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Effect of Extraction Time on Physiologically Important Constituents of Green Tea (*Camellia sinensis*) Using GC/MS

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Abstract

Two types of green tea samples Fine and Superfine were subjected to varied extraction times (20, 40, and 120 minutes) at constant temperature of 90°C. These tea extracts of were then subjected to GC-MS analysis. The phytochemical composition of these extracts showed some variations depending upon the type of sample possibly due to genetic, environmental and processing conditions. Among different time temperature combinations it was seen that a time period of 20-40 minutes was generally suitable for most of the samples for retaining a higher percentage of physiologically significant phytochemicals in the extract. Also pthalic acid, a toxic substance, was reported to be present in superfine variety, but aqueous extracts of fine tea variety did not yield any of it.

Keywords: *Camellia sinensis*; Extraction; Phytochemicals; GC-MS analysis; Caffeine; Pthalic acid

Introduction

Tea (*Camellia sinensis*) is native to the southern regions of China and parts of India, Laos, Thailand, Vietnam, and Myanmar [1]. Tea is said to have first been discovered as a drink and medicine in China around 2737 BC. It was then introduced to Japan during the early 13th century and to Europe in the 16th century, then to America, Africa and other regions of the world [1,2]. Tea is presently cultivated in over 30 countries around the world and the tea beverage is second only to water in terms of worldwide consumption [3]. It grows best in tropical and subtropical areas with adequate rainfall, good drainage, and slightly acidic soil. The worldwide popularity of tea has increased due to its potential health benefits against cardiovascular diseases and cancer as well as pharmaceutical potential such as anti-hypertensive, anti-ateriosclerotic, hypocholesteroladmic, and hypolipidemic properties mostly from activities of flavonoids present in tea [4-6]. The Green tea is also known for its unique aroma and characteristic flavor [7]. For a better understanding of the physiological and pharmacological effects of tea, it is essential to scrutinize its chemical composition. There exist volatile and non-volatile components in tea. A great deal of work about tea volatiles have been reported [8-10]. But as far as we know, the effect of time-of-extraction on volatile constituents of green tea have not yet been reported. In general, the analysis of volatile components is usually conducted by using gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS). Here we report the identification of the volatile constituents of two types of green tea using GC/MS technique and extraction behavior of physiologically important volatile components with respect to different time of extraction.

Materials and Methods

Sample collection and preparation of extract

The dried leaves of green tea samples viz., fine and superfine were purchased from the local market and stored at room temperature until use. The tea extracts of dried leaves were prepared using stirring water bath, 2 g of fresh dried leaves were extracted with 50 ml pure water at constant temperature of 90°C, for different times (20, 40 and 120 min). The extracts obtained were concentrated under reduced pressure in a rotary evaporator (Rotary equitron).

Instrument and chromatographic conditions

The concentrated pure extract was vacuum dried in a vacuum oven at 60°C to get a powdered residue. The residue was dissolved in 20 ml methanol and collected in corked glass test tubes. The extracts were analysed on a Shimadzu QP2010 Plus GC-MS system with 2010 GC. An Omega SPTm column (0.25 mm ID, film thickness 0.25 µm) was used with nitrogen as carrier gas. The injector temperature was 270°C with split ratio of 10.0. The GC oven temperature was programmed to hold at 100°C for 2 minutes and then increased to 200°C at 15°C/min and hold for 2 minutes and finally increased to 240°C at 20°C/min and hold for 18 minutes. Ion source temperature was 230°C and the interface temperature was set at 280°C. Mass spectra were collected over the range of m/z 40-650. Each compound was identified using WILEY library (8 L).

Results and Discussions

Identification and analysis various of components

GC-MS chromatograms of the aqueous extract of two types of green tea for different time of extraction are given Figures 1 and 2. The number of phytochemical constituents as depicted by the peaks varied according to the type of tea sample (possibly due to varied environmental, genetic and processing conditions) and the time of extraction. On comparison of the mass spectra of the constituents with the library (WILEY8.LIB) the different compounds were characterized and identified (Tables 1 and 2). The relative percentages of some major compounds which are present in almost in all tea types are presented in Table 3. They were identified as, Methyltetradecanoate/Myristic acid, Hexadecanoic acid/palmitic acid, Octadecanoic acid/

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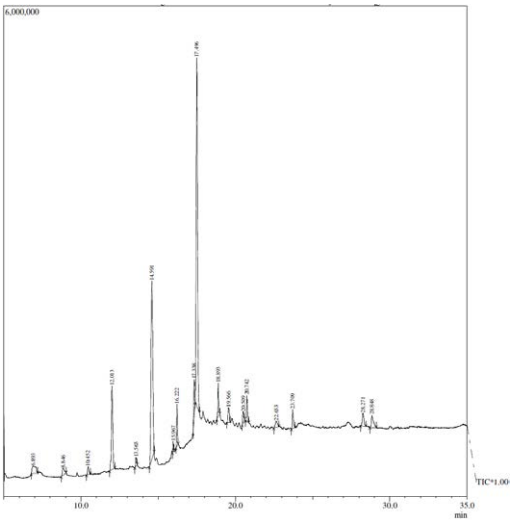
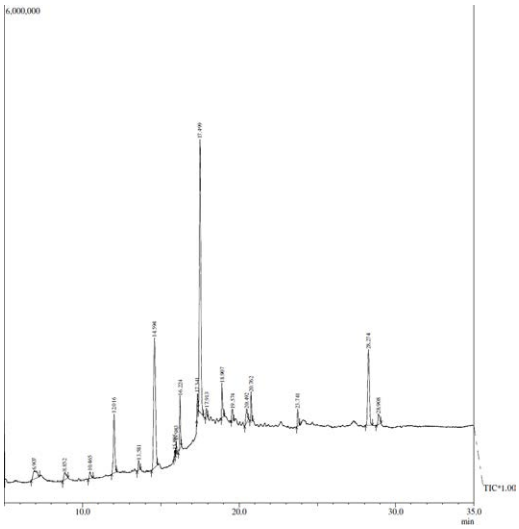
(a) Fine 20 min



(b) Fine 40 min.



(c) Fine120 min.

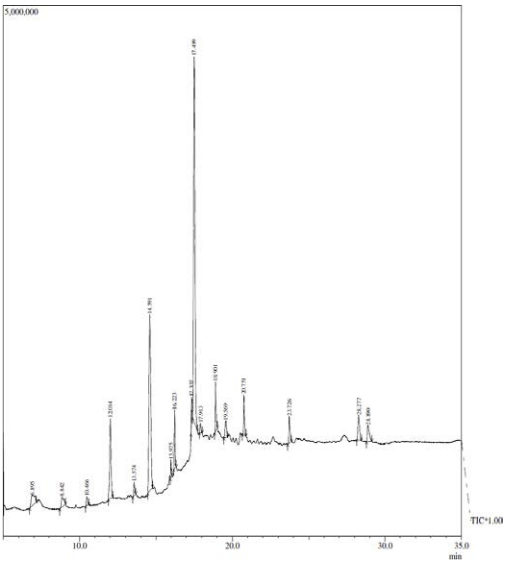
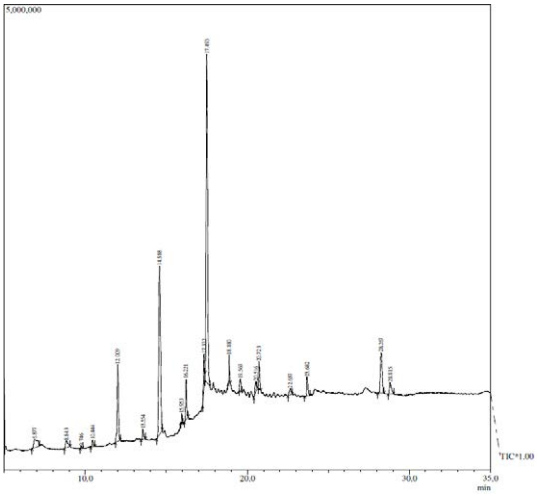
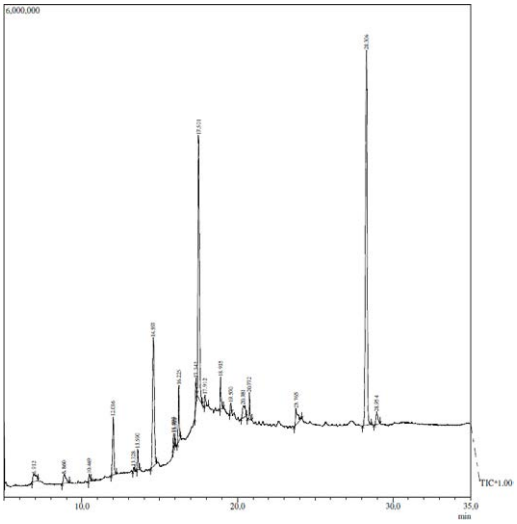


Figure 1: (a-c) Showing GCMS chromatographs of Fine green tea.

(a) Superfine 20 min.



(b) Superfine 40 min.



(c) Superfine 120 min.

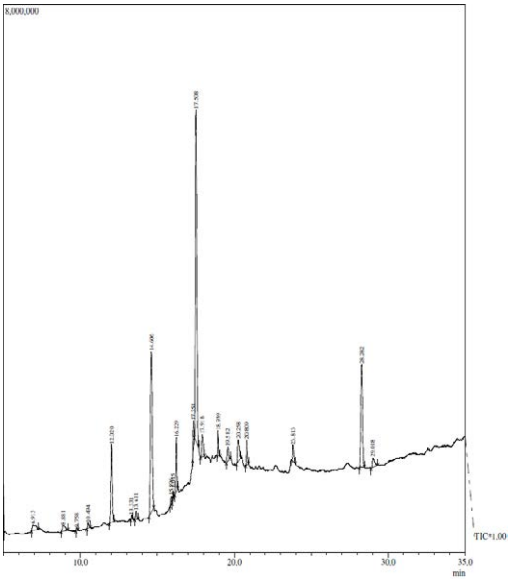


Figure 2: (a-c) Showing the GCMS chromatograph of superfine green tea.

(a): Fine 20 min.

Peak	R. time	Area%	Compound name
1	6.893	2.39	Cyclooctasiloxane
2	8.846	1.21	Cyclononasiloxane
3	10.452	0.82	Cosamethylcyclodecasiloxane
4	12.013	8.74	Methyl tetradecanoate/Myristic acid
5	13.565	0.67	Cyclononasiloxane
6	14.591	24.77	Hexadecanoic acid/palmitic acid
7	15.967	0.53	1H-Purin-6-amine
8	16.222	2.95	1,2-Benzenedicarboxylic acid
9	17.338	3.28	Octadecanoic acid/Stearic acid
10	17.496	40.41	9-Octadecenoic acid/oleic acid
11	18.893	2.23	1H-Purin-6-amine
12	19.566	1.87	9,11-Octadecadienoic acid
13	20.509	1.13	Acetic acid
14	20.742	1.96	1H-Purin-6-amine
15	22.653	1.21	Docosanoic acid
16	23.709	1.81	1H-purin-6-amine
17	28.271	1.82	1H-Purine-2,6-dione/Caffein
18	28.848	2.2	Cyclodecasiloxane

(b): Fine 40 min.

Peak	RT F2	F2A	Compound Name
1	6.907	2.88	Cyclooctasiloxane
2	8.852	1.69	Cyclononasiloxane
3	10.465	0.98	Cyclodecasiloxane
4	12.016	6.85	Methyl tetradecanoate/Myristic acid
5	13.581	0.99	Cyclononasiloxane
6	14.594	20.6	Hexadecanoic acid/palmitic acid
7	15.885	0.39	Phenol, 2-methoxy-4-propenyl
8	15.983	0.76	1H-Purin-6-amine
9	16.224	4.24	1,2-Benzenedicarboxylic acid
10	17.341	1.57	Octadecanoic acid/Stearic acid
11	17.499	35.13	9-Octadecenoic acid/oleic acid
12	17.913	0.91	9,12-Octadecadienoic Acid/Linoleic acid
13	18.907	2.57	1H-Purin-6-amine
14	19.574	1.46	Cyclopropanebutanoic acid
15	20.492	2.2	9-Octadecenoic acid
16	20.762	2.19	1H-Purin-6-amine
17	23.741	1.64	1H-Purin-6-amine
18	28.274	11.47	1H-Purine-2,6-dione/Caffein
19	28.908	1.48	Cyclononasiloxane

(c): Fine 120 min.

Peak	RTF6	F6A	Compound Name
1	6.895	2.91	Cyclooctasiloxane
2	8.842	1.94	Bis(heptamethylcyclotetrasiloxy) hexamethyltrisiloxane
3	10.466	0.97	Cyclodecasiloxane
4	12.014	7.87	Methyl tetradecanoate/Myristic acid
5	13.574	1.04	Cyclononasiloxane
6	14.591	23.21	Hexadecanoic acid/palmitic acid
7	15.975	0.88	1H-Purin-6-amine
8	16.223	3.82	1,2-Benzenedicarboxylic acid, Diethyl ester
9	17.337	1.56	Octadecanoic acid/Stearic acid
10	17.499	39.33	9-Octadecenoic acid/oleic acid
11	17.913	0.85	9,12-Octadecadienoic acid, Linoleic acid
12	18.901	2.95	1H-Purin-6-amine
13	19.569	1.97	9,11-Octadecadienoic acid,Methyl trans-9,trans-11-octadecadienoate
14	20.751	2.72	1H-Purin-6-amine
15	23.726	2.43	1H-Purin-6-amine
16	28.277	3.03	1H-Purine-2,6-dione/Caffein
17	28.89	2.54	Cyclononasiloxane

Table 1(a-c) : Showing the identification and relative percentage of volatile compounds in Fine green tea at 90°C in 20, 40 and 120 minutes time of extraction.

(a): Super fine 20 min

Peak	R. time	Area%	Name of compound
1	6.912	1.51	Cyclooctasiloxane
2	8.86	1.44	Cyclohexasiloxane
3	10.469	0.54	Cyclononasiloxane
4	12.016	4.5	Pentadecanoic acid
5	13.328	0.23	Phenol
6	13.59	1.2	Chlorocyclopentane
7	14.597	13.83	Hexadecanoic acid, Palmitic acid, methyl ester
8	15.893	0.62	Phenol
9	15.988	0.49	Cyclononasiloxane, octadecamethyl, Octadeamethyl-cyclononasiloxane, Octadecamethylcyclon
10	16.225	3.09	Phthalic acid, diethyl ester
11	17.342	1.7	Octadecanoic acid, methyl ester
12	17.501	22.99	9-Octadecenoic acid, Methyl ester, Oleic acid
13	17.912	0.85	Linoleic acid,
14	18.915	1.58	Cyclononasiloxane
15	19.57	0.77	9,12-Octadecadienoic acid (Z,Z)-, methyl ester $$ Linoleic acid, methyl ester
16	20.381	2.09	Tetradecanoic acid $$ Myristic acid,
17	20.772	1.37	Cyclodecasiloxane, eicosamethyl, Icosamethylcyclodecasiloxane
18	23.765	2.1	Cyclooctasiloxane, Hexadecamethyl
19	28.306	37.7	Caffein
20	28.954	1.41	Cyclononasiloxane, octadecamethyl

(b): Superfine 40 min

Peak	R. time	Area%	Name of compound
1	6.877	2.73	Cyclooctasiloxane, Hexadecamethyl-
2	8.843	1.44	Cyclohexasiloxane, dodecamethyl
3	9.746	0.19	Tridecanoic acid, methyl ester
4	10.444	0.72	Icosamethylcyclodecasiloxane
5	12.009	7.92	Methyl tetradecanoate, Myristic acid, methyl ester
6	13.554	1.01	Cyclononasiloxane, Octadecamethyl
7	14.588	23.87	Hexadecanoic acid, Methyl Ester
8	15.953	0.54	1H-Purin-6-amine, [(2-Fluorophenyl)methyl]
9	16.221	3.28	1,2-Benzenedicarboxylic acid, Diethyl ester, Phthalic acid
10	17.332	2.87	Octadecanoic acid; Methyl Ester; Stearic acid Methyl Ester
11	17.493	38.46	9-Octadecenoic acid, Oleic acid, methyl ester
12	18.88	1.52	1H-Purin-6-amine, [(2-Fluorophenyl)methyl]
13	19.569	1.5	Cyclopropanebutanoic acid
14	20.516	1.19	Hexadecanoic acid, (2-Phenyl-1,3-dioxolan-4-yl) methyl palmitate
15	20.723	2.05	1H-Purin-6-amine, [(2-Fluorophenyl)methyl]
16	22.657	1.03	Methyl behenate, n-Docosanoic acid methyl ester
17	23.682	1.98	1H-Purin-6-amine, [(2-Fluorophenyl)methyl]-
18	28.267	5.79	1H-Purine-2,6-dione, 3,7-dihydro-1,3,7-trimethyl-, Cafeina, Caffein, Caffine, Cafipel, Coffeine, Guaranine, Koffein
19	28.815	1.9	1H-Purin-6-amine, [(2-Fluorophenyl)methyl], Octadeamethyl-cyclononasiloxane

(c): Superfine 120 min

Peak	R. time	Area%	Name of compound
1	6.913	1.48	Cyclooctasiloxane, hexadecamethyl-
2	8.881	1.26	Cyclononasiloxane, octadecamethyl-
3	9.758	0.21	Dodecanoic acid, methyl ester
4	10.494	0.57	Icosamethylcyclodecasiloxane
5	12.02	7.25	Tetradecanoic acid, Methyl ester
6	13.331	0.3	Phenol, 2-Methoxy-4-(2-Propenyl)
7	13.611	0.68	Cyclononasiloxane, Octadecamethyl

8	14.606	21.07	Palmitic Acid, Hexadecanoic acid, Methyl ester
9	15.896	0.46	Phenol, 2-methoxy-4-(1-propenyl
10	16.015	0.49	Cyclononasiloxane, Octadecamethyl.
11	16.229	3.63	Phthalic acid, diethyl ester
12	17.351	1.95	Octadecanoic acid, Stearic acid
13	17.508	34.92	Oleic acid, methyl ester, 9-Octadecenoic acid , methyl ester
14	17.918	2.6	Linoleic acid, methyl ester
15	18.939	1.34	Cyclononasiloxane, octadecamethyl-
16	19.582	1.59	9,12-Octadecadienoic acid, methyl ester
17	20.258	2.89	Tetradecanoic acid, Myristic acid
18	20.809	1.58	Cyclodecasiloxane, eicosamethyl-
19	23.813	1.62	Cyclononasiloxane, octadecamethyl-
20	28.282	12.55	Caffein
21	29.018	1.56	Cyclononasiloxane, octadecamethyl

Table 2(a-c): Showing the identification and relative percentage of volatile compounds in superfine green tea at 90°C in 20, 40 and 120 minutes time of extraction.

Compounds present	Fine			Superfine		
Extraction time (min)	**20**	**40**	**120**	**20**	**40**	**120**
Myristic acid	8.74	6.85	7.87	7.92	2.09	2.89
Palmitic acid	24.77	20.6	23.21	23.87	13.83	21.07
Stearic acid	3.28	1.57	1.56	2.87	1.7	1.95
Oleic acid	40.41	35.1	39.33	38.46	22.99	34.92
Linoleic acid	1.87	0.91	0.85	1.5	0.85	2.6
Caffein	1.82	11.4	11.47	5.79	37.7	12.55
Phthalic acid	*	*	*	3.28	3.09	3.63
1H-Purin-6-amine	1.96	2.57	2.95	1.98	2.1	1.58

Table 3: Relative percentage of some of major compounds which are present in both tea types.

Compound name	Bioactivity	References
Methyltetradecanoate/ Myristic acid	Antioxidant, Cancer-preventive, Hypercholesterolemic, Lubricant, Nematicide	[11]
Hexadecanoic acid/ palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Anti-androgenic, Flavor Hemolytic, 5-Alpha reductase inhibitor	[11]
Octadecanoic acid/ Stearic acid	5-Alphareductase inhibitor, Cosmetic, Flavor, Hypocholesterolemic	[11]
9-Octadecenoic acid/ oleic acid	Anti-inflammatory, Anti-androgenic, Cancer preventive, Dermatitigenic, Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor	[12]
9,12-Octadecadienoic acid/Linoleic acid	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Anti-androgenic, Hypocholesterolemic Nematicide, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic, Antiacne	[13]
	Anticancerous and diuretic	[14]
1H-Purine-2,6-dione/ Caffein	Prevents: liver cirhossis, Type 2 diabetes, apnea of prematurity, bronchopulmonary dysplasia in prenmature infants. Secondary metabolic products of caffeine: *paraxanthin* increase lipolysis, *theobromine* is a vasodilator; *theophylline* acts as a muscle relaxant, anti-asthmatic.	[15-18]
Phthalic acid, diethyl ester	Can cause damage to the nervous system and reproductive system, mutagenic and carcinogenic.	[19]

Table 4: Biological activity of some phyto-components identified in the green extracts.

Stearic acid, 9-Octadecenoic acid/oleic acid, 9,12-Octadecenoic acid/Linoleic acid, 1H-PURINE-2,6-DIONE/Caffeine, pthalic acid, diethyl ester and 1H-PURINE-6-AMINE. The percentage release of compounds from these selected tea types varied according to the time of extraction employed, for example the 9-Octadecenoic acid/ oleic acid and Hexadecanoic acid/palmitic acid showed maximum peak area in 20 minutes time of extraction in Fine and Superfine tea types. 1H-PURINE-2,6-DIONE/Caffeine showed maximum peak area in 40 minutes time of extraction in Fine and Superfine varieties. Thus the best time of extraction can be considered in the range of 20-40 minutes. Further increase in time leads to decrease in the relative percentage of most of the physiologically important phytochemical compounds. Most of the compounds detected were health benefiting and physiologically important (Table 4) except for pthalic acid/diethyl ester which has been reported to show various toxic effects such as cancer and neurotoxicity etc. However pthalic acid was found in Super fine tea but was not detected in the extracts of Fine tea.

Conclusions

Present study confirmed presence of various health benefitting compounds using GC-MS. Among various times of extraction employed to tea samples, it was seen that the best time of extraction for the retention of maximum health benefiting compounds such as caffeine, linoleic acid, oleic acid, palmitic acid and etc. was in the range of 20-40 minutes. The present study also suggests presence of some toxic substances in the tea extracts such as pthalic acid which need further in detailed studies.

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