Comparative Analysis of Volatile Compounds in Tieguanyin with Different Types Based on HS–SPME–GC–MS

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Abstract: Tieguanyin (TGY) is one kind of oolong tea that is widely appreciated for its aroma and taste. To study the difference of volatile compounds among different types of TGY and other oolong teas, solid-phase microextraction–gas chromatography–mass spectrometry and chemometrics analysis were conducted in this experiment. Based on variable importance in projection > 1 and aroma character impact > 1, the contents of heptanal (1.60–2.79 µg/L), (E,E)-2,4-heptadienal (34.15–70.68 µg/L), (E)-2-octenal (1.57–2.94 µg/L), indole (48.44–122.21 µg/L), and (E)-nerolidol (32.64–96.63 µg/L) in TGY were higher than in other varieties. With the increase in tea fermentation, the total content of volatile compounds decreased slightly, mainly losing floral compounds. Heavily fermented tea contained a higher content of monoterpenoids, whereas low-fermentation tea contained higher contents of sesquiterpenes and indole, which could well distinguish the degree of TGY fermentation. Besides, the volatiles analysis of different grades of TGY showed that the special-grade tea contained more aroma compounds, mainly alcohols (28%). (E,E)-2,4-Heptadienal, (E)-2-octenal, benzeneacetaldehyde, and (E)-nerolidol were the key volatile compounds to distinguish different grades of TGY. The results obtained in this study could help enrich the theoretical basis of aroma substances in TGY.

Keywords: Tieguanyin; oolong tea; volatile compounds; aroma

1. Introduction

Oolong tea is a unique type of tea in China. Its unique floral and fruity aroma is deeply loved by consumers. Furthermore, oolong tea can improve human health because it contains rich biological functional substances, such as polyphenols, flavonoids, and amino acids. Several studies have indicated that oolong tea has the functions of anticancer, antiallergic, and improving vascular disease [1,2]. Tea variety, origin, and processing methods lead to the differences in volatile compounds among different types of oolong tea [3]. As a special tea in China, there are four famous oolong teas: Wuyi rock tea, Anxi Tieguanyin tea, Fenghuang Dancong tea, and Dongding oolong tea [4]. Wuyi rock tea is well known for its rich flavor and long-lasting fragrance, which is called “rock charm and floral fragrance” [5]. Fenghuang Dancong tea is well known for its unique floral and fruity aroma, which is traditionally divided into Youhua Xiang, Qilan Xiang, Yelai Xiang, etc. [6]. Anxi Tieguanyin tea and Dongding oolong tea have a light and elegant floral aroma. The unique biochemical composition of each cultivar greatly affects the aroma profile of oolong tea [7]. Compared with Tieguanyin (TGY), nitrogen exists in higher concentrations in Dongding oolong tea [8]. When choosing oolong tea varieties, higher terpenoid and green leaf volatile ratios may be a useful index for selecting cultivars [9]. The processing of oolong tea includes
plucking, sun-withering, indoor-withering, shaking, fixing, rolling, and drying [10]. Aroma formation can be divided into enzymatic (before the fixing procedure) and nonenzymatic stages (after the drying procedure) [11]. During the enzymatic stage, oolong tea is formed by the hydrolysis of glycosides and carotenoids, mainly including β-ionone, linalool, and nerolidol [11]. During the nonenzymatic stage, the aroma compounds mainly undergo thermochemical transformation to form large amounts of heterocyclic compounds, such as furan and pyrrole [12].

At present, gas chromatography–mass spectrometry (GC–MS) combined with solid-phase microextraction (SPME) is commonly used for the analysis of tea aroma volatiles. GC–MS has a high separation effect on volatile compounds, strong identification ability, and can provide detailed information on compounds [13]. Simultaneous distillation extraction and SPME are commonly used to extract volatile compounds from tea [14]. However, volatile compounds may be degraded during the thermal processing of simultaneous distillation extraction, whereas SPME has the advantage of being fast, simple, and convenient and has been applied to wine [15], “Marion” and “Black Diamond” blackberries [16], and tea [14]. An enormous amount of data is obtained using GC–MS analysis. Principal component analysis, partial least-squares discriminant analysis (PLS–DA), and orthogonal PLS–DA can extract relevant information and discover patterns in large series of data [17], which are widely used in tea. PLS–DA is a steady discriminant statistical method that is especially suitable for cases with large numbers of explanatory variables [18,19]. Variable importance in projection (VIP) of PLS–DA can quantify the contribution of each variable to classification. The larger the VIP value, the more significant the difference in variables between different areas of oolong tea.

There are many kinds of oolong tea, among which TGY is an important one. Different varieties and fermentation degrees will lead to different flavors and qualities of TGY. In this experiment, different varieties of oolong tea were collected to analyze the difference between TGY and other varieties. The aroma difference of TGY with different grades and fermentation degrees was also analyzed. Based on SPME extraction and GC–MS analysis, nontargeted analysis was conducted on volatile aroma substances in oolong tea samples. Combined with statistical analysis, differences in aroma substances in tea samples of different varieties (TGY vs. other oolong tea), fermentation, and grades of TGY were found. Based on this study, the aroma components of TGY oolong tea with different grades and fermentation levels could be improved, and the theoretical basis of aroma substances in TGY could be enriched.

2. Material and Methods

2.1. Tea Samples

In this study, a total of 25 tea samples were collected (Figure S1), including five special-grade TGY with low fermentation (LF-T), five special-grade TGY with heavy fermentation (HF-T), five first-grade TGY with heavy fermentation (HF-F), five other TGY samples, two Huangdan samples (HD), one Baiyaqilan sample (BYQL), and two Zhangpinshuixian samples (ZPSX). All tea samples were purchased from the local markets in Fujian, China. All tea samples were sealed in containers and stored in a −20 °C freezer for further analysis.

2.2. Chemicals and Instruments

Decanoic acid ethyl ester (analytically pure reagent, purity ≥ 99.5%) was purchased from Shanghai Guo Yao Group Chemical Reagent Co., Ltd. (Shanghai, China). Purified water used in this experiment was purchased from Hangzhou Wahaha Group Co., Ltd. (Hangzhou, China). A standard mixture of n-alkanes C8–C30 was purchased from o2si (North Charleston, SC, USA).

2.3. Tea Aroma Extraction Using SPME

The fiber was preconditioned for 5 min in the injection port of the gas chromatograph at 230 °C to remove any volatiles remaining on the fiber before each extraction. Tea samples
(0.1 g) were weighed and placed in one 20 mL headspace vial, then 5 mL of boiling distilled water and 20 mL of decanoic acid ethyl ester (5 µg/L internal standard) were added. The vials were kept in a 60 °C water bath for 5 min. After that, the SPME fiber was used for the extraction of volatiles for 60 min in a 60 °C water bath. Subsequently, the volatiles were desorbed at the injector (230 °C) of the GC–MS for 5 min [20].

2.4. GC–MS Analysis of Volatile Compounds

An Agilent 6890 gas chromatograph interfaced with an Agilent HP 5973 MSD ion trap mass spectrometer (Wilmington, DE, USA) was used for the analysis of volatiles. The separation was performed on a DB-5MS capillary column (30 m × 250 µm × 0.25 µm). The GC inlet temperature was set at 230 °C. High purity helium (99.999%) was used as the carrier gas with a constant flow of 0.544 mL/min. The temperature procedure was as follows: 40 °C for 3 min, raised to 120 °C at 2 °C/min, then held at 120 °C for 2 min, and finally raised to 230 °C at 10 °C/min and held for 2 min. For MS analysis, the electronic energy of the EI mode was 70 eV. The temperature of the ion source was set at 230 °C. The mass scan range was m/z 40–400. Each sample was analyzed in triplicate [21].

2.5. Statistical Analysis

The volatile compounds were identified using retention indices (RIs), authentic standards, or comparison with mass spectra in the National Institute of Standards and Technology library (NIST14.L). The linear RIs were determined via sample injection with a homologous series of alkanes (C₅−C₃₀) (Sigma-Aldrich (Shanghai, China)). The PLS-DA was performed using SIMCA-P 13.0 software (Umetric, Umea, Sweden). MultiExperiment Viewer software (version 4.7.4, Boston, MA, USA) was employed for heatmap analysis. ACI value calculation reference [22,23] as a standard.

3. Results and Discussion

3.1. Identification of Volatile Compounds in TGY

Volatile compounds obtained using GC–MS analysis (Figure 1) were identified using NIST14.L, combined with the retention time, indices, reference data, and data processing software. Finally, a total of 118 volatile compounds were identified, namely 18 alcohols, 13 aromatics, 23 aldehydes, 10 ketones, 18 heterocyclic compounds, 5 N-containing compounds, 22 esters, and 9 other compounds. The relative content of the identified compounds was calculated according to internal standards [20]. The analysis results showed that the main volatile compounds of TGY were (E)-nerolidol, indole, (E,E)-2,4-heptadienial, benzeneacetalddehyde, hotrienol, linalool, n-butyl acetate, hexanal, and phenylethyl alcohol. Among them, (E)-nerolidol (11.86–21.14%), indole (12.15–34.05%), and (E,E)-2,4-heptadienial (6.13–18.12%) were the three most abundant volatile compounds with the highest content in TGY samples, which was consistent with the results of previous studies [24,25]. Retention time, odor description, and type of volatile compounds are listed in Table 1.

| Retention Time (min) | Type | Odor Description | Cont |
|----------------------|------|------------------|------|
| 2.071                | 3-Methyl-furan | 594 MS, RI Roasted / | 11.353 Methyl hexoate 925 RI Fruity Pineapple |
| 2.385                | 4,7-Pentadien-1-ol | 657 MS, RI Roasted | 10.547 Acetylfuran 912 MS, RI Roasted Coffee-like |
| 2.516                | 4-Methyl-butanal | 621 MS, RI Fruity Apple-like | 9.849 (Z)-2-Heptanone 891 MS, RI Fruity |
| 2.624                | 4-Methyl-butanal | 621 MS, RI Fruity Apple-like | 9.456 (Z)-2-Heptanone 891 MS, RI Fruity |
| 2.856                | 1-Penten-3-ol | 641 MS, RI Green Grassy-green | 9.264 Styrene 887 RI Floral Floral |
| 3.060                | Pentanal | 654 MS, RI Chemical Strong, acrid, pungent odor | 8.464 1-Hexanol 868 MS, RI Green Sweet alcohol |
| 3.124                | 3-Methyl-furan | 693 MS, RI Floral Sweet | 8.237 1,3-Dimethyl-benzene 863 MS, RI Floral Sweet |
| 3.709                | 3-Methyl-butanenitrile | 693 MS, RI Floral Sweet | 8.068 Ethylbenzene 855 MS, RI Floral Aromatic |
| 4.008                | 2-Methyl-butanenitrile | 711 RI / odorless | 7.868 Ethylbenzene 855 MS, RI Floral Aromatic |
| 4.244                | 2-Methyl-butanenitrile | 711 RI / odorless | 7.600 (E)-Furfural 830 MS, RI Roasted Almond-like |
| 4.514                | Toluene | 741 MS, RI Chemical Benzene-like | 7.409 Heptanal 902 MS, RI Green Penetrating fruity |
| 5.520                | Hexanal | 801 MS, RI Green Strong, green | 7.253 2-Ethyl-2-butenal 821 RI Green Grassy green |
| 6.015                | 3-Ethyl-1H-pyrrole | 812 MS, RI Roasted | 6.414 2-Ethyl-2-butenal 821 RI Green Grassy green |
| 6.13–18.12%          | Among them, (E)-nerolidol, indole, (E,E)-2,4-heptadienial, benzeneacetalddehyde, hotrienol, linalool, n-butyl acetate, hexanal, and phenylethyl alcohol. Among them, (E)-nerolidol (11.86–21.14%), indole (12.15–34.05%), and (E,E)-2,4-heptadienial (6.13–18.12%) were the three most abundant volatile compounds with the highest content in TGY samples, which was consistent with the results of previous studies [24,25]. Retention time, odor description, and type of volatile compounds are listed in Table 1. |
Figure 1. GC–MS total ion chromatogram of aroma components in the four tea varieties sampled.

Table 1. Identified volatile compounds in *Tieguanyin*.

| Retention Time | Volatile Compounds                  | RI    | ID  | Odor Type | Odor Description                      |
|----------------|-------------------------------------|-------|-----|-----------|---------------------------------------|
| 2.071          | 3-Methyl-furan                      | 594   | MS, RI | Roasted   | Strong odor of vinegar                 |
| 2.385          | Acetic acid                         | 613   | RI   | Chemical  | Strong odor of vinegar                 |
| 2.516          | 3-Methyl-butanal                    | 621   | MS, RI| Fruity    | Apple-like                            |
| 2.624          | 2-Methyl-butanal                    | 627   | MS, RI| Roasted   |                                       |
| 2.856          | 1-Penten-3-ol                       | 641   | MS, RI| Green     | Grassy-green                          |
| 2.912          | 1-Penten-3-one                      | 645   | MS, RI| Chemical  | Penetrating                           |
| 3.060          | Pentanal                            | 654   | MS, RI| Chemical  | Strong, acrid, pungent odor            |
| 3.124          | 2-Ethyl-furan                       | 657   | MS, RI| Roasted   | Smoky burnt                           |
| 3.709          | 3-Methyl-butanenitrile              | 693   | MS, RI| /         | /                                     |
| 3.714          | Acetal                              | 693   | MS, RI| /         | Pleasant odor                         |
| 4.008          | 2-Methyl-butanenitrile              | 711   | RI   | /         | odorless                              |
| 4.244          | (E)-2-Pentenal                      | 725   | MS, RI| Green     | Pungent green                         |
| 4.514          | Toluene                             | 741   | MS, RI| Chemical  | Benzene-like                          |
| 4.709          | (Z)-2-Penten-1-ol                   | 753   | MS, RI| Green     | Green diffusive                       |
| 5.520          | Hexanal                             | 801   | MS, RI| Green     | Strong, green                         |
| 5.521          | n-Butyl acetate                     | 801   | RI   | Fruity    | Fruity                                |
| 6.015          | 3-Ethyl-1H-pyrrole                  | 812   | MS, RI| Roasted   | Grassy green                          |
| 6.414          | 2-Ethyl-2-butenal                   | 821   | RI   | Green     | Plum-like                             |
| 6.556          | n-Pentyl formate                    | 824   | RI   | Fruity    | Almond-like                           |
| 6.815          | Furfural                            | 830   | MS, RI| Roasted   | Vegetable-like                       |
| 7.600          | (E)-2-Hexenal                       | 848   | MS, RI| Green     | Sweet alcohol                         |
| 7.868          | Ethylbenzene                        | 855   | MS, RI| Floral    | Aromatic                              |
| 8.237          | 1,3-Dimethyl-benzene                | 863   | MS, RI| Floral    | Sweet                                 |
| 8.464          | 1-Hexanol                           | 868   | MS, RI| Green     | Sweet alcohol                         |
| 9.264          | Styrene                             | 887   | RI   | Floral    | Floral                                |
| 9.456          | 2-Heptanone                         | 891   | MS, RI| Fruity    | Fruity                                |
| 9.849          | (Z)-4-Heptenal                      | 900   | MS, RI| Green     | Fatty, green                          |
| 9.951          | Heptanal                            | 902   | MS, RI| Green     | Penetrating fruity                    |
| 10.547         | Acetylfuran                         | 912   | MS, RI| Roasted   | Coffee-like                           |
| 11.353         | Methyl hexoate                      | 925   | RI   | Fruity    | Pineapple                             |
| 11.353         | Methyl (Z)-3-hexenoate              | 925   | MS, RI| Fruity    | Fruity                                |
| 13.092         | (E)-2-Heptenal                      | 954   | MS, RI| Green     | Pungent green                         |
| 13.147         | Benzaldehyde                        | 955   | MS, RI| Roasted   | Almond                                |
| 13.671         | 5-Methyl-2-furancarboxaldehyde      | 963   | MS, RI| Roasted   | Caramelic                             |
| 14.148         | 1-Heptanol                          | 971   | MS, RI| Green     | Fragrant                              |
| 14.666         | 1-Octen-3-ol                       | 980   | MS, RI| Chemical  | Sweet earthy                          |
| 15.114         | 6-Methyl-5-Hepten-2-one             | 987   | MS, RI| Green     | Green citrus-like                     |
| 15.297         | β-Myrcene                           | 990   | MS, RI| Woody     | /                                     |
| 15.678         | (E,E)-2,4-Heptadienal               | 996   | MS, RI| Chemical  | Fatty, green                          |
| 15.774         | n-Butyl butanoate                   | 998   | RI   | Fruity    | Fruity, pineapple                     |
| 16.082         | Octanal                             | 1003  | MS, RI| Fruity    | Strong, fruity                        |
| 17.025         | 1,2,3-Trimethyl-benzene             | 1016  | MS, RI| Chemical  | Aromatic                              |
| 17.284         | α-Cymene                            | 1020  | MS, RI| Floral    | Aromatic                              |
Table 1. Cont.

| Retention Time | Volatile Compounds | RI | ID | Odor Type | Odor Description |
|----------------|-------------------|----|----|-----------|-----------------|
| 17.528         | D-Limonene        | 1024 | MS, RI | Fruity | Citrus odor |
| 17.720         | 1,3-Dichloro-benzene | 1027 | RI | Aromatic | |
| 17.941         | 2-Ethyl-1-hexanol  | 1030 | MS, RI | Floral | Floral |
| 18.197         | Benzyl alcohol    | 1034 | MS, RI | Fruity | Floral |
| 18.607         | Benzeneacetalddehyde | 1040 | MS, RI | Floral | Green floral and sweet |
| 19.000         | 1-Ethyl-2-formylpyrrole | 1046 | MS, RI | Roasted | Burnt smokey |
| 19.087         | β-Ocimene         | 1047 | MS, RI | Floral | |
| 19.739         | (E)-2-Octenal     | 1056 | MS, RI | Green | Fatty, green aroma |
| 20.105         | Acetophenone      | 1062 | RI | Fruity | Oranges |
| 20.652         | cis-Furan linalool oxide | 1070 | MS, RI | / | |
| 20.847         | 1-Octanol         | 1073 | MS | Floral | Penetrating Aromatic |
| 21.755         | (E)-Linalool oxide (furan) | 1086 | MS, RI | Floral | / |
| 21.790         | 2-Methoxy-phenol  | 1087 | RI | Roasted | Smoky |
| 22.729         | Linalool          | 1100 | MS, RI | Floral | Floral odor |
| 23.020         | Hotrienol         | 1105 | MS, RI | Floral | Mouldy |
| 23.451         | Phenylethyl Alcohol | 1111 | MS, RI | Fruity | Honey-like |
| 23.805         | (E)-4,8-Dimethylmona-1,3,7-triene | 1116 | RI | / | / |
| 25.190         | Benzyl nitrile    | 1136 | MS, RI | Floral | Aromatic |
| 25.882         | 5-Ethyl-6-methyl-(3(E))-hepten-2-one | 1146 | RI | / | / |
| 27.343         | trans-Linalool 3,7-oxide | 1167 | MS, RI | / | / |
| 28.265         | Octanoic acid     | 1180 | RI | Chemical | Unpleasant |
| 28.733         | a-Terpineol       | 1187 | MS, RI | Floral | Pleasant, floral |
| 28.736         | 1-Furfurylpyrrole  | 1187 | MS, RI | Roasted | Vegetable aroma |
| 28.864         | Methyl salicylate | 1189 | MS, RI | Green | Wintergreen |
| 29.059         | trans-3,7-Dimethyl-1,5-octadien-3,7-diol | 1192 | MS, RI | / | / |
| 29.257         | β-Safranal        | 1195 | MS, RI | Green | Green-floral |
| 29.969         | Decanal           | 1205 | MS, RI | Floral | Floral-fatty odor |
| 30.205         | 2,4-Dimethyl-benzaldehyde | 1208 | MS, RI | Roasted | Bitter-almond |
| 30.671         | β-Cyclocitrinal   | 1215 | MS, RI | Woody | / |
| 31.828         | (3Z)-3-Hexenyl 2-methylbutanoate | 1233 | RI | / | / |
| 32.174         | Isovaleric acid, dodecyl ester | 1238 | RI | Fruity | Fruity |
| 33.137         | β-Cyclohexomoral  | 1252 | MS, RI | / | / |
| 33.370         | Geraniol          | 1256 | MS, RI | Floral | Sweet rose odor |
| 33.693         | (E)-2-Decenal     | 1260 | MS, RI | Green | Green, fatty |
| 34.313         | Citral            | 1270 | MS, RI | Fruity | Strong lemon |
| 35.468         | Indole            | 1290 | MS, RI | Floral | Light jasmine |
| 35.982         | (2-nitroethyl)-benzene | 1294 | MS, RI | / | / |
| 36.201         | 2-Methylphthalene  | 1298 | RI | / | / |
| 40.331         | 2-Undecenal       | 1362 | MS, RI | Fruity | Orange peel |
| 40.808         | 3-hydroxy-2,2,4-trimethylpentyl isobutyrate | 1370 | MS, RI | / | Characteristic |
| 41.568         | β-Damascenone     | 1382 | MS, RI | Floral, fruity | |
| 41.855         | cis-3-Hexenyl hexanoate | 1382 | MS, RI | Green | Fruity green |
| 41.917         | n-Hexyl hexanoate | 1387 | MS, RI | Green | Herbaceous |
| 42.467         | Jasmonne          | 1396 | MS, RI | Floral | Odor of jasmine |
| 42.702         | Dodecanal         | 1399 | RI | Chemical | Fatty |
| 44.032         | Syrxfynol 104     | 1425 | MS, RI | / | / |
| 44.279         | α-Ionone          | 1430 | MS, RI | Floral | / |
| 45.256         | β-Phenylethyl butyrate | 1448 | MS, RI | Fruity | / |
| 45.422         | Octyl-cyclohexane | 1452 | RI | / | / |
| 46.481         | 3-Methyltetradecane | 1472 | RI | / | / |
| 47.168         | 1-Dodecanol       | 1485 | RI | Fruity | Sweet |
| 47.220         | α-Curcumene       | 1486 | RI | / | / |
Table 1. Cont.

| Retention Time | Volatile Compounds | RI | ID | Odor Type | Odor Description |
|----------------|--------------------|----|----|-----------|------------------|
| 47.357         | 2,6-Di-tert-butylbenzoquinone | 1489 | RI | / | / |
| 47.607         | Jasmine lactone | 1494 | MS, RI | Roasted | Coconut-fruity |
| 48.166         | α-Farnesene | 1509 | MS, RI | Fruity | Citrus, herbal, lavender-like |
| 48.355         | 2,4-Di-tert-butylphenol | 1517 | MS, RI | / | / |
| 48.512         | β-Sesquiphellandrene | 1524 | MS, RI | / | / |
| 49.664         | (E)-Nerolidol | 1571 | MS, RI | Floral | Rose apple |
| 50.324         | Txb | 1599 | MS, RI | Chemical | Musty |
| 50.342         | Cedrol | 1599 | RI | Fruity | Cedar-like |
| 51.264         | Methyl jasmonate | 1654 | MS, RI | Floral | Powerful floral-herbaceous, sweet aroma |
| 51.704         | n-Hexyl salicylate | 1680 | MS, RI | / | / |
| 53.016         | Benzyl Benzoate | 1772 | MS, RI | Floral | Faint, pleasant |
| 53.339         | Ethyl myristate | 1796 | MS, RI | Chemical | Waxy |
| 53.729         | Isopropyl myristate | 1828 | MS, RI | / | Odorless |
| 53.883         | Neophytyadiene | 1842 | MS, RI | / | / |
| 53.970         | Phytone | 1849 | MS, RI | / | / |
| 54.084         | Caffeine | 1859 | MS, RI | Odorless | / |
| 54.287         | Diisobutyl phthalate | 1876 | MS, RI | Chemical | Slight ester |
| 54.872         | Methyl palmitate | 1926 | MS, RI | Chemical | Oily, waxy, fatty |
| 54.898         | 7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione | 1928 | MS, RI | / | / |
| 55.329         | Dibutyl phthalate | 1965 | MS, RI | Floral | Slight, aromatic |
| 55.591         | Hexadecanoic acid, ethyl ester | 1987 | MS, RI | Floral | Slight, aromatic |
| 56.725         | Methyl linolenate | 2083 | MS, RI | / | / |
| 56.929         | Phytol | 2101 | MS, RI | Floral | Floral, balsam, powdery, waxy |

\* \* information was not found in the literature. \* \*: Identification methods. MS, identification based on the NIST14.L; RI, retention index. \* \*: Odor description found in the literature with database (Flavornet; https://pubchem.ncbi.nlm.nih.gov/ (accessed on 10 January 2022).

3.2. Differences of Volatile Compounds in TGY from Other Varieties of Oolong Tea

The data obtained using GC–MS analysis were analyzed with PLS–DA after data preprocessing. Figure 2A shows that there is clear discrimination between TGY and other varieties of oolong tea; HD, ZPSX, and BYQL could also be clearly separated. The PLS–DA model was confirmed by 200 permutation tests (Figure 2B). The results indicated that the model was not overfitted. Not all identified volatile compounds played an important role in the differentiation analysis of different types of oolong tea samples. To find the key differential volatiles, after PLS-DA analysis, compounds with VIP > 1 were screened out for further analysis (Figure 2C). Compounds with VIP > 1 were generally considered to be the important contributors to tea aroma difference. These compounds were divided into two groups (a and b in Figure 2C). The contents of compounds in group a were lower in TGY, including methyl jasmonate, 1-octanol, linalool, and its oxides. Methyl jasmonate has a powerful floral-herbaceous and sweet aroma, linalool has a floral aroma, and 1-octanol presents a penetrating aromatic aroma. This may be the reason why other varieties were sweeter than TGY. In group b, the content of compounds in TGY was higher, mainly including (E)-nerolidol, indole, and α-farnesene. These aromatic compounds were characteristic of oolong tea aroma [11]. Cluster analysis could distinguish TGY samples from other tea variety samples, and HD, ZPSX, and BYQL were also separated. This indicates that variety selection was very important to the aroma characteristics of oolong tea.
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Figure 2. GC–MS analysis results of Tieguanyin and other varieties. (A) The score scatter plots of PLS–DA of TGY and four other varieties. (B) Validation of the PLS–DA model. (C) Heatmap of differential substances in different varieties. TGY: Tieguanyin, HD: Huangdan, BYQL: Baiyaqilan, ZPSX: Zhangpinshuixian. Figure 2B: The vector value of R2 (0.0, 0.445) and Q2 (0.0, −0.251) from 200 permutations, which indicated that this PLS–DA model was not overfitting. Figure 2C: The contents of compounds in group a were lower in TGY, the content of compounds in group b was higher in TGY.

There were still many differential compounds screened by PLS-DA. Aroma character impact (ACI) was introduced to further screen the differential compounds. ACI is a ratio of odor-activity in a mixture and is more useful for comparing the contribution of the individual components to the overall aroma [22,23]. Therefore, ACI values of compounds (VIP > 1) were calculated, and the results are shown in Table 2. The contents of heptanal, (E,E)-2,4-heptadienal, (E)-2-octenal, indole, and (E)-nerolidol in TGY were higher than in other varieties, whereas the content of 1-octen-3-ol and linalool were lower. (E,E)-2,4-Heptadienal as fatty and oil notes, was mostly derived from lipid degradation during manufacture [26] and contained a larger quantity in high-grade green or black tea [27].
(E)-2-Octanal has a fatty, green aroma. Indole is widely distributed and plays an important role in plants and accumulates at the turnover stage of the oolong tea manufacturing process [28]. (E)-Nerolidol is a sesquiterpene presenting as an essential oil in many plants with a floral odor [29] and as a potent signal that elicits plant defenses [30]. The proportion of indole and (E)-nerolidol were higher in TGY, which might be caused by its fragrant and fruity aroma. 1-Octen-3-ol has a sweet earthy odor and is often used as mosquito bait [31]. Linalool is a mate attractant pheromone component in the bee Colletes curculiorius with a floral aroma [32]. Taken together, the data indicate that (E,E)-2,4-heptadienal, (E)-2-octenal, indole, (E)-nerolidol, 1-octen-3-ol, and linalool were key differentiating volatiles of TGY from other varieties.

Table 2. The key compounds associated with Tieguanyin and other varieties with significantly high odor-activity values (VIP > 1).

| Volatile Compounds | ACI (%) | OT (µg/L) |
|--------------------|---------|-----------|
| 3-Methyl-butanal    | 0.02    | 0.03      |
| 1-Penten-3-one      | 0.10    | 0.10      |
| (E)-2-Pentanal      | 0.00    | 0.00      |
| (E)-2-Hexenal       | 0.01    | 0.01      |
| Ethylbenzene        | 0.00    | 0.00      |
| 1-Hexanol           | 0.01    | 0.01      |
| 2-Heptanone         | 0.00    | 0.00      |
| (Z)-4-Heptenal      | 0.00    | 0.00      |
| Heptanal            | 0.54    | 0.60      |
| (E)-2-Heptanal      | 0.19    | 0.21      |
| 1-Heptanol          | 0.00    | 0.01      |
| 1-Octen-3-ol        | 0.32    | 0.34      |
| (E,E)-2,4-Heptadienal | 2.46  | 2.84      |
| 2-Ethyl-1-hexanol   | 0.00    | 0.00      |
| β-Ocimene           | 0.01    | 0.01      |
| (E)-2-Octenal       | 8.51    | 8.86      |
| cis-Furan linalool oxide | 0.00 | 0.00      |
| 1-Octanol           | 0.00    | 0.00      |
| (E)-Linalool oxide (furan) | 17.95 | 21.60    |
| Linalool            | 17.95   | 21.60     |
| β-Safranol          | 0.01    | 0.02      |
| Decanal             | 0.04    | 0.03      |
| (E)-2-Decanal       | 0.15    | 0.10      |
| Citral              | 0.00    | 0.00      |
| Indole              | 1.35    | 1.34      |
| a-Farnesene         | 0.05    | 0.04      |
| (E)-Nerolidol       | 0.20    | 0.16      |
| Methyl jasmonate    | 0.01    | 0.01      |
| n-Hexyl salicylate  | 0.00    | 0.00      |
| Methyl palmitate    | 0.00    | 0.00      |

OT: odor thresholds in water were obtained from [33]. TGY: Tieguanyin, HD: Huangdan, BYQL: Baiyaqilan, ZPSX: Zhangpinshuixian. Aroma character impact (ACI): a ratio of odor-activity in a mixture and is more useful for comparing the contribution of the individual components to the overall aroma.

3.3. Difference Analysis of Volatiles in TGY with Different Fermentation

Oolong tea fermentation mainly occurs in the withering and turnover procedures. In the fermentation process, grassy flavors were diminished, and the flowery and fruity fragrances appeared sequentially [24]. The reason was that the continuous mechanical damage during fermentation facilitated the synthesis of terpenoids, fatty acids, and benzenoid-derived compounds [34], such as trans-β-ocimene, indole, and linalool [35]. Therefore, the degree of fermentation was very important to the quality of oolong tea.

In this study, 118 volatile compounds were identified by analyzing TGY samples of different fermentation levels and classified according to aroma type and compound type (Figure 3A,B), the floral and fruity compounds were dominant in TGY. With the continuation of fermentation, the total content of compounds decreased, mainly the floral
aroma compounds. The compounds with the highest proportion in HF-T were alcohols, whereas that in LF-T were N-containing compounds. Aldehydes and alcohols were often characterized by experts with strong sensory descriptions and associated with greenery, freshness, green plants, citrusy, fatty, and sweet notes [26].

Figure 3. (A) Composition proportion of aroma of Tieguanyin with different fermentations. (B) Proportion of aroma types of Tieguanyin with different fermentations. HF: heavy fermentation, LF: low fermentation.

Through data analysis, the TGY samples with different fermentation levels were clearly separated in the PLS-DA plot (Figure 4A). To eliminate the interference of irrelevant variables and find the key compounds that affected this classification of tea samples, VIPs were used to screen compounds with significant differences among different fermentations of TGY. As the fermentation level increased (Figure 4C), the contents of (E,E)-2,4-heptadienal, n-butyl butanoate, indole, jasmine lactone, phenylethyl alcohol, benzeneacetaldehyde, (2-nitroethyl)-benzene, (E)-nerolidol, and α-farnesene decreased, whereas the content of hotrienol, benzyl alcohol, geraniol, linalool, and its oxides increased, which was consistent with previous studies [36]. Hotrienol, geraniol, and linalool are monoterpenoids, which were induced and composed by the methylyerythritol phosphate pathway. (E)-Nerolidol and α-farnesene are sesquiterpenes and were induced and composed by the mevalonic acid pathway [37]. The synthesis of these terpenes requires the same precursor, geranyl pyrophosphate, and there may be competition between the two pathways. The mevalonic acid pathway may be dominant when the fermentation degree is low. Monoterpenes were synthesized mainly through the methylyerythritol phosphate pathway at high fermentation levels. The content of indole was high in lightly fermented oolong tea, but low in heavily fermented Beauty tea or black tea [10,28], which was consistent with our study results that indole content decreased with the deepening of fermentation. In conclusion, HF-T contained a high content of monoterpenoids, whereas LF-T contained a high content of sesquiterpenes and indole. These compounds were useful for the classification of TGY with different fermentation degrees.
were screened out (Figure 6C). Compared with the special grade tea, the relative content of hotrienol, benzyl alcohol, geraniol, linalool, and its oxides increased, which was consistent with previous studies [36]. Hotrienol, geraniol, and linalool were monoterpenoids, which were induced and composed by the mevalonic acid pathway at high fermentation levels. The content of indole was high in lightly fermented oolong tea, but low in heavily fermented Beauty tea or black tea [10,28], which was consistent with our study results that indole content decreased with the deepening of fermentation. In conclusion, HF-T contained a high content of monoterpenoids, whereas the content of hotrienol, benzyl alcohol, geraniol, linalool, and its oxides in LF-T contained a high content of sesquiterpenes and indole. These compounds were useful for the classification of TGY with different fermentation degrees.

3.4. Difference Analysis of Volatiles in Different Grades of TGY

According to the tenderness, aroma, taste, and appearance, different types of tea can be classified into different grades [38]. TGY is usually classified into special grades and grades 1–4 [39]. Exploring the signature compounds of different grades of TGY could help identify the grade of TGY. Then, in this study, the differences in volatiles of TGY with different grades were analyzed. As shown in Figure 5, the total amount of aroma in the special-grade tea was higher than that in first-grade tea, especially in the floral-scented compounds. Therefore, the special-grade tea was richer in floral types under sensory evaluation, which was consistent with previous studies [27,40]. Based on the analysis of compound types, the highest proportion of volatile compounds in the special grade tea was alcohols (28%). In first-grade tea, aldehydes accounted for a higher proportion (25%), which may be caused by the oxidation of more primary alcohols into aldehydes.

The PLS–DA analysis result is shown in Figure 6. Volatile compounds with VIP > 1 were screened out (Figure 6C). Compared with the special grade tea, the relative content of benzaldehyde (volatile oil of almond), jasmine lactone (coconut-fruity odor), and α-farnesene (woody, citrus, sweet) in first-grade tea were higher, but that of acetal (pleasant odor), 2-ethyl-1-hexanol (mild, oily, sweet, slightly floral odor), benzyl alcohol (faint, aromatic, fruity odor), (E)-nerolidol (rose apple), and n-hexyl salicylate is lower. (E)-Nerolidol content was positively correlated with oolong tea grade [41,42]. The special-grade tea and first-grade tea were the highest grades of tea, and their quality evaluation was used to find out whether there was an inferior odor in the tea aroma and whether the aroma type was typical. For example, the fresh-scented TGY typically had a fresh flowers aroma, whereas that of Oriental Beauty was honey and sweet aroma. The aroma of benzaldehyde had a roasted aroma, which was not consistent with the TGY aroma type.

**Figure 4.** GC–MS analysis results of differently fermented Tieguanyin. (A) The score scatter plots of PLS–DA of TGY. (B) Validation of the PLS–DA model. (C) Heatmap of differential substances in different fermentation Tieguanyin. HF: heavy fermentation, LF: low fermentation. Figure 4B: The vector value of R2 (0.0, 0.258) and Q2 (0.0, −0.16) from 200 permutations, which indicated that this PLS–DA model was not overfitting.
Here, ACI values were also calculated to further screen out key aroma compounds related to TGY grades (Table 3). The content of $(E,E)$-2,4-heptadienal (1.68–2.19\%) and $(E)$-2-octenal (6.36–9.10\%) was higher in first-grade tea, whereas the content of benzeneacetaldehyde (0.83–1.25\%) and $(E)$-nerolidol (0.06–0.1\%) was lower. $(E,E)$-2,4-Heptadienal had a fatty and oil aroma, and its concentration was lower in the special grade tea, which was opposite to the previous results [27]. $(E)$-2-Octenal had a fatty and green aroma, and gave rise to inferior flavor. $(E)$-Nerolidol was an important contributor to oolong tea aroma, which could be regarded as one of the key odors of oolong tea quality. In general, its
content was positively correlated with oolong tea grade [42]. In conclusion, the higher grade was the grade of TGY, the more volatile compounds that were present. Furthermore, \((E,E)-2,4\)-heptadienal, \((E)-2\)-octenal, benzeneacetaldehyde, and \((E)-\)nerolidol could be used as the key volatile compounds to distinguish different grades of TGY.

Table 3. The key compounds responsible for the different grades of TGY with significantly high odor-activity values (VIP > 1).

| Volatile Compounds                  | ACI (%) | OT (µg/L) |
|-------------------------------------|---------|-----------|
|                                     | T-1     | T-2       | T-3     | T-4     | T-5     | F-1     | F-2     | F-3     | F-4     | F-5     |
| Acetal                              | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 80      |
| 3-Ethyl-1H-pyrrole                  | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 10,000  |
| Benzaldehyde                        | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 750.89  |
| \((E,E)-2,4\)-Heptadienal           | 1.23    | 1.64      | 2.09    | 1.44    | 1.26    | 2.19    | 2.17    | 1.68    | 2.05    | 2.00    | 15.4    |
| \(\alpha\)-Cymene                   | 0.06    | 0.06      | 0.06    | 0.04    | 0.03    | 0.06    | 0.04    | 0.03    | 0.03    | 0.03    | 11.4    |
| 2-Ethyl-1-hexanol                   | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 25,480  |
| Benzyl alcohol                      | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 254.6   |
| Benzeneacetaldehyde                 | 2.19    | 1.39      | 3.20    | 3.9     | 2.25    | 1.25    | 0.90    | 0.83    | 1.08    | 1.14    | 6.3     |
| \(\beta\)-Ocimene                   | 0.01    | 0.01      | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 34      |
| \((E)-2\)-Octenal                   | 5.46    | 6.31      | 7.21    | 5.77    | 4.91    | 9.10    | 7.88    | 6.36    | 7.58    | 6.83    | 0.2     |
| cis-Furan linalool oxide            | 0.01    | 0.01      | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 320     |
| 1-Octanol                           | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 125.8   |
| Hotrienol                           | 0.08    | 0.06      | 0.16    | 0.06    | 0.06    | 0.06    | 0.06    | 0.05    | 0.06    | 0.04    | 110     |
| Phenylethyl alcohol                 | 0.01    | 0.01      | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 0.01    | 564     |
| \(trans\)-Linalool 3,7-oxide        | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 190     |
| \(\beta\)-Cyclocitril                | 0.14    | 0.19      | 0.17    | 0.14    | 0.13    | 0.26    | 0.22    | 0.17    | 0.17    | 0.17    | 3       |
| \(\beta\)-Phenylenethyl butyrate    | 0.01    | 0.02      | 0.02    | 0.03    | 0.02    | 0.02    | 0.01    | 0.02    | 0.02    | 0.02    | 87      |
| \(\alpha\)-Farnesene                 | 0.10    | 0.13      | 0.12    | 0.17    | 0.11    | 0.06    | 0.07    | 0.10    | 0.09    | 0.09    | 250     |
| \((E)-\)Nerolidol                   | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 73      |
| \(n\)-Hexyl salicylate              | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 341     |
| Benzyl Benzoate                     | 0.00    | 0.00      | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 0.00    | 19,000  |

OT: odor thresholds in water were obtained from [33]. Aroma character impact (ACI): a ratio of odor-activity in a mixture and is more useful for comparing the contribution of the individual components to the overall aroma.

4. Conclusions

In this study, a combination of SPME–GC–MS and chemometrics analysis provided a convenient and reproducible method for differential analysis of oolong tea samples. The content of heptanal, \((E,E)-2,4\)-heptadienal, \((E)-2\)-octenal, indole, and \((E)-\)nerolidol in TGY was higher than in other varieties, whereas the content of \(1\)-octen-3-ol and linalool was lower than in other varieties. With the extension of fermentation, HF contains a high content of monoterpenoids, whereas LF contains a high content of sesquiterpenes and indole. \((E,E)-2,4\)-Heptadienal, \((E)-2\)-octenal, benzeneacetaldehyde, and \((E)-\)nerolidol were the key volatile compounds to distinguish different grades of TGY. \((E)-\)nerolidol, \((E,E)-2,4\)-heptadienal, and \((E)-2\)-octanal were important compounds contributing to the aroma quality of TGY. The results enriched the theoretical basis of aroma substances in TGY and could also provide theoretical guidance for consumers to choose tea.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/foods11111530/s1. Figure S1: Information on the tea samples.

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