Communication

Absolute Quantitative Volatile Measurement from Fresh Tea Leaves and the Derived Teas Revealed Contributions of Postharvest Synthesis of Endogenous Volatiles for the Aroma Quality of Made Teas

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Abstract: Characteristic aroma is a well-appreciated feature contributing to tea quality. Although extensive studies have been made to investigate aroma biosynthesis and gene expressions during tea making processes, it remains unclear whether the endogenous volatile biosynthesis during postharvest tea processing contributes to the aroma quality of made tea. To critically evaluate this question, we used the same batch of fresh tea leaves and produced three different types of tea with different degrees of fermentation (green tea, oolong tea, and black tea). Total volatiles were extracted by solvent-assisted-flavor evaporation, then quantified by gas chromatography-flame ionization detector combined with response factor correction for quantitative measurement. Compared with fresh tea leaves, the volatile profiles of the made teas were dramatically altered, with significant loss for the majority of endogenous volatiles and simultaneous gain for non-endogenous volatiles. By calculation of odor-activity values, the potential volatiles contributing to the aroma characteristics of each tea type were identified. Our data suggest that postharvest synthesis of endogenous volatiles did not contribute to the aroma quality of made tea.

Keywords: solvent-assisted-flavor evaporation; GC-FID; FID response factor; endogenous volatile; fresh leaves; green tea; oolong tea; black tea

1. Introduction

Aroma is determined by the concentration and composition of various volatiles. Tea aroma characteristics affect its quality, consumer preference, and commercial value. Based on the degrees of fermentation, tea can be classified into three large categories: non-fermented green tea, semi-fermented oolong tea, and fully fermented black tea [1,2]. Tea making starts with fresh tea leaves as the raw material, which has a grassy odor. During tea processing the volatile profiles are altered and eventually form the characteristic aroma [3–6]. Volatiles retained in the made teas are either derived from fresh tea leaves or newly synthesized during postharvest tea making. Here, for clarity, the volatiles existed in the preharvest tea leaves are named as endogenous volatiles (EVs) of fresh tea leaves. Accordingly, the non-endogenous volatiles (NEVs) refer to those that are not detected from the fresh tea leaves but present in made teas.

The quality of tea depends first and foremost, on the quality of the raw material and rational application of technological processes. EVs are affected by the tea cultivar, growing environment, and agronomic practices [7–10]. It is generally assumed that EVs synthesis during tea making processes contribute significantly to the aroma characteristics of made teas; extensive efforts have been invested to characterize EVs biosynthesis pathways and related gene expressions during postharvest tea making, in the hope to find new strategies to improve the aroma quality of made teas [11–17]. In most of these studies, volatiles were measured in a relative- or semi- quantitative manner; volatiles of fresh tea leaves versus those of made teas were not quantitatively extracted and compared. Thus, it remains unclear whether these synthesized EVs really contribute to the aroma quality of made teas. In this study, we started with the same batch of fresh tea leaves (*Camellia sinensis* cv *Tieguanyin*), and made three different types of tea (green tea, oolong tea, and black tea) according to respective standard tea processing methods (Supplementary Figure S1). Their volatiles were extracted by a solvent-assisted-flavor evaporation, then quantified by gas chromatography-flame ionization detector (GC-FID) combined with FID response factor correction. We found that compared to fresh tea leaves, the volatile profiles of made teas were dramatically altered; the majority of the EVs contents were reduced, while many NEVs accumulated simultaneously. The total volatile contents of made teas were significantly lower than those of the fresh tea leaves. By calculating the odor-activity value (OAV), the potential volatiles contributing to the aroma characteristics of each tea type were identified. Our data suggest that EVs synthesis during postharvest tea processing did not make significant contributions to the aroma quality of made tea.

2. Materials and Methods

2.1. Sample and Chemicals

One bud and three leaves were plucked from *Camellia sinensis* cv *Tieguanyin* on 29 April 2017, then processed into green tea, oolong tea, and black tea according to respective standard tea making methods (Supplementary Figure S1). The tea leaves were withered under sunlight for 30 min, then moved indoors to cool down. To make green tea, tea leaves were heat deactivated. To make oolong tea, leaves were subjected to turning-over followed by spreading. This turning-over/spreading cycle was repeated four times, the time for the first turning-over to the fourth turning-over lasted for 3-, 4-, 8-, and 10- min, respectively. The spreading lasted for 2–3 h following each turning-over treatment. After the completion of the 4th turning-over/spreading cycle, the tea leaves were heat deactivated. Heat deactivation was conducted in an electric-powered pan, the leaf temperature was monitored, and the deactivation was stopped once leaf temperature reached to 80–85 °C. The leaves were rolled for 35 min, followed by a drying treatment. To make black tea, tea leaves were withered indoors for 18 h, then rolled for one hour, followed by fermentation for 4 h before drying. The drying was performed at 100 °C for 10 min followed by 90 °C for 1 h. Before tea making, a portion of the fresh tea leaves were frozen in liquid nitrogen as the control sample.

Diethyl ether (analytical grade, 99.9%) and anhydrous sodium sulfate (analytical grade, 99%) were ordered from Sino Pharm, Shanghai, China; ethyl caprate (GC ≥ 98%) was purchased from Sigma, St. Louis, MO, USA.

2.2. Volatile Extraction

Tea volatiles were extracted as before [18]. The water contents from the fresh tea leaves varied between 65% to 75% depending on the tenderness of the tea leaves, germplasms and plucking seasons. In this study, 8 g of fresh tea leaves and 2 g of dry tea were used for volatile extraction. Tea leaves were frozen with liquid nitrogen and powdered, the tea powder was transferred into a glass stoppered flask. Forty milliliters of diethyl ether and 64.5 μg of ethyl caprate internal standard were added. To absorb water, 8 g (for fresh tea leaves) or 0.5 g (for dry tea) anhydrous sodium sulfate was also added. The tea powder was extracted under stirring (200 rpm) for 2 h. The organic phase was collected, then distilled

through a solvent-assisted-flavor-evaporation device (SAFE). The detailed SAFE operation followed the method described by Chen et al. (2020) [18]. The volume for the distillation flask and the receiver flask was 500 mL. The volatile distillate was collected, aliquot into 8-mL glass tubes, concentrated in a rotary evaporator to about 1.5 mL, then transferred into labeled 2-mL GC vials, and further concentrated in a rotary evaporator. The volume was closely monitored; the concentration step was stopped immediately once the volume was slightly below the 500 μL marker line. A small volume of diethyl ether was added to make the total volume reach 500 μL. Fresh leaves and oolong tea had four biological replicates, whereas green tea and black tea had three biological replicates.

2.3. Volatile Identification and Quantification

Volatiles were analyzed as described before [18,19]. Briefly, 1 μL of sample was injected into a capillary column (RXi-5SiIMS column, 30 m $\times$ 0.25 mm $\times$ 0.25 μm) in a splitless mode for GC-MS and GC-FID analysis. The oven temperature program was initiated at 50 °C for 2 min, raised 5 °C min^{-1} to 180 °C, and held 2 min; then raised 10 °C min^{-1} to 230 °C, and held 5 min before returning to 50 °C for the next sample. Injector, ion source, interface, and FID temperatures were 250, 230, 270, and 230 °C, respectively. The flow rates of hydrogen, nitrogen, and zero air were 40, 30, and 400 mL min^{-1}, respectively, and helium was used as the carrier gas with a flow rate of 1.0 and 1.7 mL min^{-1} for GC-MS and GC-FID, respectively. Zero air is a highly purified air in which almost all the hydrocarbon is removed. The electron ion source was set at 70 eV, and the chromatograms were recorded by monitoring the total ion current in the mass range of 45–600 m/z. Since each compound has its own ionization and fragmentation in the MS detector [20], thus, various volatiles cannot be reliably quantified by comparing with a single internal standard. In this study, MS data was used for volatile identification by comparing MS spectral with the NIST14 database, and further confirmed by their reported retention index; volatiles were quantified from FID data by normalizing to the internal standard peak area. To calibrate the FID response differences for individual volatiles, FID response factors relative to the internal standard were calculated and used for further normalization (Supplementary Table S1). The fresh leaf water contents were determined by the National Standard of P. R. China (GB/T 5009-2016), then used to convert fresh leaf weight into dry mass. The volatile contents were normalized to dry mass. To convert the retention time into the retention index, n-alkanes (C9–C25) were analyzed under the same condition, and the data was used to convert the retention time into the retention index (RI) by the formula: RI = $100 \times [(tR - tRz)/(tR(z + 1) - tRz) + z]$. Where z is the carbon number of the alkane standard, tRz and tR(z + 1) represent the retention times of z and z + 1 alkane standards, and tR is the retention time of tea volatile that falls between tRz and tR(z + 1).

2.4. Calculation of Volatile Concentration from Tea Infusion and Odor-Activity Value

Organoleptic assessment was made according to the National Standard of P. R. China (GB/T 23776-2018)—Methodology for Sensory Evaluation of Tea. In a standard organoleptic assessment by a trained tea taster, the oolong tea infusion was prepared by extracting 5 g of oolong dry tea in 110 mL of boiling water; in contrast, the green tea or black tea infusion was prepared by extracting 3 g of dry tea in 150 mL of boiling water. To evaluate the volatile contribution to the aroma characteristics of tea infusion, ideally their concentrations in the tea infusion are directly measured. However, to fully recover volatiles from the simulated standard tea infusion preparation is technically challenging, since a considerable amount of volatiles easily evaporate into the air space and get lost; to add this complexity, it has been reported that during tea infusion preparation, under the action of hot water, some new volatiles are formed from its glycosidically bound precursors [21]. To avoid these issues, we assumed that all the volatiles from dry tea were extracted into the tea infusion during the tea infusion preparation. Based on the absolute volatile contents from dry tea and the tea mass to water ratio, the volatile concentrations in the tea infusion could be

calculated. The odor detection threshold in water was obtained from literature, then used to calculate odor activity value.

2.5. Statistical Analysis

A Student's t-test with two-tailed equal variance was performed to determine the significance ($p < 0.05$).

3. Results and Discussion

The volatiles from fresh tea leaves, green tea, oolong tea, and black tea were extracted in diethyl ether, then separated from non-volatile substances and analyzed by GC-MS and GC-FID (Supplementary Figures S2 and S3). In total; 31, 32, 27, and 31 volatiles were identified from respective samples (Table 1). Among the 31 EVs of fresh tea leaves, there were 19, 15, and 14 volatiles detected from green tea, oolong tea, and black tea, respectively. Among the 32 green tea volatiles, there were 17 and 15 volatiles commonly detected from oolong tea and black tea, respectively. There were 14 volatiles commonly detected between oolong tea and black tea (Table 1, Supplementary Figure S4).

Among the 31 EVs detected from fresh tea leaves, nine volatiles were below the detection threshold from these three types of made tea: phenylethyl alcohol, germacrene D, nerolidol isobutyrate, γ-cadinene, trans-calamenene, *cis*-cadina-1(2),4-diene, and three unidentified volatiles. Meanwhile, nine volatiles from fresh tea leaves were commonly detected from these three types of made teas. In four of them, β-*cis*-ocimene, *cis*-linalool oxide (furanoid), *trans*-linalool oxide (furanoid), and linalool, their contents from made teas only accounted for 0.8–14% of that from fresh tea leaves. The other five volatiles, including *E*-nerolidol, (*Z*)- 3-hexenyl benzoate, hexyl benzoate, neophytadiene, and caffeine, were not significantly different from that of fresh tea leaves except that the *E*-nerolidol contents from green tea and oolong tea as well as the neophytadiene and caffeine contents from black tea were significantly higher than that of the fresh tea leaves. The remaining 13 volatiles were either detected from only one or two types of made teas; their contents were significantly lower than that of fresh tea leaves except *cis*-jasmone and β-caryophyllen, which were similar between fresh tea leaves and green tea or oolong tea (Table 1). The total volatile contents for fresh tea leaves, green tea, oolong tea, and black tea were 76.13 ± 11.66, 7.73 ± 0.71, 5.77 ± 0.74, 7.80 ± 1.83 µg. g^{-1} dry weight (DW), respectively. These data demonstrated that the tea making processes were characterized by the significant loss for most of the EVs. Thus, their synthesis during postharvest tea making processes would not contribute significantly to the aroma quality of made tea products. It remains unclear why and how these EVs were lost during tea making processes. A detailed quantitative volatile measurement at each processing step is required to resolve this issue. It is obvious that the abundant volatiles from fresh tea leaves were lost more compared to less abundant volatiles (Table 1). We speculated that there are two factors that could affect volatile retention. One was that the leaf cell structure was disrupted by the tea making process, which may reduce the leaf's volatile holding capacity, and thus result in the evaporation of the abundant volatiles. On the other hand, drying should result in significant volatile loss since it was performed at 90–100 °C, which was above the boiling points for many tea volatiles. Under this pathway, the amounts of volatile loss would depend on the drying temperature, time, and the volatility of individual tea aroma.

Table 1. Volatile contents from fresh tea leaves, green tea, oolong tea, and black tea.

RT (min)	RI$_{lit}$	RI$_{exp}$	Identification	Boiling Point (°C)	Aroma Descriptions	Contents (µg. g^{-1} DW)			
						Fresh Leaves (*n* = 4)	Green Tea (*n* = 3)	Oolong Tea (*n* = 4)	Black Tea (*n* = 3)
8.966		960	unknown 1	nf	nf	2.45 ± 1.19	nd	nd	nd
9.344		983	unknown 2	nf	nf	6.47 ± 2.05	nd	nd	nd
10.31		1022	unknown 3	nf	nf	1.26 ± 0.27	nd	nd	nd
10.631	1028.9	1031	β-cis-ocimene	175.2	Fruity, floral	4.71 ± 1.48 [a]	0.10 ± 0.03 [b]	0.11 ± 0.03 [b]	0.05 ± 0.01 [c]
11.388	1075.1	1079.4	cis-linalool oxide (furanoid)	188	Fresh, floral	3.55 ± 0.51 [a]	0.12 ± 0.03 [b]	0.12 ± 0.03 [b]	0.41 ± 0.10 [c]
11.876	1083.3	1080.1	trans-linalool oxide (furanoid)	222.6	flower	5.94 ± 0.98 [a]	0.13 ± 0.04 [b]	0.13 ± 0.03 [b]	0.86 ± 0.21 [c]
12.272	1099	1095.7	Linalool	199	flower, lavender	23.14 ± 2.09 [a]	0.23 ± 0.06 [b]	0.19 ± 0.04 [b]	1.06 ± 0.27 [c]
12.427	1106.8	1104	Hotrienol	80–84	Fruity	nd	nd	0.42 ± 0.07 [a]	nd
12.691	1113	1109	(E)-4,8-dimethylnona-1,3,7-triene	81	nf	3.47 ± 0.81 [a]	0.14 ± 0.03 [b]	nd	nd
13.383	1128	1124.3	Benzyl nitrile	190.7	pungent smell	0.32 ± 0.03 [a]	0.17 ± 0.04 [b]	0.20 ± 0.08 [b]	nd
14.475	1114.9	1113.1	Phenylethyl alcohol	219	Fresh, rose aroma	0.14 ± 0.02 [a]	nd	nd	nd
14.604	1164.7	1162	(Z)-3-Hexenyl butanoate	98	Aroma like apple and grass	0.45 ± 0.09 [a]	0.27 ± 0.06 [b]	nd	0.22 ± 0.07 [b]
14.677	1171	1174	trans-pyranoid linalool oxide	201–202	Earthy	nd	nd	nd	0.41 ± 0.07 [a]
14.865	1180	1178	Dodecane	216	nf	0.88 ± 0.19 [a]	nd	0.10 ± 0.02 [b]	0.14 ± 0.03 [b]
15.107	1187	1184.1	Methyl salicylate	223.3	Minty flavor	11.04 ± 1.51 [a]	0.08 ± 0.02 [b]	nd	0.45 ± 0.12 [c]
15.386	1189	1188	2,6-dimethyl-3,7-octadiene-2,6-diol	284	pungent and bad smell	nd	nd	0.11 ± 0.03 [a]	nd
16.231	1191.5	1190	Hexyl butanoate	205	Green, fruity	0.56 ± 0.10 [a]	nd	0.08 ± 0.02 [b]	0.08 ± 0.03 [b]
16.252	1200.9	1203	4-methylpentyl 2-methylbutanoate	nf	nf	nd	nd	nd	0.12 ± 0.03 [a]
16.418	1236.3	12,331	Hexyl 2-methyl butanoate	217–219	Green, fruity	nd	nd	nd	0.11 ± 0.03 [a]

Table 1. *Cont.*

RT (min)	RI$_{lit}$	RI$_{exp}$	Identification	Boiling Point (°C)	Aroma Descriptions	Contents (µg. g^{-1} DW)			
						Fresh Leaves (*n* = 4)	Green Tea (*n* = 3)	Oolong Tea (*n* = 4)	Black Tea (*n* = 3)
16.899	1253	1250	(E)- geraniol	230	rose, floral	2.30 ± 0.77 [a]	0.14 ± 0.06 [b]	nd	nd
17.22	1249	1254	1,3-bis(1,1-dimethylethyl)benzene	106–107	nf	nd	nd	0.09 ± 0.01	nd
17.595	1260	1261	2,5-dihydro-2,5-dimethoxyfuran	160–162	nf	nd	nd	0.12 ± 0.02	nd
18.033	1298.4	1301.6	Indole	253–254	mothball, burnt	5.19 ± 0.81 [a]	0.73 ± 0.15 [b]	0.78 ± 0.18 [b]	nd
20.516	1333	1330	cis-3-hexenyl hexanoate	115	Tender, fresh and clean aroma	1.05 ± 0.14 [a]	0.26 ± 0.06 [b]	nd	nd
20.679	1368	1370	trans-2-hexenyl hexanoate	79	fruity	nd	0.23 ± 0.05 [a]	0.20 ± 0.02 [a]	0.51 ± 0.11 [b]
20.753	1381	1378	Hexyl hexanoate	246	fruity	nd	0.06 ± 0.02 [a]	0.12 ± 0.03 [b]	0.19 ± 0.04 [c]
20.837		1382	unknown 4	nf	nf	nd	nd	nd	0.10 ± 0.11
21.124	1394.6	1396.8	cis-Jasmone	248	sweet, flower	0.15 ± 0.01	0.18 ± 0.03	0.18 ± 0.04	nd
21.743	1413.5	1410.7	β-caryophyllen	262–264	nf	0.45 ± 0.07	0.43 ± 0.66	nd	nd
21.751	1420.1	1418	(E)-caryophyllene	266–268	nf	nd	nd	nd	0.05 ± 0.01 [a]
22.36	1453.1	1451	α-humulene	nf	nf	nd	nd	nd	0.06 ± 0.01 [a]
22.361	1455.9	1451.6	(E)-β-farnesene	271	Floral	nd	nd	0.01 ± 0.03 [a]	nd
22.468	1468.2	1463.3	(E)-2-dodecenal	93	nf	nd	nd	nd	0.05 ± 0.00 [a]
22.696	1472.8	1472	1-dodecanol	255–259	grease	nd	nd	nd	0.07 ± 0.03 [a]
23.367	1480.6	1478.6	Germacrene D	279.7	floral	0.11 ± 0.02 [a]	nd	nd	nd
23.382	1485.9	1484	trans-β-ionone	239	seaweed, violet, flower, raspberry	nd	0.17 ± 0.17 [a]	nd	0.06 ± 0.01 [a]
23.455	1504.1	1499.4	α-farnesene	260	Floral, fresh	nd	0.31 ± 0.23 [a]	0.39 ± 0.03 [a]	nd
23.471		1502	Nerolidol isobutyrate	361.9	Sweet, rose-like	0.23 ± 0.08 [a]	nd	nd	nd
23.482	1510	1507	Tridecanal	132–136	nf	nd	nd	nd	0.16 ± 0.02 [a]
23.73	1513.1	1512	γ-cadinene	272	Floral, fresh, sweet	0.05 ± 0.06 [a]	nd	nd	nd
23.9	1517.8	1518	cis-jasmin lactone	130	sweet, flower	nd	0.07 ± 0.01 [a]	nd	nd

Table 1. *Cont.*

RT (min)	RI$_{lit}$	RI$_{exp}$	Identification	Boiling Point (°C)	Aroma Descriptions	Contents (µg. g^{-1} DW)			
						Fresh Leaves (*n* = 4)	Green Tea (*n* = 3)	Oolong Tea (*n* = 4)	Black Tea (*n* = 3)
24.205	1523.2	1520	δ-cadinene	279	nf	nd	nd	0.09 ± 0.01 [a]	nd
24.311	1528	1524	trans-calamenene	285–286	nf	0.21 ± 0.04 [a]	nd	nd	nd
24.323 [b]	1529	1526	Naphthalene	217.9	Tar, camphoric and greasy odor	nd	0.12 ± 0.02 [a]	nd	0.09 ± 0.01 [b]
24.431		1527.2	cis-hexahydro-8a-methyl-1,8(2H,5H)-naphthalenedione	nf	nf	nd	0.08 ± 0.02	0.07 ± 0.00	nd
24.637	1531	1532	Cis-cadina-1(2),4-diene	137.9	nf	0.11 ± 0.04 [a]	nd	nd	nd
24.644	1532	1533.4	(E)-γ-bisabolene	262	nf	nd	0.03 ± 0.00 [a]	nd	nd
24.998	1535	1536	Dihydroactinidiolide	296.1	Floral, rose-like	nd	nd	nd	0.11 ± 0.06 [a]
25.149	1533.3	1537.3	α-cadinene	271	green	0.08 ± 0.02 [a]	0.04 ± 0.01 [b]	nd	nd
25.308 [c]	1541	1543	E-nerolidol	276	Slight neroli-like, rose-like and sweet flavor	0.47 ± 0.14 [a]	1.17 ± 0.10 [b]	1.03 ± 0.18 [b]	0.51 ± 0.08 [c]
25.324	1550.9	1547	Germacrene B	287.2	nf	nd	0.11 ± 0.03 [a]	nd	nd
25.626 [c]	1572.9	1568	(3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene	293.2	nf	0.52 ± 0.09 [a]	nd	0.13 ± 0.01 [b]	0.20 ± 0.04 [c]
25.773	1562	1570	cis-3-hexanyl n-octanoate	292.5	nf	nd	nd	0.14 ± 0.02 [a]	nd
25.928	1569.5	1573	(Z)-3-hexenyl benzoate	105	Green, herb-like	0.06 ± 0.08	0.08 ± 0.01	0.08 ± 0.00	0.07 ± 0.01
26.297	1581.8	1580	Hexyl benzoate	272	Wood, green	0.07 ± 0.05 [a]	0.11 ± 0.01 [a]	0.13 ± 0.01 [b]	0.14 ± 0.04 [b]
26.667	1588	1590.1	E-2-hexenyl benzoate	165–166	nf	nd	0.04 ± 0.00 [a]	nd	nd
27.048	1655	1650	Methyl jasmonate	302.9	Sweent, jasmine-like	nd	1.04 ± 0.74 [a]	nd	nd
27.676	1670	1672	cis-3-hexenyl salicylate	145	Flower, green	nd	0.08 ± 0.01 [a]	nd	nd
27.677	1676.3	1673.2	1-tetradecanol	289	Wax-like	nd	nd	nd	0.06 ± 0.01 [a]
28.337	1673	1675	α-cadinol	137–139	Sweet, baking aroma	nd	nd	nd	0.05 ± 0.00 [a]
29.903 [b]	1827	1823.1	Neophytadiene	128	nf	0.09 ± 0.04 [a]	0.07 ± 0.02 [a]	0.06 ± 0.01 [a]	0.41 ± 0.19 [b]
31.359	1830	1834	Hexadecanal	151	nf	nd	nd	nd	0.05 ± 0.02 [a]
32.162 [b]	1842	1840	Caffeine	178	nf	0.64 ± 0.07 [a]	0.85 ± 0.20 [a]	0.62 ± 0.39 [a]	0.94 ± 0.20 [b]
32.985	1927	1931	n-hexadecanoic acid methyl ester	185	nf	nd	0.09 ± 0.02 [a]	0.05 ± 0.04 [a]	nd
Total						76.13 ± 11.66 [a]	7.73 ± 0.71 [b]	5.77 ± 0.74 [c]	7.80 ± 1.83 [b]

nf: not found; nd: not detected; RT: retention time in minutes; RI$_{lit}$: retention index from literature; RI$_{exp}$: retention index from this study. The different superscript letters from the same row indicate statistically significant ($p < 0.05$). Data are expressed as mean ± standard deviation.

A major difference between green tea and oolong tea making technology was the "turning over" step of oolong tea (Supplementary Figure S1). Some volatile contents from oolong tea, including hotrienol, dodecane, 2,6-dimethyl-3,7-octadiene-2,6-diol, hexyl butanoate, 1,3-bis(1,1-dimethylethyl) benzene, 2,5-dihydro-2,5-dimethoxyfuran, hexyl hexanoate, (*E*)-β-farnesene, δ-cadinene, (3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene, and *cis*-3-hexanyl n-octanoate, were significantly higher than that of green tea (Table 1). This suggested that the "turning over" treatments could activate these volatile syntheses. Meanwhile, other volatile contents from oolong tea, including (*E*)-4,8-dimethylnona-1,3,7-triene, (*Z*)-3-hexenyl butanoate, methyl salicylate, (*E*)-geraniol, *cis*-3-hexenyl hexanoate, β-caryophyllen, trans-β-ionone, *cis*-jasmin lactone, naphthalene, (*E*)-γ-bisabolene, α-cadinene, germacrene B, E-2-hexenyl benzoate, methyl jasmonate, and *cis*-3-hexenyl salicylate, were lower than that of green tea, suggesting that the "turning over" step could also facilitate these volatile's evaporation.

There were 13, 12, and 17 volatiles detected from green tea, oolong tea, and black tea respectively, but below the detection limit from fresh tea leaves (Table 1, Supplementary Figure S2). These data demonstrated that postharvest tea processing facilitate NEVs formation and accumulation. Interestingly, oolong tea was generally regarded to be more aromatic than green tea or black tea [22]. However, its total aroma contents were significantly lower than that of green tea or black tea (Figure 1). Thus, the total volatile contents did not positively correlate with their aroma quality. Other factors, such as the volatile compositions, could play a larger role to influence tea's aroma characteristics.
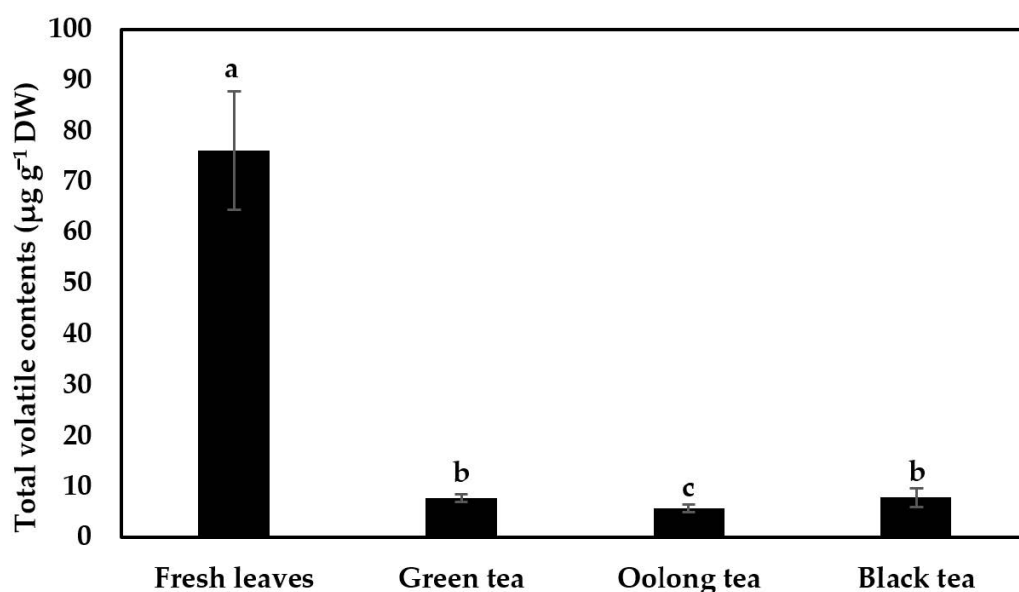
Figure 1. Total volatile contents from fresh leaves, green tea, oolong tea, and black tea. Data are expressed as average ± standard deviation. Different letters indicates statistically significant ($p < 0.05$).

To identify which volatiles could confer an aroma note in a standard sensory evaluation, the odor-activity value (OAV) was calculated. The odor activity values (OAVs) are the ratios of concentration found in the food matrix to its odor detection threshold [23]. The odor detection threshold is the lowest concentration of a certain odor compound that is perceivable by the human sense of smell. The OAV is commonly used to estimate odor potency, their values from fresh tea leaves and made teas are listed in the order of retention time from low to high in Table 2. The volatiles with OAV > 1 were generally regarded as contributors to the aroma characteristics. Thus, linalool, (*E*)-geraniol, indole, *cis*-jasmone, *trans*-β-Ionone, and α-farnesene could be the potential contributors to the aroma quality of *Tieguanyin* green tea; linalool, indole, *cis*-jasmone, and α-farnesene could be the potential contributors to the aroma quality of *Tieguanyin* oolong tea; linalool, *trans*-linalool oxide

(pyranoid), 1-dodecanol, and *trans*-β-ionone could be the potential contributors to the aroma quality of *Tieguanyin* black tea. Interestingly, among these potential aroma contributors, linalool, (*E*)-geraniol, and indole are EVs. Although their contents in the made teas were significantly lower than that of fresh tea leaves, they still retained a sufficient amount contributing to the aroma characteristics in the made tea. On the other hand, *tran*-β-ionone, α-farnesene, *trans*-linalool oxide (pyranoid), and 1-dodecanol, which were NEVs produced in sufficient amount during tea processing, become aroma contributors.

Although a 100% volatile recovery from dry tea to tea infusion is not plausible, considering that most of these OAVs were larger than two, thus, even with a 50% recovery rate, the OAVwould still be larger than one, and contributed to the characteristic aroma of tea infusion. Although linalool, (*E*)-geraniol, and indole were present in fresh leaves at much higher contents compared to made teas (Table 1), the fresh tea leaves did not show the pleasant aroma as the made tea did. One possibility could be that fresh tea leaves also contained other unpleasant volatiles which created masking effects. Removing or reducing these endogenous unpleasant volatiles could be an important aspect for tea processing; another possibility could be that some volatiles showed totally different characteristics in a concentration-dependent manner. One well-characterized example was indole; at very low concentrations indole had a flowery smell [39], however, in high concentrations it smelled animal-like [40]. Under our experimental conditions, only about 14% of the indole from fresh tea leaves was retained in green tea or oolong tea (Table 1). However, their OAV values in green tea and oolong still were in the ranges of 14.7–35.2 (Table 2), thus, it could confer a flowery smell to green tea or oolong tea. Four contributing volatiles, including *trans*-pyranoid linalool oxide, 1-dodecanol, *trans*-β-ionone, and α-farnesene were only detected from certain types of made tea and with large difference in odor-activity value, suggesting that tea processing technologies determined specific NEVs formation and retention, and thus shaped the characteristic aroma for each tea type.

Previously Zhang et al. (2013) applied simultaneous distillation extraction method and isolated volatiles from green teas, oolong teas, and black teas with various origins, then semi-quantified by two-dimensional GC-TOFMS, through multivariate data analysis the compounds with a significant difference were defined [41]. Several volatiles were commonly detected from these two studies; they can be divided into two groups. The first group showed similar changing trends among green tea, oolong tea and black tea between these two studies, including cis-linalool oxide (furanoid), trans-linalool oxide (furanoid), benzyl nitrile, methyl salicylate, 2,6-dimethyl-3,7-octadien-2,6-diol, indole, cis-jasmone, α-farnesene, and nerolidol; in contrast, the second group showed different changing trends among green tea, oolong tea, and black tea between these two studies, including (E)-Hotrienol, (E)-4,8-dimethyl-nona-1,3,7-triene, phenylethyl alcohol, cis-3-hexenyl hexanoate, jasmin lactone, and methyl jasmonate. Since in the previous study green tea, oolong tea and black tea came from different sources, and different cultivars may be used to produce them, this could account for the difference between these two studies. To support this notion, even within same tea type large variations have been reported in previous study [41].

Table 2. Aroma contents from tea infusion and odor-activity value (OAV) of green tea, oolong tea, and black tea.

Compound	Threshold (μg. L^{-1})	Ref.	Concentration in Tea Infusion (μg. L^{-1})			OAV		
			Green Tea	Oolong Tea	Black Tea	Green Tea	Oolong Tea	Black Tea
β-cis-ocimene	34	[24]	2.0	5.1	1.0	<1	0<1	<1
cis-linalool oxide (furanoid)	320	[25]	2.4	5.3	8.2	<1	<1	<1
trans-linalool oxide (furanoid)	320	[25]	2.7	6.1	17.2	<1	<1	<1
Linalool	1	[24]	4.6	8.9	21.3	4.6	8.9	21.3
Hotrienol	110	[26]	nd	18.9	nd	<1	<1	<1
(E)-4,8-dimethylnona-1,3,7-triene	nf		2.8	nd	nd	nf	nf	nf
Benzyl nitrile	1000	[25]	3.3	9.3	nd	<1	<1	<1
(Z)-3-hexenyl butanoate	500	[24]	5.4	nd	4.4	<1	<1	<1
trans- linalool oxide (pyranoid)	0.025	[27]	nd	nd	8.2	<1	<1	329.0
Dodecane	nf		nd	4.4	2.8	nf	nf	nf
Methyl salicylate	40	[28]	1.5	nd	9.0	<1	<1	<1
2,6-dimethyl-3,7-octadiene-2,6-diol	89		nd	5.2	nd	<1	<1	<1
Hexyl butanoate	607	[29]	nd	3.8	1.6	<1	<1	<1
4-methylpentyl 2-methylbutanoate	nf		nd	nd	2.3	nf	nf	nd
Hexyl 2-methyl butanoate	nf		nd	nd	2.2	nf	nf	nf
(E)- geraniol	1.1	[30]	2.8	nd	nd	2.6	<1	<1
1,3-bis(1,1-dimethylethyl)benzene	81		nd	3.9	nd	<1	<1	<1
2,5-dihydro-2,5-dimethoxyfuran	13	[24]	nd	5.5	nd	<1	<1	<1
Indole	1	[31]	14.7	35.3	nd	14.7	35.2	<1
cis-3-hexenyl hexanoate	16	[25]	5.1	nd	nd	<1	<1	<1
trans-2-hexenyl hexanoate	781	[32]	4.7	9.1	10.1	<1	<1	<1
Hexyl hexanoate	820	[32]	1.1	5.6	3.9	<1	<1	<1

Table 2. *Cont.*

Compound	Threshold (µg. L^{-1})	Ref.	Concentration in Tea Infusion (µg. L^{-1})			OAV		
			Green Tea	Oolong Tea	Black Tea	Green Tea	Oolong Tea	Black Tea
unknown 4	nf		nd	nd	2.0	nf	nf	nf
cis-Jasmone	1.9	[33]	3.7	8.4	nd	1.9	4.4	<1
β-caryophyllen	150	[24]	8.5	nd	nd	<1	<1	<1
(E)-caryophyllene	64	[34]	nd	nd	0.9	<1	<1	<1
α-humulene	390	[24]	nd	nd	1.2	<1	<1	<1
(E)-β-farnesene	87		nd	0.6	nd	<1	<1	<1
(E)-2-dodecanal	20	[25]	nd	nd	1.1	<1	<1	<1
1-dodecanol	0.5	[35]	nd	nd	1.4	<1	<1	2.7
trans-β-ionone	0.2	[25]	3.4	nd	1.1	16.9	<1	5.7
α-farnesene	0.1	[36]	6.1	17.8	nd	61.0	178	<1
γ-cadinene	nf		1.4	nd	nd	nf	nf	nf
cis-jasmin lactone	2000	[37]	nd	nd	3.2	<1	<1	<1
δ-cadinene	120	[37]	nd	4.1	nd	<1	<1	<1
trans-calamenene	nf		2.4	nd	1.8	nf	nf	nf
Naphthalene	300		1.6	3.1	nd	<1	<1	<1
8a-methylhexahydro-1,8(2H,5H)-naphthalenedione	nf		nd	nd	2.3	nf	nf	nf
(R)-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	nf		0.8	nd	nd	nf	nf	nf
α-cadinene	nf		0.7	nd	nd	nf	nf	nf
E-nerolidol	15	[38]	2.27	nd	nd	<1	<1	<1
Germacrene B	nf		23.4	47.0	10.2	nf	nf	nf
(3E,7E)-4,8,12-trimethyltrideca-1,3,7,11-tetraene	nf		nd	6.0	4.0	nf	nf	nf
cis-3-hexanyl n-octanoate	nf		nd	6.2	nd	nf	nf	nf
(Z)-3-hexenyl benzoate	4.5		1.6	3.6	1.4	<1	<1	<1
Hexyl benzoate	nf		2.2	6.0	2.9	nf	nf	nf

Table 2. *Cont.*

Compound	Threshold (µg. L^{-1})	Ref.	Concentration in Tea Infusion (µg. L^{-1})			OAV		
			Green Tea	Oolong Tea	Black Tea	Green Tea	Oolong Tea	Black Tea
E-2-hexenyl benzoate	nf		nd	nd	1.2	nf	nf	nf
Methyl jasmonate	70	[38]	nd	nd	0.9	<1	<1	<1
cis-3-hexenyl salicylate	nf		0.9	nd	nd	nf	nf	nf
1-tetradecanol	5000	[37]	20.8	nd	nd	<1	<1	<1
α-cadinol	nf		1.7	nd	nd	nf	nf	nf
Neophytadiene	nf		1.4	2.6	8.3	nf	nf	nf
Hexadecanal	75	[25]	nd	nd	1.1	<1	<1	<1
Caffeine	29,000	[39]	17.0	28.3	18.8	<1	<1	<1
n-hexadecanoic acid methyl ester	1000	[25]	1.8	2.3	nd	<1	<1	<1

nd: not detected; nf: not found.

4. Conclusions

In this study, we successfully produced green tea, oolong tea, and black tea from the same batch of fresh leaves by following respective standard tea processing methods; tea volatiles were then extracted and quantified by GC-FID, further calibrated by FID response factor, and compared with that of fresh leaves. We found that total volatile contents from made teas were significantly reduced compared to the contents of fresh tea leaves; most of the EVs from made teas were lower in amounts than those of fresh tea leaves. Meanwhile, specific NEVs were produced during tea making processes. Calculation of odor-activity values identified that some EVs and NEVs both contributed to the characteristic aroma of made teas. Our data suggest that the postharvest endogenous volatile synthesis during tea making processes were not important to shape the characteristic aroma of made tea.

Supplementary Materials: The following are available online at https://www.mdpi.com/2076-3417/11/2/613/s1, Figure S1: Diagram presentation of standard tea making processes for green tea, oolong tea, and black tea, Figure S2: GC-MS chromatography of fresh tea leaves, green tea, oolong tea and black tea, Figure S3: GC-FID chromatography of fresh tea leaves, green tea, oolong tea and black tea, Figure S4: Volatile distributions among fresh tea leaves, green tea, oolong tea, and black tea, Table S1: Tea volatile FID response factor relative to ethyl caprate.

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