Hindawi
Journal of Food Processing and Preservation
Volume 2023, Article ID 3046129, 13 pages
https://doi.org/10.1155/2023/3046129

Research Article

Study on the Effects of Processes on Aroma Compounds in Rizhao Green Tea Based on the Gas Chromatography-Ion Mobility Spectrometry

Ao Sun[1], **Chun Li**[1], **Suyuan Lv**[1], **Jianhua Gao**[2], **Yuanzhi Xia**[3] and **Yue Geng**[1]

[1]*Key Laboratory of Food Nutrition and Safety of SDNU, Provincial Key Laboratory of Animal Resistant Biology, College of Life Science, Shandong Normal University, Jinan 250014, China*
[2]*Shandong Rizhao Shenggushan Tea Farm Co., Ltd, Rizhao 276800, China*
[3]*Jinan Three Thousand Tea Grower Co., Ltd, Jinan 250000, China*

Correspondence should be addressed to Yue Geng; gengy@sdnu.edu.cn

Received 13 September 2022; Revised 8 November 2022; Accepted 9 November 2022; Published 30 March 2023

Academic Editor: Fabiano A.N. Fernandes

Tea processing plays an important role in the formation of tea aroma. In order to explore the composition and variation in aroma during Rizhao green tea processing, gas chromatography-ion mobility spectrometry was employed to investigate the formation and changes of tea aroma in four processing stages. A total of 62 volatile compounds were identified, including alcohols, aldehydes, ketones, esters, terpenes, and oxygen compounds. Relative odor activity value was used to identify 17 characteristic aroma compounds. The main aromatic compounds of chestnut-like aroma were linalool, pentanal, hexanal, heptanal, octanal, nonanal, 2-methylbutanal, 3-methylbutanal, 3-methylthiopropanal, and methyl 2-methylbutanoate. There were significant changes in the volatile components at different processing stages. Samples of tea leaves and green tea had special aroma characteristics, while samples of fixated and rolled leaves had similar aroma compounds. The results are beneficial for the improvement of the flavor and quality of Rizhao green tea. *Practical Application*. Tea is the world's most consumed nonalcoholic beverage, with a multitude of health-promoting benefits, such as antioxidant, antiobesity, and antiallergic activities. At present, the research on the aroma compounds of Rizhao green tea mainly focuses on the aroma characteristics of finished tea and the aroma differences of different kinds of commercial tea. This study explored the effects of the processes on aroma compounds, and the results provide a theoretical reference for the improvement of tea processing technology, tea quality control, and evaluation methods.

1. Introduction

According to the fermentation degree, tea can be classified as green, white, yellow, oolong, black, or dark tea [1]. Rizhao green tea is a type of green tea produced in Rizhao in the Shandong Province of China. Fresh leaves, buds, and tender stems of the tea plants (*Camellia sinensis* (L.) O. Kuntze) are processed, without fermentation, through high-temperature fixation, rolling, and drying techniques [2]. The city of Rizhao has a warm, temperate, and humid monsoon climate, adequate light, large temperature differences between day and night, weakly acidic soil, and high organic matter content. These unique climatic and geographical conditions are conducive to the growth of tea trees and the accumulation of metabolites. Therefore, Rizhao green tea has unique and excellent qualities, such as strong buds and leaves, which can endure repeated brewing, yellow-green soup, chestnut-like aroma, and is gaining increasing worldwide popularity that is known as "the first tea in the northern region of the Yangtze river" [3].

Flavor substances are important quality characteristics of tea. There are great differences in the composition and content of flavor substances in tea with different fermentation degrees [4]. In addition, these tea flavor substances differ due to chemical changes in processing [5]. Tea aroma is one of the important factors that determine the quality of

tea and is detected by human olfactory organs [6]. Previous studies have shown that the volatile components of Rizhao green tea include hydrocarbons, alcohols, and esters with special floral and fruity aromas, indoles, and furans with roasted aromas. Among them, cis-3-hexenol, linalool, hexanal, 3-methylbutanal, and 2-pentylfuran provide the chestnut-like aroma of Rizhao green tea [7].

Tea aroma quality is determined via sensory evaluation, electronic nose, gas chromatography-olfactometry, and odor activity values. Sensory evaluation relies on human sensory organs and has uncertainty and instability caused by human error [8]. Instrumental analysis methods, such as chromatography and mass spectrometry, require complex sample pretreatment and cannot achieve rapid detection in the field [9]. Relative odor activity value (ROAV) is a new method of evaluating the contribution of different threshold compounds to the overall aroma, and it can be used to determine the contribution of volatile substances to the aroma characteristics of samples [10]. It has been used to identify key aroma compounds in foods [11, 12].

Gas chromatography-ion mobility spectroscopy (GC-IMS) is a new gas chromatography separation and detection technology that has emerged recently. It does not require sample pretreatment and achieves nondestructive and rapid detection [13], making it suitable for trace detection of volatile components [14]. GC-IMS first separates the complex volatile components of green tea by gas phase. The components flowing out at different retention times collide with the reverse drift gas molecules, and the secondary separation is carried out [15]. By obtaining the ion drift time and ion peak intensity, each component can be qualitatively and quantitatively analyzed. At present, GC-IMS is applied in traditional Chinese medicine [16], clinical examination [17], food [18] and environmental analysis [19].

There is no systematic report on the changes and formation mechanism of aroma compounds in Rizhao green tea during processing. In this study, GC-IMS technology was used to collect and analyze the volatile components of Rizhao green tea during processing, and the key odorants were screened by the ROAV method to explore the influence of processing on the aromatic substances in Rizhao green tea. The analysis of volatile components during tea processing is important to study the effects of the processes on aroma compounds in Rizhao green tea and the improvement of Rizhao tea flavor and quality.

2. Materials and Methods

2.1. Experimental Materials and Instruments. Rizhao green tea samples were collected on the first week of May in 2021 at Shenggushan Tea Farm Co., Ltd. (Rizhao, Shandong, China). The fresh tea leaves with one or two leaves and one bud were repeatedly collected and screened by tea makers to ensure the samples are more representative in appearance, colour, shape, and other aspects, and then converted into commercial premium green tea of first grade, according to the standard process of withering, fixation, rolling, and drying. Postharvested samples were spread out from 4 h to 6 h. Following this step, tea samples were fixed at 230°C for 1 min to reach a relative humidity of 60%, rolled at room temperature for 35 min, and dried at 90°C for 20 min to produce Rizhao green tea. Tea samples ($n = 3$) from four key processing stages were named as tea leaves (TL), fixated leaves (FL), rolled leaves (RL), and green tea (GT) and were placed in centrifuge tubes, packed with sealing film, and transported to the laboratory at low temperature. The tea samples from each processing step were crushed with a household blender for 7–8 s and stored at −20°C.

The volatile metabolites of Rizhao green tea were analyzed using the FlavourSpec® GC-IMS system (G.A.S. Department of Shandong Haineng Science Instrument Co., Ltd., Shandong, China) equipped with an autosampler unit (CTC Analysis AG, Zwingen, Switzerland), Laboratory Analytical Viewer analysis software, GC × IMS Library Search software, and the NIST and IMS built-in databases.

2.2. GC-IMS Analysis. Samples (1.5 g) from different processing stages were weighed into 20-mL head space bottles and incubated at 50°C for 15 min with rotation at 500 rpm. Then, 500 μL of the headspace sample was injected into the heated injector (85°C). After injection, the samples were detected by GC-IMS instrument. Each sample was analyzed at least in triplicate.

The GC-IMS conditions were as follows: an MXT-5 capillary column (5 m × 0.53 mm, 1 m) was used at a column temperature of 60°C. The carrier gas was pure helium (99.999%) for 0–20 min. and with the following linear gradient: the initial carrying flow rate of carrying was 2 mL/min, held for 2 min and then increased to 100 mL/min for 18 min. The IMS drift tube was 110°C, and the drift gas was pure helium (99.999%) at 150 mL/min for 20 min.

2.3. Data Analysis. The GC-IMS data analysis software included VOCal and three plug-ins, which were used to analyze the samples from different perspectives. The reporter and Gallery Plot plug-ins were used to obtain the differences in the spectra of the tea samples and generate fingerprints of the volatile compounds. The dynamic principal component analysis (PCA) plug-in and K-fingerprint analysis were used to perform dynamic PCA for clustering analysis and similarity analysis of samples. VOCal was mainly used to analyze spectrograms and to obtain qualitative and quantitative information. The NIST and IMS databases were built into the software for qualitative analysis of the materials. Volatile compound content is the normalized relative peak volume (%), and the results are presented as the mean of triplicate measurements.

2.4. Statistical Analysis. The processed data were then exported to SIMCA 14.1 (Umetrics AB, Umea, Sweden) software for multivariate statistical analysis, including PCA, partial least-squares discriminant analysis (PLS-DA), and orthogonal partial least-squares discriminant analysis (OPLS-DA). A cross-validation plot of the OPLS-DA model was generated with 200 permutation tests. After multivariate statistical analysis, the differences between groups were tested with the Student t-test ($p < 0.05$), and univariate

statistical analysis was performed by one-way ANOVA using SPSS Statistics 26.0 (IBM Corp., Armonk, NY).

2.5. ROAV Calculation. The ROAV was used to evaluate the contribution of volatile compounds to the overall aroma and was calculated according to the formula [12]:

$$\mathrm{ROAV}_A = \mathrm{OAV}_A/\mathrm{OAV}_{\mathrm{stan}} \times 100 = C_A/T_A \times T_{\mathrm{stan}}/C_{\mathrm{stan}} \times 100. \quad (1)$$

Where OAV_A represents the odor activity value of A, $\mathrm{OAV}_{\mathrm{stan}}$ represents the odor activity value for the compounds that contribute the most to the overall flavor. C_A (%) and T_A (mg/kg) represent the relative content and the sensory threshold of each volatile component, respectively, C_{stan} represents the relative content of the compound that contributes the most to the overall flavor (%), and T_{stan} represents the sensory threshold for the compounds that contributes the most to the overall flavor (mg/kg).

3. Results and Discussion

3.1. Qualitative and Multivariate Statistical Analysis of Rizhao Green Tea Processing. The volatile compounds in the Rizhao green tea samples from four key processing stages, namely, tea leaves (TL), fixated leaves (FL), rolled leaves (RL), and green tea (GT), were analyzed by GC-IMS technology. As shown in Figure 1, the qualitative and quantitative analyses of Rizhao green tea at different processing stages were carried out by VOCal software, which is built in to the GC-IMS. A total of 85 volatile substances were detected (Table S1, list of the 85 volatile compounds in the process of Rizhao green tea). Sixty-two volatile substances, including monomers and dimers, were identified through the NIST database and IMS database built into the software. The compounds include 15 alcohols, 20 aldehydes, 13 ketones, 10 esters, 2 terpenes, and 2 oxygen compounds.

Rizhao green tea samples from four processing stages were analyzed by multivariate statistical analysis. The results are shown in Figure 2. In the score scatter plot of PCA (Figure 2(a)), the cumulative contribution rate of the two principal components was 79%, which could distinguish TL and GT successfully, and a close aggregation was seen between the FL and RL samples, indicating the differences between and similarities of their volatile component compositions. The results of PLS-DA (Figure 2(b)) and OPLS-DA (Figure 2(c)) are consistent with those of PCA. The cross-validation with 200 permutation verifications (Figure 2(d)) indicate that the OPLS-DA model is reliable.

Figure 2(e)shows the Euclidean distance between samples. It can be seen from the figure that the distance between FL and RL was the closest, and the distance between TL and GT was the farthest, indicating that the volatile substances in Rizhao green tea at different processing stages were quite different, and some substances showed certain regularity, that is, the volatile components of TL and GT were relatively special, while the volatile components of FL and RL were highly similar.

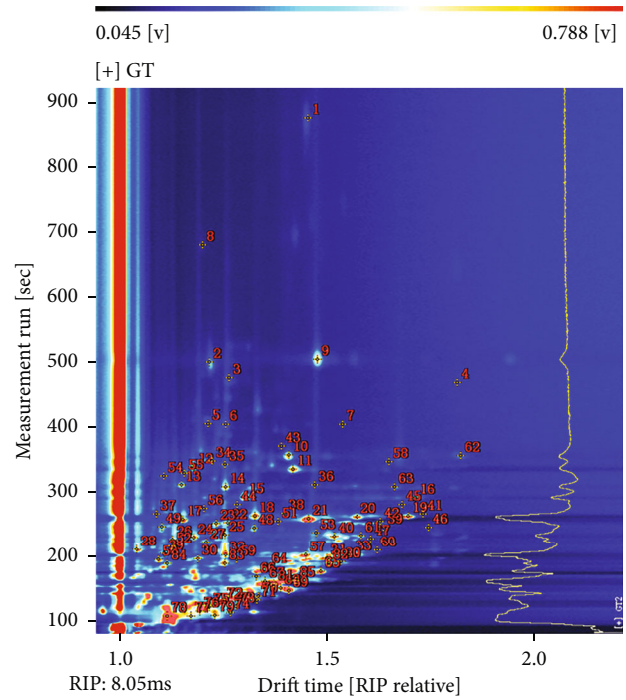

Figure 1: Qualitative analysis of Rizhao green tea with different processing.

3.2. Spectral Analysis and Fingerprint of Volatile Compounds. The GC-IMS instrument-supported reporter plug-in could directly compare the differences in the spectra between samples. Figure 3(a) is a three-dimensional spectrum of volatile substances in samples, in which the ordinate is the retention time of gas chromatography, and the abscissa is the ion migration time. Figure 3(b) shows the comparison plots of volatile components in the samples. With the TL sample as the reference, the different volatile substances were evaluated by comparing the spectrum colors of the other three types of samples. The darker the red was, the more the corresponding substance concentration was higher than that in TL. The darker the blue, the reverse was true. It could be seen that there are great differences in volatile substances between the four groups, and the most volatile substances in TL are significantly higher than those in the other three groups. The contents of volatile organic compounds in FL and RL were similar in the abscissa of 1.2–1.75 and the ordinate of 250–420 s.

Figure 4 shows the fingerprints of volatile compounds in Rizhao tea at different processing stages. Comparing the composition and changes of aroma in different processing stages, the results are consistent with the results of the difference comparison plots and multivariate statistical analysis. The volatile compounds in the red frame included (E)-ocimene, linalool, linalool oxide monomer, and its dimer, benzene acetaldehyde monomer and its dimer, alpha-phellandrene, methyl salicylate, 2-pentyl furan, and benzaldehyde monomer and its dimer, 3-methylthiopropanal monomer and its dimer, n-hexanol monomer and its dimer, (E)-2-hexenyl, (E)-2-pentenal, heptan-2-ol, and methyl hexanoate monomer and its dimer, 3-methyl-1-pentanol, 2-

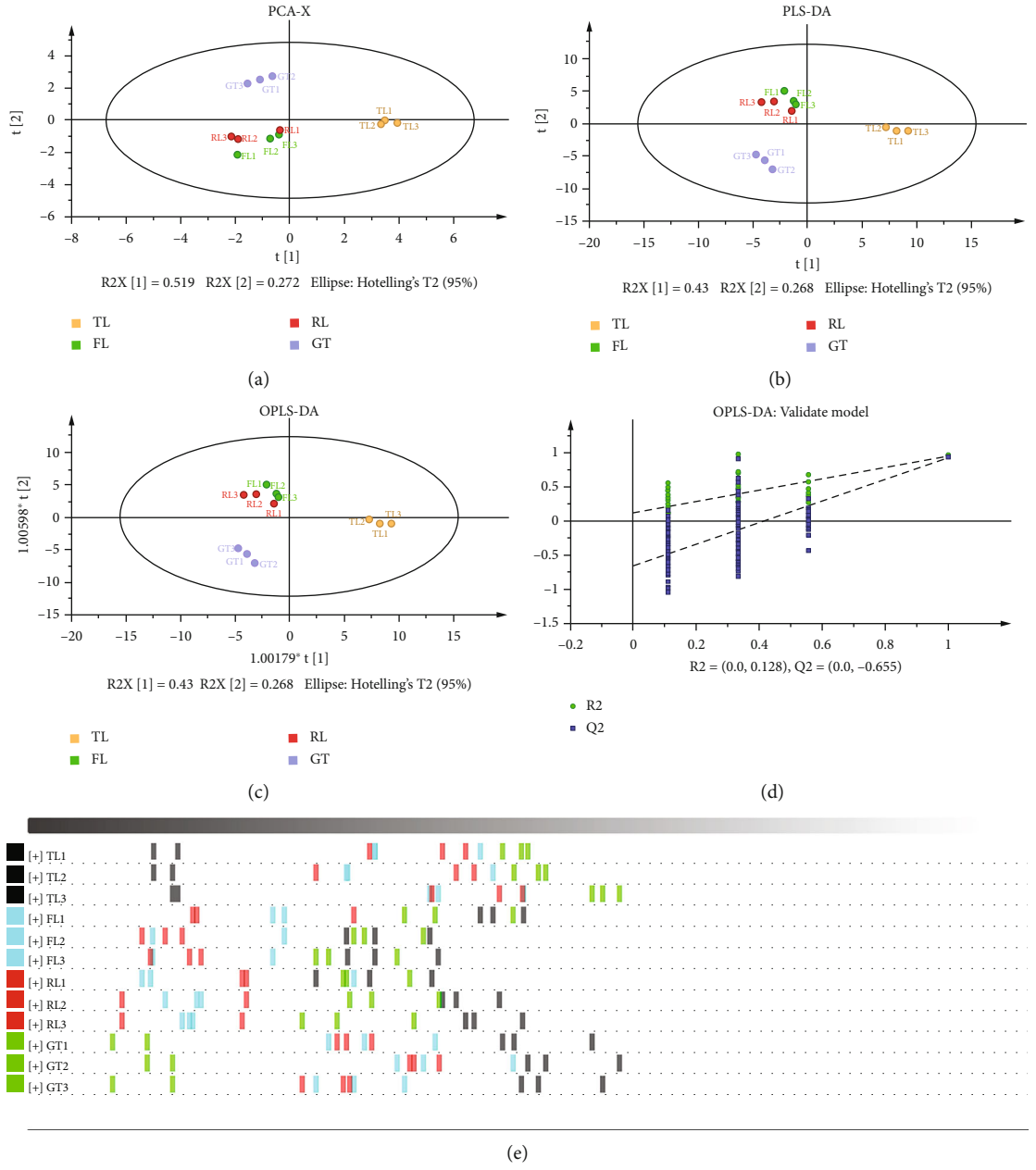

Figure 2: Multivariate statistical analyses of Rizhao green tea at different processing stages. (a) PCA score plot, (b) PLS-DA score plot, (c) OPLS-DA score plot, (d) cross-validation plot of the OPLS-DA model with 200 permutation tests, and (e) Euclidean distance of tea samples.

butanol, ethyl acetate, isoamyl acetate, 3-pentanone, methyl acetate, butanal, and (E)-2-hexen-1-ol monomer and its dimer, 2-heptanone monomer and its dimer, 2-methylbutanal, 3-methylbutanal, and unknown substances that were not identified numbered 1, 10, 20, 21, 22. Their relative contents in tea samples were significantly higher than that in other processing stages, and they were identified as special volatile substances in TL.

The volatile substances in the yellow frame included methyl-5-hepten-2-one, oct-1-en-3-ol, 2-butoxyethanol, 3-hydroxybutan-2-one, methyl isobutyl ketone, 2-butanone, acetone, ethyl formate, methyl 2-methylbutanoate, 2-pentanone, and 1-pentanol monomer and its dimer, and unknown substances 11, 13, 14, 15, 17, 18. Their relative contents were significantly higher than those of the samples in other processing stages, and they were identified as unique volatile substances in the FL and RL samples.

The volatile compounds in the green box are 2-hexanone monomer and its dimer, nonanal, (E)-hept-2-enal monomer and its dimer, octanal monomer and its dimer, cyclohexanone monomer and its dimer, heptanal monomer and its dimer, (E)-3-hexen-1-ol, butyl acetate, 2-furanmethanol, hexanal monomer and its dimer, pentanal, and unknown substances 2, 3, 4, 5, 6, 7, 8, 16, 19, 23. Their relative contents were significantly higher than those of the samples in other processing points, and they are specific to the GT sample.

In summary, linalool, phellandrene, methyl salicylate, methyl hexanoate, benzaldehyde, benzene acetaldehyde, 2-

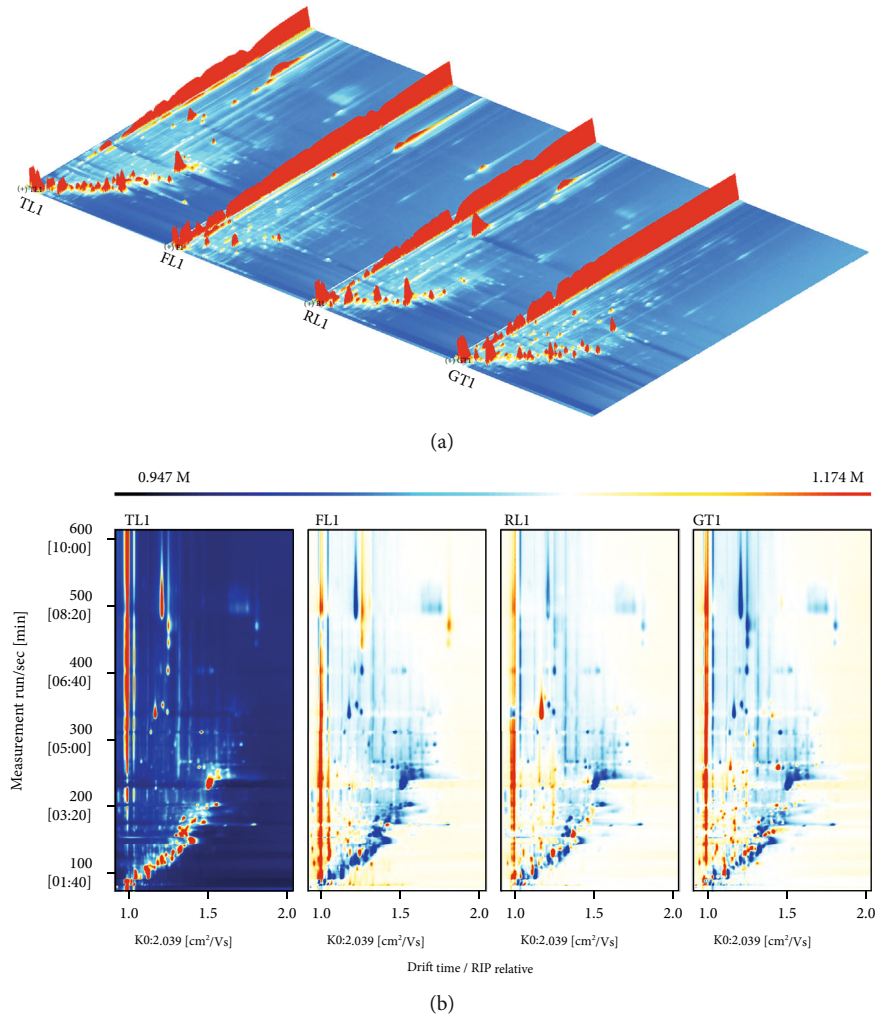

Figure 3: The 3D spectral (a) and differences analysis plots (b) of Rizhao green tea with different processing.

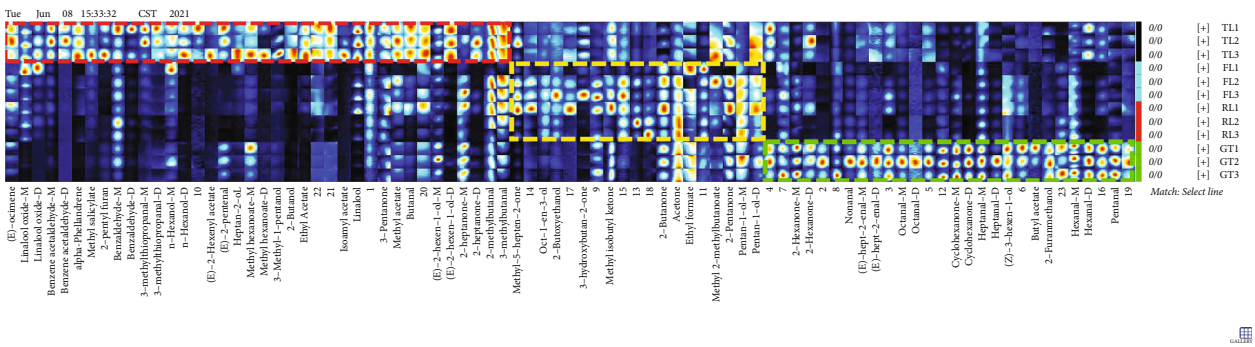

Figure 4: Fingerprint analysis of volatile compounds of Rizhao green tea samples obtained from different processing stages.

heptanone, hexanol, 2-pentylfuran, and other substances had the highest relative content in the TL. The relative contents of pentanal, hexanal, heptanal, octanal, nonanal, and other substances in the GT samples were the highest; the contents of acetone, butanone, and pentanone were the highest in the FL or RL samples.

3.3. Changes in Volatile Compounds in Rizhao Green Tea during Processing. There are two sources of tea aroma compounds: one is inherent in fresh leaves and the other is generated from precursors during manufacturing through enzymatic and thermophysical activities [20]. The original free aroma compounds in fresh leaves are not only few in

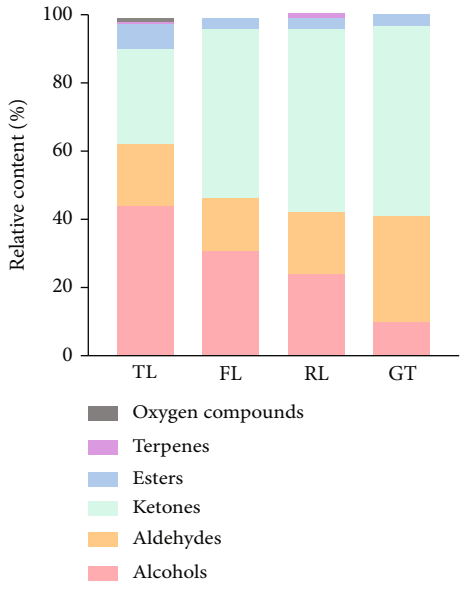

Figure 5: Changes of volatile compounds in different processes.

species but are also very small in retention after processing. Therefore, processing is the main reason for tea aroma production [21]. As shown in Figure 5, the volatile organic compounds in Rizhao green tea vary greatly at different processing stages. Alcohols accounted for 44.00% of the total aroma in the TL. However, the content gradually reduced after processing. In the GT stage, they accounted for less than 10.00%. Aldehydes were relatively stable and increased significantly only at the final stage of green tea processing. This is due to the Strecker reaction and phospholipid-induced lipid degradation [22]. The proportion of ketones increased gradually with the advance of the processing stage, especially from the TL stage to the FL stage. Compared with other stages, esters accounted for a higher proportion in TL. Ester content decreased significantly from TL to FL and then stabilized. The proportion of terpenes and oxygen compounds was always less than 1.00%.

Fresh leaves contain a variety of esters, such as (E)-2-hexenyl acetate and methyl salicylate. After high-temperature fixation, the aroma compounds in fresh tea leaves changed fundamentally. Most of the low-boiling grass aroma substances were lost, and the contents of high-boiling aldehydes, alkenes, and aromatic hydrocarbons increased significantly, such as 2-methylbutanal, 3-methylbutanal, and nonanal. With the increase in temperature and the decrease in water content, a variety of enzymatic reactions occur, which greatly increase the types of aroma compounds in tea. At the same time, a large number of volatile compounds were produced in the dehydration reaction of sugar, amino acids, and pectin, forming roasted and fresh aromas [23]. Tea fixation led to the dissipation of volatile metabolites with low boiling point as well as green flavor. Fixation also promoted the formation of ketones, aldehydes, and other metabolites with chestnut, floral, and fruity aromas.

Rolling can damage the cells of fresh leaves, causing the overflow of tea juice and increasing the leaching rate of tea. This leads to the further transformation and degradation of biochemical components within the tea. However, most aroma compounds show a decreasing trend during shaping after rolling. Unrolled tea is often floral. Most of the rolled tea is green, and the aroma concentration and freshness are lower than unrolled tea. Catechins inhibited the formation of terpenic alcohols, such as geraniol and linalool, at the subcellular level, catechins are mainly located in the chloroplasts and vacuoles [24]. When the cells were distorted and the outer membrane of the chloroplast was broken due to rolling, polyphenols were mixed with various inclusions located in the cytoplasm, which may affect the formation and transformation of aromatic substances in rolling tea [25].

The thermophysical and chemical effects of the drying process are important for the development of green tea aroma. After the drying process, the content of aromatic substances in the products changed significantly, compared with fresh leaves and fixated leaves. The contents of aliphatic alcohols and aldehydes, with green flavors and low boiling points, continued to decrease. Esters, aroma compounds, terpenes with clean or floral aromas, and furfural are the products of the Maillard reactions and impart a pleasant and roasted burnt flavor. The contents of these compounds greatly increased. Some original components of lower content in fixated leaves increased significantly during the drying stage, such as pentanal, heptanal, nonanal, and cyclohexanone, which greatly enriched the aroma compounds in tea.

The aroma compounds in Rizhao green tea underwent tremendous changes through three key processes, including fixation, rolling, and drying. These changes are related to the enzymatic reactions and chemical reactions during processing.

3.4. Identification of Key Odorants Responsible for Rizhao Green Tea during Processing. The variations in the content of volatile organic compounds, the aroma sensory threshold [26] and the ROAV of the samples are shown in Table 1.

The common aroma types of green tea include pekoe-flavor, tender-flavor, delicate-flavor, chestnut-flavor, and high fired-flavor [27]. Rizhao green tea's chestnut-like aroma is determined by the relative content of volatile components and sensory threshold. Only some compounds contributed significantly to the overall flavor of Rizhao green tea. By calculating the ROAV of the finished green tea, namely the GT sample, the main aromatic substances were determined. It is generally believed that the volatile substances with ROAV > 1 are key flavor substances in the sample, and the volatile substances with $0.1 < \text{ROAV} \leq 1$ play a role in modifying the overall flavor of the sample. We selected the ROAV of octanal, which contributed the most to the flavor of the sample, as 100, and calculated the relative ROAV of each component. The key odorants compounds in Rizhao green tea included linalool (ROAV:6.5), pentanal (ROAV:7.7), hexanal-M (ROAV:5.6), hexanal-D (ROAV:12), heptanal-M (ROAV:2.4), heptanal-D (ROAV:1.5), octanal-M (ROAV:100), octanal-D (ROAV:16), nonanal (ROAV:1.1), 2-methylbutanal

Table 1: Relative content, aroma sensory threshold, and ROAV of volatile substances in tea samples.

Type	Serial number	Name of compound	Relative content[1]/%				Aroma sensory threshold[2] (mg/kg)	ROAV
			TL[3]	FL	RL	GT		
Alcohols (15)	1	2-Butanol	1.210 ± 0.276*a*	0.093 ± 0.041*b*	0.133 ± 0.045*b*	0.140 ± 0.017*b*	5.1	0.0004
	2	Pentan-1-ol-M	0.107 ± 0.007*b*	0.603 ± 0.024*ab*	0.920 ± 0.238*a*	0.690 ± 0.093*a*	5	0.0018
	3	Pentan-1-ol-D	0.453 ± 0.042*a*	0.563 ± 0.135*a*	0.697 ± 0.052*a*	0.595 ± 0.128*a*	5	0.0016
	4	n-Hexanol-M	0.500 ± 0.078*a*	0.697 ± 0.482*a*	0.257 ± 0.043*a*	0.323 ± 0.073*a*	0.7	0.0061
	5	n-Hexanol-D	0.997 ± 0.078*a*	0.177 ± 0.082*b*	0.093 ± 0.007*b*	0.133 ± 0.009*b*	0.7	0.0025
	6	3-Methyl-1-pentanol	0.237 ± 0.091*a*	0.050 ± 0.000*a*	0.053 ± 0.003*a*	0.083 ± 0.007*a*	\	\
	7	Heptan-2-ol	0.313 ± 0.125*a*	0.227 ± 0.142*a*	0.060 ± 0.010*a*	0.113 ± 0.003*a*	0.1	0.0147
	8	(E)-2-Hexen-1-ol-M	0.550 ± 0.017*a*	0.677 ± 0.325*a*	0.423 ± 0.098*a*	0.927 ± 0.062*a*	\	\
	9	(E)-2-Hexen-1-ol-D	10.127 ± 0.595*a*	0.720 ± 0.015*b*	0.430 ± 0.222*b*	0.627 ± 0.254*b*	\	\
	10	(Z)-3-Hexen-1-ol	0.073 ± 0.009*c*	0.363 ± 0.069*b*	0.463 ± 0.038*b*	0.710 ± 0.030*a*	0.2	0.0473
	11	Oct-1-en-3-ol	0.113 ± 0.015*b*	0.290 ± 0.092*ab*	0.457 ± 0.074*a*	0.110 ± 0.010*b*	0.007	0.2
	12	Linalool	18.777 ± 0.316*a*	10.313 ± 2.668*ab*	8.680 ± 2.829*b*	1.847 ± 0.150*b*	0.0038	6.5
	13	2-Furanmethanol	0.127 ± 0.027*b*	0.087 ± 0.017*b*	0.127 ± 0.013*b*	0.790 ± 0.040*a*	1	0.0105
	14	Linalool oxide-M	6.017 ± 0.366*a*	13.253 ± 7.962*a*	7.990 ± 2.393*a*	0.993 ± 0.029*a*	\	\
	15	Linalool oxide-D	1.797 ± 0.280*a*	3.197 ± 2.422*a*	0.827 ± 0.303*a*	0.160 ± 0.006*a*	\	\
Aldehydes (20)	1	Butanal	1.013 ± 0.063*a*	0.323 ± 0.114*b*	0.430 ± 0.201*b*	0.360 ± 0.042*b*	0.06	0.0800
	2	Pentanal	0.730 ± 0.147*b*	0.743 ± 0.041*b*	1.157 ± 0.157*b*	4.633 ± 0.387*a*	0.008	7.7
	3	Hexanal-M	0.423 ± 0.020*b*	1.200 ± 0.012*ab*	1.823 ± 0.473*a*	2.083 ± 0.202*a*	0.005	5.6
	4	Hexanal-D	1.810 ± 0.403*b*	0.177 ± 0.009*c*	0.290 ± 0.078*c*	4.487 ± 0.047*a*	0.005	12
	5	Heptanal-M	0.737 ± 0.111*a*	1.047 ± 0.332*a*	1.317 ± 0.347*a*	1.823 ± 0.147*a*	0.01	2.4
	6	Heptanal-D	0.167 ± 0.044*a*	0.147 ± 0.024*a*	0.277 ± 0.067*a*	1.147 ± 0.478*a*	0.01	1.5
	7	Octanal-M	0.163 ± 0.012*b*	0.180 ± 0.025*b*	0.227 ± 0.067*b*	0.747 ± 0.198*a*	0.0001	100
	8	Octanal-D	0.047 ± 0.007*a*	0.047 ± 0.007*a*	0.047 ± 0.009*a*	0.123 ± 0.058*a*	0.0001	16
	9	Nonanal	0.190 ± 0.010*a*	0.443 ± 0.088*a*	0.633 ± 0.178*a*	1.273 ± 0.509*a*	0.015	1.1
	10	2-Methylbutanal	3.753 ± 0.204*a*	4.033 ± 1.360*a*	4.770 ± 0.905*a*	4.087 ± 0.068*a*	0.001	54
	11	3-Methylbutanal	2.427 ± 0.061*a*	2.887 ± 1.056*a*	3.480 ± 0.868*a*	1.963 ± 0.276*a*	0.002	13
	12	(E)-2-Pentenal	0.823 ± 0.168*a*	0.080 ± 0.031*b*	0.087 ± 0.018*b*	0.847 ± 0.084*a*	2.7	0.0042
	13	(E)-Hept-2-enal-M	0.167 ± 0.018*b*	0.240 ± 0.010*b*	0.387 ± 0.090*b*	0.937 ± 0.071*a*	\	\
	14	(E)-Hept-2-enal-D	0.080 ± 0.015*ab*	0.037 ± 0.007*b*	0.043 ± 0.012*ab*	0.120 ± 0.030*a*	\	\
	15	Benzaldehyde-M	0.473 ± 0.111*a*	0.553 ± 0.064*a*	0.813 ± 0.270*a*	0.427 ± 0.062*a*	0.3	0.0191
	16	Benzaldehyde-D	0.773 ± 0.296*a*	0.187 ± 0.022*a*	0.260 ± 0.067*a*	0.163 ± 0.015*a*	0.3	0.0071

Table 1: Continued.

Type	Serial number	Name of compound	Relative content[1]/% TL[3]	FL	RL	GT	Aroma sensory threshold[2] (mg/kg)	ROAV
	17	Benzene acetaldehyde-M	2.237 ± 0.170*a*	0.847 ± 0.091*b*	0.883 ± 0.116*b*	0.403 ± 0.068*b*	0.009	0.6
	18	Benzene acetaldehyde-D	0.387 ± 0.045*a*	0.130 ± 0.042*b*	0.080 ± 0.017*b*	0.067 ± 0.003*b*	0.009	0.1
	19	3-Methylthiopropanal-M	0.303 ± 0.087*a*	0.197 ± 0.077*a*	0.133 ± 0.037*a*	0.083 ± 0.007*a*	0.00004	27
	20	3-Methylthiopropanal-D	0.250 ± 0.056*a*	0.070 ± 0.012*b*	0.077 ± 0.013*b*	0.123 ± 0.003*ab*	0.00004	40
Ketones (13)	1	Acetone	2.807 ± 0.308*a*	6.900 ± 0.869*ab*	13.157 ± 3.826*b*	9.750 ± 0.524*ab*	100	0.0013
	2	2-butanone	1.320 ± 0.113*a*	8.413 ± 1.669*b*	7.903 ± 0.058*b*	6.460 ± 0.299*b*	3	0.0287
	3	3-Hydroxybutan-2-one	1.737 ± 0.077*a*	2.410 ± 1.264*a*	0.327 ± 0.050*a*	0.490 ± 0.057*a*	0.75	0.0087
	4	2-Pentanone	5.220 ± 0.925*a*	6.433 ± 2.983*a*	6.703 ± 2.315*a*	9.160 ± 0.311*a*	0.3	0.4
	5	3-Pentanone	3.090 ± 0.046*a*	2.967 ± 0.999*a*	4.683 ± 0.146*a*	3.747 ± 0.254*a*	\	\
	6	Methyl isobutyl ketone	1.673 ± 0.043*a*	1.877 ± 0.469*a*	2.077 ± 0.307*a*	2.637 ± 0.281*a*	\	\
	7	2-Hexanone-M	0.117 ± 0.009*b*	0.360 ± 0.032*b*	0.377 ± 0.030*b*	1.087 ± 0.146*a*	0.09	0.2
	8	2-Hexanone-D	0.903 ± 0.225*b*	0.297 ± 0.099*b*	0.393 ± 0.228*b*	1.980 ± 0.210*a*	0.09	0.3
	9	2-Heptanone-M	0.157 ± 0.062*b*	0.563 ± 0.166*ab*	0.720 ± 0.040*a*	0.970 ± 0.125*a*	0.2	0.0647
	10	2-Heptanone-D	1.017 ± 0.255*a*	0.160 ± 0.076*b*	0.147 ± 0.087*b*	0.297 ± 0.015*b*	0.2	0.0193
	11	Methyl-5-hepten-2-one	6.853 ± 2.510*a*	11.637 ± 3.657*a*	10.623 ± 4.402*a*	1.720 ± 0.021*a*	0.1	0.2
	12	Cyclohexanone-M	0.167 ± 0.030*b*	0.693 ± 0.162*b*	0.787 ± 0.187*b*	2.500 ± 0.434*a*	\	\
	13	Cyclohexanone-D	0.533 ± 0.144*b*	0.337 ± 0.107*b*	0.307 ± 0.055*b*	6.620 ± 0.697*a*	\	\
Esters (10)	1	Ethyl formate	0.243 ± 0.009*a*	0.593 ± 0.299*a*	0.443 ± 0.026*a*	0.763 ± 0.034*a*	6.6	0.0016
	2	Methyl acetate	1.243 ± 0.078*a*	0.967 ± 0.420*a*	0.970 ± 0.140*a*	0.780 ± 0.012*a*	\	\
	3	Ethyl acetate	3.830 ± 0.638*a*	0.573 ± 0.205*b*	0.770 ± 0.093*b*	0.383 ± 0.023*b*	0.1	0.0507
	4	Methyl 2-methylbutanoate	0.163 ± 0.041*a*	0.163 ± 0.085*a*	0.150 ± 0.050*a*	0.100 ± 0.025*a*	0.0002	6.7
	5	Butyl acetate	0.017 ± 0.003*a*	0.027 ± 0.003*a*	0.033 ± 0.007*a*	0.137 ± 0.059*a*	0.2	0.0093
	6	Methyl hexanoate-M	0.087 ± 0.024*ab*	0.050 ± 0.006*b*	0.063 ± 0.017*b*	0.197 ± 0.047*a*	\	\
	7	Methyl hexanoate-D	0.137 ± 0.074*a*	0.033 ± 0.009*a*	0.040 ± 0.010*a*	0.043 ± 0.003*a*	\	\
	8	Isoamyl acetate	0.233 ± 0.100*a*	0.070 ± 0.006*a*	0.070 ± 0.006*a*	0.063 ± 0.003*a*	0.005	0.2
	9	(E)-2-Hexenyl acetate	0.347 ± 0.143*a*	0.137 ± 0.033*a*	0.100 ± 0.017*a*	0.107 ± 0.003*a*	\	\
	10	Methyl salicylate	0.650 ± 0.222*a*	0.477 ± 0.180*a*	0.347 ± 0.088*a*	0.257 ± 0.023*a*	0.06	0.0578
Terpenes (2)	1	Alpha-Phellandrene	0.807 ± 0.091*a*	0.137 ± 0.035*b*	0.163 ± 0.046*b*	0.037 ± 0.003*b*	\	\
	2	(E)-Ocimene	0.430 ± 0.047*a*	0.297 ± 0.039*a*	0.433 ± 0.037*a*	0.313 ± 0.056*a*	\	\

Table 1: Continued.

Type	Serial number	Name of compound	Relative content[1]/%				Aroma sensory threshold[2] (mg/kg)	ROAV
			TL[3]	FL	RL	GT		
Oxygen compounds(2)	1	2-Pentyl furan	0.980 ± 0.289*a*	0.110 ± 0.006*b*	0.163 ± 0.041*b*	0.257 ± 0.012*b*	0.0048	0.7
	2	2-Butoxyethanol	0.047 ± 0.003*a*	0.180 ± 0.044*a*	0.180 ± 0.047*a*	0.073 ± 0.007*a*	2.6	0.0004

[1] Relative content of compounds in samples are represented as mean ± standard error of mean (mean ± SEM). [2] "\" represents the threshold values of compounds that were not measured in water. All aroma sensory thresholds were obtained from Van Gemert, L. J. (2011). Compilations of Flavour Threshold Values in Water and Other Media (2nd ed). Tang, E., et al., trans. Beijing, Science Press, 2015.[3] The letters represent the significant difference ($p < 0.05$) between different process of Rizhao green tea.

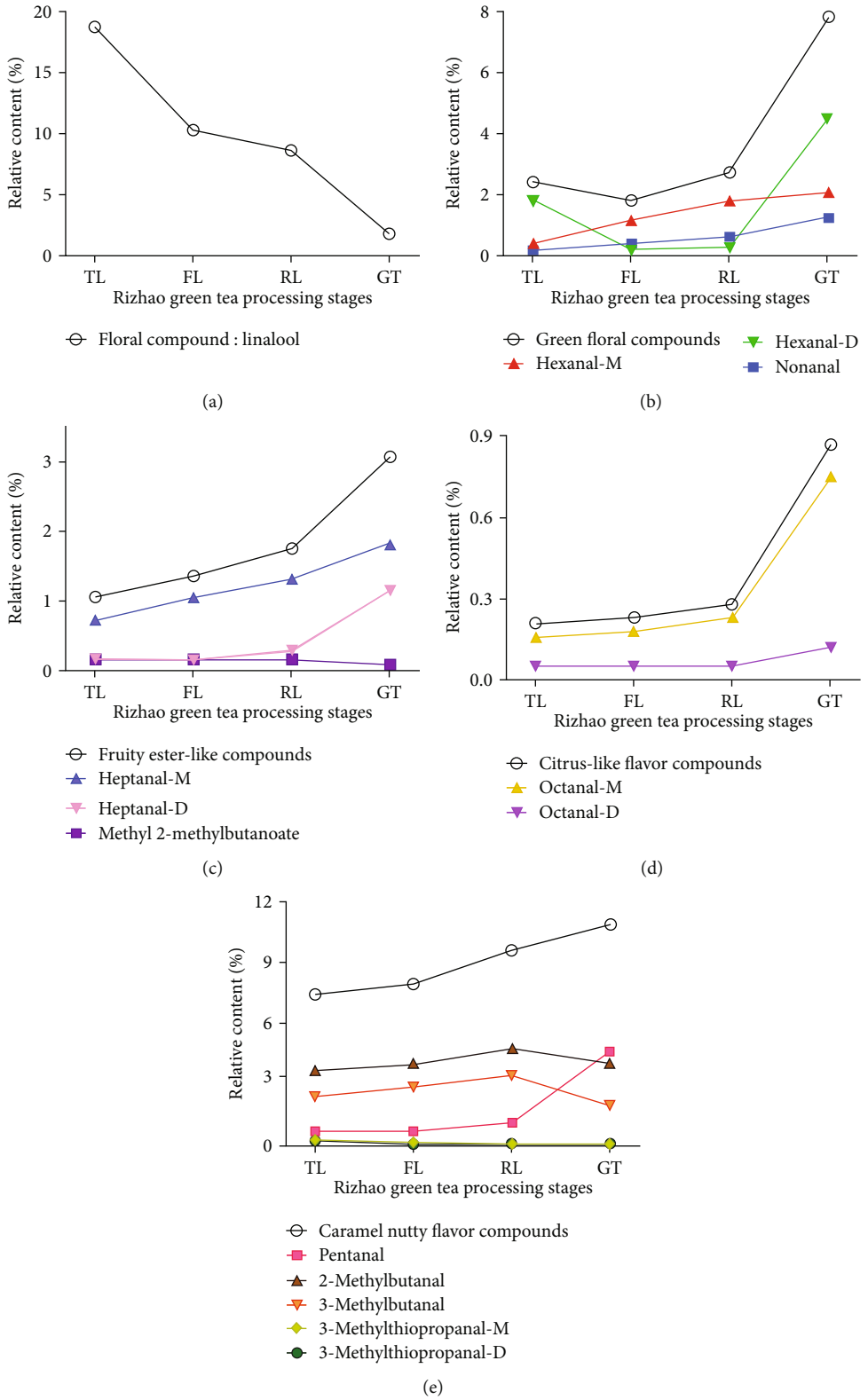

Figure 6: Change trend of key odorant compounds in processes. (a) Floral compound, (b) green compounds, (c) fruity ester-like compounds, (d) citrus-like flavor compounds, and (e) caramel nutty flavor compounds.

(ROAV:54), 3-methylbutanal (ROAV:13), 3-methylthiopropanal-M (ROAV:27), 3-methylthiopropanal-D (ROAV:40), and methyl 2-methylbutanoate (ROAV:6.7). Some volatile compounds also had an effect on the aroma characteristics of Rizhao green tea, such as oct-1-en-3-ol (ROAV:0.2), benzeneacetaldehyde-M (ROAV:0.6), 2-

pentanone (ROAV:0.4), 2-hexanone-M (ROAV:0.2), 2-hexanone-D (ROAV:0.3), methyl-5-hepten-2-one (ROAV:0.2), isoamyl acetate (ROAV:0.2), and 2-pentyl furan (ROAV:0.7).

According to the flavor wheel classification method [28], 14 kinds of aroma compounds with a ROAV value greater than 1 were classified into five categories according to their flavor characteristics: green, fruity ester-like, citrus-like, floral, and caramel nutty flavors. Figure 6 shows the change trend of the key odorant compounds of different aroma types in each processing stage. It can be seen that the processing procedure has a great influence on the aroma compounds. The content of linalool, a floral aromatic substance, was significantly reduced in the fixation and drying stages. The contents of aromatic substances with green, fruity ester-like, and citrus-like flavors increased with processing, contributing to the strong and clean flavor of green tea. The increase in aromatic substances with caramel nutty flavor presents a cocoa, nutty flavor as well as a chestnut-like aroma with a roasted burnt flavor.

3.5. Discussion. In Rizhao green tea products, ketones and aldehydes accounted for the largest proportion, followed by alcohols and esters. This was similar to previous research results. Li et al. [3] found that the aroma compounds in Rizhao green tea were mainly alcohols, aldehydes, acids, and phenols. Aldehydes, esters, and olefins also played a role. Liu [7] speculated that the aroma compounds in Rizhao curly green tea and Rizhao pelleted green tea were mainly hydrocarbons, esters, and alcohols, followed by ketones and aldehydes. The authors showed that the tea contains a large number of volatiles from saturated and unsaturated fatty acids, including alcohols and aldehydes. Saturated and unsaturated aldehydes and alcohols (especially C6, C9) are important contributors to the characteristic aroma of clean flavor [29].

The ROAV results showed that 17 kinds of volatile compounds had an effect on the aroma characteristics of Rizhao green tea products. Among them, linalool, pentanal, hexanal, heptanal, octanal, nonanal, 2-methylbutanal, 3-methylbutanal, 3-methylthiopropanal, and methyl 2-methylbutanoate were the key flavor compounds. Zhang [27] have reported that the contribution of linalool, hexanal, heptanal, nonanal, and other key aroma components to the characteristic aroma of chestnut-like green tea was preliminarily confirmed. In our study, the main flavor substances of Rizhao green tea are aldehydes and alcohols. Nevertheless, this is different from the results reached by He et al. [21], who reported that esters with floral or fruity flavors were more beneficial to the formation of aroma in fragrant green tea. Aldehydes are closely related to the formation of tea aroma. Low molecular aliphatic aldehydes have a strong pungent odor. With the increase in the relative molecular mass, the irritation degree of the odor decreases, and a pleasant aroma gradually appears. Among them, hexanal and heptanal with grassy aroma have low boiling point and high threshold [30], which is perceived to be one of the important reasons for the slight grassy aroma in green teas with chestnut-like aroma. Nonanal has rose and waxy aromas, and 2-methylbutanal has malty and cocoa aromas [31]. The threshold of saturated alcohols was higher than that of unsaturated alcohols, and it contributes to the flavor of tea at high concentrations. Unsaturated alcohols have a relatively low threshold and contribute greatly to tea flavor. For example, 1-octen-3-ol has a mushroom-like aroma [30]. Terpene alcohols have a high boiling point, which play an important role in the formation of tea aroma. Linalool belongs to monoterpene alcohols, with a floral and fruity flavor [32]. In addition, benzene acetaldehyde has honey and fruity flavors, and 2-pentylfuran has a bean aroma and a strong burned flavor, which may be the main contributor to the aroma of green tea with a heavy flavor [33]. The interaction between these volatile compounds constitutes the unique flavor characteristics of Rizhao green tea, which is consistent with previous studies [34].

The GC-IMS results show that the composition and content of volatile compounds in Rizhao green tea from the four key processing stages were significantly different. The results of the difference comparison plots show that the characteristic aromatic substances in tea leaves, fixated leaves, and rolled leaves were quite different from those in green tea, which was related to the treatment temperature at different processing stages and various enzymatic and biochemical reactions in tea processing. Tea aroma compounds are generated from four main pathways: carotenoid degradation, the oxidation of tea lipids, glycosides as precursors, and the Maillard reaction or the Stecker degradation reaction pathways [35]. Linalool is a monoterpene alcohol, with a boiling point of 199–200°C, which mainly exists in the tea plants in the form of glucosides. After tea leaves are picked, the glycosides are hydrolyzed by glucosidase into free linalool. β-D-Glucoside is the precursor of linalool and its oxidation products [36]. 2-Methylbutanal, methylthiopropanal, and benzene acetaldehyde were degraded by the Strecker reaction, and the corresponding precursors were isoleucine, methionine, and phenylalanine, respectively. Hexanal, heptanal, octanal, nonanal, and 1-octene-3-ol are the key aroma compounds derived from fatty acids in Rizhao green tea. The C9–C12 saturated aldehydes have good aroma at high dilution.

The above results revealed the change trend of volatile organic compounds in Rizhao green tea at different processing stages. We explored the formation mechanism of aromatic substances during processing, and the results provided a theoretical reference for the improvement of tea processing, tea quality control, and evaluation methods. Due to the incomplete data in the current GC-IMS spectral library, twenty-three volatile substances remain undetermined. In the future, further analysis will be carried out, in combination with gas chromatography-mass spectrometry and electronic nose. In addition, in order to retain the original components of tea leaves and other samples to the maximum extent, relatively mild temperature (50°C) was used for incubation. In the future, an incubation temperature (80°C–90°C) closer to the usually water temperature of tea infusion can be used for research.

4. Conclusion

In this paper, we employed GC-IMS technology to explore the evolution of volatile metabolites during Rizhao green

tea processing. A total of 62 volatile components were detected, including alcohols, aldehydes, ketones, esters, terpenes, and oxygen compounds. The drying processes had the greatest influence on the evolution of the volatile metabolites, followed by fixation. Further analysis clarified the contribution of volatile substances to the aroma characteristics of samples. Ten compounds including linalool, pentanal, hexanal, heptanal, octanal, nonanal, methyl 2-methylbutanoate, 2-methylbutanal, 3-methylbutanal, and 3-methylthiopropanal were ultimately identified as the key odorants of the chestnut-like aroma of Rizhao green tea by the ROAV method. The result of the flavor wheel classification method showed that the contents of aromatic substances with green, fruity ester-like, caramel nutty, and citrus-like flavors exhibited a significant increasing trend from fresh leaves to the finished teas, whereas aromatic substances with floral flavors exhibited the opposite trend. This initial progress provides a theoretical basis for further research on the directional adjustment and control of tea aroma.

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

Conflicts of Interest

The authors declare no potential conflicts of interest regarding the research, authorship, and publication of this article.

Supplementary Materials

Table S1 Qualitative analysis result of volatile substances. *(Supplementary Materials)*

References

[1] Q. Chen, Y. Zhu, W. Dai et al., "Aroma formation and dynamic changes during white tea processing," *Food Chemistry*, vol. 274, pp. 915–924, 2019.

[2] K. Wang, *Find the Differential Metabolites of Rizhao Green Tea Based on Metabolomics Technology and Explore the Applications*, Shandong Normal University, 2017.

[3] W. Li, H. Gao, Q. Fan, J. Wang, and X. Tang, "Comparison of the quality and aroma components of Rizhao green tea harvested in summer dried under different roasting conditions," *Modern Food Science and Technology*, vol. 36, no. 11, pp. 255–262, 2020.

[4] Y. Liu, H. Wang, J. Huang, and P. Lei, "Application of GC-IMS in qualitative analysis of volatile compounds in green tea," *China Tea Processing*, vol. 1, pp. 55–59, 2020.

[5] M. Wong, S. Sirisena, and K. Ng, "Phytochemical profile of differently processed tea: a review," *Journal of Food Science*, vol. 87, no. 5, pp. 1925–1942, 2022.

[6] H. Yin, *Studies on Characteristic Volatile Components of Green Tea with Chestnut Aroma*, Chinese Academy of Agricultural Sciences, 2018.

[7] R. Liu, L. Ding, Q. Liang, X. Ding, and Z. Chen, "Comparison of aroma components between Rizhao pelleted green tea and Rizhao curly green tea," *Science and Technology of Food Industry*, vol. 36, no. 21, pp. 323–326, 2015.

[8] J. Jiang, P. Xie, F. Ren, Z. Lai, and A. Ou, "Flavor analysis of Xinhui citrus Pu'er tea based on gas-phase ion migration Spectrum and headspace solid phase microextraction," *Science and Technology of Food Industry*, vol. 41, no. 12, pp. 214–220, 2020.

[9] Y. Luo, Y. Wei, L. Lin, F. Lin, F. Su, and W. Sun, "Origin discrimination of Fujian white tea using gas chromatography-ion mobility spectrometry," *Transactions of the CSAE*, vol. 37, no. 6, pp. 264–273, 2021.

[10] D. Li, N. Tian, Z. Tian et al., "Analysis of typical aromatic matters in cigarettes using headspace-gas chromatography-ion mobility spectroscopy combined with relative odor activity value," *Journal of Instrumental Analysis*, vol. 40, no. 8, pp. 1164–1170, 2021.

[11] L. Wang, Z. Chen, B. Han et al., "Comprehensive analysis of volatile compounds in cold-pressed safflower seed oil from Xinjiang, China," *Food Science & Nutrition*, vol. 8, no. 2, pp. 903–914, 2020.

[12] Y. Zhu, J. Chen, X. Chen, D. Chen, and S. Deng, "Use of relative odor activity value (ROAV) to link aroma profiles to volatile compounds: application to fresh and dried eel (*Muraenesox cinereus*)," *International Journal of Food Properties*, vol. 23, no. 1, pp. 2257–2270, 2020.

[13] J. Jin, M. Zhao, N. Zhang, T. Jing, H. Liu, and C. Song, "Stable isotope signatures versus gas chromatography-ion mobility spectrometry to determine the geographical origin of Fujian Oolong tea (*Camellia sinensis*) samples," *European Food Research and Technology*, vol. 246, no. 5, pp. 955–964, 2020.

[14] J. Yin, M. Wu, R. Lin et al., "Application and development trends of gas chromatography-ion mobility spectrometry for traditional Chinese medicine, clinical, food and environmental analysis," *Microchemical Journal*, vol. 168, article 106527, 2021.

[15] R. Lin, R. Deng, and L. Xu, "Feasibility study of green tea flavor identification based on GC-IMS technology," *Guangdong Chemical Industry*, vol. 44, no. 23, pp. 19–21, 2017.

[16] Z. Yuan, H. Qu, M. Xie et al., "Direct authentication of three Chinese materia medica species of the Lilii Bulbus family in terms of volatile components by headspace-gas chromatography-ion mobility spectrometry," *Analytical Methods*, vol. 11, no. 4, pp. 530–536, 2019.

[17] S. Liu, J. Yu, M. Fu, X. Wang, and X. Chang, "Regulatory effects of hawthorn polyphenols on hyperglycemic, inflammatory, insulin resistance responses, and alleviation of aortic injury in type 2 diabetic rats," *Food Research International*, vol. 142, article 110239, 2021.

[18] S. Gu, W. Chen, Z. Wang, and J. Wang, "Rapid determination of potential aflatoxigenic fungi contamination on peanut kernels during storage by data fusion of HS-GC-IMS and fluorescence spectroscopy," *Postharvest Biology and Technology*, vol. 171, article 111361, 2021.

[19] X. Yang, R. Jiao, X. Zhu et al., "Profiling and characterization of odorous volatile compounds from the industrial fermentation of erythromycin," *Environmental Pollution*, vol. 255, article 113130, 2019.

[20] Q. Chen, Y. Zhu, Y. Liu et al., "Black tea aroma formation during the fermentation period," *Food Chemistry*, vol. 374, article 131640, 2022.

[21] Y. He, B. Cao, M. Liang, and D. Fu, "Research on the changes of aroma constituents in fragrant green tea during processing," *China Tea Processing*, vol. 2, pp. 51–56, 2016.

[22] H. Wang, J. Hua, Q. Yu et al., "Widely targeted metabolomic analysis reveals dynamic changes in non-volatile and volatile metabolites during green tea processing," *Food Chemistry*, vol. 363, article 130131, 2021.

[23] M. Wang, W. Ma, J. Shi, Y. Zhu, Z. Lin, and H. Lv, "Characterization of the key aroma compounds in Longjing tea using stir bar sorptive extraction (SBSE) combined with gas chromatography-mass spectrometry (GC-MS), gas chromatography-olfactometry (GC-O), odor activity value (OAV), and aroma recombination," *Food Research International*, vol. 130, article 108908, 2020.

[24] S. Chen, H. Liu, X. Zhao et al., "Non-targeted metabolomics analysis reveals dynamic changes of volatile and non-volatile metabolites during oolong tea manufacture," *Food Research International*, vol. 128, article 108778, 2020.

[25] L. Sun, M. Dong, J. Gu, D. Yang, and M. Wang, "Research progress in flavor chemistry of famous green teas," *Journal of Food Science and Technology*, vol. 37, no. 1, pp. 62–71, 2019.

[26] L. J. Van Gemert, *Compilations of Flavour Threshold Values in Water and Other Media (2nd Ed)*, E. Tang, Ed., Science Press, Beijing, 2011.

[27] M. Zhang, H. Yin, Y. Deng et al., "Analysis of key odorants responsible for different chestnut-like aromas of green teas based on headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry and odor activity value," *Food Science*, vol. 41, no. 2, pp. 244–252, 2020.

[28] H. Chen and B. Sun, *The Technology of Food Flavoring*, Chemical Industry Press, Third Edition edition, 2017.

[29] Z. Yang, S. Baldermann, and N. Watanabe, "Recent studies of the volatile compounds in tea," *Food Research International*, vol. 53, no. 2, pp. 585–599, 2013.

[30] Y. Shi, Y. Zhu, W. Ma et al., "Research Progress on the volatile compounds of premium roasted green tea," *Journal of Tea Science*, vol. 41, no. 3, pp. 285–301, 2021.

[31] Q. Peng, H. Zhou, X. Duan et al., "Changes of main quality components of Hainan Bosha green tea during processing," *China Tea*, vol. 43, no. 2, pp. 45–49, 2021.

[32] Z. Liu, *Analysis of the Formation and Chang of Flavor Substances in the Process of Hanzhon Green Tea*, Shanxi University of Technology, 2020.

[33] Y. Yang, J. Chen, Y. Jiang et al., "Aroma dynamic characteristics during the drying process of green tea by gas phase electronic nose and gas chromatography-ion mobility spectrometry," *LWT*, vol. 154, article 112691, 2022.

[34] Y. Zhu, H. Lv, C. Shao et al., "Identification of key odorants responsible for chestnut-like aroma quality of green teas," *Food Research International*, vol. 108, pp. 74–82, 2018.

[35] C. Ho, X. Zheng, and S. Li, "Tea aroma formation," *Food Science and Human Wellness*, vol. 4, no. 1, pp. 9–27, 2015.

[36] P. Yin, J. Wang, Z. Wang et al., "Progress research of the characteristic aroma for the green tea," *Journal of Food Safety and Quality*, vol. 12, no. 18, pp. 7324–7332, 2021.