ISSN : 0974 - 7508 Volume 9 Issue 1

Trade Science Inc.

Natural Products

An Indian Journal

Full Paper

NPAIJ, 9(1), 2013 [16-21]

Comparison investigation of essential oils in tea (*Camellia sinensis* L. *var. sinensis*) clones

Akbar Norastehnia[1]*, Maryam Ghorbani[2]

[1]Department of Biology, Faculty of Science, University of Guilan, Rasht, (IRAN)

[2]Department of Biology, Islamic Azad University of Tonekabon Branch, Tonekabon, (IRAN)

E-mail: norasteh@guilan.ac.ir

ABSTRACT

Periodic seasonal variations in quality of Iranian tea over various growth flushes are reported. To characterize tea flavor, volatile components two clones of tea (*Camellia sinensis* L. *var. sinensis*) were isolated by hydro-distillation through a Clevenger system, and analyzed by GC-MS. A total 79 compounds were tentatively identified in tea clones by matching mass spectra and Kovats Index with the literature data. Flavor profile analysis revealed qualitative seasonal variations in aroma complex. Linalool and geraniol recorded maximum content during spring harvest and continuing decrease with progress in season, showing a minimum during summer harvest and minor improvement through autumn harvest in some cases. Results suggest increasing crop productivity during spring and fall harvests could enhance profitability.

KEYWORDS

Aroma profile;
Clevenger system;
Seasonal variation;
Tea clones.

INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the wordås oldest beverages and processed from tender shoots (two and a bud). Most of the quality parameters inherent in the tea shoots are determined by the germplasm characteristics and agro-climatic conditions[1-3].

Flavor is the most important parameter in evaluation of the quality of made tea. Flavor of tea comprises of taste and aroma. Taste comes from non-volatile constituents, while aroma is due to volatile constituents. Over 500 volatile flavor components have been identified from tea. Flavor is dependent on the availability of precursors present in tea shoot, stimulation of conditions during tea manufacture for their liberation and retention of flavor components in the product[4]. Very complex mixtures of carbonyl, terpenoid and other volatiles create tea aroma. Precursors of monoterpene alcohols like geraniol and linalool which give flowery aroma to tea are present in the form of non-volatile, which are liberated by hydrolytic breakdown of bound forms in disrupted tea shoot[4]. Agro-climatic conditions, clonal variations and geographical origin/locations greatly affect the aroma constituents of tea[2,4-6]. Precursors of monoterpene alcohols like geraniol and linalool which give flowery aroma to tea are present in the form of non-volatile, which are liberated by hydrolytic breakdown of bound forms in disrupted tea shoot[4]. Geographical and clonal variations affect the aroma constituents of tea. Teas of different varieties and from different countries of origin were observed to have different terpene indices[7]. It was reported that assamica cultivars contained high levels of linalool and sinensis cultivars produced mainly geraniol, while hybrids of assamica and sinensis varieties showed intermediate characteris-

tics[4]. The terpene index, i.e. the ratio of the gas chromatographic peak areas of linalool and linalool plus geraniol was also reported to be the characteristic of a cultivar or clone[4,7]. Tea from different clones grown under Kangra conditions showed variation in quality when evaluated on the basis of taste and aroma[8]. In this study two clones of tea (*Camellia sinensis var. sinensis*) were investigated in order to seasonal variations in quality of aroma components over various growth flushes. Different compounds present in tea (*C. sinensis* var. *sinensis*) extracts were investigated and their variety was compared; as a result, our study was a purely qualitative study. In order to avoid separate studies on tea samples after different processing pathways, only compounds influencing tea aroma and taste were studied, before any processing.

EXPERIMENTAL

Experimental biological material

Diethyl ether and Sodium sulfate.

Plant material

The aerial parts of two tea clones (KEN & DN) *Camellia sinensis var*. sinensis were plucked from Tea Research Station of Lahijan (Guilan, Iran) (altitude 34.2 m amsl, latitude 37° 11å S, longitude 50° 0å E) during summer and autumn 2009 as well as in spring 2010. A voucher specimen was deposited in the Herbarium of Guilan University (GUH, number 4039).

Methods

(a) Extraction of essential oil

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[9].

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 4C in the dark until further analysis as described below.

(b) Gas chromatography-mass spectrometry (GC-MS) analysis of aroma compounds

GC-MS analysis was carried out using an Agilent 6890N coupled to an 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, 280ºC; injected volume, 1 μl; splitless mode; the oven temperature program was 50ºC for 2 min, increased at 3ºC/min to 250ºC and held at 250ºC for 5 min. The mass range was 30-600 m/z.

(c) Compound identification

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[10]. 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.

RESULT AND DISCUSSION

Volatile components of two tea clones (KEN and DN) (*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 two tea clones (KEN & DN) are shown in TABLES 1, 2 and 3.

In this study three different components (40, 46 and 28) were identified in the aroma of clone KEN in spring, summer and autumn, respectively and were found to represent 92, 31.84 and 62.12% of the total composition (TABLE 1, 2 & 3). Also clone DN included 29, 25 and 36 aroma compounds in investigated seasons and total composition in these samples were 85.18, 49.99 and 63.37%, respectively (TABLE 1, 2 & 3). GC–MS profile of the extracted aroma showed the presence of a wide range of compounds, including monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, alkanes, alcohols, acids, aldehydes, ketones and esters.

Qualitative differences were observed in terpenoid and non-terpenoid flavor components. The major con-

Full Paper

stituents of tea clone KEN in spring, summer and autumn detected were geraniol (1.57-40.27%), linalool (2.51-21.75%), methyl salicylate (2.63-6.61%), octadecane (0.45-6.45%), cyclohexanone (5.72%), phytol (1.62-4.10%), pentacosane (1.18-3.66%) and tetracosane (1.63-3.06%). Other components, such as *trans* linalool oxide (furanoid) (0.88-2.84%), heneicosane (1.45-2.75%), tricosane (0.09-2.66%), nonadecane (0.45-2.33%), docosane (1.11-1.91%), heptadecane (0.56-1.78%) geranial (1.36%), β-myrcene (1.44%) and nerol (0.10-1.07%), were detected in lower amounts.

The major components of tea clone DN in spring, summer and autumn were linalool (12.73-22.20%), geraniol (6.42-17.83%), cyclohexanone (15.32%), phytol (1.08-10.95%), bornyl acetate (0.18-7.%), *trans* linalool oxide (furanoid) (2.49-6.71%), methyl salicylate (2.24-3%) and tetracosane (0.85-2.15%), other components present in appreciable contents were: tricosane (1.76-2.04%), *cis* linalool oxide (furanoid) (0.69-1.76%), pentacosane (1.63-1.72%) docosane (0.11-1.49%), heneicosane (1.15-1.40%), hexadecane (0.41-1.28%), geranial (1.28%), hexacosane (1.22-1.26%), heptadecane (0.31-1.15%) and z-nerolidol (1.11%).

TABLE 1 : Identified aroma compounds of tea clone KEN and DN in spring.

Peak No.	Compounds	KI[a]	Peak Area Percent	
			KEN	DN
1	Cyclohexanone	952	5.72	15.32
2	*β*-Myrcene	991	1.44	-
3	3,4,5-Trimethyl Isothiazole	996	0.07	0.72
4	Limonene	1029	0.55	-
5	Benzyl alcohol	1032	0.78	-
6	(E)-*β*-Ocimene	1050	0.60	-
7	*Trans* linalool oxide (furanoid)	1073	2.48	-
8	*Cis* linalool oxide (furanoid)	1087	0.59	1.06
9	Linalool	1097	21.75	22.20
10	Citronella	1153	0.12	-
11	Methyl salicylate	1192	6.61	2.66
12	Myrtenol	1196	0.25	-
13	Nerol	1230	1.07	-
14	Neral	1238	0.64	-
15	Geraniol	1253	40.27	13.86
16	Geranial	1267	1.36	1.28
17	*α*-Cubebene	1351	0.08	-
18	*α*-Copaene	1377	0.12	0.71
19	*Cis*-3-Hexenyl hexanoate	1384	0.09	0.59
20	*β*-Bourbonene	1388	0.10	-
21	Tetradecane	1400	0.25	0.78
22	*β*-Caryophyllene	1425	0.15	0.73
23	(E)-*α*-Ionone	1430	0.15	-
24	*α*-Humulene	1455	0.21	0.53
25	Germacrene-D	1485	0.12	-
26	(E)-*β*-Ionone	1489	0.11	-
27	Cadina-1,4-diene(*Cis*)	1496	0.20	-
28	Pentadecane	1500	-	0.81
29	Z-Nerolidol	1533	-	1.11
30	*α*-Calacorene	1546	0.13	-
31	Hexadecane	1600	0.13	0.87
32	*α*-Cadinol	1654	0.08	0.43
33	2-pentadecanone,6,10,14-trimethyl	1681	0.09	0.69
34	Heptadecane	1700	-	0.45
35	(Z,E)-Farnesol	1701	0.58	-
36	Octadecane	1800	0.07	0.63
37	Nonadecane	1900	-	0.52
38	Methyl palmitate	1922	0.17	0.52
39	Phytol	1943	4.10	10.95
40	Isophytol	1948	0.19	0.51
41	Methyl linoleate	2096	0.14	-
42	Heneicosane	2100	-	1.30
43	9,12-Octadecadienoic acid(Z,Z)	2146	0.21	-
44	9,12,15-Octadecatrienoic acid, methyl ester(Z,Z,Z)	2158	0.14	-
45	Docosane	2200	-	0.87
46	Tricosane	2300	0.09	2.04
47	Tetracosane	2400	-	0.85
48	Pentacosane	2500	-	1.63
49	Heptacosane	2700	-	0.56
	Total % composition		92	85.18

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

The variability in these constituents may be attributed to factors such as origin of the material, crop husbandry, plucking standard, method of processing, grades and most important, their genotype[11]. The teas from different origins can be marked by the composition of volatile flavor compounds. Gulati and Ravindranath (1996) explained seasonal variation of the volatile fla-

vor compounds in Kangra teas[12]. Gulati et al., (1999) studied the aroma profiles with respect to clonal variations in Kangra teas. However, they identified 10 volatile flavor compounds[13].

TABLE 2 : Identified aroma compounds of tea clone KEN and DN in summer.

Peak No.	Compounds	KI[a]	Peak	Area Percent
			KEN	DN
1	Camphene	954	0.02	-
2	Benzaldehyde	960	0.03	-
3	Benzyl alcohol	1032	0.07	-
4	Acetophenone	1065	0.04	-
5	*Trans* linalool oxide (furanoid)	1073	0.88	6.71
6	*Cis* linalool oxide (furanoid)	1087	0.31	1.76
7	Linalool	1097	2.51	12.73
8	Terpineol	1148	-	0.22
9	*Cis* linalyl oxide (pyranoid)	1174	-	0.44
10	Methyl salicylate	1192	2.63	3.00
11	Myrtenal	1193	0.03	-
12	Dodecane	1200	0.06	0.32
13	*β*-Cyclocital	1223	0.05	-
14	Nerol	1230	0.10	0.17
15	Geraniol	1253	1.57	6.42
16	Bornyl acetate	1289	0.10	0.18
17	Indole	1291	0.07	-
18	Tridecane	1300	0.58	0.41
19	1-Undecanol	1370	0.12	-
20	(E)-*β*-Damascenone	1385	0.02	-
21	*β*-Bourbonene	1388	0.03	-
22	Tetradecane	1400	0.17	0.75
23	(E)-*β*-Damascone	1414	0.05	-
24	*β*-Caryophyllene	1425	0.08	-
25	(E)-*α*-Ionone	1430	0.08	-
26	(E)-*β*-Ionone	1489	0.01	-
27	Pentadecane	1500	0.33	1.19
28	(E-E)-*α*-Farnesene	1506	0.14	-
29	*δ*-Cadinene	1523	0.04	-
30	Z-Nerolidol	1533	0.34	-
31	*α*-Calacorene	1546	0.04	-
32	Hexadecane	1600	0.39	1.28
33	Heptadecane	1700	1.78	1.15
34	(Z,E)-Farnesol	1701	0.06	-
35	Octadecane	1800	0.45	0.98
36	Benzyl salycilate	1866	0.38	-
37	Nonadecane	1900	0.45	0.80
38	Methyl palmitate	1922	0.18	-
39	Phytol	1943	3.45	1.24
40	Heneicosane	2100	1.45	1.40
41	Docosane	2200	1.91	1.49
42	Tricosane	2300	2.66	1.99
43	Tetracosane	2400	3.06	2.15
44	Pentacosane	2500	3.66	1.72
45	Hexacosane	2600	0.56	1.22
46	Dibuthyl phthalate	2630	0.19	-
47	Heptacosane	2700	0.12	0.27
48	Nonacosane	2900	0.59	-
	Total % composition		31.84	49.99

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

It can be understand from 1, 2 and 3 tables which all components of the tables with some exceptions have highest essential oils variety in spring. Decreasing in the essential oils variety in the cases of geraniol, β-ionone, methyl salicylate, nerol and limonene is caused decrease in tea flavor quality in the summer and fall compared to spring, but high quality of tea sometimes related to some component like types of linalool which are considered as terpenoid alcohols of aromatic flowers with high boiling point which are belived[5] their drop off is caused better quality of tea. It may be because of that reduce in some volatile compounds is accompanied with elevator of pleasant aroma[14]. So, according to their role in diminish of the tea quality, their decrease can be a positive point for summer and fall harvest, although this may be has not accepted by all researches[5].

On the other hand there is a complex of component which we know them as essential oils such as phytol, α-cadinol, β-myrcene, farnesol, geranial, δ-cadinene, palmitic acid and methyl palmitate that some of them can increase tea quality by consolidation of aroma in it[15]. TABLES of 1, 2 and 3 clear a continuous reduce of component variety in three spring, summer and autumn harvesting respectively as they decrease from spring to autumn. The decreasing in number of essential oils can be related to seasonal condition of their synthesis. It may be some instrument and conditions of their synthesis are not prepared in summer and autumn so they cannot be reproduced. The matter is caused we found higher variety of essential oils in spring against summer and fall.

Full Paper

TABLE 3 : Identified aroma compounds of tea clone KEN and DN in autumn.

Peak No.	Compounds	KI[a]	Peak Area Percent	
			KEN	DN
1	Camphene	954	-	0.39
2	β-Pinene	979	-	0.23
3	Benzyl alcohol	1032	0.52	-
4	*Trans* linalool oxide (furanoid)	1073	2.84	2.49
5	*Cis* linalool oxide (furanoid)	1087	0.96	0.69
6	Linalool	1097	11.18	13.28
7	Benzeneethanol	1107	-	0.57
8	Camphor	1146	-	0.51
9	Terpineol	1148	0.27	0.39
10	*Cis* linalyl oxide (pyranoid)	1174	-	0.49
11	Methyl salicylate	1192	3.43	2.24
12	Myrtenal	1193	-	0.39
13	Dodecane	1200	0.79	-
14	Nerol	1230	0.34	0.37
15	Neral	1238	0.90	0.56
16	Geraniol	1253	18.61	17.83
17	Bornyl acetate	1289	-	7.30
18	Thymol	1290	-	0.24
19	Tridecane	1300	0.18	-
20	β-Cubebene	1388	-	0.06
21	β-Bourbonene	1388	-	1.01
22	*Cis*-Jasmone	1393	-	0.29
23	Tetradecane	1400	0.44	-
24	(E)-α-Ionone	1430	0.33	-
25	Germacrene-D	1485	-	0.15
26	Pentadecane	1500	0.49	0.23
27	δ-Cadinene	1523	0.14	0.22
28	α-Calacorene	1546	0.14	-
29	Spathulenol	1578	-	0.29
30	Caryophyllene oxide	1583	-	2.16
31	Globulol	1585	-	0.47
32	Hexadecane	1600	0.56	0.41
33	Heptadecane	1700	0.56	0.31
34	Octadecane	1800	6.41	1.87
35	Nonadecane	1900	2.33	0.37
36	Phytol	1943	1.62	1.08
37	9-Octadecenoic acid	2004	0.23	-
38	Heneicosane	2100	2.75	1.15
39	Docosane	2200	1.11	0.11
40	Tricosane	2300	1.92	1.76
41	Tetracosane	2400	1.63	2.06
42	Pentacosane	2500	1.18	-
43	Hexacosane	2600	-	1.26
44	Heptacosane	2700	-	0.14
45	Nonacosane	2900	0.26	-
	Total % composition		62.12	63.37

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

CONCLUSIONS

The results presented here demonstrate that variations in quality of unprocessed green tea leaves may be related to differences in vegetative 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.

ACKNOWLEDGMENTS

The authors acknowledge for the plant material provided by Tea Research Institute of Lahijan, Iran.

REFERENCES

[1] G.W.Sanderson; Tea Quart, **35**, 101-109 **(1964)**.
[2] T.Takeo, P.K.Mahanta; Journal of the Science of Food and Agriculture, **34**, 307-310 **(1983)**.
[3] P.L.Fernandez, F.Pablos, M.J.Martin, A.G.Gonzales; Food Chemistry, **76**, 483-489 **(2002)**.
[4] T.Takeo; Phytochemistry, **20**, 2145-2147 **(1981)**.
[5] J.B.Cloughley, R.T.Ellis, S.Pendlington, P.Humphrey; Journal of Agriculture and Food Chemistry, **30**, 842-845 **(1982)**.
[6] V.Fernando, G.R.Roberts; Journal of the Science of Food and Agriculture, **35**, 71-76 **(1984)**.
[7] T.Takeo; Japan Agricultural Research Quarterly, **17**,120-124 **(1983)**.
[8] T.Balasaravanan, P.K.Pius, R.R.Kumar, N.Muraleedharan, A.K.Shasany; Plant Science, **165**, 365-372 **(2003)**.
[9] E.Derwich, Z.Benziane, A.Boukir, L.Benaabidate; Chemical Bulletin of "POLITEHNICA" Univer-

sity of Timisoara, **54(68)**, 85-88 **(2009)**.

[10] R.P.Adams; Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy, Allured Publishing Corporation, Carol Stream, Illinois; USA, **(2001)**.

[11] B.B.Borse, L.Jagan Mohan Rao, S.Nagalakshmi, N.Krishnamurthy; Food Chemistry, **79**, 419-424 **(2002)**.

[12] A.Gulati, S.D.Ravindranath; Journal of the Science of Food and Agriculture, **71**, 231-236 **(1996)**.

[13] A.Gulati, M.B.Tamang, S.D.Ravindranath; Journal of Plantation Crops, **27**, 175-178 **(1999)**.

[14] E.S.Kim, J.J.Liang, Q.F.Sun, J.L.Lu, Y.Du, C.Lin; Food Chemistry, **103**, 1263-1267 **(2007)**.

[15] M.Kawakami, T.Yamanishi; Agricultural and Biological Chemistry, **47(9)**, 2077-2083 **(1983)**.