Quantitatively Unravelling the Impact of High Altitude on Oolong Tea Flavor from *Camellia sinensis* Grown on the Plateaus of Tibet

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Abstract: The plateaus of Tibet have a unique climate that poses a great challenge for local agriculture. To promote sustainable development in Tibet, an elite tea variety has been introduced. However, the modifications of tea flavors in response to the climate of the plateaus are unknown. In this study, volatile organic compounds (VOCs) and other taste substances of tea planted in its original location (OOT) and in Tibet (TOT) were systematically analyzed and compared. The volatile components in TOT and OOT showed a slight difference, and principal component analysis revealed that the characteristic aroma compounds distinguishing tea grown in Tibet from tea grown in Guangdong were hotrienol and benzyl alcohol. In terms of taste substances, TOT exhibited higher levels of water extractable compounds, including polyphenols and amino acids, but lower levels of caffeine than OOT, which implies that TOT may taste better than OOT. To our knowledge, this is the first study to describe the changes in aroma and flavor profiles of tea induced by high altitude systematically, which will provide a basis for reference during the introduction and cultivation of tea crops to the plateaus of Tibet.

Keywords: *Camellia sinensis*; oolong tea; volatile organic compounds (VOCs); taste substances; Tibet

1. Introduction

Tibet, located at the top of the world, has a unique plateau climate. The territory comprises a total area of 1.23 million km². Nevertheless, the region is extremely mountainous, and only a small portion supports human settlement and farming [1]. The area used for agricultural purposes in Tibet amounts to 0.45 km², corresponding to 0.42% of the total area. Income in the agricultural sector is limited due to low land utilization and extreme climate. Improving the use of its limited arable land is critical for promoting sustainable development in the highlands and mountainous areas of Tibet.

Tea made from the tender leaves of *Camellia sinensis* is among the world’s three major beverages. Tea’s high polyphenol content, caffeine, theanine, and terpenoids collectively contribute to the charming flavors of tea and to its health benefits [2,3]. Tea is categorized into three major types, green, black, and oolong, which are determined by the employed processing technologies [4]. Oolong tea has distinctive features; its sweet, floral and fruity aroma sets it apart from green and black teas [5,6]. Traditional oolong teas originate from Fujian, Guangdong, and Taiwan. To meet the amplified demand of oolong tea, more and more varieties suitable for the manufacture of oolong tea have been introduced to other
regions for China. ‘Wuyedancong’, originating from Chaozhou in Guangdong province, is an elite variety suitable for oolong tea that has been cultivated in Medog, Tibet since 2019 [7].

Cultivation of crops on plateaus, which have dramatic changes in climate, modifies flavors profiles [8,9]. For example, coffee and grapes cultivated at high elevation affected both the abundance of volatile organic compounds (VOCs) and the aroma profile of the processed products [9,10]. Generally, tea grown at high elevation fetching a higher price than that grown in lowland regions, but few scientific reports have documented these effects. In particular, how the plateau climate affects the quality of tea planted on the highest elevation region on Earth, Tibet, is still unknown.

In this study, tea cultivar ‘Wuyedancong,’ planted in its origin Chaozhou, Guangdong and in Medog, Tibet, were processed into oolong tea using the same technology. The aromatic components and taste substances of origin oolong tea (OOT) and Tibet oolong tea (TOT) were systematically analyzed and compared. The results will provide a reference for monitoring the introduction and cultivation of the crop into plateau areas, in an effort to maintain efficient and sustainable development in Tibet.

2. Materials and Methods
2.1. Plant Materials and Reagents

The cultivar ‘Wuyedancong’ was planted in Chaozhou, Guangdong Province (23.93° N, 116.70° E) and Medog, Tibet (29.49° N, 95.26° E). In the spring of 2021, the first two leaves and buds were plucked and processed to oolong tea. The manufacturing processes of oolong tea is withering, rolling, kneading and drying. The processed tea samples were stored in airtight canisters.

The reference standards, decanoic acid ethyl ester, catechin ©, epicatechin (EC), epigallocatechin (EGC), epicatechin-3-O-gallate (ECG) and epigallocatechin-3-O-gallate (EGCG), and formic acid were purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Caffeine was obtained from Beijing North Weiye Institute of Measuring and Testing Technology (Beijing, China). Acetonitrile and methanol (HPLC-grade) were purchased from Spectrum Chemical Manufacturing Co., Ltd. (Shanghai, China). Alkane standard solutions (C₈–C₄₀) for linear retention index (RI) calculation were obtained from TanMo Quality Testing Technology Co., Ltd. (Beijing, China). Internal standard solutions were prepared in dichloromethane prior to use. Ultrapure water was prepared by a Barnstead GenPure Pro system (Thermo Fisher Scientific, Waltham, MA, USA).

2.2. Electronic Nose Measurements

Fragrance identification of two tea samples using an PEN3 electronic nose (AIRSENSE Analytics GmbH, Schwerin, Germany) was performed according to previous research [11]. The sensor performance is described in Table 1. About 2 g tea powder was added into a headspace enrichment injection bottle attached to the electronic nose, and extracted at 80 °C for 40 min. After extraction, the collection needle and invigorating needle were quickly inserted and odor data were collected. The collection time was 100 s, and data were collected once per second.

Table 1. Selectivity of electronic nose sensors.

| No. | Sensor Name | Selectivity                              |
|-----|-------------|------------------------------------------|
| 1   | W1C         | Aromatic components (e.g., benzene)      |
| 2   | W5S         | Nitrogen oxides                          |
| 3   | W3C         | Aromatic compounds, ammonia              |
| 4   | W6S         | Hydrides                                  |
| 5   | W5C         | Short chain alkane, aromatic compounds    |
| 6   | W1S         | Methane                                  |
| 7   | W1W         | Inorganic sulfides                       |
| 8   | W2S         | Alcohols, aldehydes and ketones          |
| 9   | W2W         | Aromatic compounds, organic sulfides     |
| 10  | W3S         | Long chain alkanes                       |
2.3. Analysis of Volatile Compounds by GC-MS

Volatile compounds were extracted and analyzed using headspace solid phase microextraction/gas chromatography-mass spectrometry (HS-SPME/GC-MS) [12]. Briefly, 2.0 g tea and 0.0864 g ethyl decanoate were added to a 40 mL headspace vial and quickly sealed. After preheating for 15 min at 80 °C, a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 µm inner diameter, 2 cm length) (Supelco, Darmstadt, Germany) was inserted into the headspace vial, and extraction proceeded for 40 min at 80 °C. After extraction, the SPME fiber was injected into the GC inlet for 3 min at 250 °C.

GC-MS was carried out on an Agilent 1890B gas chromatograph coupled with a 5977A mass spectrometer (Agilent, Santa Clara, CA, USA). Compounds were separated on an HP-5MS column (30 m × 0.25 mm × 0.25 µm film thickness). Samples were injected in splitless mode. The carrier gas was high purity helium (purity ≥ 99.99%) that was maintained at a column flow rate of 1.0 mL/min. The heating program had an initial temperature of 50 °C for 1 min, increase to 220 °C for 5 min at the rate of 5 °C/min. The ion source temperature was 230 °C and the electron impact (EI) ionization was 70 eV. The solvent delay time was 4 min. The scan range was 30–400 atomic mass unit (amu). The analyses were performed in triplicate.

To qualitatively identify the volatile components, retention index (RI) and mass spectral match factors were used. RI was calculated by comparing retention times of a series of n-alkanes (C₉–C₂₁) to the values provided in the NIST 11 database that used the same capillary column as:

\[ RI = 100n + 100 \frac{RT(x) - RT(n)}{RT(n + 1) - RT(n)} \]

where RT(x) is the retention time of compound x, and RT(n) and RT(n + 1) are the retention times of the alkanes with carbon number n and n + 1 immediately eluting before and after compound x, respectively. Compound matches were accepted when the calculated RI was less than 15, or if the mass spectral match factor was more than 90.

2.4. Calculation of Wickremasinghe-Yamanishi Ratio and Owuor’s Flavor Index

The Wickremasinghe-Yamanishi ratio is the ratio of the sum of the gas chromatographic peak areas of compounds eluting before linalool to the gas chromatographic peak areas of linalool and the compounds eluting after it [13]. The Wickremasinghe-Yamanishi ratio is calculated from the classified data in Supplemental Table S1. Owuor’s flavor index is the ratio of the volatile substances with sweet flowery aromas (Group II) to the sum of the contents of aroma substances with grassy aromas (Group I); The Owuor’s flavor index is calculated from the classified data in Supplemental Table S2 [14].

2.5. Determination of Water Extractable Components

Water extracts were measured using the suggested protocol from the Chinese National Standard GBT8305-2013 with minor changes [15]. Briefly, 1.5 g tea powder was extracted with 225 mL ultrapure water at 100 °C for 45 min. The analyzed solution was composed of extract, washing solution, and ultrapure water mixed to 250 mL. 50 mL of the solution were poured into a pre-dried and weighed (m₁, accurate to 0.0001 g) evaporating dish, evaporated to dryness on a boiling water bath, dried in an oven at 120 °C for 3 h and weighed (m₂, accurate to 0.0001 g). The water extracts of the samples were calculated as:

\[ \text{water extracts} (%) = \frac{m₂ - m₁}{0.3} \times 100\% \]

The analyses were performed in triplicate.

2.6. Determination of Free Amino Acids

Free amino acids were measured using the suggested protocol from the Chinese National Standard GBT8314-2013 with minor changes [16]. Briefly, 1.5 g tea powder was extracted with 225 mL ultrapure water at 100 °C for 45 min. This test solution was composed of extract, washing solution and ultrapure water mixed to 250 mL. To 1 mL of the solution, 0.5 mL pH 8.0 buffer (22.71 mg/mL disodium hydrogen phosphate dodecahydrate
and 0.46 mg/mL potassium dihydrogen phosphate in ultrapure water) and 0.5 mL 2% ninhydrin (diluted in ultrapure water with 0.8 mg/mL stannous chloride) were added. The mixture was incubated in a boiling water bath for 15 min, and ultrapure water was added to 25 mL after cooling. Absorbance was measured at 570 nm using a UV spectrophotometer (Shimadzu Instruments, Suzhou, China). The free amino acids of the samples were calculated as: free amino acids (%) = c/6w × 100%, where c represents the mass concentration obtained from the linear regression equation based on the absorbance value, and w is the sample dry matter content. The analyses were performed in triplicate.

2.7. Quantification of Caffeine Contents

Analysis of caffeine contents was carried out on an HPLC (Alliance E2695, Waters) coupled to a 2489 UV/Vis detector (Waters Technologies, Milford, MA, USA) [17]. 30 mL of 1.5% magnesium oxide in ultrapure water (w/v) was added to 0.10 g tea power in a 50 mL centrifuge tube and extracted for 30 min at 100 °C. After centrifugation at 13,000 × g for 10 min, 1 mL of the supernatant was filtered through a 0.22 µm nylon membrane (Jinteng experimental equipment Co., Ltd., Tianjin, China). 10µL of the filtrate was injected into an XSelect HSS C18 SB column (4.6 × 250 mm, 5 mm, Waters Technologies, Milford, MA, USA) with a column temperature of 35 ± 1 °C and at a flow rate of 0.9 mL/min. Caffeine was eluted under isocratic conditions: 30% A (100% methanol) and 70% B (ultrapure water) and detected at 280 nm. Caffeine content was identified by comparison of its retention time and absorption spectrum with the standard. The analyses were performed in triplicate.

2.8. Determination of Tea Polyphenols

Tea polyphenols were measured using the suggested protocol from the Chinese National Standard GBT8313-2018 with minor changes [18]. Briefly, 1.5 g tea powder was extracted with 225 mL ultrapure water at 100 °C for 45 min, and then brought to 250 mL with ultrapure water. To 1 mL of the extraction solution, 4 mL ultrapure water, 5 mL ferrous tartrate solution and 15 mL pH 8.0 buffer (20.32 mg/mL disodium hydrogen phosphate dodecahydrate and 1.39 mg/mL potassium dihydrogen phosphate in ultrapure water) were added, and the absorbance of the mixed solution was measured at 540 nm using a UV spectrophotometer (Shimadzu Instruments, Suzhou, China). Tea polyphenols were calculated as: tea polyphenols (%) = 65.23 A/w × 100%, where A is the absorbance and w is the dry matter content of the sample.

2.9. Quantification of Catechin Contents

The contents of C, EC, EGC, ECG, and EGCG were analyzed by Waters Alliance Series HPLC system (Waters Technologies, Milford, MA, USA) [19]. Briefly, 0.20 g tea powder in 8 mL 70% methanol were sonicated (Xinzhi Biological Technology Co., Ltd., Ningbo, China) for 30 min and centrifuged at 13,000 rpm for 10 min. One mL of the supernatant was filtered through a 0.22 µm Millipore membrane and then injected into an XSelect HSS C18 SB column (4.6 × 250 mm, 5 mm, Waters Technologies, Milford, MA, USA) maintained at 25 ± 5 °C. A gradient elution program, using mobile phases A (0.1% aqueous formic acid) and B (100% acetonitrile), was performed as follows: 0–5 min, 8–25% B; 14–30 min, 8% B. Catechins were detected at 280 nm, and the flow rate was 1 mL/min. The content of C, EC, EGC, ECG and EGCG were identified by comparison of their retention time and absorption spectrum with those of an authentic standard. The analyses were performed in triplicate.

2.10. Statistical Analysis

Statistical significance was evaluated with the coefficients of variation (CV%) and analysis of variance (ANOVA) using Excel 2020 (Microsoft, Redmond, WA, USA) and GraphPad Prism 9.0 (GraphPad Software, San Diego, CA, USA). Principal component analysis (PCA) of the volatile components was performed in GraphPad Prism 9.0. Samples were considered significantly different when p < 0.05.
3. Results and Discussion

3.1. Aroma-Active Compounds Identified by an Electronic Nose

The aroma of tea is the comprehensive effect of various volatile compounds. The electronic nose is widely used for odor analysis. PCA of the electronic nose results show that the two axes were 83.99% for PC1 and 13.05% for PC2 (Figure 1). According to electronic nose analysis, the aromas of the two tea samples were very similar and had no significant difference. Different from GC-MS, the electronic nose does not provide specific information on quantitative differences between samples, and replace by matching the sensory database of instrument [20–22]. Therefore, the slight difference between samples could not be distinguished by the electronic nose.

![Figure 1. Principle component analysis of aroma-active compounds of TOT and OOT analyzed by an electronic nose.](image)

3.2. Volatile Organic Compounds Identified by GC-MS

HS-SPME-GC-MS analysis was used to investigate the VOCs of OOT and TOT (Supplemental Figure S1). A total of 104 VOCs were identified by mass spectrometry and retention index. Although the variety of volatiles was abundant, they were detected in trace levels. Volatile compounds were divided into six categories: alcohols, alkenes, aldehydes, ketones, esters, and others (Supplemental Figure S2) [23]. The main compounds were alcohols, with 49.81% and 45.71% of total volatiles in TOT and OOT, respectively. Alkenes were 24.44% and 30.19% and esters were 6.85% and 6.65% of the total. The content of aldehydes in TOT was significantly higher than that of OOT, while the content of alkenes and other components of TOT were significantly lower (Figure 2A and Figure S2).

The Wickremasinghe-Yamanishi ratio and Owuor’s flavor index, both known to correlate with sensory evaluations of tea, were used to evaluate the aroma quality of the samples [13,14]. The Wickremasinghe-Yamanishi ratio is as follows: the smaller the index, the higher the quality; while the larger the Owuor’s flavor index indicate the higher the aroma quality. The Wickremasinghe-Yamanishi ratios of TOT and OOT were 0.33 and 0.30, respectively, but were not significantly different (Figure 2B and Supplemental Table S1). The Owuor’s flavor index of TOT and OOT were 39.67 and 29.06, respectively. The Owuor’s flavor index of TOT was significantly higher than that of OOT, which implied that the aroma quality of TOT was better than OOT (Figure 2C and Supplemental Table S2).
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The Owuor’s flavor index of TOT and OOT were 39.67 and 29.06, respectively. The Owuor’s flavor index of TOT was significantly higher than that of OOT, which implied that the aroma quality of TOT was better than OOT (Figure 2C and Supplemental Table S2).

Figure 2. Characteristics of the volatile components of TOT and OOT. (A) The differences in six volatile categories between TOT and OOT. (B) The Wickremasinghe-Yamanishi ratio of TOT and OOT. t-test was used to identify significant differences (ns = not significant; * p < 0.05). (C) The Owuor’s flavor index of TOT and OOT. TOT: Tibet oolong tea; OOT: Origin oolong tea.

3.3. Key Volatile Compounds Identified in OOT and TOT

The key volatile compounds of OOT and TOT are summarized in Table 2, and their relative contents are illustrated in a heatmap (Figure 3A). Among the 40 volatile compounds commonly shared by TOT and OOT, the main aroma components were linalool, hotrienol, linalool oxide III, indole, β-myrcene and β-ocimene. Indole, linalool, and their oxides were the dominant aroma components of oolong tea, consistent with previous research [24,25]. Isoeugenol was only detected in OOT, while α-cedrene, α-curcumene and o-cymene were detected exclusively in TOT at trace levels. Twenty volatiles were significantly different between TOT and OOT, including three alcohols (benzyl alcohol, linalool and nerolidol), nine alkenes (1,3,5,7-cyclooctatetraene, α-farnesene, α-copaene, styrene, β-caryophyllene, β-myrcene, (E)-β-farnesene, limonene and β-ocimene), one aldehyde (benzene acetaldehyde), three ketones (trans-β-ionone, (Z)-jasmine and α-ionone), one ester (hexanoic acid hexyl ester) and three other types of substances (indole, p-xylene and 1-(1H-pyrrol-2-yl)-ethanone). The differences between samples mainly occurred with alkenes.
Figure 3. Analysis of key volatile compounds in OOT and TOT. (A) Heatmap showing differences in 45 key volatile compounds. Red and green represent positive and negative fold changes. (B) Differences in 16 key aroma compounds in TOT and OOT. Relative content in the vertical scale is shown by means ± SD (n = 3). t-test was used to identify significant differences (* p < 0.05; ** p < 0.01).

Due to the large number of volatiles, we focused on 16 major volatiles that play key roles in tea fragrance [15,26,27]. The contents of benzene acetaldehyde (floral, rose, cherry-like aroma), \((Z)\)-jasmone (sweet, flower, violet-like aroma), copaene (wood aroma), \(\beta\)-caryophyllene (wood aroma) and benzyl alcohol (sweet, floral, rose-like, caramel-like aroma) were significantly higher in TOT than in OOT (Figure 3B). Benzyl alcohol is hydrolyzed from glycosidic precursors in tea leaves under acidic or enzymatic actions to produce aroma compounds [28]. The environmental conditions at high altitudes were...
extremely favorable for the accumulation of benzyl alcohol glycosides in tea leaves. However, the contents of indole (floral, animal-like aroma), β-myrcene (wood, resinous, musty aroma), linalool (floral, sweet, grape-like, woody aroma), α-farnesene (wood, green, floral, herbal aroma), β-ocimene (warm, floral, herbal, sweet aroma) and 1,3,5,7-cyclooctatetraene (solvent-like aroma) in TOT were significantly lower than those in OOT (Figure 3B). Indole has a low aroma threshold and high odor activity value that greatly contributes to the floral aromas of oolong tea [25]. Linalool and its oxides are the main aroma contributors among green, black and oolong teas [29–32]. The content of α-farnesene, nerolidol, indole and benzyl alcohol increase significantly during the rolling step of oolong tea processing [33]. In this study, α-farnesene, nerolidol and indole showed the same trend in the two samples, indicating that their content may be simultaneously induced by stress.

Interestingly, some volatile compounds, such as α-cedrene (wood and spice aroma), α-curcumene (herbal aroma) and O-cymene (aromatic aroma), that had never been detected in ‘Wuyedancong’ tea before were found in TOT. O-Cymene is a collinear component of the five fragrance types of oolong tea [25,34,35]. Generally, the content of α-cedrene decreases during the processing steps of withering, rolling and roasting [35–37]. α-Curcumene is a sesquiterpene rarely detected in tea, and only some samples of longjing tea from different regions contained α-curcumene [38]. The finding suggests that the synthesis of α-cedrene, α-curcumene and O-cymene might be induced by certain environmental factors of the plateau.

### Table 2. Summary of volatile compounds in TOT and OOT.

| No. | Compounds                    | Relative Contents a | Aroma Description b               | RI (Cal) c/ RI (Ref) d | I Method ε |
|-----|------------------------------|---------------------|-----------------------------------|------------------------|------------|
|     | TOT                          | OOT                 |                                   |                        |            |
| 1   | Benzyl alcohol               | 2.60 ± 0.10         | 1.04 ± 0.13                       | Sweet, floral, rose-like, caramel-like 1 | 1035/1034  | MS, RI    |
| 2   | Linalool oxide I             | 1.19 ± 0.12         | 1.52 ± 0.23                       | Sweet, floral, creamy 1 | 1075/1078  | MS, RI    |
| 3   | Linalool oxide II            | 1.38 ± 0.15         | 1.46 ± 0.30                       | Floral 2               | 1090/1094  | MS, RI    |
| 4   | Linalool                     | 1.27 ± 0.12         | 2.51 ± 0.36                       | Floral, sweet, grape-like, woody 1 | 1101/1101  | MS, RI    |
| 5   | Hotrienol                    | 9.22 ± 0.79         | 7.26 ± 1.07                       | Fresh, floral, fruity 1 | 1107/1107  | MS, RI    |
| 6   | Linalool oxide III           | 1.26 ± 0.14         | 1.82 ± 0.24                       | Musty, wood, sweet, tea-like, citrus 1 | 1171/1178  | MS, RI    |
| 7   | Geraniol                     | 0.75 ± 0.05         | 0.69 ± 0.09                       | Rose-like, sweet, honey-like 1 | 1255/1255  | MS, RI    |
| 8   | Nerolidol                    | 1.09 ± 0.33         | 3.92 ± 0.47                       | Floral, apple, green 1 | 1567/1535  | MS, RI    |
| 9   | Cedrol                       | 0.15 ± 0.08         | 0.09 ± 0.02                       | Wood, floral 1         | 1609/1593  | MS, RI    |
| 10  | τ-Muurolol                   | 0.23 ± 0.08         | 0.21 ± 0.00                       | Herb, weak spice 2     | 1648/1640  | MS, RI    |
| 11  | α-Cadinol                    | 0.14 ± 0.05         | 0.16 ± 0.01                       | Herb, wood 2           | 1660/1663  | MS, RI    |
|     | Total alcohols               | 19.28 ± 0.83        | 20.68 ± 2.88                      |                        |            |

### Alkene

| No. | Compounds                    | Relative Contents a | Aroma Description b               | RI (Cal) c/ RI (Ref) d | I Method ε |
|-----|------------------------------|---------------------|-----------------------------------|------------------------|------------|
|     | TOT                          | OOT                 |                                   |                        |            |
| 1   | 1,3,5,7-Cyclooctatetraene    | 0.28 ± 0.08         | 1.48 ± 0.17                       | Solvent-like 1         | -/-        | MS        |
| 2   | Styrene                      | 0.31 ± 0.06         | 0.65 ± 0.11                       | Balsamic, gasoline 2   | 913/989    | MS, RI    |
| 3   | β-Myrcene                    | 1.24 ± 0.25         | 2.28 ± 0.34                       | Wood, resinous, musty 1 | 992/988    | MS, RI    |
| 4   | D-Limonene                   | 0.78 ± 0.16         | 0.45 ± 0.03                       | Citrus, lemon, orange-like, green 1 | 1029/1030  | MS, RI    |
| 5   | β-Ocimene                    | 1.74 ± 0.18         | 2.64 ± 0.39                       | Warm, floral, herbal, sweet 1 | 1049/1046  | MS, RI    |
| No. | Compounds                          | Relative Contents | Aroma Description | RI (Cal) / RI (Ref) | I Method |
|-----|-----------------------------------|-------------------|-------------------|--------------------|----------|
|     |                                   | **TOT**           | **OOT**           |                    |          |
| 6   | 1,3,8-p-Menthatriene              | 0.77 ± 0.03       | 0.66 ± 0.09       | Turpentine         | 1131/1119| MS, RI  |
| 7   | α-Copaene                         | 0.68 ± 0.05       | 0.33 ± 0.03       | Wood, spice        | 1379/1367| MS, RI  |
| 8   | α-Cedrene                         | 0.19 ± 0.02       | -                 | Wood, spice        | 1418/1408| MS, RI  |
| 9   | β-Caryophyllene                   | 0.79 ± 0.09       | 0.44 ± 0.03       | Fried, Spice, wood | 1424/1417| MS, RI  |
| 10  | (E)-β-Farnesene                   | 0.28 ± 0.08       | 0.56 ± 0.07       | Wood, citrus, sweet| 1458/1443| MS, RI  |
| 11  | Alloaromadendrene                 | 0.15 ± 0.06       | 0.16 ± 0.01       | Wood               | 1466/1457| MS, RI  |
| 12  | γ-Murolene                        | 0.27 ± 0.06       | 0.19 ± 0.01       | Herb, wood, spice  | 1481/1474| MS, RI  |
| 13  | α-Curcumene                       | 0.30 ± 0.06       | -                 | Herbal             | 1486/1481| MS, RI  |
| 14  | α-Murolone                        | 0.37 ± 0.09       | 0.20 ± 0.01       | Wood               | 1504/1497| MS, RI  |
| 15  | α-Farnesene                       | 0.86 ± 0.25       | 3.36 ± 0.49       | Wood, green, floral| 1511/1507| MS, RI  |
| 16  | γ-Cadinene                        | 0.20 ± 0.05       | 0.12 ± 0.00       | Wood               | 1519/1511| MS, RI  |
| 17  | α-Calacorene                      | 0.25 ± 0.06       | 0.14 ± 0.00       | Wood               | 1548/1542| MS, RI  |
|     | **Total alkene**                  | **9.46 ± 0.39**   | **13.66 ± 1.76**  |                    |          |

### Aldehydes

| No. | Compounds                        | **RI (Cal)** | **RI (Ref)** | I Method |
|-----|----------------------------------|--------------|--------------|----------|
| 1   | Furfural                         | 0.43 ± 0.00  | -/835        | MS        |
| 2   | Benzaldehyde                      | 1.00 ± 0.06  | 963/961      | MS, RI    |
| 3   | Benzene acetaldehyde             | 0.52 ± 0.02  | 1044/1043    | MS, RI    |
| 4   | Decanal                          | 0.43 ± 0.11  | 1206/1206    | MS, RI    |
|     | **Total aldehydes**              | **2.38 ± 0.19** | **1.63 ± 0.25** |          |

### Ketones

| No. | Compounds                         | **RI (Cal)** | **RI (Ref)** | I Method |
|-----|-----------------------------------|--------------|--------------|----------|
| 1   | (Z)-Jasmine                       | 1.00 ± 0.18  | 1402/1396    | MS, RI   |
| 2   | α-Ionone                          | 0.08 ± 0.02  | 1400/1421    | MS, RI   |
| 3   | Geranylacetone                    | 0.59 ± 0.14  | 1454/1452    | MS, RI   |
| 4   | (E)-β-Ionone                      | 0.55 ± 0.14  | 1489/1469    | MS, RI   |
|     | **Total ketones**                 | **2.22 ± 0.48** | **2.11 ± 0.23** |          |

### Esters

| No. | Compounds                         | **RI (Cal)** | **RI (Ref)** | I Method |
|-----|-----------------------------------|--------------|--------------|----------|
| 1   | Methyl salicylate                 | 0.34 ± 0.04  | 1196/1187    | MS, RI   |
| 2   | Hexanoic acid, hexyl ester        | 0.33 ± 0.05  | 1387/1385    | MS, RI   |
| 3   | Jasmine Lactone                   | 1.41 ± 0.28  | 1486/-       | MS        |
| 4   | dihydroactinidiolide              | 0.57 ± 0.15  | 1536/1525    | MS, RI   |
|     | **Total esters**                  | **2.65 ± 0.52** | **3.01 ± 0.33** |          |

### Other components

| No. | Compounds                         | **RI (Cal)** | **RI (Ref)** | I Method |
|-----|-----------------------------------|--------------|--------------|----------|
| 1   | p-Xylene                          | 0.36 ± 0.02  | -/870        | MS, RI   |
| 2   | α-Cymene                          | 0.36 ± 0.06  | 1025/1021    | MS, RI   |
| 3   | Ethanone, 1-(1H-pyrrol-2-yl)-Indole| 0.74 ± 0.02  | 1064/1063    | MS, RI   |
| 4   | (E)-Isoeugenol                    | 1.26 ± 0.17  | 1296/1293    | MS, RI   |
| 5   |                                  | 2.72 ± 0.20  | 1452/1453    |          |
|     | **Total others**                  | **4.15 ± 0.70** |             |          |

*a* Relative content: the material peak area/internal standard peak area (based on triplicate analyses). *b* Aroma description: the material peak area/internal standard peak area (based on triplicate analyses). *c* RI (Cal): retention index of compounds calculated using the homologous series of n-alkanes. *d* RI (Ref): the published retention index of compounds in NIST 14 library. *e* I Method: MS, mass spectrum comparison using NIST 14 library; RI, retention index in agreement with literature values.
3.4. PCA of Volatile Compounds

PCA of the volatile compounds in TOT and OOT (Figure 4) showed that ‘Wuyedancong’ planted in different areas had different fragrances. Consistent with previous research, the characteristic aroma substances of OOT were terpenes, such as nerolidol (point 3), α-farnesene (point 4) and linalool (point 6) [40,41], while those of TOT were hotrienol (point 1) and benzyl alcohol (point 2). Hotrienol is one of the main sources of the fruity aroma in Camellia sinensis, and it is a key compound used to distinguish different aroma types of tea [42]. In this study, hotrienol could be used to distinguish tea planted in Medog, Tibet from tea planted in Chaozhou, Guangdong (origin). The content of benzyl alcohol also showed extremely significant differences in tea samples from the two regions, which suggests the sweet, floral, rose-like and caramel-like aromas of benzyl alcohol would allow one to intuitively distinguish tea planted in Medog from tea planted in Chaozhou. However, more data are needed to determine whether those differences could be used to differentiate ‘Wuyedancong’ planted in other regions.

Figure 4. PCA biplot of volatile substance of TOT and OOT. Each point represents a volatile substance: 1, hotrienol; 2, benzyl alcohol; 3, nerolidol; 4, α-farnesene; 5, 1,3,5,7-cyclooctatetraene; 6, linalool; 7, β-ocimene; 8, β-myrcene; 9, indole; 10, linalool oxide III.

3.5. Taste Substances in TOT and OOT

The water extract of tea (i.e., the total amount of substances soluble in hot water), which determines the thickness of tea soup and the quality and intensity of its taste, is considered to be one of the important indicators of tea quality [43,44]. The main substances in the water extract, including polyphenols such as caffeine and catechins (EC, ECG, EGC, EGCG and C) and free amino acids, were significantly higher in TOT than in OOT (Figure 5, Supplemental Table S3). Total free amino acids, which endow sweet and umami flavors and are important precursors to aroma compounds [45], were significantly higher in TOT than OOT.

Tea polyphenols are one of the main substances contained in tea, accounting for 20–35% of the dry weight of tea leaves, and catechins account for more than 70% of the total polyphenols [45]. The four substances EGCG, EGC, ECG, and EC account for about 90% of total catechins [28]. As one of the most important functional components of tea, catechins have strong antioxidant effects and can effectively reduce the risk of cardiovascular disease and cancer [2,32]. The content of total polyphenols was significantly higher in TOT than in OOT. Most of the catechins were significantly higher in TOT, except EC, which was not significantly different between two tea samples. The only component that was higher in OOT than TOT was caffeine. Caffeine is one of the most bitter components of tea, being about twice as bitter as EGCG [3]. Taken together, the taste of TOT is expected to be better than the taste of OOT, due to the lower content of caffeine and the higher amount of free amino acids that likely balance the bitter effect of the polyphenols.
In this study, the volatile components and the flavor components of tea planted in Tibet and in its original location were systematically analyzed. The volatile components in TOT and OOT showed a slight difference that could not be distinguished by the electronic nose. Through HP-SPME/GC-MS analysis, we found the contents of benzene acetaldehyde, (Z)-jasmine, copaene, β-caryophyllene and benzyl alcohol were significantly higher in TOT than in OOT, while the contents of indole, β-myrcene, linalool, nerolidol, α-farnesene, β-octomere and 1,3,5,7-cyclooctatetraene were significantly lower in TOT than in OOT. PCA revealed that the characteristic aroma substances that can distinguish the teas from Tibet and Guangdong were hotrienol and benzyl alcohol. In terms of taste substances, TOT exhibited higher contents of water extractable compounds, polyphenols and amino acids and lower contents of caffeine than OOT, which implies that TOT might have a better flavor than OOT. This study shows that the content of volatiles and flavor compounds is greatly affected by environmental conditions. The results will provide a reference for evaluating the introduction and cultivation of this crop in the plateau areas of Tibet.

4. Conclusions

In this study, the volatile components and the flavor components of tea planted in Tibet and in its original location were systematically analyzed. The volatile components in TOT and OOT showed a slight difference that could not be distinguished by the electronic nose. Through HP-SPME/GC-MS analysis, we found the contents of benzene acetaldehyde, (Z)-jasmine, copaene, β-caryophyllene and benzyl alcohol were significantly higher in TOT than in OOT, while the contents of indole, β-myrcene, linalool, nerolidol, α-farnesene, β-octomere and 1,3,5,7-cyclooctatetraene were significantly lower in TOT than in OOT. PCA revealed that the characteristic aroma substances that can distinguish the teas from Tibet and Guangdong were hotrienol and benzyl alcohol. In terms of taste substances, TOT exhibited higher contents of water extractable compounds, polyphenols and amino acids and lower contents of caffeine than OOT, which implies that TOT might have a better flavor than OOT. This study shows that the content of volatiles and flavor compounds is greatly affected by environmental conditions. The results will provide a reference for evaluating the introduction and cultivation of this crop in the plateau areas of Tibet.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8060539/s1, Figure S1: Total ion chromatograms of volatile components of TOT and OOT. Figure S2: The six volatile categories of TOT (A) and OOT (B). Table S1: The substance groupings for calculating the Wickremasinghe-Yamanishi ratio. Table S2: Aroma components of Groups I and Group II of Owuor’s flavor index. Table S3: The contents of taste components of TOT and OOT.

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