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CHARACTERIZATION OF THE KEY AROMATIC CONSTITUENTS IN TEA FLOWERS OF ELITE CHINESE TEA CULTIVARS

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ABSTRACT

The volatiles in flowers of twenty-three elite tea cultivars were obtained by simultaneous distillation extraction, from which seventy-nine aromatic constituents were identified using gas chromatography coupled with mass spectrometry. The major detected constituents found were acetophenone, linalool, 1-hexanol, 2-pentanol, methyl salicylate, 4-methyl-2-hexanone, alpha-methyl-benzene methanol, cis-linaloloxide, acetic acid, Z-3-Hexen-1-ol, S-2-heptanol, and hexanal. Both acetophenone and linalool were determined from every cultivar, and each volatile accounted for more than 20 % of the total. Several of the cultivars analysed contained some special constituents which were not detected from other cultivars.

Keywords: elite tea plant cultivar; tea flowers; volatiles; aroma constituents

INTRODUCTION

Usually tender tea buds and leaves of Camellia sinensis are plucked and processed into high grade tea of commerce, as a beverage. During the past several years, a few tea companies in China and India have begun to process the fresh tea flowers into dry ones for drinking and for blending into specialty teas, though normally, after blooming the large number of tea flowers wither away without being harvested. Up to date the tea growing area in China fills around 1.33×10^{6} ha, occupying the first position in the world, and throughout the area dozens of well-bred tea plant cultivars or elite selections (clones) have been cultivated. In China, from September to November, tea flowers bloom and attract bees and wasps. The bees swarm into tea gardens to collect nectar from the flowers and produce honey. Thus the tea flowers have become one of main honey fountains in China. As well as bees, several braconid wasps, Apanteles spp. also flit between tea flowers.

So far there has been little understanding or knowledge about the aroma of tea flowers, though You et al. (1990) identified twenty six constituents and confirmed the major constituents from the volatiles of flowers of a little leaf cultivar (Zaobaidan), a mid leaf cultivar (Zhongcha) and a large leaf cultivar (Linguidaye). They also provided a formula for calculating tea flower terpene index (You et al., 1992). We chose twenty-three elite tea cultivars with a spread of growing area, analyzed their aromatic composition, and discussed the aromatic characteristics so as to provide reference to the further utilization of tea flowers.

MATERIALS AND METHODS

Plant Materials. The blossoming process of tea flowers was divided into four stages i.e. tight bud, split bud, bursting and full bloom (You et al., 1990). As the aroma type profile during the split stage was like those during bursting and during full blooming stages, therefore, the tea flowers during bursting were used as the experimental material in this study. Tea flowers

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were provided from the national tea plant idioplasm resource garden located in Tea Research Institute of Chinese Academy of Agricultural Sciences. Flowers used were from cultivars Anjibaicha (AJBC), Baihaozao (BHZ), Cuifeng (CF), Fudingdabaicha (FDDBC), Fuyun 6 (FY6), Guihong 4 (GH4), Huangjingui (HJG), Jinmianqilan (JMQL), Longjing 43 (LJ43), Longjingchangye (LJCY), Maoxie (MX), Qianmei (QM), Qinxinqilan (QXQL), Ruanzhiwulong (RZWL), Tieguanying (TGY), Wuniuzao (WNZ), Xicha 11 (XC11), Yingshuang (YS), Zhenong 113 (ZN113), Zhenong 117 (ZN117), Tieluohan (TLH), Aijiaowulong (AJWL), and Zhenghedabaicha (ZHDBC). These tea plants growing in the idioplasm resource garden originated from throughout Chinese tea plants growing region.

Essential Oil Preparation. The essential oils of tea flowers were prepared by simultaneous distillation extraction method (SDE). Put 50 g fresh tea flowers into a 2 L flask, infuse with 1,000 ml boiling distilled water, then add 1 ml of 10 - 4 g/ml decanoic acid ethyl ester as an internal standard. Put 50 ml of distilled ether into the extraction flask. After tea flowers were distilled for 20 min, a little anhydrous sodium sulfate was added into ether extraction solution, which was placed into a refrigerator overnight.

Sperarации and Identification of Aromatic Constituents in Essential Oils. Essential oils were analyzed by gas chromatography / mass spectrometry (GC/MS). GC/MS analysis was carried out using a HP 5890 GC coupled with 5972 MSD. A quartz capillary (PEG-20M) column (50 m ´ 0.20 mm Ø, with 0.25 mm film thickness) was used with helium as the carrier gas. The oven temperature was increased from 50°C up to 250°C at a rate of 4°C per minute and then kept constant at 200°C for 5 minutes. Injector temperature was 200°C, and interface temperature was 250°C. No split. MS was taken at 70 eV. The overnight collected essential oil was concentrated to about 20 ml under a stream of nitrogen, 1 ml of which was immediately injected into a GC/MS. The identification of all constituents was confirmed by GC/MS library, and the library search was carried out using the Wiley GC/MS Library. At the same time, the identification of the partial constituents was confirmed by retention time and peak enhancement on co-injection with authentic commercial samples. The relative content of every constituent was the ratio of its measured peak area to the internal standard peak area.

RESULTS AND DISCUSSION

Major constituent volatiles were acetophenone and linalool: these were detected from all of the twenty-three subject cultivars. Acetophenone comprised between 20 % and 30 % of the total amount of aroma; it was highest at 38 % (in cultivar LJ43), and lowest at 3 % (in LJC). Linalool comprised between 2 % and 10 % of total amount of aroma, the highest was 26.3 % (in TGY), and the lowest was 1.7 % (in XC11). Other major volatile aroma constituents followed as: 1-hexanol, 2-pentanol, methyl salicylate, 4-methyl-2-hexanone, alpha-methylbenzene methanol, cis-linaloloxide, acetic acid, Z-3-hexen-1-ol£¬S-2-heptanol, and hexanal. The benzaldehyde content was high in several cultivars. Some cultivars had special constituents. For example, seven constituents in cultivar MX were not detected in other cultivars, i.e. heptanal, 3-carene, 3-methyl-pyridine, 1-heptanol, benzyl alcohol, Z-3-tetradecene, 2-methoxy-benzoic acid methyl ester. However, the amount of these special constituents was low. **Table 1** compares the levels of all 79 aroma volatiles detected in the 23 tea cultivars of this study.

Although containing the same constituents of tea flower aroma, many other fresh plant flowers also contain abundant esters and consequently have a heavy perfume. Tea flowers have only a light faint scent, for the lack of esters. The main constituents of tea flower aroma have been determined in tea leaf aroma, too. However, the finger prints of tea flower aroma were different from those of typical tea leaf aroma (Jiang et al., 2005; Miao et al., 2003; Schuh et al., 2006; Wang et al., 1993; Yamanishi, 2004). The high grade teas of commerce each have a perfume based upon its aroma profile but while the constituents are similar to those of tea flowers, the ratio of constituents is different. Like tea leaves, tea flowers contain linalool, linaloloxide, geraniol and nerol and so on, which contribute to their tea flower aroma. Therefore, the dry tea flowers smell somewhat of tea but have a certain scent of their own.

While the tea flowers blossom, some braconid wasps, Apanteles spp. also flit between the flowers. This suggests that benzaldehyde is released from certain cultivars and attracts these wasps (Han et al., 2002). The bees certainly like tea flowers, for the aroma of tea flowers always attracts bees. If bioassay by olfactometer or electroantennogram were used to investigate this behaviour, some responsible aromatic constituents may be discovered in the flowers.

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Table 1 Volatiles* from Tea Flowers of Twenty-Three Elite Tea Cultivars

C> V*	AJBC	BHZ	CF	FDDBC	FY6	GH4	HJG	JMQL	LJ43	LJCY	MX	QM	QXQL	RZWL	TGY	WNZ	XC11	YS	ZN113	ZN117	AJWL	TLH	ZHDBC
1	0.037			0.067	0.093	0.110	0.064	0.043	0.006		0.034	0.151							0.017				
2	0.530	0.460	0.073		0.057	0.214	0.758	1.162	0.104	0.243			0.055	0.226		0.108	0.019	0.630		0.710			
3	0.030																						
4	0.034	0.062	0.023	0.078	0.188		0.050			0.056	0.012	0.128		0.040	0.208	0.004				0.027			
5	0.743	0.257	0.390	0.385	0.179	0.170	0.486	0.242	0.188	0.248	0.035	1.026	0.041	0.111	0.159		0.360	0.494	0.376	0.523			0.179
6	0.159	0.083						0.177	0.103	0.065		0.282				0.035							
7	0.276		0.334		1.011		0.293	0.177	0.208	0.170						0.045	0.461	0.307	0.395	0.391			
8	0.091	0.260	0.096		0.567	0.216	0.127	0.168	0.101	0.117	0.045	0.282	0.136	0.127	0.255	0.037	0.104	0.073	0.175	0.106	0.089		
9	0.127																						
10	0.155	0.316	0.330	0.309	0.496	0.948	0.864	1.509	0.504	0.072	0.604	0.930	0.542	1.063	2.932	0.081	0.145	0.398	0.270	0.391	1.076	0.614	0.405
11	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1.429	3.252	1.640	2.313	2.804	1.689	0.779	2.097	1.945	0.088	0.513	2.213	2.070	0.847	2.826	0.367	1.502	1.510	1.497	1.357	0.244	0.408	0.670
13	0.073																					0.082	0.106
14	0.283	0.317	0.117	0.237	0.195		0.088	0.154	0.094				0.117	0.056	0.368	0.002	0.403			0.120			
15	0.177	0.628						0.135			0.153		0.551				0.556	0.109					
16	1.271	1.774	2.073	2.304		4.500	1.592	1.009			0.432	2.647	0.671	1.045	1.718	0.034	3.336	0.660		1.359			
17		0.053	0.023							0.213													
18		0.035			0.196				0.013						0.008	0.008	0.063						
19		0.477									0.079									0.182			
20		0.147			0.761			0.167	0.107	0.041		0.104		0.075	0.133	0.037				0.099	0.162	0.127	0.047
21		0.324	0.227	0.148	0.706	0.635			0.051	0.144	0.038	0.439	0.348	0.085	0.284		0.385		0.152	0.071			
22		0.160			1.766					0.082	0.128		0.137			0.162							
23			0.022																0.042				
24			0.013				1.105		0.027														
25			0.145			0.068	0.041	0.050				0.107									0.160		
26			0.012	0.079			0.024	0.004	0.045	0.043		0.153	0.030	0.036		0.008							
27			0.038		0.211		0.073	0.084		0.143		0.129		0.040	0.217	0.014	0.049	0.025					
28			0.271			1.473				0.058		1.244			0.548	0.101					0.215	0.814	0.484
29			0.073											0.128	0.325		0.097						
30				0.070								0.139						0.021					

31			0.691		0.080					0.044	0.440	0.174	0.128		0.043							
32			0.273														0.024					0.026
33			0.240					0.037		0.101		0.034						0.156				
34				0.142															0.061	0.061		0.026
35				0.433																		
36				0.801	0.197	0.183								0.014						0.267		0.155
37				0.862																		
38					0.611		0.396			0.042			0.182									
39					0.784					0.067												
40					1.076						0.524											
41					0.811			0.046	0.143	0.098					0.032		0.243	0.132				
42					0.424	0.038																
43						0.050										0.044						
44						0.041		0.032		0.035										0.023		
45						0.156											0.126				0.092	
46						0.086	0.039									0.107				0.096		
47							0.126	0.070														
48								0.095														
49									0.331					0.142					0.053	0.976	1.635	2.678
50								0.012														
51										0.022												
52										0.032												
53										0.069												
54										0.033												
55										0.094												
56										0.054												
57										0.069												
58											4.242											
59												0.038										
60												0.579										

61												0.114										
62												0.103										
63																	0.169					
64																		0.046				
65																		1.926				
66																			0.101			
67																				0.119		
68																				0.310	0.501	
69																				0.102		
70																				0.343		0.172
71																				0.188		
72																				1.696		
73																				0.221		0.093
74																					0.144	0.094
75																					0.214	
76																					0.788	1.478
77																					0.133	
78																					0.18	
79									0.023													

***** V, VOLATILES: . 1, Ethyl acetate. 2, Ethanol. 3, 2-Butanol. 4, Hexanal. 5, 2-Pentanol. 6, *E*-2-Hexenal. 7, *S*-2-Heptanol. 8, 1-Hexanol. 9, *Z*-1-Hexen-1-ol. 10, linalool. 11, Decanoic acid,ethyl ester. 12, Acetophenone. 13, beta-Myrcene. 14, Benzene methanol,alpha-methyl. 15, Phenylethyl alcohol. 16, 2-Hexanone,4-methyl. 17, Butanal. 18, 1-Penten-3-ol. 19, 2-Hexenal. 20, Z-3-Hexen-1-ol. 21, Methyl salicylate. 22, 1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl. 23, 2-Pentanone. 24, 1-Octanol. 25, Butanal,3-methyl. 26, Acetic acid. 27, cis-Linaloloxide. 28,Nerol. 29, E- 2-Octenal. 30, Cyclopentanol. 31, 2-Heptanol. 32, 1-Butanol. 33, Nonanal. 34, E-2-Penten-1-ol. 35, 2-Butanol,3-methyl. 36, Furan,2,5-dihydro. 37, Pyridine,4-methyl. 38, 2-Furanmethanol,5-ethenyltetrahydro-al. 39, alpha-Farnesene. 40, trans-Linaloloxide. 41, geraniol. 42, 3-Cyclohexene-1-methanol,alpha,alpha. 43, 3-Hexanol. 44, Limonene. 45, Z-3-Hexenal. 46, Octanal. 47, 2-Furanmethanol. 48, 1,6-Octadiene,3,5-dimethyl,[Z]. 49, Benzaldehyde. 50, 3-Pentanol. 51, Heptanal. 52, 3-Carene. 53, Pyridine,3-methyl. 54, 1-Heptanol. 55, Benzyl alcohol. 56, Z-3-Tetradecene. 57, Benzoic acid,2-methoxy,methyl ester. 58, 7-Octen-2-ol,2-methyl-6-methylene. 59, Pentanal. 60, Naphthalene,1,2,3,4,4a,5,6,8a-octahydro. 61, Camphene. 62, Nerol. 63, Salicylate acid. 64, 2-Pentanol,4-methyl. 65, 2-Heptanone,4-methyl. 66, cis-s-Hexenyl acetate. 67, Z-4-Heptenal. 68, 2-Hexanol. 69, E-4-Heptenal. 70, 4-methyl-3-Penten-1-ol. 71, Z-2-Nonenal. 72, Pyrrolidine. 73, E- 4-Hepten-2-ol. 74, Pyridine. 75, 2-Pentenal,2-methyl. 76, 2-Hexanone,5-methyl. 77, 1,5,7-Octatrien-3-ol,2,6-dimethyl. 78,1-Undecanol. 79, Cyclobutanol