); pure water was purchased from Wahaha Group Company (Hangzhou, China); anhydrous ethanol was from Tianjin Damao Chemical Reagent Factory (Tianjin, China); linalool, geraniol, nerolidol, 2-ethyl-3,6-dimethylpyrazine, and indole were purchased from Shanghai Zhenzhun Bio-technology Co. Ltd. (Shanghai, China); SAFE apparatus was purchased from Chongqing Xinville Glass Co. Ltd. (Chongqing, China); Supelco® SPME fiber needles (divinylbenzene/carboxylic acid/polydimethylsiloxane, 50/30 μm) and sampling handles were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA).

2.3. Sensory evaluation

Quantitative descriptive analysis (QDA) is a sensory evaluation method in which the assessors quantitatively analyze and describe the characteristics of a sample (Wang et al., 2021a). In this study, twelve trained panelists (six males and six females) were invited. All team members received two months of training in odor identification, which involved sniffing different foodstuffs-primarily vegetables and fruits-and aroma standards. The aroma standards were first dissolved in ethanol and then diluted with water to a concentration 100 times of their common thresholds. The panel assessed the tea infusions and identified seven key odor attributes: flowery, clean and fresh, fruity, chestnut, honey, roasty, and green. These attributes were quantified against compound standards representing each odor attribute: flowery (*trans*--beta-Ionone), fruity (geraniol), clean and fresh (nonanal), chestnut (cooked chestnut), honey (benzeneacetaldehyde), roasty (2-acetylpyrazine), and green (hexanal). The intensity of each odor was rated on a scale of 0–3.0 for weak, 3.0–5.0 for medium, and 5.0–7.0 for strong, with the results visualized on a radar chart. For the evaluation, 5.0 g of each tea sample was accurately weighed and brewed in 150 mL of boiling water for 5 min. The infusion was then filtered through a 400-mesh gauze, and 25 mL of the filtrate was transferred to a 50 mL brown olfactory probe flask.

2.4. SPME extraction of volatiles from tea infusion

The desired internal standard solution was prepared by dissolving 17.8 mg of ethyl decanoate in 10 mL of anhydrous ethanol. After that, 56.2 μL of the solution was added into 10 mL of deionized water.

The SPME method was based on a previous research detailed by Wu et al. (2022) with minor modifications, aligning with the tea infusion preparation described in Section 2.3. Specifically, 10 mL of the filtered tea infusion was transferred into a headspace flask. To this, 5 μL of the internal standard solution was added, along with 3.0 g of sodium chloride, to ensure a thorough mixing. The mixture was then uniformly heated and stirred in a water bath at 50 °C for 10 min, followed by the SPME fiber exposure to the headspace for 40 min to adsorb the volatile compounds.

2.5. SAFE for the extraction of volatile compounds in tea samples

The internal standard solution was prepared by dissolving 17.8 mg of ethyl decanoate in 10 mL of anhydrous ethanol. Subsequently, 2.809 mL of this solution was further diluted in 10 mL of deionized water.

Using the method proposed by Huang et al. (2022), this study incorporated the internal standard solution into the tea infusion preparation process, as outlined in Section 2.3. Specifically, 5 μL of the internal standard solution was introduced to 150 mL of the tea infusion. After thorough fusion, the mixture underwent extraction through the SAFE unit at 40 °C under a vacuum of 10^{-2} Pa. Following the SAFE extraction, the volatiles were further isolated using 30 mL of dichloromethane, which was repeated three times. The collected extract was

then dried using anhydrous sodium sulfate to remove any residual water content. Finally, the extract was concentrated to 100 μL by nitrogen purging and stored at −20 °C in a refrigerator for measurement.

2.6. Analysis of volatile compounds by GC-MS

Volatile compounds were characterized using an Agilent 7890B GC equipped with a 5977B mass spectrometer. These compounds were separated using DB-5MS (30 m × 0.25 mm, 0.25 μm film thickness) and DB-FFAP (30 m × 0.25 mm, 0.25 μm film thickness) capillary columns. The flow rate was 1.00 mL/min with helium as carrier gas (purity >99.99%). The volatiles extracted *via* SPME were analyzed in the splitless injection mode. The GC oven temperature was programmed as follows: For samples extracted *via* SPME, the temperature started at 40 °C (held for 5 min), then increased to 160 °C at a rate of 4 °C/min (held for 35 min), and finally reached 280 °C at 10 °C/min (held for 5 min), totaling a 52-min run. For samples extracted *via* SAFE, the temperature started at 40 °C (held for 5 min), increased to 100 °C at 4 °C/min, then to 190 °C at 3 °C/min, and finally to 280 °C at 15 °C/min (held for 5 min) for 61 min. At the end of the experimental method, the GC-MS automatically decreases the column chamber temperature to its initial value (40 °C). The mass spectrometer was operated in positive ionization mode, with ion scanning range of 30–350 *m/z* ratio at 70 eV. The linear retention index (RI) was determined using a series of n-alkanes (C_6–C_{40}).

2.7. Gas chromatography-olfactometry-mass spectrometry (GC-O-MS) combined with aroma extract dilution analysis (AEDA)

Following the method described in a previous study (Shen et al., 2023), MS analyses were performed using an Agilent 7890B equipped with an MS detector and an olfactory detection port (ODP3 Gerstel, Germany). The effluent from the analytical column was bifurcated, with equal parts directed to the MS detector (250 °C) and the olfactory detection port (230 °C), using helium as the carrier gas at a velocity of 40 cm/s. Different injection techniques were employed for SPME and SAFE samples. Initially, SPME fibers were inserted into the GC injection port and desorbed for 5 min. As for SAFE extracts, 2 μL was injected into the GC injection port using an injection needle. The GC oven temperatures and the conditions of the ion-selective detector were maintained as described in Section 2.5.

In order to accurately capture the odor properties of different volatile compounds, sniffing analyses were conducted involving three panelists to examine the SPME and SAFE extracts by GC-*O*-MS. Extracts obtained from the two methods were examined separately. For SPME extracts, odor activity was diluted by adjusting the split ratio of the column oven (1:1, 3:1, 7:1, etc.) until odors were no longer perceptible at the sniffing port, thus to calculate the flavor dilution (FD) factor. Conversely, SAFE extracts underwent stepwise dilution with dichloromethane at 1:1 (v/v). Each dilution was then subjected to GC-O detection from high to low concentrations until odors were not perceptible at the sniffing port. The FD factor for each identified volatile compound was determined accordingly. An aroma compound was considered identified if detected by at least two of the three panelists.

2.8. Screening of key aroma compounds by FD factor and odor activity value

The odor activity value (OAV) was determined by comparing the concentration of volatile compounds with their odor thresholds in water. It is generally accepted that an OAV ≥1 indicates that aroma compounds significantly contribute to the formation of tea aroma (Liu et al., 2023; Zhu, Niu, & Xiao, 2021). Given the wide variety of aroma standards, the absolute quantification of all compounds is impractical. Therefore, in the study, volatile compounds were quantified using an internal standard method, using ethyl decanoate as an internal standard, to facilitate comparison across tea samples. Aroma compounds meeting the criteria of relative odor activity values ≥ 1 and FD ≥ 8 were identified as key aroma compounds and were again subjected to absolute quantification (Table S2), which provided accurate references for the final tests of aroma recombination and deletion analyses.

2.9. Aroma recombination and omission tests

To validate of the key aroma compounds identified, an aroma recombination experiment was conducted following the method described in a previous study (Zhang et al., 2023). Specifically, the nine identified differential compounds (FD ≥ 8, OAV ≥1) at their corresponding assay concentrations were dissolved into brown bottles containing 25 mL of purified water to replicate the aromatic profile of the original tea infusion. The aroma recombination and control samples (original tea infusion) were then incubated in a water bath at 40 °C. Their aromatic profiles were evaluated according to the QDA criteria outlined in Section 2.3.

The aroma omission tests were designed to characterize the contribution of each compound to the aroma of the tea infusion (Cui et al., 2022). In these tests, different blend models were prepared by omitting one of the nine aroma compounds at a time, all of which had an OAV ≥1. The impact of each compound's omission on the aromatic profile was subsequently assessed by twelve trained panelists using a triangular testing method. The significance level discrimination of the triangular tests was based on the method described in a study (Wei et al., 2024).

2.10. Statistical analysis

Statistical analysis was conducted using Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Partial Least Squares-Discriminant Analysis (PLS-DA) through SIMCA-P software (V14.1, Umetrics, Umea, Sweden). In the PLS-DA model, variables with a Variable Importance in Projection (VIP) score ≥1 were identified as volatile differential compounds across different samples. Furthermore, analysis of variance (ANOVA) and the least significant difference (LSD) tests were performed using SPSS Statistics 24.0 to determine the significance levels ($p < 0.05$) of volatile compound concentrations. In addition, the ratio of the concentration of each compound in water to its threshold was used to characterize the odor activity value of key aroma compounds. Data visualization was performed using Origin 2021b and TBtools.

3. Results and discussion

3.1. Differences in aromatic profiles of Anjibai tea with different fixation methods

To explore the impact of different fixation methods on the aroma characteristics of Anjibai tea, a QDA was first conducted to evaluate the aroma attributes and intensity of the samples from a sensory evaluation perspective. Seven odor attributes relevant to Anjibai tea-flowery, clean and fresh, fruity, chestnut, honey, roasty, and green-were selected for QDA analysis. As shown in Fig. 1, there were significant differences in the odor attributes among the three tea samples. Specifically, the CMF samples exhibited an obvious green aroma, while the RHF samples were distinguished by stronger chestnut and baked aromas. Furthermore, the REF samples outperformed the others in terms of clear, flowery, honey, and fruity aromas. These significant differences may be attributed to different fixation methods applied, which alter the content of volatiles in the tea leaves, consequently affecting their aroma quality (Qi et al., 2016).

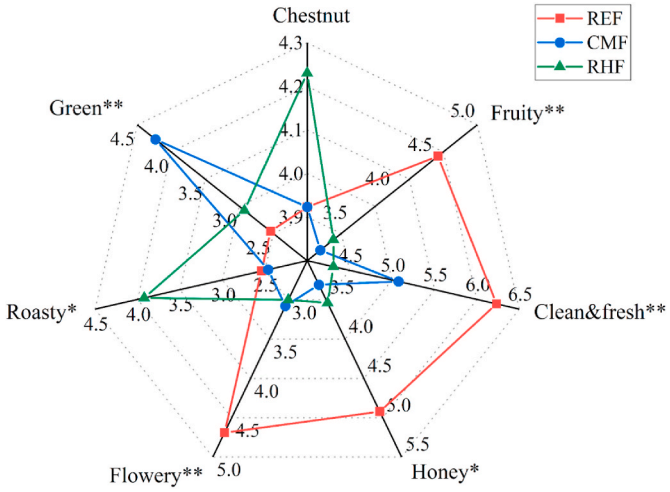

Fig. 1. QDA radar map for Anjibai tea processed *via* roller-electrical fixation (REF), carding machine fixation (CMF), and roller-hot air fixation (RHF). "*", Significant ($p \leq 0.05$); "**", highly significant ($p \leq 0.01$).

3.2. Volatile compound composition of Anjibai tea with three fixation methods

The volatile compounds of Anjibai tea were extracted using two methods: SPME and SAFE. Subsequent detection and analysis of these compounds were performed by GC-MS, followed by quantification using ethyl decanoate as an internal standard. The retention indices were determined by comparing the retention times of volatile compounds with n-alkanes, and mass spectra and retention indices were matched with the NIST 2017 database, resulting in the identification of 112 volatile compounds (Table S3). Among the three tea samples, a total of 108 (REF), 109 (CMF), and 107 (RHF) volatile compounds were identified, respectively. Based on their chemical structure, these compounds were classified into 8 categories: 24 alcohols, 28 esters, 16 aldehydes, 11 ketones, 7 acids, 9 terpenes, 3 aromatics, 14 heterocyclic compounds. Notably, alcohols, esters, aldehydes, and ketones exhibited the greatest diversity, aligning with findings from previous studies (Shi et al., 2022).

In order to further reveal the differences in volatile compound profiles among tea samples subjected to different fixation treatments, a multivariate statistical analysis was performed. Initially, an unsupervised PCA separated the tea samples effectively (Fig. 2A), indicating obvious differences in volatile compound profiles associated with each fixation method. HCA further differentiated CMF samples into one category, while REF and RHF samples were classified into one category (Fig. 2B), suggesting similarity in their volatile profiles, which may be due to the higher fixation temperature employed in the two methods (Wang et al., 2020b). Subsequently, supervised PLS-DA modeled the relationship between volatile compound profiles and sample categories, enabling sample category prediction (Fig. 2C). This model is validated through a permutation test, confirming no overfitting and affirming its suitability for further analysis (Fig. 2D).

3.3. Identification and screening of differential aroma compounds of Anjibai tea with three fixation methods

The complexity of volatile compounds in tea samples necessitates a screening process to identify those crucial for tea aroma formation, as not all detected compounds contribute significantly to the tea's aromatic profile (Yin et al., 2022a). Utilizing the model described in Section 3.2, the volatile compounds with a VIP score ≥1 were selected. Combined with ANOVA ($p < 0.05$), a total of 48 volatile compounds exhibiting significant differences were identified. These differences were mainly attributed to the hydrolysis of glycosides, the Maillard reaction and the degradation of carotenoids and lipids in tea leaves (Feng et al., 2019).

The heat map (Fig. 3) analysis revealed that the content of differentiated compounds was significantly higher in REF than in RHF and

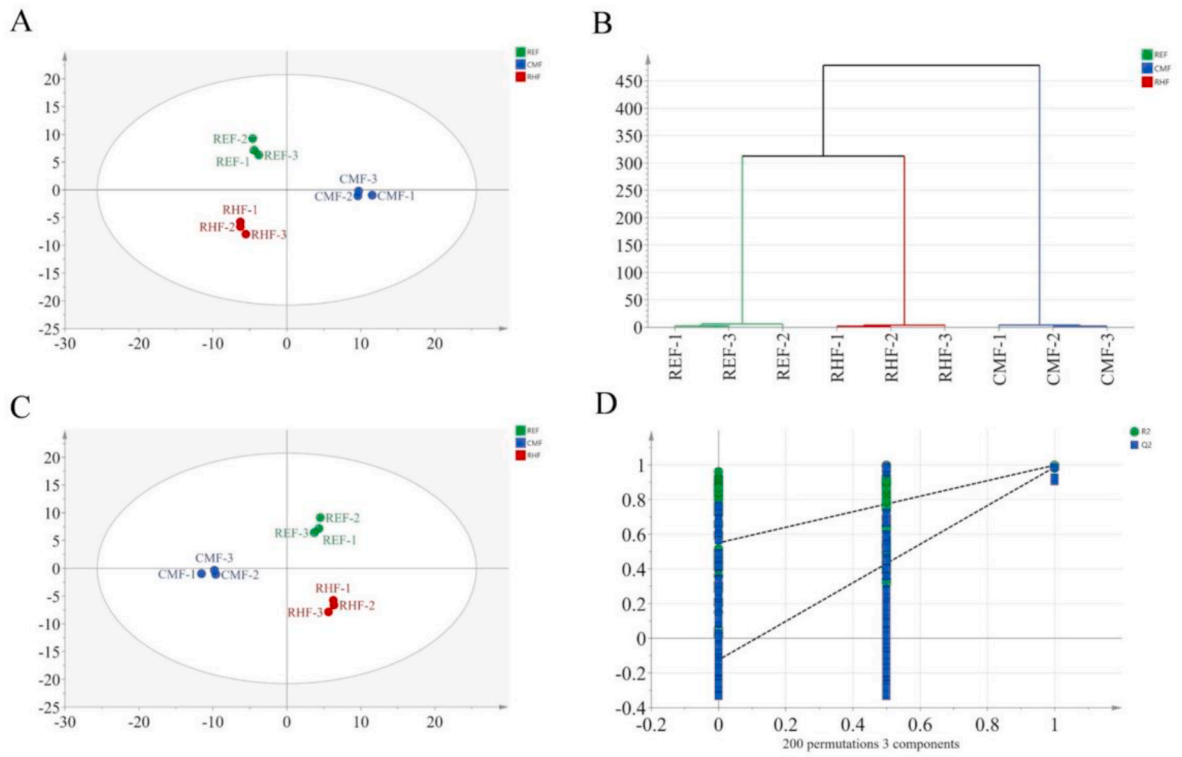

Fig. 2. Multivariate statistical analysis of volatile components in Anjibai tea with different fixation methods: A, PCA results, which $R^2X = 0.889$, $Q^2 = 0.815$; B, HCA results. C, PLS-DA results, which $R^2X = 0.921$, $R^2Y = 0.997$, $Q^2 = 0.985$. D, Permutation test results, with Q^2 intercepts at 0.582 and R^2 intercept at −0.174.

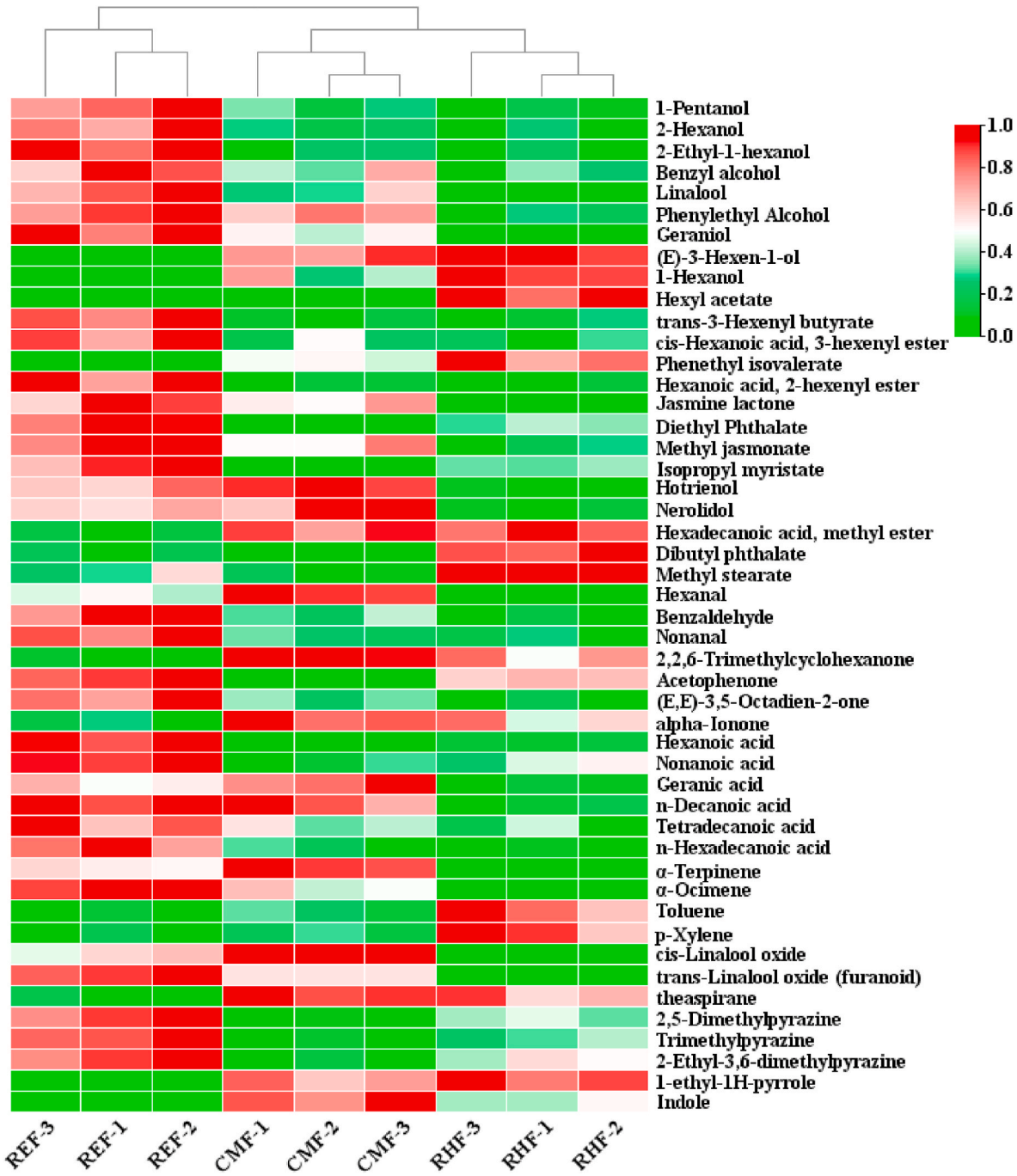

Fig. 3. Heat map of relative concentrations of differentiated volatile compounds in Anjibai tea with different fixation methods: roller-electrical fixation (REF), carding machine fixation (CMF), roller-hot air fixation (RHF).

CMF. Among them, benzyl alcohol, linalool, phenylethanol, and geraniol were more abundant in REF. Benzyl alcohol and phenylethanol originated from the biosynthesis pathway of phenylpropanes and benzenes are the main contributors to the floral and fruity aromas of tea (Zhai et al., 2022). Geraniol and linalool, mainly produced by the enzymatic hydrolysis of glycosides, are important contributors to the formation of aroma in tea, and their biosynthesis is facilitated by geraniol and linalool synthases, respectively, with geranyl pyrophosphate as a precursor (Yang, Baldermann, & Watanabe, 2013). Esters, notably hexyl acetate, phenethyl isovalerate, and methyl hexadecanoate, showed higher concentrations in RHF samples. These compounds usually present fruity and fatty aromas, which are crucial for the tea's flavor profile, suggesting the significant role of esters in defining tea aroma quality (Zhai et al., 2022). Moreover, hexanal, a product of lipid degradation abundant in CMF samples, usually contributes a grassy aroma to the tea infusion. Its higher content in CMF samples suggests a unique processing attribute of this fixation method, where lower drying temperatures might significantly enhance hexanal concentration. This observation is consistent with findings from previous research (Wang et al., 2011).

3.4. Screening of aroma-active compounds from the Anjibai tea with three fixation methods

GC-O olfactory detection combined with the AEDA technique is one of the most commonly used methods in tea flavor analysis (Feng, Li, Li,

Wan, & Yang, 2020). Through stepwise dilution, extracts were evaluated by trained panelists who recorded the highest dilution at which each analyte's odor was perceptible, thereby determining the FD factor (Zhang et al., 2021). A total of 35 key aroma compounds (Table 1) were identified with FD factors ≥8, which had high aroma activity in tea infusion and were the main contributors to the aroma formation of Anjibai tea.

In REF samples, linalool (FD: 64) and geraniol (FD: 256) were important contributors to the aroma quality of green tea. Linalool, a terpene alcohol widely present in tea, is associated with tea quality and exhibits significant antioxidant activity (Qin et al., 2013). Although geraniol shares the same precursor with linalool, it offers a pronounced rose aroma, contrasting linalool's floral and citrus aromas, aligning with the results of a previous study (Yanagimoto, Ochi, Lee, & Shibamoto, 2003; Yin et al., 2022a). In RHF samples, E-nerolidol (FD: 128), formed by carotenoids' non-enzymatic degradation, presented a woody or floral aroma, contributing to the formation of the tea flavor. However, its concentration significantly decreased after fixation (Cui et al., 2022). 1-octen-3-ol (FD: 64), formed by the degradation of linoleic acid in tea leaves, showed high activity in CMF samples. It is characterized by mushroom and hay odors, indicating its role in green tea's aroma (Ho, Zheng, & Li, 2015).

Meanwhile, the study revealed that volatile compounds, despite their high concentrations, exhibited low FD factors, indicating that a compound's contribution to tea's flavor depends not only on its concentration but also on its interaction with the surrounding matrix. Synergistic or additive effects among compounds with similar structural or aromatic characteristics intensified specific odors. Conversely, a masking effect might occur between compounds of different structures, potentially reducing or even nullifying the perceptibility of certain aromas (Wang et al., 2020c; Zhu et al., 2016).

3.5. Screening of key aroma compounds by OAVs

By integrating the 48 significantly different volatiles identified in Section 3.3 with the 35 aroma compounds with FD factors ≥8 in Section 3.4, the comparative analysis revealed 20 common volatile compounds, including linalool, phenylethanol, geraniol, nerolidol, hexanal, nonanal, benzaldehyde, methyl jasmonate, isopropyl myristate, isoamyl caprylate, isoamyl caprylate, ethyl benzene, nonanoic acid, geranylgeranic acid, decanoic acid, 3,5-octadien-2-one, α-ionone, *cis*-linalool oxide, teaspoonane, 2-ethyl-3,6-dimethylpyrazine, and indole. These compounds were identified as drastically active in the tea infusion, and were representative of the differences among the three tea samples. Upon reviewing relevant literature (Guo et al., 2021; Guo, Ho, Schwab, & Wan, 2021; Zhai et al., 2022) for thresholds, OAVs were calculated, and nine key aroma compounds with an OAV ≥1 were identified, namely linalool, geraniol, nerol, phenethyl isovalerate, hexanal, nonanal, decanoic acid, 2-ethyl-3,6-dimethylpyrazine, and indole. Table S4 lists their chemical structural formulas, chemical formulae, and class information.

Among these nine key compounds (Fig. 4), hexanal and indole exhibited higher concentrations in CMF samples. Hexanal is known for its green aroma, suggesting that the lower fixation temperature in CMF may inhibit the dissipation of the green aroma in tea, thus influencing the aroma quality of the tea leaves. Indole, formed from tryptophan by the action of tryptophan indole cleavage enzyme, presents a camphoraceous odor at high concentrations but exhibits a floral aroma at low concentrations (Yvon & Rijnen, 2001). GC-O analysis indicated that indole contributed a subtle floral aroma in the tea samples, suggesting a relatively low concentration in Anjibai Tea. It was found that the CMF samples had a higher concentration of indole, which might be attributed to the increased damage to the tea leaves during the CMF process (Zeng et al., 2016). The concentration of 2-ethyl-3,6-dimethylpyrazine, characterized by its roasty aroma, was significantly higher in the REF and RHF samples than in the CMF samples, probably due to the higher temperature during fixation. Additionally, the highly active signature

Table 1
Aroma compounds smelled at the GC-O sniffing port.

No	CAS	Compounds[a]	Odor description[b]	FD[c]		
				REF	CAM	RHF
1	78-70-6	Linalool	Citrus-like, flowery	64	32	32
2	1960-12-8	Phenylethyl Alcohol	Floral, rose-like	512	128	128
3	106-24-1	Geraniol	Rose-like, citrus-like	256	64	32
4	142-50-7	Nerolidol	Woody, flowery	512	256	256
5	77-53-2	Cedrol	Woody	16	8	32
6	481-34-5	α-Cadinol	Herbal, woody	32	4	4
7	3391-86-4	1-Octen-3-ol	Mushroom-like	16	64	32
8	40716-66-3	E-Nerolidol	Woody, flowery	64	32	128
9	66-25-1	Hexanal	Green, grassy	4	16	4
10	4313-3-5	(*E,E*)-2,4-Heptadienal	Fatty, flowery	16	4	4
11	124-19-6	Nonanal	Citrus-like, soapy	16	4	8
12	112-31-2	Decanal	aldehyde-like, fatty and citrus-like	64	8	16
13	121-33-5	Vanillin	Vanilla-like, sweet	16	4	64
14	590-86-3	3-methylbutanal	Malty, alcohol	2	8	4
15	505-57-7	2-Hexenal	Green apple-like, bitter almond-like	8	4	8
16	100-52-7	Benzaldehyde	Bitter almond-like, marzipan-like	32	16	32
17	141-27-5	α-Citral	Citrus, lemon-like	16	32	32
18	119-36-8	Methyl salicylate	Mint-like	16	8	8
19	1211-29-6	Methyl jasmonate	Jasmine-like, flowery	64	16	32
20	110-27-0	Isopropyl myristate	Faint oily, fatty	64	32	16
21	2349-14-6	Methyl geranate	Fresh green, rose-like	256	128	64
22	31501-11-8	(*Z*)-Hexanoic acid, 3-hexenyl ester	Fruity, waxy, green, fatty, winey	8	4	8
23	140-26-1	Phenethyl isovalerate	Fruity, floral, ripe rose-like	8	8	16
24	112-05-0	Nonanoic acid	Moldy, pungent	64	32	32
25	459-80-3	Geranic acid	Musty, green, woody	64	128	64
26	334-48-5	Decanoic acid	Soapy, musty	16	8	4
27	30086-02-3	(*E,E*)-3,5-Octadien-2-one	Fatty, musty	32	16	16
28	141-79-7	4-Methyl-3-penten-2-one	Honey-like, card board-like, nutty	8	16	4
29	127-41-3	α-Ionone	Flowery, violet-like	16	8	4
30	5989-33-3	cis-Linalool oxide	Woody, flowery	32	32	16
31	14009-71-3	trans-Linalool oxide (pyranoid)	Woody, flowery	16	8	8
32	36431-72-8	Theaspirane	Woody, cooling, minty, herbal	32	64	64
33	586-62-9	Terpinolene	Fresh, woody, sweet, piney, citrus	16	4	4
34	13360-65-1	2-Ethyl-3,6-dimethylpyrazine	Roasted potato, cocoa-like, nutty	32	8	16

(*continued on next page*)

Table 1 (*continued*)

No	CAS	Compounds[a]	Odor description[b]	FD[c]		
				REF	CAM	RHF
35	120-72-9	Indole	Floral, mothball-like	8	16	8

Note.
[a] Compounds were identified in the three Anjibai teas infusions.
[b] Odor description of each odorant at the sniffing port.
[c] FD: Flavor dilution factor.

compounds, such as linalool, geraniol, nerolidol, and nonanal, were significantly higher in REF than in the other two fixation methods, indicating that REF enhances the aroma quality of Anjibai tea.

3.6. Aroma recombination and omission analysis

The aroma recombination experiment involved blending nine key aroma compounds an OAV ≥1, namely linalool, geraniol, nerolidol, phenethyl isovalerate, hexanal, nonanal, decanoic acid, 2-ethyl-3,6-dimethylpyrazine, and indole (Fig. S3). There was no significant difference between the recombination aromatic profile and the original tea infusion's aroma, thereby validating the accuracy of our findings.

The omission tests further elucidated the contribution of each key aroma compound to the aroma of Anjibai tea. Conducted on three tea samples, these tests allowed 12 trained panelists to identify the absence of six compounds: nerolidol, geraniol, linalool, hexanal, 2-ethyl-3,6-dimethylpyrazine, and indole, which were main contributors to the flowery, honey, and roasty aromas of the tea samples (Table 2). Among them, the absence of nerolidol and geraniol was accurately detected by all panelists, which indicated that nerolidol and geraniol were the most active aroma compounds in Anjibai tea. The panelists also noted a significant reduction in the green aroma of the CMF samples upon the omission of hexanal, suggesting that hexanal is an important compound contributing to the formation of green aroma characteristic of this fixation method. This comprehensive analysis *via* omission tests clarified that nerolidol, geraniol, linalool, hexanal, 2-ethyl-3,6-dimethylpyrazine, and indole were the key factors for the formation of flowery, honey, roasty, and green aromas of Anjibai tea.

4. Conclusion

In this study, first time by SPME and SAFE, combined with GC-MS and GC-O, were employed to explore the aroma quality characteristics of Anjibai tea produced by different fixation methods. Through separation, extraction, and analyses of the volatile compounds in tea samples, nine key aroma compounds were identified as significant to the aromatic profile of Anjibai tea, namely linalool, geraniol, nerolidol, phenethyl isovalerate, hexanal, nonanal, decanoic acid, 2-ethyl-3,6-dimethylpyrazine, and indole. The results showed that our previous hypotheses were basically valid, and the green aroma in the CMF tea samples was due to the accumulation of hexanal content because of the lower fixation temperature. RHF tea samples showed the roasted aroma, which is due to the double heating effect of hot air and cylinder wall, which promotes the production of pyrazines. It was also found that REF favoured the accumulation of linalool and geraniol in tea leaves, thus improving the aroma quality of Anjibai tea. The results of the study provide a theoretical basis for the selection of Anjibai tea fixation method and the improvement of tea quality. In addition, considering the complex chemistry of tea broth, the perception of aroma intensity, in addition to being directly affected by the concentration of aroma

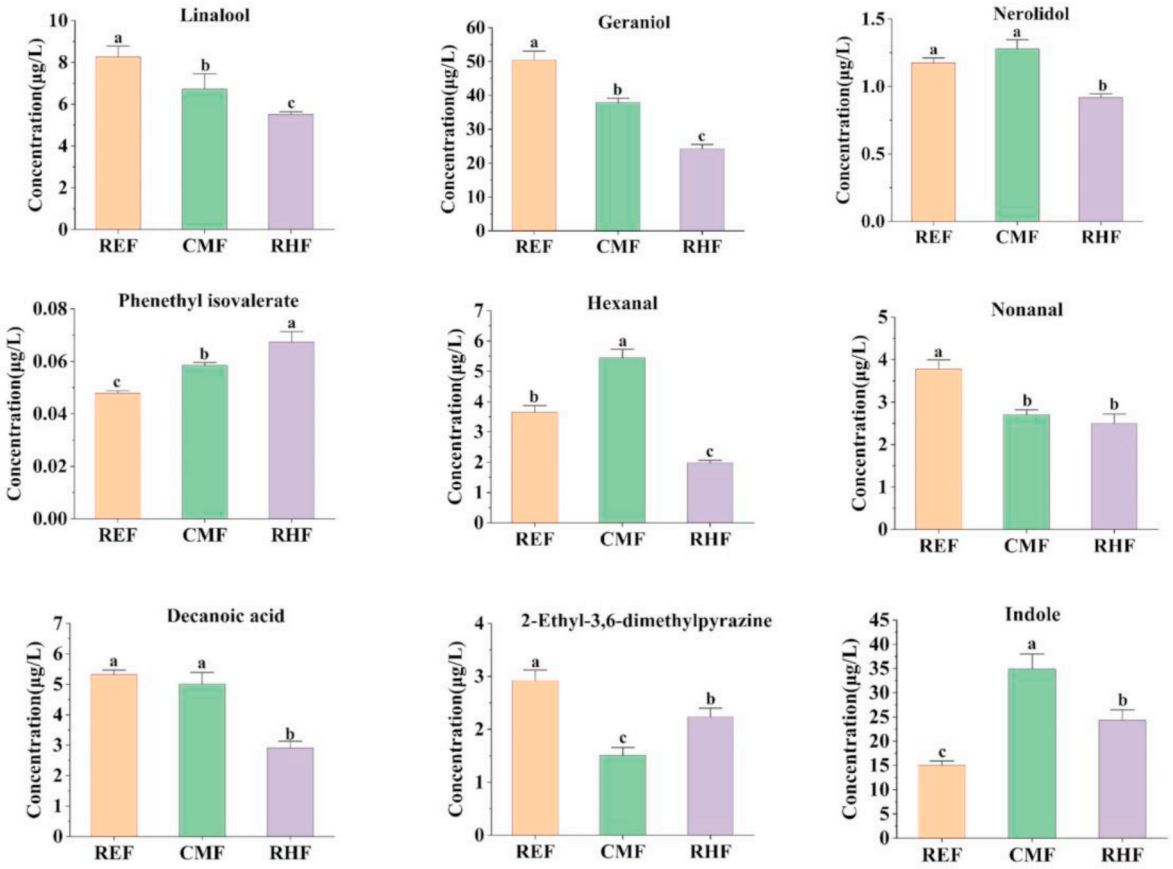

Fig. 4. Relative concentrations of key differential compounds in Anjibai tea with different fixation methods: roller-electrical fixation (REF), carding machine fixation (CMF), roller-hot air fixation (RHF). Different letters indicate significant differences at the 0.05 level.

Table 2
Omission test results of each key aroma-active compound in Anjibai tea according to omission experiments.

Key aroma-active compounds	Number of correct judgments[a]			Description of odor difference
	REF	CMF	RHF	
Nerolidol	12***	12***	12**	Less floral and sweet
Geraniol	12***	12**	12**	Less floral and sweet
Linalool	11**	10**	9**	Less citrus-like
Hexanal	9**	12***	10**	Less green
2-Ethyl-3,6-dimethylpyrazine	9**	8*	10**	Less roasted
Indole	8*	10**	9**	Less floral
Phenethyl isovalerat	5	5	7	
Nonanal	5	6	4	
Decanoic acid	4	4	3	

[a] Number of correct judgments from 12 panelists in the omission test. "*" = significant ($\alpha < 0.05$); "**" = highly significant ($\alpha < 0.01$); "***" = very highly significant ($\alpha < 0.001$); "-" = the compounds were not detected. Three types of processing for Anjibai teas: roller-electrical fixation (REF), carding machine fixation (CMF), roller-hot air fixation (RHF).

compounds, the enantiomeric forms of the matrix and specific molecules should not be ignored, which will be the focus of our subsequent research.

Ethical statements

Ethical permission, to conduct a human sensory study, was granted by our institution. Participants gave informed consent *via* the statement "I am aware that my responses are confidential, and I agree to participate in this sensory evaluation" where an affirmative reply was required to enter the sensory evaluation. They were able to withdraw from the sensory evaluation at any time without giving a reason. The tea products evaluated were safe for consumption.

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CRediT authorship contribution statement

Dongzhou Xia: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Jixin Zhang:** Software, Methodology, Formal analysis. **Zhichao Xiong:** Resources, Methodology, Investigation, Data curation. **Wenjing Huang:** Resources, Methodology, Investigation, Data curation. **Yuming Wei:** Visualization, Supervision, Investigation. **Wanzhen Feng:** Visualization, Supervision, Investigation. **Junlan Huang:** Visualization, Supervision, Investigation. **Jingming Ning:** Resources, Project administration, Funding acquisition.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2024.116430.

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