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Research Article
Influence of Different Sampling Site and Storage Duration on Volatile Components of Korean Green Tea

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Abstract
The purpose of the study was to analyse the volatile components of tea leaves and green tea depending on sampling site and storage duration. Volatile components of fresh tea leaves and green tea of Suncheon and Namwon were detected by headspace-GC/MS. The mean annual rainfall and air temperature of Suncheon is higher than that of Namwon. A total of 57 different constituents were identified and qualitative and quantitative differences were observed among the samples. Fresh tea leaves and green tea of Namwon contained more components than that of Suncheon. Composition of volatile component of green tea was changed by the storage duration, namely the longer storage duration, the smaller was the number of volatile components. Percentage of some volatile components in the green tea was decreased with storage duration, the content of some components have gone with storage duration.

Key words: Green tea, volatile component, sampling site, storage duration, headspace-GC/MS

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tea is processed from tender shoot (two and a bud) of *Camellia sinensis* (L.) O. Kuntze. Quality of tea has three dimensions, i.e., physical appearance, infusion and flavour. Most of the quality parameters inherent in the tea shoots are determined by germplasm characteristics and agro-climatic conditions. Over 500 volatile flavour components have been identified from tea. Flavour is dependent on the availability of precursors present in tea shoot, stimulation of conditions during tea manufacture for their liberation and retention of flavour component in the product (Rawat and Gulati, 2008).

Green tea is a popular beverage worldwide with most frequent consumption in Asian countries. Black tea is allowed to completely oxidize, oolong tea undergoes partial enzymatic oxidation. In contrast, for green tea, oxidation is stopped directly after harvest before drying. The tea contains large amounts of polyphenols, monomeric flavonoids of the catechin family. Green tea has recently gained scientific interest for its potential health benefits. The main interest focuses on the effects of green tea on cancer prevention due to its high content of polyphenol (Honow *et al*., 2010). The composition of volatile essential oils is usually predominated by the presence of terpenoid compounds. The same as other important natural products such as phenolics, flavonoids and coumarins; terpenonids are secondary metabolites with various biological activities (Zwenger and Basu, 2008). Aroma is one of the critical aspects of tea quality which can determine acceptance or rejection of a tea before it is tasted. All the data reported so far shows that more than 630 compounds have been reported responsible in tea aroma. It is generally believed that the characteristics of various kinds of tea consist of a balance of very complicated mixtures of aroma compounds in tea (Chaturvedula and Prakash, 2011). The aim of the present study was to determine the volatile components of fresh tea leaves and green tea depending on sampling site and storage duration.

MATERIALS AND METHODS

Experimental sites: The present experiment was conducted to study the difference in volatile components of green tea manufactured by tea leaves plucked two sites. One experimental site, Suncheon, is located between 35°00 58"N, 127°12 25"E to 35°00 58"N, 127°12 31"E. The mean annual rainfall and air temperature is 1531.3 mm and 12.6°C. The other site, Namwon, is located between 35°20 04"N, 127°16 09"E to 35°20 09"N, 127°16 15"E. The mean annual rainfall and air temperature is 1380.4 mm and 12.3°C.

Sample preparation: The leaves of *Camellia sinensis* were plucked on 11 May, 2013 from cultivated field of Suncheon and Namwon, Korea. Fresh leaves were sealed in plastic bags and transported to laboratory for analysis of volatile components. And the freshly plucked tea leaves were pan-fried to prevent fermentation. The pan temperature was set at 300°C and then decreased to 200°C and then these were rolled and dried. The step was achieved in ten times. The manufactured green tea was kept at 20°C and analyzed at an interval 10 days. A 3.7 g of each sample was placed in glass headspace vials. The headspace vials were then sealed with aluminum crimp cap with PTEE/silicone septum and immediately analyzed as described below.

Headspace-GC/MS instrument and conditions: A Teledyne tekmar HT3™ (Teledyne Tekmar, USA) was used to perform the experiment. The conditions were as follows: Oven temperature 100°C, transfer line temperature 100°C, platen/sample temperature 80°C, mixing time 2.00 min, pressurize time 2.00 min and injection time 2.00 min. The GC-MS analysis was conducted using a GC-2010 (Shimadzu, Japan) coupled with a GCMS-QP2010plus (Shimadzu, Japan). The separation was carried out using a HP-5MS capillary column (30 m$\times$0.35 mm$\times$0.2 µm). The injector temperature was set at 200°C and the column temperature program started at 35°C, which was held for 10 min and then increased to 120°C at 2°C min^{-1} then to 200°C at 5°C min^{-1} and finally to 250°C at 5°C min^{-1}. Purified helium was applied at a flow rate of 1.2 µL min^{-1} as the carrier gas. The GC-MS transfer line temperature was 250°C and the mass spectrometer was operated in Electron Impact (EI) mode with an ion source temperature of 200°C, interface temperature of 250°C and mass range of m/z 33-400.

The volatile components were identified by comparison of their retention indices and mass spectra fragmentation patterns with the literature values (Wiley 275 and NIST 21). Each volatile compound was quantified based on the ratio of the peaks obtained from the total ion chromatogram and was expressed as percent peak area relative to total peak area from MS analyses of the whole materials (Chang and Kim, 2013; Choi *et al*., 2013).

RESULTS AND DISCUSSION

The main difference of three types of tea is the degree of fermentation. Green, oolong and black teas are recognized as unfermented, semi-fermented and fully-fermented tea, respectively. One important purpose of fermentation is to enhance the flavor including taste and aroma (Wang *et al*., 2011; Zhang *et al*., 2013). The volatile profile varies with the tea manufacturing process (Yang *et al*., 2013; Kim *et al*., 2011). The influence of sampling site and storage duration on volatile components of green tea is reported here for the first time. Weather conditions influence the essential oil composition in aromatic plants. The content and composition of active plant principles is known to depend considerably on extrinsic and intrinsic factors, including soil and climatic conditions, plant ontogenesis and season of harvest (Kakaraparthi *et al*., 2014). Day temperatures and geographical conditions influence the aroma biogenesis in tea (Rawat and Gulati, 2008). In Suncheon and Namwon region, early growth flush coincides with moderate humidity and warm weather conditions that favour faster growth of tea tree. The variations in volatile components and their percentages are now studied in relation to storage duration (10-30 days) (Table 1). Variation was observed in the number of volatile components by storage duration. In general the longer storage duration, the smaller was the number of volatile components. Lowest the number of volatile components was observed in Suncheon tea stored in 30 days. Previous study revealed that the composition of volatile component of Korean green tea was dependent on the harvest time (Choi *et al*., 2013).

Table 1: Volatile components of tea leaves and green tea of Suncheon and Namwon

			Peak area (%)									
			Samples collected in Suncheon					Samples collected in Namwon				
				Storage duration of green tea (days)					Storage duration of green tea (days)			
RT	RI	Compound name	Fresh leaves	0	10	20	30	Fresh leaves	0	10	20	30
2.050	656	Propylmethyl ketone	12.63	1.97	3.25	-	-	11.83	2.73	4.19	-	-
2.367	665	3-hydroxy-pentene	2.81	-	-	-	-	3.84	0.71	-	-	-
2.475	668	Isopropyl-acetate	0.27	0.67	-	-	-	0.39	-	-	-	-
2.633	673	Isobutyl-formate	0.53	0.74	-	-	-	0.88	-	-	-	-
2.900	681	n-pentanal	14.97	2.31	3.57	4.26	11.75	10.86	2.44	3.62	-	-
3.042	685	2-methyl-butyraldehyde	13.90	0.89	1.66	-	6.70	10.46	0.86	1.45	3.04	5.63
3.400	696	3-hydroxy-pentene	0.87	-	1.16	-	2.66	0.83	-	0.90	4.54	6.89
3.642	703	Pentan-3-one	0.29	3.00	3.93	4.40	20.89	1.49	3.02	4.39	7.76	10.37
4.492	728	Allyl acetate	0.76	-	-	-	-	4.41	-	-	-	6.51
4.667	733	Isopentyl alcohol	1.14	-	-	-	-	1.93	-	-	-	-
4.775	736	Sec-butyl-carbinol	1.44	-	-	-	-	0.59	-	-	-	-
5.550	759	Toluene	-	3.07	-	-	-	-	4.34	0.30	0.95	-
5.733	765	Methyl L-lactate	-	1.02	0.82	-	-	-	-	-	-	-
5.917	770	Pentyl alcohol	-	0.58	-	-	-	0.09	0.43	1.17	3.39	5.26
6.058	774	Acetylbutyryl	-	1.75	-	-	-	-	-	0.70	-	-
6.592	790	α-octane	-	-	1.39	-	-	-	1.00	0.89	-	-
7.008	801	n-octane	-	0.57	-	-	-	1.58	0.90	0.93	-	-
7.217	804	n-hexanal	0.21	1.93	2.30	3.63	3.62	0.47	2.46	3.26	5.46	6.46
7.533	808	Ethyl-pyruvate	-	-	1.31	2.02	-	-	0.57	-	-	-
8.650	823	Furfural	-	2.07	-	-	2.63	-	-	-	-	-
9.900	839	Ethyl-lactate	-	-	-	-	-	-	-	-	1.09	1.12
11.525	860	Butyrone	1.90	-	1.71	-	-	4.16	0.48	-	-	1.06
13.042	880	n-hexanol	-	-	-	-	-	0.87	1.71	-	-	-
13.250	883	Allyl-thiocyanate	-	-	-	4.58	-	-	0.68	-	-	-
13.592	887	2,5-dimethyl-pyrazine	-	-	-	5.80	-	-	0.51	-	-	-
14.242	896	4-heptyl alcohol	-	-	-	3.64	-	-	-	-	-	-
14.575	900	Heptan-2-ol	-	-	-	1.71	-	-	-	-	0.94	-
15.025	905	n-heptanal	0.11	2.80	3.39	5.16	4.44	0.75	5.14	5.95	9.30	7.81
19.708	960	Tetrahydro-citronellene	0.42	1.33	1.01	-	-	0.56	1.85	2.08	-	-
22.208	988	Decane	0.53	0.81	-	-	-	-	1.59	-	-	-
22.592	993	2-pentyl-furan	0.81	-	0.80	-	-	0.33	1.02	1.62	-	-
23.450	1003	Limonene	0.27	13.63	0.85	-	-	-	-	0.63	1.28	1.15
23.767	1031	2-ethyl-hexanol	4.16	9.84	11.82	3.30	-	2.03	11.4	10.52	4.80	
26.617	1042	γ-terpinene	-	-	0.79	-	-	-	0.60	-	-	1.09
26.833	1045	Phenylacetaldehyde	0.26	0.72	0.80	-	-	0.23	1.17	-	-	-
27.617	1054	Isoamyl-pyruvate	2.74	6.48	8.11	6.04	-	2.06	8.01	8.30	3.39	0.96
28.342	1063	Nonylol	1.35	3.89	6.54	3.05	-	0.71	5.93	4.51	5.03	1.07

Table 1: Continue

			Peak area (%)									
			Samples collected in Suncheon					Samples collected in Namwon				
				Storage duration of green tea (days)					Storage duration of green tea (days)			
RT	RI	Compound name	Fresh leaves	0	10	20	30	Fresh leaves	0	10	20	30
29.175	1073	n-undecane	2.91	2.88	4.99	-	-	2.44	4.33	4.64	2.05	0.82
30.108	1085	Isopentyl-butyrate	0.41	-	2.33	3.78	-	-	2.86	4.39	-	-
30.108	1087	Cis-linalool oxide	5.02	1.25	-	-	-	7.08	0.81	1.47	-	1.05
31.408	1101	Linalool	25.25	2.88	11.58	8.33	9.01	20.57	11.36	8.41	8.23	6.94
36.558	1170	α-terpineol	0.49	27.23	2.45	-	-	0.65	5.14	2.01	2.82	-
38.208	1192	Methyl-salicylate	2.80	-	2.32	-	-	3.01	2.41	1.14	1.29	-
40.175	1220	Benzosulfonazole	0.92	-	3.16	4.62	3.01	1.05	4.12	3.29	3.73	2.78
40.358	1222	δ-hezalactone	-	-	-	1.76	-	-	-	0.71	0.92	-
41.992	1246	2,5-dimethyl-furanone	-	-	-	-	-	-	-	-	-	2.62
42.900	1259	Nerol	0.64	0.61	-	-	-	0.32	3.38	1.01	-	-
45.708	1299	Methyl nonyl carbinol	-	-	0.81	-	-	-	0.56	-	-	-
47.750	1331	Triacetin	-	0.73	-	-	-	-	-	-	-	-
48.117	1336	Ethyl-acetaldehyde	-	3.81	-	-	2.92	0.28	-	0.86	-	1.19
50.350	1371	Butyl-butyryllactate	-	-	-	-	-	-	0.46	-	-	-
51.033	1381	n-tetradecane	-	-	-	-	-	-	-	0.67	-	1.17
57.650	1516	δ-cardinene	1.05	0.54	10.44	19.98	29.05	3.26	5.02	12.68	21.91	25.00
64.617	1759	α-bisabolol oxide A	-	-	0.83	-	-	-	-	-	-	1.48
66.500	1842	Neophytadiene	-	-	-	-	-	-	-	1.20	2.37	-
68.367	1930	Methyl-hexadecanoate	-	-	4.97	11.69	3.32	-	-	2.01	5.71	1.26
73.475	2203	n-docosane	-	-	0.89	2.25	-	-	-	-	-	0.31
Monoterpene hydrocarbons			1.22	15.77	2.65	-	-	0.56	4.04	2.71	1.28	2.24
Oxygenated monoterpenes			31.40	31.97	14.03	8.33	9.01	28.62	20.69	12.9	11.05	7.99
Oxygenated sesquiterpene			1.05	0.54	11.27	19.98	29.05	3.26	5.02	12.68	21.91	26.48
Others			66.33	51.72	72.05	71.69	61.94	67.56	70.25	71.71	65.76	63.29
Total			100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

The volatile components of tea leaves and green tea of two sites were compared, the change of the volatile components of green tea by the storage duration was also studied, the variation in the volatile components of tea leaves and green tea depends on the sampling site and storage duration, RT: Retention time and RI: Retention index

The identified volatile components and the percentages of tea leaves and green tea sampled in Namwon and Suncheon are shown in Table 1, together with their variation by storage duration. In the volatile components of tea leaves plucked in Suncheon and Namwon, 31 and 32 compounds were identified, in which 1.22 and 0.56% of which were monoterpene hydrocarbons, 31.40 and 28.62% oxygenated monoterpenes, 1.05 and 3.26% oxygenated sesquiterpenes and 66.33 and 67.56% other compounds. One example of terpene derivatives is terpenoids. The amount of terpenoids, along with other phytochemicals could be involved in antibacterial activity (Compean and Yanalvez, 2014). The main volatiles present in tea leaves of Suncheon and Namwon were linalool (25.25 and 20.57%), n-pentanal (14.97 and 10.86%), 2-methyl-butyraldehyde (13.90, 10.46%), propylmethyl ketone (12.63 and 11.83%) and cis-linalool oxide (5.02 and 7.08%). In the volatile component of green tea of Suncheon and Namwon, 28 and 35 compounds were identified, in which 15.77 and 4.04% of which were monoterpene hydrocarbons, 31.97 and 20.69% oxygenated monoterpenes, 0.54 and 5.02% oxygenated sesquiterpenes and 51.72 and 70.25% other compounds. The main components of green tea of Suncheon were α-terpineol (27.23%), limonene (13.63%), 2-ethyl-hexanol (9.84%) and isoamyl-pyruvate (6.84%), whereas the main components of Namwon green tea was linalool (11.36%), 2-ethyl-hexanol (11.04%), isoamyl-pyruvate (8.01%), nonylol (5.93%) and n-heptanal (5.14%). Lomonene is a potential active antioxidant components (Wang *et al*., 2015). Linalool was recognized as one of the key odorants in several studies of tea leaves, tea infusion and green tea and presented as contributor to floral odour note of teas (Kraujalyte *et al*., 2016; Tontul *et al*., 2013) and the main volatile component in high quality green tea (Kato and Shibamoto, 2001). Linalool was found as main component in Namwon green tea and Suncheon green tea stored in 10 days. The percentage of δ-cardinene, pentan-3-one and n-heptanal in green tea of Suncheon and Namwon was increased with storage duration. The content of α-terpineol, 2-ethyl-hexanol and toluene of green tea have gone with storage duration. This study showed that sampling site and storage duration can significantly affect the volatile components of green tea as, Tontul *et al.* (2013) pointed out that the volatile composition of the green tea powders notably changed with respect to the tea clone, shading level and shoot period.

CONCLUSION

In this study, the volatile components of tea leaves and green tea from two sites (Suncheon and Namwon) was compared, the changes of the volatile components of green tea by the storage duration was also studied. The tea leaves and green tea of Namwon contained more volatile components compared with that of Suncheon and the number of volatile component of green tea was decreased by storage duration. The variation in the volatile component of tea leaves and green tea depending on the sampling site and storage duration.

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