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Discrimination Based on Volatile Compounds and Differential Analysis of Chinese Dark Tea

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Abstract

Simultaneous distillion and extraction (SDE)/gas chromatographymass spectrometry (Gc-Ms) detection coupled with partial least squares-discriminate analysis (PLS-DA) were used to discriminate Chinese dark teas (CDTs). A total of 84 volatile compounds were taken to conduct the cluster analysis. 9 kinds of CDTs could be divided into 3 groups in consistent with the manufacturing processes. Besides, ingredients that were related with the quality of CDT, such as free amino acid, soluble sugar and catechines were analyzed. Differential trends were disclosed between Pu-Er raw tea and Pu-Er ripe tea as well as raw dark green tea (RDGT).

Keywords: Chinese dark tea, volatile compounds, clustering analysis, free amino acid, soluble sugar, catechines

1. Introduction

Chinese dark tea (CDT) (Zhang et al., 2013), which was characterized with the post-fermentation, was popular among those who take tea as their favorite beverage. Pharmaceutical research pronounced Chinese dark tea therapeutic benefits in the treatment with hyperlipemia (Li et al., 2012), cardiovascular disease (CVD) (Xu et al., 2010), diabetes (Zhang et al., 2009; Li et al., 2014) and etc (Lv et al., 2007; Hou et al., 2010; Xiong et al., 2012; Liu et al., 2014). Generally, manufacture of CDTs was beginning with the fixation, rolling, pile-fermentation and drying sequentially. Then the raw dark green tea (RDGT) was obtained (Xia, 2016). After which, with steaming and pressing, the RDGT was compressed into brick, cake or other types. However, CDTs were consisted of different categories of teas, such as RDGT, Pu-Er raw tea, Pu-Er ripe tea, and so on. Each of them was prepared through respective process, so the connotation of chemical compositions would be distinguished and the subsequent derivation of them would be disparate as well (Lv et al., 2013). Distinguished with Pu-Er raw tea, there was pile fermentation during the manufacture process of Pu-Er ripe tea as well as RDGT. Moreover, loose tea distinguished from compressed tea in autoclaved typing.

Discrimination of diverse dark teas was the pursuit of researchers as well as consumers. However, several precise means could be employed. Liquid chromatography-mass spectrometry was employed to investigate the metabolic changes of Pu-Er tea during the pile-fermentation. And discrimination between the raw material group and pile-fermentation process groups or the final product group were obtained (Chen et al., 2013). Using electronic nose and ultrasound-assisted extraction-dispersive liquid-liquid microextraction-gas chromatography-mass spectrometry, Ye and co-workers discriminated raw Pu-Er tea from ripened Pu-Er tea (Ye et al., 2016). Combined with chemometrics methods, GC-MS identification of volatile compounds is fast and ideal for discrimination of Pu-Er teas with different processing technologies and storage times (Lv et al., 2015a). Taking 77 volatile compounds into PCA, Pu-Er green tea could be clearly distinguished from regular green tea. Meanwhile, GC-MS fingerprints were used to demonstrate the production sites of Pu-Er green tea in Yunnan (Lv et al., 2015b). At the other hand, aged Pu-Er tea were clustered away from ripened Pu-Er tea using principal component analysis (PCA) and hierarchical cluster analysis (HCA) based on comparison of ten major constituents (Yi et al., 2015).

Herein, comprehensive analyses were taken up to elucidate the different succession of quality-related constituents between RDGT in bulk, Pu-Er raw tea and Pu-Er ripe tea in cake type. These results highlight our current understanding of the exact objective and accurate analytical method to discriminate CDTs varieties.

2. Material and Methods

All the tea samples were commercially obtainable from Colors of Yunnan Tea CO. LTD. All the solvents and chemicals were commercially available. RDGT in bulk aged for 3, 2, 1 years, Pu-Er raw tea, which was in chitsu pingcha aged for 7, 5, 3 years and Pu-Er ripe tea which was in chitsu pingcha aged for 8, 7, 6 years were chosen to conduct the research. Listed in Table I, each of them was numbered in order to simplify the experiment (Table 1).

Table 1. Tea sample of CDTs

	Raw dark green tea			**Pu-Er ripe tea**			**Pu-Er raw tea**		
Storage time (Year)	3	2	1	8	7	6	7	5	3
Number	A1	A2	A3	B1	B2	B3	B4	B5	B6

All of the tea samples were ground to pass through 30-60 mesh and sealed for future use. Tea samples were weighted on analytical balance (Metiler Toledo, ME-104E). UV absorption spectrum was obtained from Shimadzu UV-3600. According to GB/T 8314-2013, free amino acids were detected at the wavelength of 570 nm. Anthrone colorimetry method was adopted to determine the content of soluble total sugar.

GC-MS data were collected from Agilent 7890A-5975C. Capillary column of Agilent DB-5Ms (30 m × 0.25 mm inner diameter, 0.25 μm film thickness) was chosen to conduct the detection. The stepped temperature program was set as below: An initial temperature of 50 °C was held for 2 min and then increased to 85 °C at 5 °C min^{-1}; this temperature was held for 2 min and then increased to 110 °C at 2 °C min^{-1}; and then increased to 130 °C at 7 °C min^{-1}; heating up to 230 °C at 5 °C min^{-1}, this temperature was maintained for 8 min. The injector temperature was 230 °C. Helium (percentage purity > 99.999%) was used as the carrier gas. The column flow rate was 1 mL min^{-1} and the split ratio was 1:1. The mass spectrometer was used under the following conditions: ionisation voltage, 70 eV; ion source temperature, 230 °C; interface temperature, 230 °C. The mass scan range was 30-400 atomic mass units (amu). Mass spectral identification was achieved by comparing spectra with the commercial mass spectral databases NIST-14. Components were tentatively identified by agreement of their retention times and mass spectra with published data and, if available, with those of authentic compounds.

3. Experimental

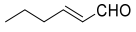
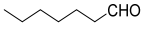
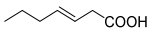
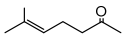
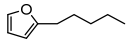
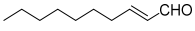
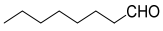
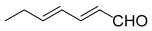
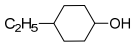
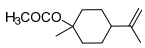
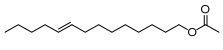
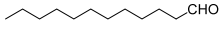
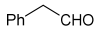
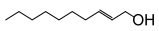
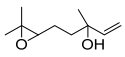
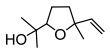
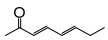
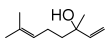
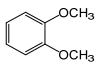
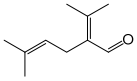
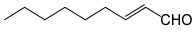
Simultaneous distillion and extraction was used to absorb the volatile compounds. 10 g of the tea sample were sealed in the tube, and 300 ml boiling purity water were added. After balancing for 10 min, 50 ml distilled ether was added into the tube on the other side to extract the volatile compounds. Refluxing for 50 mins, then the heater was removed. After separation of the water, organic phase was dried with Na_2SO_4. Then filtrated and concentrated to 1-2 ml for GC-MS detection. The temperature of the injection port was set at 230 °C. The data was collected and analyzed on the Agilent ChemStation.

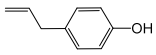
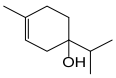
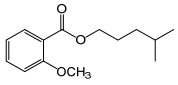
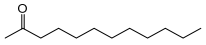
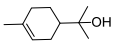
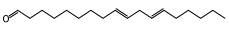
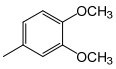
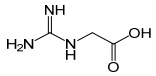
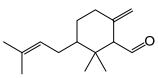
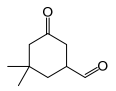
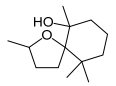
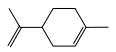
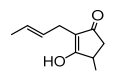
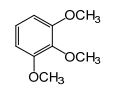
4. Results and Discussion

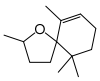
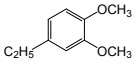
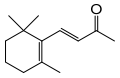
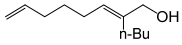
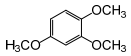
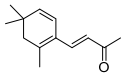
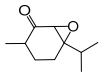
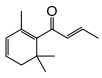
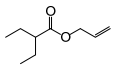
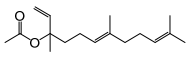
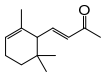
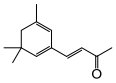
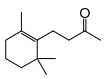
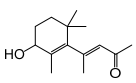
4.1 Difference in the Constituent of Volatile Compounds

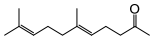
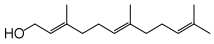
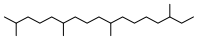
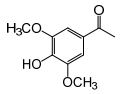
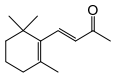
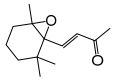
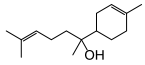
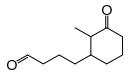
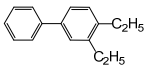
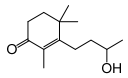
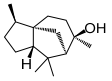
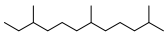
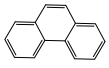
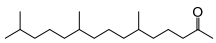
Listed in Table 2, ingredients of the volatile compounds in RDGT, Pu-Er raw tea and Pu-Er ripe tea were distinguished. 84 compounds were isolated, among which there were 13 aldehydes, 19 ketones, 6 esters, 16 alcohols, 8 alkoxides and 12 alkanes. Furthermore, 9 alkenyl aldehydes and 14 ketenes could be isolated. Particularly worth mentioning is that linalool was not detected in Pu-Er ripe tea in spite of the highest account in RDGT and Pu-Er raw tea. Also, alcohols in RDGT and Pu-Er raw tea were dominate while alkoxides in Pu-Er ripe tea were the maximum compounds. Remarkably, naphthalene and naphthalene were also detected in Pu-Er ripe tea as well as Pu-Er raw tea, but none in RDGT. Interestingly, alkoxides in RDGT aged for 3 years was 5.93%, about 60 times more than RDGT aged for 1 year.

Table 2. Volatile compounds isolated from CDTs

NO.	Name	Chemical structure	Rt. (min)	Proportion in volatile compounds (%)								
				B1	B2	B3	B4	B5	B6	A1	A2	A3
1	(E)-hex-2-enal		8.46	-	-	-	0.32	0.08	-	-	-	-
2	Heptanal		10.822	0.87	2.09	1.37	1.06	1.11	-	0.89	0.79	1.00
3	benzaldehyde		13.77	4.40	5.95	6.55	2.55	1.59	1.76	3.24	3.17	4.66
4	(E)-hept-3-enoic acid		14.581	0.28	0.62	0.15	0.62	0.92	0.62	-	-	-
5	6-methylhept-5-en-2-one		14.971	0.42	0.30	0.16	0.19	0.25	-	0.31	0.27	0.15
6	2-pentylfuran		15.251	0.34	0.79	1.10	0.87	0.88	0.56	-	-	0.33
7	(E)-dec-2-enal		15.656	0.21	0.33	0.33	0.21	0.14	-	0.09	-	-
8	Octanal		15.998	-	0.29	0.29	0.56	0.68	0.37	1.07	-	0.35
9	(2E,4E)-hepta-2,4-dienal		16.469	1.41	1.27	1.89	-	-	-	-	-	-
10	4-ethylcyclohexan-1-ol		16.489	-	-	-	0.71	0.22	-	-	-	-
11	1-methyl-4-(prop-1-en-2-yl)cyclohexyl acetate		17.472	-	0.25	0.30	0.80	1.03	0.74	0.24	0.37	0.21
12	(E)-tetradec-9-en-1-yl acetate		17.761	0.31	0.42	0.42	-	-	-	0.62	0.65	0.43
13	Dodecanal		17.845	-	-	-	0.59	0.86	0.48	-	-	-
14	2-phenylacetaldehyde		18.325	2.88	6.10	1.88	4.95	2.27	2.30	3.09	1.61	4.81
15	(E)-dec-2-en-1-ol		19.222	0.64	0.87	0.75	0.51	0.57	-	0.38	0.50	-
16	acetophenone		19.61	0.37	0.47	0.33	-	0.26	-	0.90	0.89	0.65
17	5-(3,3-dimethyloxiran-2-yl)-3-methylpent-1-en-3-ol		20.019	2.60	4.50	6.16	2.19	1.53	1.52	1.86	4.09	3.19
18	2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol		21.042	3.22	7.32	9.15	3.26	2.64	2.54	1.60	3.79	4.76
19	(3E,5E)-octa-3,5-dien-2-one		21.419	1.10	0.76	0.72	0.32	0.53	0.25	-	-	-
20	3,7-dimethylocta-1,6-dien-3-ol		21.9	-	-	-	48.91	39.08	34.84	22.99	30.98	29.55
21	5-methyl-2-(prop-1-en-2-yl)cyclohexan-1-ol		22.112	2.53	1.63	3.46	-	-	-	-	-	-
22	propane-1,2,3-triol		22.5	5.24	2.23	0.73	-	-	-	13.17	13.41	-
23	1,2-dimethoxybenzene		24.651	4.78	5.09	5.49	0.12	0.12	-	0.98	-	-
24	5-methyl-2-(propan-2-ylidene)hex-4-enal		25.163	-	-	-	0.08	0.04	-	-	0.13	-
25	6,6-dimethyl-2-methylenebicyclo[3.1.1]heptan-3-ol		25.265	1.06	0.99	0.79	0.14	0.12	-	-	-	-
26	(E)-non-2-enal		25.747	0.83	0.64	0.79	0.17	0.23	-	-	0.15	0.14

27	4-allylphenol		26.65	0.22	0.60	0.18	-	-	-	-	-	-
28	1-isopropyl-4-methylcyclohex-3-en-1-ol		27.186	-	-	-	0.64	1.04	0.66	0.28	0.41	0.25
29	Naphthalene		27.19	0.62	0.66	0.26	-	-	-	-	-	-
30	4-methylpentyl 2-methoxybenzoate		27.77	0.49	0.87	0.66	-	0.06	-	0.42	0.16	0.21
21	dodecan-2-one		27.892	-	-	-	0.40	0.12	-	-	-	-
32	2-(4-methylcyclohex-3-en-1-yl)propan-2-ol		28.227	3.73	4.29	7.49	8.21	13.70	8.89	4.11	4.29	1.99
33	9,12-octadecadienal		28.833	0.59	0.70	0.39	-	-	-	-	-	-
34	2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-ol		28.858	-	-	-	0.20	0.30	-	0.32	-	0.34
35	2,6,6-trimethylcyclohex-1-ene-1-carbaldehyde		29.536	1.27	1.16	0.90	0.62	0.97	0.56	1.37	1.43	1.11
36	1,5,5-trimethyl-6-methylenecyclohex-1-ene		30.216	0.19	0.16	0.18	0.33	0.39	0.34	0.49	1.06	0.55
37	1,2-dimethoxy-4-methylbenzene		30.752	1.76	2.28	1.07	-	-	0.09	-	-	-
38	carbamimidoylglycine		30.988	-	-	-	0.24	0.36	0.23	0.11	-	-
39	2,2-dimethyl-3-(3-methylbut-2-en-1-yl)-6-methylenecyclohexane-1-carbaldehyde		31.931	-	0.14	0.17	0.12	0.22	0.13	0.39	0.33	0.29
40	3,3-dimethyl-5-oxocyclohexane-1-carbaldehyde		32.486	0.31	0.29	0.41	0.30	0.45	0.21	0.39	0.35	0.45
41	2,6,10,10-tetramethyl-1-oxaspiro[4.5]decan-6-ol		33.043	0.28	0.33	0.41	0.22	0.35	0.43	1.25	1.23	1.35
42	1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene		33.771	0.79	-	0.22	-	-	-	-	-	-
43	bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene		34.469	-	1.12	0.72	-	0.31	0.18	-	0.23	0.20
44	(3Z,7E)-12-oxabicyclo[9.1.0]dodeca-3,7-diene		34.755	-	-	-	-	0.22	0.10	0.18	-	0.25
45	(E)-2-(but-2-en-1-yl)-4-hydroxy-3-methylcyclopent-2-en-1-one		35.176	0.35	0.27	-	-	-	0.13	0.34	0.39	0.68
46	1,2,3-trimethoxybenzene		35.419	12.19	10.76	13.90	-	-	-	4.66	0.31	1.77

47	2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene		35.818	-	-	-	0.19	0.25	0.09	-	-	0.10
48	4-ethyl-1,2-dimethoxybenzene		36.198	3.83	3.90	2.51	-	-	0.07	0.11	-	0.11
49	(E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one		36.749	0.69	0.68	0.74	0.22	1.03	0.51	1.59	1.04	-
50	(E)-2-butylocta-2,7-dien-1-ol		37.464	-	-	-	0.59	0.30	0.12	-	-	-
51	7-methoxy-1,1-dimethyl-1,2-dihydronaphthalene		38.241	0.48	0.44	0.55	0.35	0.10	-	0.19	0.13	0.13
52	2,2,4-trimethyl-2H-chromene		38.384	--	-	-	-	0.06	0.39	0.18	0.17	0.10
53	1,2,4-trimethoxybenzene		39.356	2.75	1.62	0.27	-	-	-	-	-	-
54	(E)-4-(2,4,4-trimethylcyclohexa-1,5-dien-1-yl)but-3-en-2-one		39.659	0.46	0.49	0.43	-	-	-	0.35	0.76	0.20
55	6,8,8-trimethyl-3-methyleneoctahydro-1H-3a,7-methanoazulene		39.697	-	-	-	3.49	0.59	0.25	-	-	-
56	6-isopropyl-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one		39.884	0.62	1.07	0.72	-	-	-	-	-	-
57	(E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one		39.91	-	-	-	0.23	0.03	0.36	0.21	0.22	0.22
58	1-isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydronaphthalen-4a(2H)-ol		40.111	0.19	0.48	0.39	-	-	-	-	-	-
59	allyl 2-ethylbutanoate		41.557	-	-	-	0.28	0.28	-	-	-	-
60	(E)-3,7,11-trimethyldodeca-1,6,10-trien-3-yl acetate		41.77	0.71	-	-	-	0.08	-	-	-	-
61	3,6,8,8-tetramethyl-2,3,4,7,8,8a-hexahydro-1H-3a,7-methanoazulene		42.177	0.51	-	-	-	0.38	-	-	-	-
62	(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one		42.62	2.52	2.05	2.13	1.21	1.16	0.85	1.35	1.32	0.30
63	(E)-4-(3,3,5-trimethylcyclohexa-1,5-dien-1-yl)but-3-en-2-one		42.946	2.42	1.27	1.92	0.53	0.03	0.22	0.18	-	-
64	4-(2,6,6-trimethylcyclohexa-1-en-1-yl)butan-2-one		43.347	0.21	0.12	0.31	-	-	-	0.22	-	-
65	(E)-4-(3-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl)pent-3-en-2-one		43.638	0.19	0.17	0.23	-	-	-	-	-	-

66	1,2,3,4-tetramethoxybenzene		43.836	0.13	0.17	-	-	-	-	-	-	-
67	(E)-6,10-dimethylundeca-5,9-dien-2-one		44.357	2.49	1.77	1.66	0.71	1.03	0.67	0.76	0.87	0.66
68	(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol		44.862	-	-	-	0.78	0.13	0.07	0.16	0.14	0.15
69	2,6,10,15-tetramethylheptadecane		45.264	0.60	-	0.41	0.26	0.58	0.60	1.28	0.24	0.39
70	1-(4-hydroxy-3,5-dimethoxyphenyl)ethan-1-one		45.79	2.36	1.49	-	-	-	-	-	-	-
71	(E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one		46.101	2.59	2.08	2.23	1.56	1.28	0.87	1.98	2.51	1.45
72	(E)-4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one		46.295	0.95	0.66	0.31	0.31	0.52	0.25	0.55	0.44	0.28
73	6-methyl-2-(4-methylcyclohex-3-en-1-yl)hept-5-en-2-ol		47.319	-	0.68	-	0.39	0.20	0.20	0.32	0.34	0.25
74	4-Methyldocosane		47.706	0.50	0.30	0.30	0.17	0.42	0.51	0.34	0.39	0.16
75	2-hydroxy-3-isopropyl-6-methylcyclohex-2-en-1-one		48.133	1.12	0.93	0.48	-	-	-	0.49	0.37	-
76	4-(2-methyl-3-oxocyclohexyl)butanal		48.14	-	-	-	0.50	1.01	0.69	-	-	-
77	4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one		48.828	1.34	0.79	0.67	0.35	0.49	0.44	0.61	0.25	-
78	3,4-diethyl-1,1'-biphenyl		50.594	0.51	0.41	-	-	-	-	-	-	-
79	3-(3-hydroxybutyl)-2,4,4-trimethylcyclohex-2-en-1-one		50.858	0.68	0.54	0.53	-	0.17	-	0.68	0.59	0.39
80	Nonadecane		53.645	-	0.38	-	0.36	0.63	0.21	-	-	-
81	Cedrol		54.057	7.31	3.56	3.75	-	2.79	0.52	-	-	-
82	2,6,10-trimethyldodecane		59.387	-	0.60	-	0.85	1.09	0.57	0.52	-	-
83	Phenanthrene		62.849	2.26	1.71	0.97	0.36	0.61	0.45	-	-	-
84	6,10,14-trimethylpentadecan-2-one		66.75	2.99	2.76	4.23	2.55	7.48	16.67	5.08	3.25	27.01
				97.69	97.66	95.51	96.65	96.36	83.51	82.89	84.02	91.57

Taking these 84 volatile compounds as variables, partial least squares-discriminate analysis was conducted to predict the category of the dark tea. Shown as Figure 1, Pu-Er raw tea and Pu-Er ripe tea as well as RDGT could be distinguished faultlessly in accordance with the traditional classification of CDT. And the correlation analysis

of compounds showed that 2,6,10,10-tetramethyl-1-oxaspiro[4.5]decan-6-ol, acetophenone, (E)-tetradec-9-en-1-yl acetate, (E)-hept-3-enoic acid, dodecanal, 4-(2-methyl-3-oxocyclohexyl)butanal, (E)-2-(but-2-en-1-yl)-4-hydroxy-3-methylcyclopent-2-en-1-one, 2,2-dimethyl-3-(3-methylbut-2-en-1-yl)-6-methylenecyclohexane-1-carbaldehyde, 2,6,6-trimethylcyclohex-1-ene-1-carbaldehyde and 2-(4-methylcyclohex-3-en-1-yl)propan-2-ol gave the top ten variable importance value. The variable importance values of the volatile compounds were listed in Table 3.

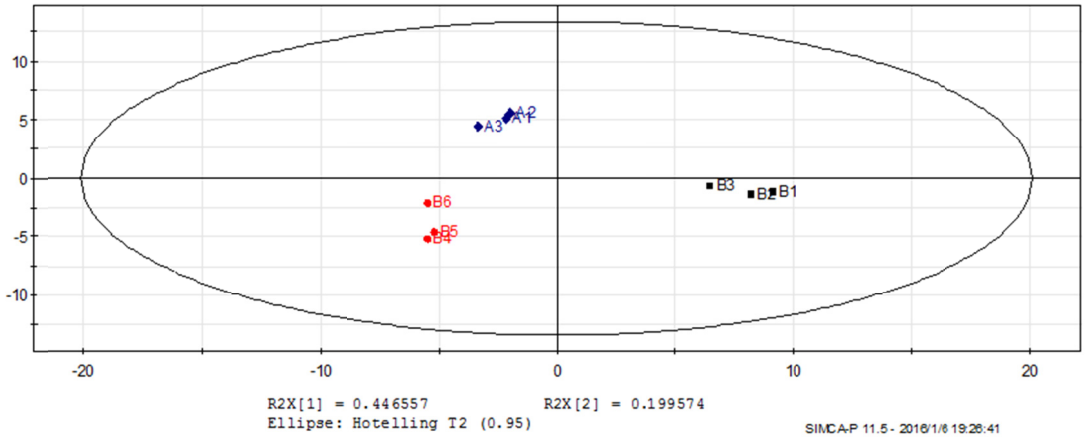


Figure 1. Discrimination of CDTs

As known, pile fermentation was regarded as an oxidative process. Comparing the content of alcohols and aldehydes as well as ketones in Pu-Er raw tea and Pu-Er ripe tea, it was intuitive that oxidation was the dominate transformation during the pile fermentation (Table 3). Existence of amount alcohols especially linalool in Pu-Er raw tea indicated the similar of Pu-Er raw tea to green tea (NO. 20, Table 3). Alkoxides, which was related to the stale flavor of CDT, occupied very great proportion in the volatile compounds of RDGT and Pu-Er ripe tea. On the contrary, only sparse alkoxides were detected from Pu-Er raw tea. And this result illustrated from another aspect that methylation mediated by microorganism taken place in the pile fermentation. Generally, augmentation of esters and ketenes accomplished with the subduction of alcohols along with the storage time in all these 9 CDTs as well as alkoxides in RDGT and Pu-Er ripe tea confirmed the oxidation and esterification during post fermentation attached CDT the longer the storage time the better the quality (Table 3).

Table 3. Variable importance values (VIP) of the volatile compounds

Name of the volatile compounds	VIP
2,6,10,10-tetramethyl-1-oxaspiro[4.5]decan-6-ol	1.54471
acetophenone	1.51515
(E)-tetradec-9-en-1-yl acetate	1.47905
(E)-hept-3-enoic acid	1.42608
dodecanal	1.32897
4-(2-methyl-3-oxocyclohexyl)butanal	1.31365
(E)-2-(but-2-en-1-yl)-4-hydroxy-3-methylcyclopent-2-en-1-one	1.30158
2,2-dimethyl-3-(3-methylbut-2-en-1-yl)-6-methylenecyclohexane-1-carbaldehyde	1.29795
2,6,6-trimethylcyclohex-1-ene-1-carbaldehyde	1.2944
2-(4-methylcyclohex-3-en-1-yl)propan-2-ol	1.27715
3-(3-hydroxybutyl)-2,4,4-trimethylcyclohex-2-en-1-one	1.27447
2-pentylfuran	1.27398
carbamimidoylglycine	1.24257
1-methyl-4-(prop-1-en-2-yl)cyclohexyl acetate	1.24019

(3E,5E)-octa-3,5-dien-2-one	1.21713
1,5,5-trimethyl-6-methylenecyclohex-1-ene	1.17806
Nonadecane	1.16222
(E)-2-butylocta-2,7-dien-1-ol	1.15233
(E)-4-(2,4,4-trimethylcyclohexa-1,5-dien-1-yl)but-3-en-2-one	1.14528
propane-1,2,3-triol	1.12216
2,6,10,10-tetramethyl-1-oxaspiro[4.5]dec-6-ene	1.11826
1-isopropyl-4-methylcyclohex-3-en-1-ol	1.10842
6,6-dimethyl-2-methylenebicyclo[3.1.1]heptan-3-ol	1.10178
Phenanthrene	1.0991
2,6,10-trimethyldodecane	1.09651
allyl 2-ethylbutanoate	1.08414
(E)-4-(3-hydroxy-2,6,6-trimethylcyclohex-1-en-1-yl)pent-3-en-2-one	1.08096
1,2-dimethoxybenzene	1.07282
(2E,4E)-hepta-2,4-dienal	1.07017
(E)-non-2-enal	1.06803
(E)-dec-2-enal	1.06381
4-ethyl-1,2-dimethoxybenzene	1.06093
(E)-4-(3,3,5-trimethylcyclohexa-1,5-dien-1-yl)but-3-en-2-one	1.05513
9,12-octadecadienal	1.05221
6-isopropyl-3-methyl-7-oxabicyclo[4.1.0]heptan-2-one	1.04842
3,7-dimethylocta-1,6-dien-3-ol	1.04822
1,2,3-trimethoxybenzene	1.03638
1,2-dimethoxy-4-methylbenzene	1.03192
5-methyl-2-(prop-1-en-2-yl)cyclohexan-1-ol	1.03057
benzaldehyde	1.01487
(E)-6,10-dimethylundeca-5,9-dien-2-one	1.01414
Cedrol	1.01381
1-isopropyl-4,7-dimethyl-1,3,4,5,6,8a-hexahydronaphthalen-4a(2H)-ol	1.00773
naphthalene	1.00456
'(E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	1.00388
4-methylpentyl 2-methoxybenzoate	1.00184
(E)-4-(2,6,6-trimethylcyclohex-2-en-1-yl)but-3-en-2-one	0.968378
7-methoxy-1,1-dimethyl-1,2-dihydronaphthalene	0.964548
4-ethylcyclohexan-1-ol	0.94432
2-hydroxy-3-isopropyl-6-methylcyclohex-2-en-1-one	0.939252
dodecan-2-one	0.939173
4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one	0.912251
(E)-hex-2-enal	0.91149
6,8,8-trimethyl-3-methyleneoctahydro-1H-3a,7-methanoazulene	0.906313
4-allylphenol	0.897966
(E)-1-(2,6,6-trimethylcyclohexa-1,3-dien-1-yl)but-2-en-1-one	0.889626
1,2,4-trimethoxybenzene	0.852967
(E)-dec-2-en-1-ol	0.827381
4-(2,6,6-trimethylcyclohexa-1-en-1-yl)butan-2-one	0.82483
5-(3,3-dimethyloxiran-2-yl)-3-methylpent-1-en-3-ol	0.824278
3,4-diethyl-1,1'-biphenyl	0.821748
2-methyl-5-(prop-1-en-2-yl)cyclohexan-1-ol	0.819692
1,2,3,4-tetramethoxybenzene	0.819488
1-(4-hydroxy-3,5-dimethoxyphenyl)ethan-1-one	0.801718

2,2,4-trimethyl-2H-chromene	0.760263
2-(5-methyl-5-vinyltetrahydrofuran-2-yl)propan-2-ol	0.758959
3,3-dimethyl-5-oxocyclohexane-1-carbaldehyde	0.757164
(3Z,7E)-12-oxabicyclo[9.1.0]dodeca-3,7-diene	0.725399
(2E,6E)-3,7,11-trimethyldodeca-2,6,10-trien-1-ol	0.725317
heptanal	0.717593
6-methylhept-5-en-2-one	0.712737
1-methyl-4-(prop-1-en-2-yl)cyclohex-1-ene	0.696311
bicyclo[4.4.1]undeca-1,3,5,7,9-pentaene	0.671335
(E)-4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)but-3-en-2-one	0.607334
4-Methyldocosane	0.592105
3,6,8,8-tetramethyl-2,3,4,7,8,8a-hexahydro-1H-3a,7-methanoazulene	0.569998
6,10,14-trimethylpentadecan-2-one	0.55094
(E)-3,7,11-trimethyldodeca-1,6,10-trien-3-yl acetate	0.538597
octanal	0.516773
5-methyl-2-(propan-2-ylidene)hex-4-enal	0.505328
2,6,10,15-tetramethylheptadecane	0.497495
(E)-4-(2,6,6-trimethylcyclohex-1-en-1-yl)but-3-en-2-one	0.438949
2-phenylacetaldehyde	0.363846
6-methyl-2-(4-methylcyclohex-3-en-1-yl)hept-5-en-2-ol	0.278908

4.2 Trends of Quality-Related Ingredients

Content of amino acid in tea was the decisive factor for fresh and mellow. Shown as Figure 2, the content of free amino acids in RDGT as well as Pu-Er ripe tea decreased as the accumulation of storage time. Whereas, free amino acids consisted in Pu-Er raw tea were incremental with storage time. Meanwhile, comparing B2 with B4, it could be concluded that Pu-Er ripe tea and Pu-Er raw tea prepared from the same fresh leaves at the same time distinguished with each other in the content of amino acids. Furthermore, it is obvious that Pu-Er raw tea contained more dose of free amino acids than Pu-Er ripe tea integrally.

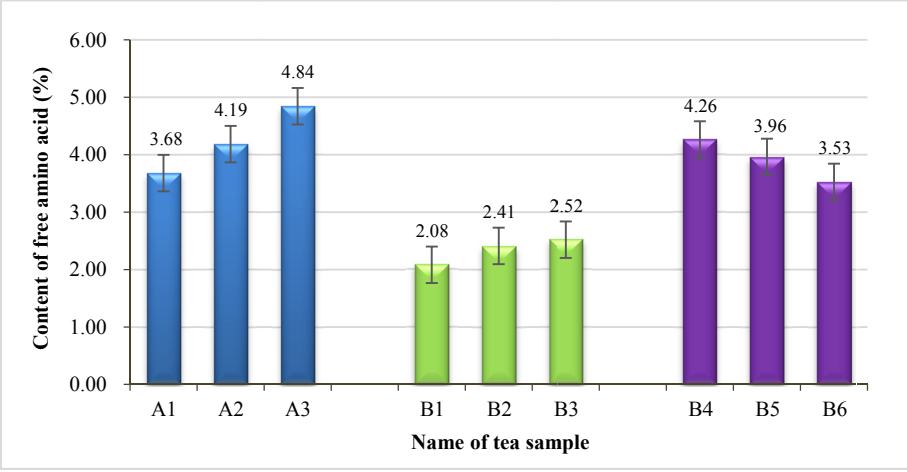


Figure 2. Difference of free amino acid contained in CDTs

Soluble total sugar in tea was another indicator that affects the quality of CDT. From Figure 3, it could be found that in raw dark green tea as well as Pu-Er raw tea, content of soluble total sugar was augmented with the storage time. In contrast, the content decreased as the time in Pu-Er ripe tea. By the way, the content of soluble total sugar in CDT was at a high level than in green tea.

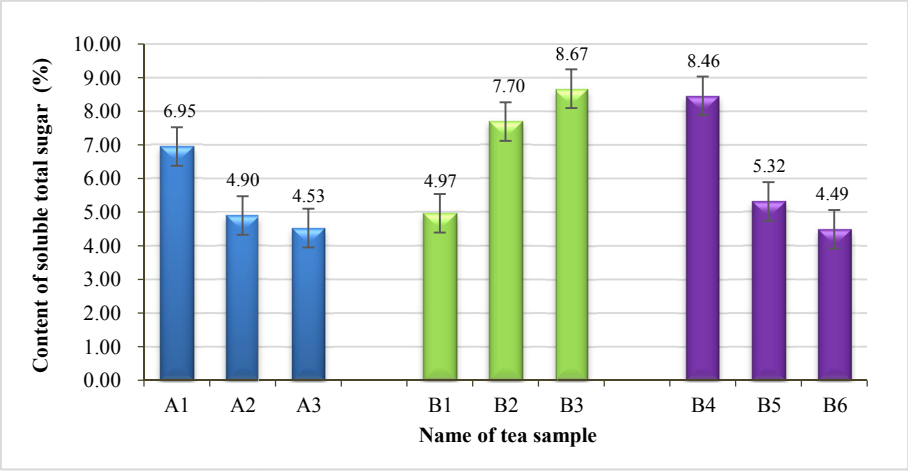


Figure 3. Difference of soluble total sugar contained in CDTs

At the other hand, high performance liquid chromatography (HPLC) was used to analyse the constituents of catechins. Shown as in Figure 4, the content of epi-gallo-catechin-gallate (EGCG), gallo- catechin-gallate (GCG), epi-catechin gallate (ECG) and catechin gallate (CG) were accumulated as the storage time of RDGT. Only the content of EGCG and GC in Pu-Er ripe tea showed the trend of accumulation with storage time, similar as RDGT. In contract, the content of gallo-catechin (GC), catechin (C) and ECG in Pu-Er raw tea was a descending series accompanying with time. It is worth to noted that the total content of catechines in RDGT and Pu-Er raw tea were much higher than in Pu-Er ripe tea. The content of catechines in CDTs were enclosed in Table 4.

Table 4. Contents of catechines in CDTs

Sample	Content(‰)							
	GC	EGC	C	EGCG	EC	GCG	ECG	CG
A1	47.17	25.07	16.16	96.01	81.01	63.59	13.39	5.48
A2	85.00	39.41	31.23	56.72	0.05	14.17	1.56	0.13
A3	75.11	29.71	29.71	47.52	0.15	3.15	1.10	0.09
B1	4.34	0.41	6.47	4.30	0.44	0.16	0.23	0.09
B2	3.82	0.17	1.32	0.31	0.02	0.57	0.21	0.51
B3	2.45	0.92	2.19	0.25	0.04	0.58	0.19	0.31
B4	28.94	4.97	43.75	91.06	0.12	14.44	0.12	0.85
B5	34.16	22.11	54.50	9.00	183.30	45.49	4.77	0.13
B6	35.74	21.71	55.59	99.94	4.36	14.70	7.86	1.37

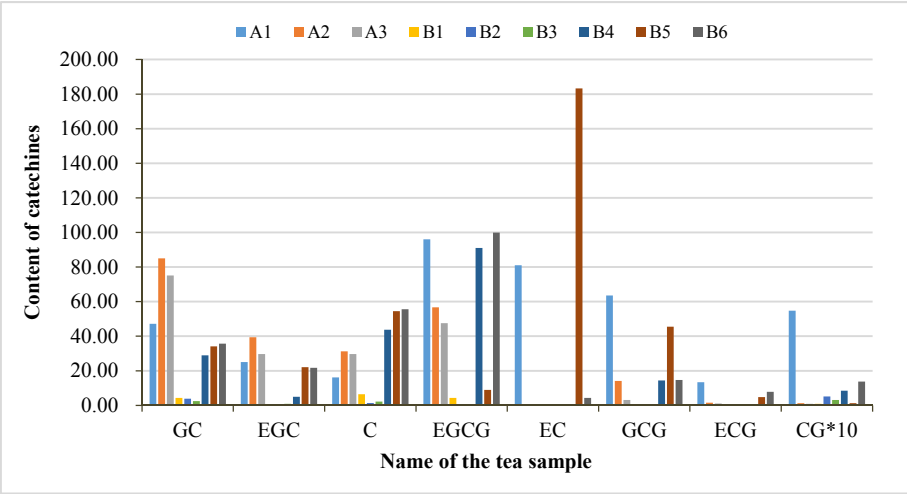


Figure 4. Contents of catechines in CDTs

At the same time, sensory evaluation was conducted according to GB/T 14487-2008 as well as GB/T 23776-2009 and the results were disclosed as below in Table 5. Overall, both Pu-Er raw tea and Pu-Er ripe tea obtained higher score than RDGT.

Table 5. Sensory evaluation of CDTs

Sample	Score of sensory evaluation					
	Shape 25%	Color 10%	Aroma 25%	Taste 30%	Leaf 10%	Total
A1	85	86	83	83	86	84.1
A2	80	86	84	82	83	82.5
A3	75	90	80	85	80	81.3
B1	86	93	88	88	76	86.8
B2	90	92	92	88	78	88.9
B3	84	91	86	82	78	84
B4	92	86	86	86	85	87.4
B5	80	92	80	82	86	82.4
B6	84	89	85	82	82	84

According to literature, microorganism played important role in the post-fermentation of CDT (Fu et al., 2012; Zhao & Liu, 2014; Zhou et al., 2014). Decline of the content of free amino acids in Pu-Er ripe tea as well as RDGT gave an evidence, at least partially, that reproduction of microorganism during the post fermentation would consume free amino acids (A1, A2, A3 and B1, B2, B3, Figure 2). The difference of free amino acids contents between Pu-Er raw tea and Pu-Er ripe tea demonstrated that breeding of microorganism during the pile fermentation consumed free amino acids dramatically (B2 and B4, Figure 2). Without the pile fermentation, free amino acids reserved commendably in Pu-Er raw tea (B4, B5 and B6, Figure 2).

It is well known that the material for CDT was coarse and old, so the content of cellulose in CDT was much higher than the other teas. When it turned to soluble total sugar, the tendency was increasing which benefited from the hydrolysis of cellulose consisted in the old leaves. In respect to the attenuation of soluble total sugar in Pu-Er ripe tea, consumption by microorganism was much stronger than the hydrolysis of cellulose (B1, B2 and B3, Figure 3).

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