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COMPARATIVE INVESTIGATION OF VOLATILE AROMA COMPOUNDS IN SELECTED TEA CLONES (*CAMELLIA SINENSIS* L.)

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Summary: Seasonal and clonal variations in aroma compounds of Iranian native clones of green tea were studied. Aromatic compounds were extracted by hydro-distillation using a Clevenger system. The aroma constituents were analyzed by gas chromatography-mass spectrometry (GC-MS). Differences in quality of experimental clones in terms of aroma composition during various seasons were recorded. Substantial differences in the chemical composition of samples were found to be related to seasonal variations and genetic differences within clones. The main compounds of clone 100 were linalool (2.55-20.07%), geraniol (1.14-20.00%), *trans* linalool oxide (furanoid) (0.64-15.50%) and heneicosane (0.27-12.30%). On the other hand, clone 578 contained methyl salicylate (12.60%), *trans* linalool oxide (furanoid) (10.80%), phytol (1.80-9.17%) and linalool (8.74%) as main constituents. Finally the major compounds found in clone 444 were linalool (7.62-48.58%), methyl salicylate (1.45-9.34%), tricosane (0.53-5.28%) and eicosane (0.53-4.39%). Therefore, clones 100 and 444 are recommended as preferred clones for their quality of specific aroma and flavor components.

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Keywords: Aroma compounds; *Camellia sinensis* L.; Clone; GC-MS.

Abbreviations: GC-MS – gas chromatography-mass spectrometry.

INTRODUCTION

Tea obtained from apical leaves and buds of *Camellia sinensis* (L.) Kuntze is one of the most popular beverages, well known for its flavor and aroma. Differences in tea aroma and taste could be related to several factors. These include geographical variations (Borse et al., 2002; Takeo and Mahanta, 1983; Yamanishi et al., 1968), seasonal changes (Sharma et al., 2011; Erturk et al., 2010; Cloughley et al., 1982), genetic variations (Magoma et al., 2000), processing during manufacture (Shoae Hassani et al., 2008) and biotic injury (Dong et al., 2011). It

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is expected, therefore, that vegetatively propagated cultivars (VPC), clones of *Camellia sinensis*, planted at least a decade ago, should exhibit differences in chemical composition in comparison to other green teas. There may also be variations in flavoring components among these Iranian clones, and differences in harvest times may affect composition as well.

In this study, a survey of the compounds present in tea extracts of *C. sinensis* var. *sinensis* was conducted and variation between clones was determined. Compounds influencing tea aroma and taste were studied in unprocessed tea samples, thus avoiding variations resultant from differences in processing methods. In determining the relative abundance of compounds known to affect tea aroma and taste, a qualitative comparison of tea clones has been generated.

MATERIALS AND METHODS

The aerial parts of three tea clones (100, 578 and 444) *Camellia sinensis* var. *sinensis* were plucked from Tea Research Station of Lahijan (province of Guilan, Iran) (altitude 34.2 m amsl, latitude 37° 11' S, longitude 50° 0' E) during the summer and autumn 2009 as well as in spring 2010. A voucher specimen was deposited in the Herbarium of Guilan University (GUH, number 4038). 50 g fresh tea shoots (*C. sinensis*) consisting of one apical bud and two adjoining leaves were picked**.** Samples were minced and immediately hydrodistilled for 3 h using a modified Clevenger-type apparatus (Derwich et al., 2009).

Aroma-associated compounds were isolated by steam distillation under vacuum followed by solvent extraction of the distillate with diethyl ether. Sodium sulfate was used for dehydration and the compounds were stored at 4°C in the dark until further analysis as described below.

GC-MS analysis was carried out using Agilent 6890N coupled to Agilent 5973B MS. Samples were analyzed on a capillary column HP-5MS (30 m × 0.25 mm, film thickness 0.5 μm) with electron impact ionization (70 eV). The carrier gas was helium with a flow rate of 1 ml/min. Injector and detector temperatures, 280°C; injected volume, 1 μl; splitless mode; the oven temperature program was 50°C for 2 min, increased at 3°C/min to 250°C and held at 250°C for 5 min. The mass range was 30-600 m/z.

The aroma-associated constituents of the tea samples were identified in comparison with their Kovats index, calculated in relation to the retention time of a series of lineary alkanes (C8-C38) with those of reference products comparing with their Kovats index and those of chemical components gathered by Adams (Adams, 2001). Further identification was made by matching their recorded mass spectra with those stored in the WILEY7n.L mass spectral library. The composition of aromas was reported as a relative percentage of the total peak area.

RESULTS AND DISCUSSION

Volatile components of three Iranian tea clones (100, 444 and 578) (*Camellia sinensis* var. *sinensis*) were compared in seasonal harvests (August and December 2009; May 2010). The results obtained from the analysis of the aroma compounds of three tea clones (100, 578 & 444) are shown in Tables 1, 2 and 3.

Table 1 (Part I). Aroma compounds identified in tea clone 100. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
1	Thiazole,4-methyl	819	-	0.24	-
2	Hexanol	871	0.06	-	-
3	Heptanal	902	0.55	-	-
4	Cyclopentanone,2-methyl		0.47	-	-
5	Cyclohexanone	952	7.06	-	-
6	*β*-Myrcene	991	0.58	-	-
7	3,4,5-Trimethyl Isothiazole	996	0.12	-	-
8	Limonene	1029	0.30	0.32	-
9	Benzyl alcohol	1032	0.89	-	-
10	Phenylacetaldehyde	1042	0.33	-	-
11	*Trans* linalool oxide (furanoid)	1073	15.50	2.53	0.64
12	*Cis* linalool oxide (furanoid)	1087	3.75	1.26	0.25
13	Furfuryl alcohol	1087	-	0.12	-
14	Linalool	1097	20.07	8.14	2.55
15	Nonanal	1101	-	0.17	0.26
16	1,5,7-octatrien-3-ol,3,7-dimethyl		0.21	-	-
17	Benzeneethanol	1107	1.36	-	-
18	*Cis* linalyl oxide (pyranoid)	1174	0.19	-	-
19	Methyl salicylate	1192	4.39	1.14	0.38
20	Dodecane	1200	-	0.45	0.12
21	Nerol	1230	0.47	-	-
22	Neral	1238	0.95	-	-
23	Geraniol	1253	20.00	1.14	2.01
24	(2E)-Decenal	1264	-	-	0.18
25	Geranial	1267	0.17	-	-
26	Indol	1291	0.29	-	-
27	Tridecane	1300	-	0.37	0.13
28	*α*-Copaene	1377	0.13	-	-
29	*Cis*-Jasmone	1393	0.31	-	-
30	Tetradecane	1400	0.27	0.50	0.27
31	*β*-Caryophyllene	1425	0.09	-	-
32	(E)-*α*-Ionone	1430	-	-	0.07
33	(E)-*β*-Ionone	1489	0.08	-	-
34	Pentadecane	1500	-	0.85	0.40
35	*δ*-Cadinene	1523	0.57	0.37	0.06

Table 1 (Part II). Aroma compounds identified in tea clone 100. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
36	*Cis*-calamenene	1540	0.09	-	-
37	*α*-Calacorene	1546	0.03	-	-
38	Hexadecane	1600	0.13	0.60	0.49
39	*β*-Eudesmol	1651	-	1.95	-
40	*α*-Cadinol	1654	0.18	0.16	-
41	2-pentadecanone,6,10,14-trimethyl	1681	0.11	-	-
42	3,6,6-Trimethylcyclohexa-2-en-1-ol		0.87	-	-
43	Heptadecane	1700	0.07	0.10	0.46
44	(Z,E)-Farnesol	1701	1.39	0.47	0.21
45	Benzyl benzoate	1760	-	0.19	-
46	Octadecane	1800	0.13	1.42	4.45
47	Benzothiazole	1873	0.17	-	0.32
48	Nonadecane	1900	0.27	3.73	2.10
49	Methyl palmitate	1922	0.28	0.12	-
50	Phytol	1943	6.23	-	4.37
51	Isophytol	1948	0.17	-	-
52	Palmitic acid	1957	0.15	-	-
53	Eicosane	2000	0.16	1.10	0.53
54	9-Octadecenoic acid	2004	-	0.12	-
55	Heneicosane	2100	0.27	12.30	1.30
56	Docosane	2200	0.15	1.31	2.02
57	Tricosane	2300	0.26	4.37	3.63
58	Tetracosane	2400	0.12	1.64	4.19
59	Pentacosane	2500	0.14	2.72	3.02
60	Hexacosane	2600	-	0.92	0.47
61	Dibutyl phthalate	2630	-	0.61	-
62	Octacosane	2800	0.26	0.24	-
	Total % composition		90.79	51.67	34.88
	Monoterpene hydrocarbons		0.88	0.32	-
	Oxygenated monoterpenes		61.10	13.07	5.45
	Sesquiterpene hydrocarbons		0.91	0.37	0.06
	Oxygenated sesquiterpenes		1.57	2.58	0.21
	Alkanes		2.23	32.62	23.58
	Others		24.10	2.71	5.58

[a]KI: Kovats Index was determined by GC-MS on a HP-5MS column.

Table 2 (Part I). Aroma compounds identified in tea clone 578. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
1	2,4-Pentanedione	700	-	0.58	-
2	1-Methyl butanol	741	0.07	-	-
3	2-Pentanol	771	0.77	-	-
4	2-Butanol,3-methyl	774	0.32	-	-
5	*β*-Myrcene	991	1.07	-	-
6	3,4,5-trimethyl Isothiazole	996	1.43	-	-
7	Acetic acid		6.84	-	-
8	Benzyl alcohol	1032	-	1.68	-
9	(E)-*β*-Ocimene	1050	-	-	3.34
10	*Trans* linalool oxide (furanoid)	1073	-	10.80	-
11	*Cis* linalool oxide (furanoid)	1087	-	4.96	0.80
12	Linalool	1097	-	8.74	-
13	Benzeneethanol	1107	-	1.05	-
14	Terpineol	1148	-	0.31	-
15	*Cis* linalyl oxide (pyranoid)	1174	-	1.33	-
16	Methyl salicylate	1192	-	12.60	-
17	Dodecane	1200	-	0.40	-
18	Nerol	1230	-	0.50	-
19	Geraniol	1253	-	2.23	-
20	Bornyl acetate	1289	-	0.62	-
21	Tetradecane	1400	0.29	0.58	-
22	3,4-dihydro-*β*-ionone	1421	-	0.33	-
23	Pentadecane	1500	-	0.53	-
24	Tridecanal	1510	0.58	-	-
25	Hexadecane	1600	-	0.78	-
26	Heptadecane	1700	-	0.77	1.07
27	(Z,E)-Farnesol	1701	-	3.08	1.43
28	Octadecane	1800	-	0.90	0.76
29	Nonadecane	1900	-	0.76	0.85
30	Methyl palmitate	1922	-	0.26	0.80
31	Phytol	1943	-	1.80	9.17
32	Palmitic acid	1957	3.21	-	-
33	Eicosane	2000	-	0.95	1.25
34	9-octadecenoic acid	2004	2.05	-	-
35	Heneicosane	2100	-	1.82	5.17

Table 2 (Part II). Aroma compounds identified in tea clone 578. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
36	Docosane	2200	-	2.08	0.58
37	Tricosane	2300	-	2.98	5.20
38	Tetracosane	2400	-	2.83	0.86
39	Pentacosane	2500	-	2.79	7.59
40	Dibuthyl phthalate	2630	-	-	3.98
41	Octacosane	2800	-	0.21	-
	Total % composition		16.63	69.25	42.85
	Monoterpene hydrocarbons		1.07	-	3.34
	Oxygenated monoterpenes		-	29.49	0.80
	Oxygenated sesquiterpenes		-	3.08	1.43
	Alkanes		0.29	18.38	23.33
	Others		15.27	15.30	13.95

[a]KI: Kovats Index was determined by GC-MS on a HP-5MS column.

Table 3 (Part I). Aroma compounds identified in tea clone 444. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
1	Cyclohexanone	952	6.36	-	-
2	2-Hexanol,3-methyl		7.34	-	-
3	1-Octen-3-ol	979	0.52	-	-
4	*Cis*-3-Hexenyl acetate	1005	-	0.27	-
5	Limonene	1029	0.47	-	-
6	Acetic acid		3.02	-	-
7	*Trans* linalool oxide (furanoid)	1073	4.14	0.51	-
8	*Cis* linalool oxide (furanoid)	1087	1.28	0.23	-
9	Linalool	1097	48.58	7.62	8.57
10	Benzeneethanol	1107	0.02	-	-
11	Terpineol	1148	-	0.16	-
12	Methyl salicylate	1192	9.34	1.45	-
13	Dodecane	1200	-	0.16	-
14	Geraniol	1253	1.15	0.20	-
15	Tridecane	1300	-	0.21	-
16	2-Undecanone,6,10-dimethyl	1370	0.36	-	-

Table 3 (Part II). Aroma compounds identified in tea clone 444. Data are presented as a relative percentage of the total peak area.

Peak No.	Compounds	KI[a]	Harvest time		
			Spring	Summer	Autumn
17	*Cis*-3-Hexenyl hexanoate	1384	0.29	-	-
18	(E)-*β*-Damascenone	1385	-	0.23	-
19	*Cis*-Jasmone	1393	0.51	-	-
20	Tetradecane	1400	-	0.47	1.42
21	(E)-*β*-Damascone	1414	-	0.11	-
22	3,4-dihydro-*β*-ionone	1421	-	0.11	-
23	Pentadecane	1500	-	0.82	2.42
24	*δ*-Cadinene	1523	0.81	0.18	-
25	Z-Nerolidol	1533	-	0.62	-
26	Hexadecane	1600	-	1.02	3.09
27	Heptadecane	1700	-	1.01	2.90
28	(Z,E)-Farnesol	1701	0.91	-	-
29	Octadecane	1800	-	1.07	2.66
30	Nonadecane	1900	-	0.94	5.13
31	Methyl palmitate	1922	0.33	0.31	-
32	Phytol	1943	0.89	-	-
33	Palmitic acid	1957	0.31	-	-
34	Eicosane	2000	0.53	4.39	3.63
35	1-Octadecanol	2078	-	0.26	-
36	Heneicosane	2100	-	2.11	3.71
37	Docosane	2200	-	2.74	3.56
38	Tricosane	2300	0.53	4.09	5.28
39	Tetracosane	2400	-	4.90	-
40	Pentacosane	2500	-	0.37	-
41	Hexacosane	2600	-	0.42	5.73
42	Dibutyl phthalate	2630	-	0.32	-
43	Octacosane	2800	-	1.39	2.22
	Total % composition		87.69	38.69	50.32
	Monoterpene hydrocarbons		0.47	-	-
	Oxygenated monoterpenes		55.15	8.99	8.57
	Sesquiterpene hydrocarbons		0.81	0.18	-
	Oxygenated sesquiterpenes		0.91	0.62	-
	Alkanes		1.06	26.11	41.75
	Others		29.29	2.79	-

[a]KI: Kovats Index was determined by GC-MS on a HP-5MS column.

The aroma profile of tea clone 100 in spring, summer and autumn (Table 1) was dominated by terpenoids, such as Linalool (2.55-20.07%), which was present in the highest amounts, followed by geraniol (1.14-20.00%) and *trans* linalool oxide (furanoid) (0.64-15.50%). Other compounds including heneicosane (0.27-12.30%), phytol (4.37-6.23%), cyclohexanone (7.06%), octadecane (0.13-4.45%), tricosane (0.26-4.37%), methyl salicylate (0.38-4.39%) and tetracosane (0.12-4.19%) were detected in somewhat lower amounts. The samples of clone 578 (Table 2) showed a smaller number of aroma associated compounds in the GC-MS profile. The major compounds in this clone were methyl salicylate (12.60%), *trans* linalool oxide (furanoid) (10.80%), phytol (1.80-9.17%), linalool (8.74%), pentacosane (2.79-7.59%) and acetic acid (6.84%). Results of the assay of aroma-associated compounds in clone 444 are presented in Table 3. This clone contained high levels of linalool (7.62-48.58%); slightly higher levels of methyl salicylate (1.45-9.34%), 2-hexanol-3-methyl (7.34%), cyclohexanone (6.36%), tricosane (0.53-5.28%) and eicosane (0.53-4.39%) were also recorded.

Our finding suggests that mostly linalool, trans linalool oxide (furanoid), and geraniol are the dominant volatiles in the three studied tea clones. Similar observations have been reported for high-grown teas from Africa (Cloughley et al., 1982) and India (Yamanishi et al., 1968). Despite similar observation of the composition and frequency of volatiles in tea clones, the tea clones that we have studied showed seasonal changes in the concentration and composition of volatiles.

As shown in Table 1, clone 100 showed the highest variation of volatile components in the spring. Most of the constituents were found to be absent or rarely presented in summer and autumn. Although the decrease in various components e.g. geraniol, β-Ionone, methyl salicylate, nerol and limonene causes a reduction in tea quality during summer and fall seasons, a parallel decrease in others, such as linalool, may be accompanied by an increase in desirability (Cloughley et al., 1982).

As shown in Tables 1, 2 and 3, some important and effective components, such as geraniol, were not present in clones 444 and 578. Phytol and β-myrcene, which were detectable in clones 100 and 578, were less frequently detected or not present in clone 444. In addition, some of the compounds, such as palmitic acid, did not show any variation in their frequency in the above-mentioned clones at all. There were also compounds like farnesol which behaved completely differently in these three clones. Nevertheless, in terms of flavoring compounds and according to their alterations in the three seasons, clone 444 was more similar to clone 100 and had a relative dominance to clone 578 in spring and autumn harvesting. As the agronomic practices were identical for all clones, it is probable that the observed differences were related to changes in their gene structure, resulting in their different phenology. These differences may be related to time since the individual clones were first propagated; the clones may be divided into early clones (including clones 100 and 444) and semi-late clones (including clone 578). In earlier studies, it was demonstrated that oxygenated terpenoids were more effective than

other terpenoid hydrocarbons in aroma and flavor (Bousbia et al., 2009). From a qualitative perspective, clones 100 and 444 have much more oxygenated terpenoids than other terpenoids compared to clone 578, which is an added advantage.

CONCLUSIONS

The results presented here demonstrate that variations in quality of unprocessed green tea leaves may be related to differences in vegetatively propagated cultivars (clones) and the season of harvesting. Agronomic conditions were identical for all clones tested. The phenotypic variations observed may quite possibly have resulted from genetic differences acquired during the years over which they have been planted since originally cloned. In Iranian farms, clones 100 and 444 can be recommended as preferred clones because of the quality and quantity of specific aroma and flavor components.

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