Ahmad et al., *Cogent Food & Agriculture* (2015), 1: 1106387
http://dx.doi.org/10.1080/23311932.2015.1106387

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FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

Effect of extraction time on antioxidants and bioactive volatile components of green tea (*Camellia sinensis*), using GC/MS

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Received: 03 September 2015
Accepted: 01 October 2015
Published: 23 November 2015

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Reviewing editor:
Fatih Yildiz, Middle East Technical University, Turkey

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Abstract: Two green tea types, leaf grade and sanding, were extracted at different time intervals: 20, 40, and 120 min at a constant temperature of 50°C. The extracts were analyzed by GC/MS technique. The major compounds identified were myristic acid, palmitic acid, stearic acid, oleic acid, 1H-purine-2,6-dione, caffeine, linoleic acid, diethyl ester, and 1H-purine-6-amine. Stearic acid, palmitic acid, linoleic acid, and myristic acid were more abundantly present in the leaf-grade variety than sanding. However, some levels of acetic acid, cyclobutanol, hexadecanoic acid, octadecanoic acid, 9-octadecenoic acid, and caffeine were also found in both the tea types. Most of the volatile compounds were detected between 20–40-min time of extraction. The 40-min time of extraction also showed the maximum content of polyphenols and antioxidants in both the tea types. Thus, 40 min was suggested as the most suitable time for maximum extraction of bioactive volatiles, antioxidants, and polyphenols from green tea.

Subjects: Bioscience; Environment & Agriculture; Food Science & Technology

Keywords: *Camellia sinensis*; extraction; GC–MS; antioxidants; polyphenols

1. Introduction

Green tea (*Camellia sinensis*) is native to the southern regions of China and some parts of India, Laos, Thailand, Vietnam, and Myanmar (Balentine, Harbowy, & Graham, 1998). Tea was first known to be

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PUBLIC INTEREST STATEMENT

The article reports about the extraction time required to release maximum bioactive components from green tea. This could be helpful for industrialists to optimize the process for extracting tea components. In the present era, the general public is more prone to various diseases like cancer, diabetes, cardiovascular diseases and others. Green tea is reported for its health-promoting activities such as anti-proliferative, anti-hypertensive, anti-atherosclerotic, hypo-cholesterolemic, and hypo-lipidemic activities, which are mostly attributed to the flavonoids, antioxidants, and total phenols present in it. The article reports that processing time effects the type and amount of compounds released from green tea. The consumers can process green tea at the domestic level, at suggested time for extraction, and can avail maximum health benefits from it.

discovered as a medicinal drink in China around 2737 BC. It was then introduced to Japan during the thirteenth century and to Europe in the sixteenth century, then to America, Africa, and other regions of the world (Chan, Fong, Cheung, Huang, & Chen, 1992; Graham, 1992). 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 (Chan et al., 1992). It grows best in tropical and subtropical areas with adequate rainfall, good drainage, and slightly acidic soil. The worldwide popularity of tea has increased not only because of its characteristic aroma and flavor, but also due to its health-promoting activities such as anti-proliferative, anti-hypertensive, anti-atherosclerotic, hypo-cholesterolemic, and hypo-lipidemic activities, which are mostly attributed to the flavonoids, antioxidants, and total phenols present in it (Ahmad et al., 2014, 2015; Chen, Zhu, Tsang, & Huang, 2001; Cheng, 2006; Kim, Goodner, Park, Choi, & Talcott, 2011). Various studies about the tea volatiles have already been reported (Amanda, Teobaldo, Ivan, & Edward, 1996; Bilia, Flamini, Taglioli, Morelli, & Vincieri, 2002; Kumar, Satyanarayan, Gopikrishna, & Solomon, 2012; Shah et al., 2015),

It is essential to determine the volatile as well as non-volatile components present in green tea for a better understanding of the physiological, pharmacological, and flavor-imparting properties of tea. To the best of the knowledge of the authors, the effect of extraction time on the volatile constituents of green tea has not been reported yet. In general, the analysis of volatile components is usually conducted using gas chromatography and mass spectrometry (GC–MS). The present study analyzes the effect of extraction time on the bioactive volatile components, antioxidants, and polyphenolic contents of green tea.

2. Materials and methods

2.1. Sample collection and preparation of the extract

Two green tea types, viz. leaf grade and Sanding, were purchased from the local market and stored at room temperature until use. To extract the sample, 2 g of each green tea variety were extracted in 50-ml distilled water at a constant temperature of 50°C, for different times 20, 40, and 120 min. The extracts obtained were concentrated under reduced pressure in a rotary evaporator (Rotary Equitron).

2.2. Instrument and chromatographic conditions

The concentrated pure extracts were 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 analyzed on a GC–MS system Shimadzu QP2010 Plus with 2010 GC. An Omega SPTm column 0.25 mm ID, film thickness of 0.25 µm was used with nitrogen as the 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 min and then increased to 200°C at 15°C/min and held for 2 min, and finally increased to 240°C at 20°C/min and held for 18 min. 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.

2.3. Free radical scavenging activity

The antioxidant activity of the tea extract was determined according to the method of Gani et al. (2014) with some modifications. Eighty microliters of the sample were mixed with 200 µL of 0.05% DPPH in a total volume of 4-ml methanol and allowed to react in the dark for 30 min. The results were expressed as percent inhibition using the relation.

$$\text{Percent inhibition} = (\text{Absorbance of control} - \text{Absorbance of sample})/\text{Absorbance of control} \times 100$$

2.4. Determination of total phenol content

Total phenol content of the extract was determined according to the method of Jan et al. (2015) with modifications. One hundred and fifty microliters of the extract, 2,400 µL of nanopure water, and

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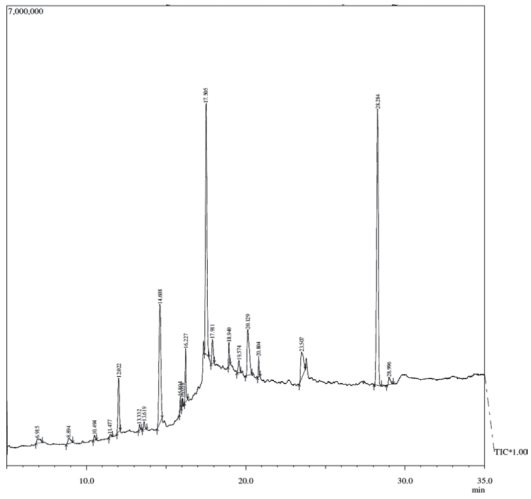
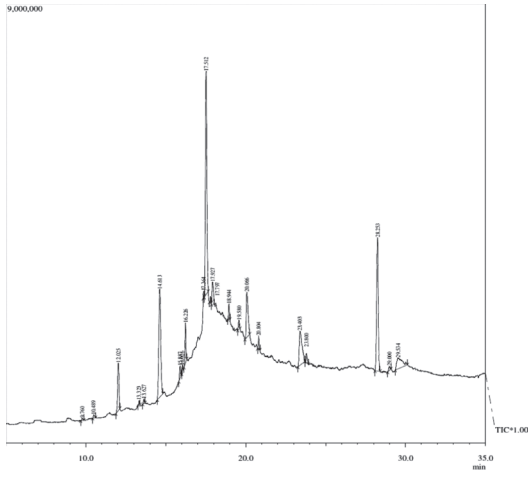
Figure 1. (a)–(c) Showing GC–MS chromatograph of leaf-grade tea type at different extraction times.

(a) Leaf grade 20 minutes



(b) Leaf grade 40 minutes



(c) Leaf grade 120 minutes

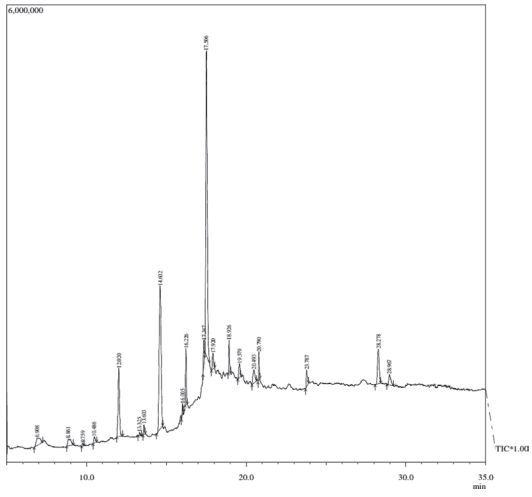


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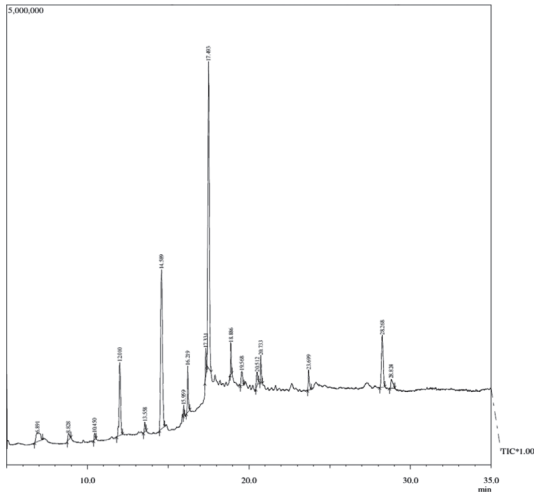
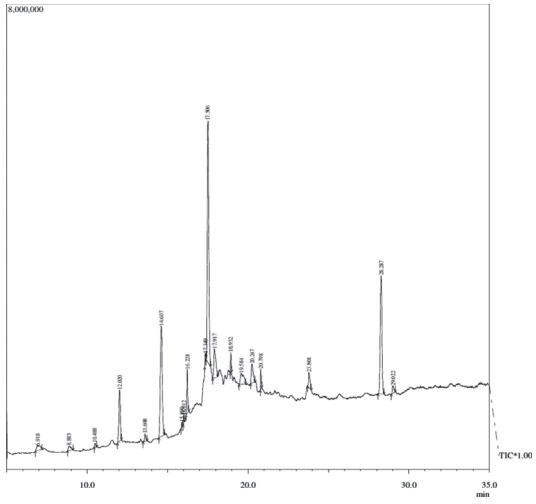
Figure 2. (a)–(c) Showing GC–MS chromatograph of sanding-grade tea type at different extraction times.

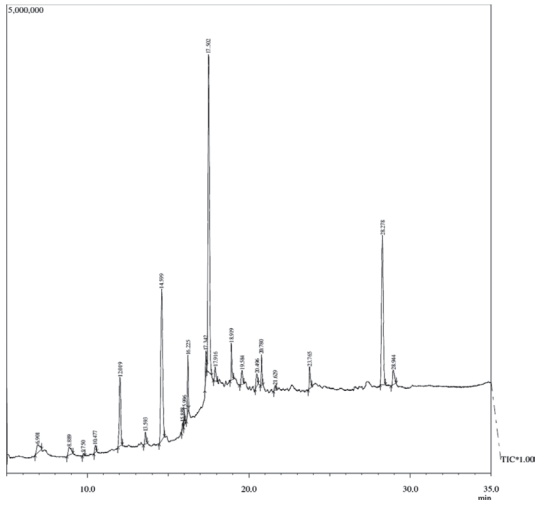
(a) Sanding 20 minutes



(b) Sanding 40 minutes



(c) Sanding 120 minutes



150 µL of 0.25 N folin ciocalteu's reagent were combined and then mixed well by shaking. The mixture was allowed to react for 3 min then 300 µL of 1 N Na_2CO_3 solution was added and mixed well again by shaking. The solution was incubated at room temperature in the dark for 2 h. The absorbance was measured at 725 nm using a spectrophotometer and the results were expressed as milligram of gallic acid equivalents (GAE) per gram of the extract.

Table 1. (a)–(c) Showing the identification and relative percentage of volatile compounds in leaf-grade type of green tea at 50°C in 20-, 40-, and 120-min time of extraction

Peak	*R* Time	Area %	Name of compound
(a) Leaf grade, 20 min			
4	12.01	8.19	Heptadecanoic acid and methyl ester
5	13.558	0.8	Cyclononasiloxane and octadecamethyl
6	14.589	24.43	Palmitic Acid and hexadecanoic acid
7	15.959	0.67	1H-purine-6-amine
8	16.219	3.85	Phthalic Acid
9	17.331	2.24	Stearic acid, methyl ester, octadecanoic acid, and methyl ester
10	17.493	36.94	9-octadecenoic acid, methyl ester, and oleic acid
12	19.568	1.95	12-octadecadienoic acid, methyl ester, and linoleic acid
13	20.512	1.64	Tetradecanoic acid and myristic acid
16	28.268	7.42	Caffein
(b) Leaf grade, 40 min			
4	12.02	5.77	Methyl heptadecanoate
6	14.607	18.31	Palmitic acid, methyl ester, hexadecanoic acid, and methyl ester
7	15.89	0.34	Phenol, 2-methoxy-4-(1-propenyl)
8	16.012	0.35	1H-purine-6-amine
9	16.228	3.73	Phthalic Acid
10	17.349	0.57	Octadecanoic acid, methyl ester, stearic acid, and methyl ester
11	17.506	31.24	9-octadecenoic acid, methyl ester, oleic acid, and methyl ester
12	17.917	5.57	Linoleic acid and 9,12-octadecadienoic acid
14	19.584	3.36	13,16-octadecadienoic acid and methyl ester
15	20.247	3.23	Tetradecanoic acid and myristic acid
18	28.287	18.08	Caffein
(c) Leaf grade, 120 min			
3	9.75	0.16	Methyl hexadecanoate
5	12.019	6.41	Methyl heptadecanoate
7	14.599	19.22	Methyl hexadecanoate, palmitic acid, and methyl ester
8	15.888	0.38	Phenol, 2-methoxy-4-(1-propenyl)-
9	15.996	0.73	1H-purine-6-amine,
10	16.225	3.45	Phthalic acid
11	17.342	1.61	Methyl octadecanoate, stearic acid, and methyl ester
12	17.502	33.09	9-octadecenoic acid, methyl ester, oleic acid, and methyl ester
13	17.916	1.43	9,12-octadecadienoic acid, methyl ester, and linoleic acid
15	19.584	1.49	9,12-octadecadienoic acid and methyl ester
16	20.496	1.42	Tetradecanoic acid
18	21.629	0.43	12,15-octadecadiynoic acid and methyl ester
20	28.278	17.8	Caffein

Table 2. (a)–(c) Showing the identification and relative percentage of volatile compounds in sanding type of green tea at 40°C in 20-, 40-, and 120-min time of extraction

Peak	*R* Time	Area %	Compound name
(a) Sanding, 20 min			
4	11.477	0.25	Carbamic acid, phenyl ester, and phenyl carbamate
5	12.022	4.26	Methyl tetradecanoate and tetradecanoic acid
6	13.332	0.37	Phenol and 2-methoxy-4-2-propenyl
8	14.608	13.98	Hexadecanoic acid and methyl ester
9	15.894	0.76	Phenol, 2-methoxy-4-(1-propenyl)
11	16.227	3.45	1,2-benzenedicarboxylic acid and diethyl ester
12	17.505	24.29	9-octadecenoic acid (Z) and oleic acid
13	17.911	2.38	Cis-9,cis-12-octadecadienoic acid, and cis-linoleic acid
15	19.574	1.28	Cyclopropanebutanoic acid,
16	20.129	6.88	Tetradecanoic acid and myristic acid
17	20.804	1.21	1H-purine-6-amine
18	23.507	4.74	Hexadecanoic acid and palmitic acid
19	28.284	30.62	1H-purine-2,6-dione and 3,7-dihydro-1,3,7-trimethyl
(b) Sanding, 40 min			
1	9.76	0.19	Dodecanoic acid, methyl ester, and lauric acid
3	12.025	4.83	Tetradecanoic acid and methyl ester
4	13.323	0.26	Phenol and 2-methoxy-4-(2-propenyl)
6	14.613	16.61	Hexadecanoic acid and methyl ester
7	15.887	1.04	Phenol, 2-methoxy-4-(1-propenyl), phenol, and 2-methoxy-4-propenyl
8	16.026	0.36	1H-purine-6-amine
9	16.226	3.14	1,2-benzenedicarboxylic acid and diethyl ester
10	17.364	0.27	Octadecanoic acid and methyl ester
11	17.512	26.58	9-octadecenoic acid, methyl ester, and oleic acid
12	17.797	0.29	9-octadecenoic acid and octadec-9-enoic acid
13	17.927	2.82	Methyl octadeca-9,12-dienoate, and octadeca-9,12-dienoic acid
15	19.58	0.83	Cyclopropanebutanoic acid
16	20.066	6.93	Tetradecanoic acid and myristic acid
17	20.804	0.95	1H-purine-6-amine
18	23.403	8.19	n-Hexadecanoic acid, hexadecanoic acid, n-Hexadecoic acid, and palmitic acid
19	23.8	0.94	1H-purine-6-amine
20	28.253	18.69	1H-purine-2,6-dione and 3,7-dihydro-1,3,7-trimethyl
21	29	0.43	1H-purine-6-amine
21	29.534	5.07	Oleic Acid, 9-octadecenoic acid, and delta.(Sup9)-cis-oleic acid
(c) Sanding, 120 min			
3	9.759	0.24	Tridecanoic acid and methyl ester
5	12.02	7.71	Methyl ester, myristic acid, methyl ester, and methyl myristate
6	13.325	0.19	Phenol, 2-methoxy-4-(2-propenyl), 4-allyl-2-methoxy-phenol, and 1,3,4-eugenol
8	14.602	22.22	Hexadecanoic acid, methyl ester, palmitic acid, and methyl ester
9	16.005	0.67	1H-purine-6-amine, [(2-fluorophenyl)methyl]
11	17.347	2.12	Octadecanoic acid, methyl ester, stearic acid, and methyl ester

(*Continued*)

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Table 2. (*Continued*)

Peak	*R* Time	Area %	Compound name
12	17.506	38.18	9-octadecenoic acid, methyl ester, and oleic acid
13	17.92	1.81	10,13-octadecadienoic acid and methyl ester
14	18.926	2.01	1H-purine-6-amine and [(2-fluorophenyl)methyl]
15	19.579	1.72	Cyclopropanebutanoic acid and methyl ester
16	20.493	2.37	9-octadecenoic acids
18	23.787	1.79	1H-purine-6-amine and [(2-fluorophenyl)methyl]
19	28.278	5.16	Cafeina, caffein, caffine, cafipel, coffeine, guaranine, and koffein

2.5. Stastical analysis

Analysis was carried out in triplicates and results were represented as means ± standard deviation. The data were assessed by analysis of variance (ANOVA) and significant differences were considered at $p \leq 0.05$ using Duncan's Multiple Range Test in SPSS software (version 16.0.).

3. Results and discussion

3.1. Identification and analysis of various components

GC–MS chromatograms of aqueous extract of the different types of green tea viz. leaf-grade and Sanding samples for different time periods of extraction are given in Figures 1 and 2. The number of volatile constituents as depicted by the peaks varied to some extent 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, different compounds were characterized and identified (Tables 1 and 2). The relative percentages of some major compounds which are present, in almost, in both the samples are presented in Table 3. They were identified as myristic acid, palmitic acid, stearic acid, oleic acid, 1H-purine-2, 6-dione, caffeine, linoleic acid, diethyl ester, and 1H-purine-6-amine. However, compounds such as stearic acid, palmitic acid, linoleic acid, and myristic acid were more abundantly present in the leaf grade than sanding. Varying levels of acetic acid, cyclobutanol, hexadecanoic acid, octadecanoic acid, 9-octa-

Table 3. Relative percentages of some major compounds present in both types of samples

Tea type	Compounds present	20-min	40-min	120-min
Sanding	Myristic acid	$6.88^{a} \pm 0.07$	$6.93^{a} \pm .09$	$7.71^{b} \pm 0.08$
	palmitic acid	$13.98^{a} \pm 0.13$	$16.61^{b} \pm 0.17$	$22.22^{c} \pm 0.12$
	Stearic acid	*	$0.27^{a} \pm 0.12$	$2.12^{a} \pm 0.06$
	oleic acid	$24.29^{a} \pm 0.22$	$26.58^{b} \pm 0.06$	$38.18^{c} \pm 0.12$
	Linoleic acid	$2.38^{b} \pm 0.10$	$2.82^{c} \pm 0.12$	$1.72^{a} \pm 0.04$
	Caffein	$30.62^{c} \pm 0.22$	$18.69^{b} \pm 0.12$	$5.16^{a} \pm 0.22$
	1H-purin-6-amine	$1.21^{a} \pm 0.02$	$1.21^{a} \pm 0.03$	$1.91^{b} \pm 0.07$
Leaf grade	Myristic acid	$1.64^{b} \pm 0.07$	$3.23^{c} \pm .22$	$1.42^{a} \pm 0.06$
	palmitic acid	$24.43^{c} \pm 0.23$	$18.31^{a} \pm 0.06$	$19.22^{b} \pm 0.08$
	Stearic acid	$2.24^{c} \pm 0.07$	$0.57^{a} \pm 0.03$	$1.61^{b} \pm 0.07$
	oleic acid	$36.94^{c} \pm 0.12$	$31.24^{a} \pm 0.08$	$33.09^{b} \pm 0.32$
	Linoleic acid	$1.95^{b} \pm 0.08$	$5.57^{c} \pm 0.22$	$1.43^{a} \pm 0.31$
	Caffein	$7.42^{a} \pm 0.09$	$18.08^{b} \pm 0.05$	$17.8^{b} \pm 0.19$
	1H-purin-6-amine	$2.1^{a} \pm 0.09$	$1.95^{a} \pm 0.05$	$1.76^{a} \pm 0.13$

Notes: The values are mean ± SD of three independent determinations.

Means followed by different superscripts within a row are significantly ($p \leq 0.05$) different.

*Not detected.

decenoic acid, and caffeine were also found in both the tea types. The amount of compounds released in the extract was observed to be influenced by the type of tea and time of extraction employed: for example, 9-octadecenoic acid, oleic acid, hexadecanoic acid, and palmitic acid showed the maximum peak area at 20 min in leaf-grade tea, while in sanding, the maximum peak area was seen at 120 min of extraction. Similarly, 1H-purine-2, 6-dione and caffeine showed the maximum peak area at 40 min of extraction in leaf-grade tea, whereas sanding showed the maximum peak area at 20 min of extraction. Further increase in extraction time leads to a decrease in the relative percentage of most of the physiologically important volatile compounds. Most of the compounds detected were health benefiting and physiologically important as mentioned in Table 4.

3.2. Total phenolic content

The effect of extraction time on the polyphenolic content of green tea extracts is depicted in Table 5. The results revealed that with increase in time of extraction from 20 to 40 min in both types of green tea, the content of polyphenols in the extract increased significantly from 120.79 to 137.59 GAE/g and 116.59 to 131.37 GAE/g, respectively, but further increase in time of extraction up to 120 min decreased the polyphenols to 118.76 and 127.45 GAE/g, respectively. Hence, the maximum amount of total phenols was observed at 40 min of extraction and thus suggesting it as the most efficient extraction time for maximum release of phenolic components in both the types of green tea. The decrease in total polyphenols at prolonged time of extraction may be due to the thermal degradation of the polyphenols. Thermal degradation of heat-susceptible phenolic compounds leads to the decrease in the total phenolic content (Randhir, Kwon, & Shetty, 2008). The decrease in total phenolic content could be due to the alteration in molecular structure of phenolic compounds, which may lead to the decrease in extractability due to the degree of polymerization (Altan, McCarthy, & Maskan, 2009). Perva-Uzunalić et al. (2006) also showed the tendency of catchins to degrade during prolonged extraction in some green tea types (Fanning Belas and China) and also found highest extraction efficiency at 80°C for 20–30 min of extraction.

Table 4. Biological activity of some phytocomponents identified in the green extracts

Compound name	Bioactivity	References
Methyltetradecanoate/Myristic acid	Antioxidant, Cancer-preventive, Hypercholesterolemic, Lubricant, Nematicide	Gopalakrishnan and Vadivel (2011)
Hexadecanoic acid/Palmitic acid	Antioxidant, Hypocholesterolemic Nematicide, Pesticide, Anti androgenic, Flavor, Hemolytic,5-Alpha reductase inhibitor	Gopalakrishnan and Vadivel (2011)
Octadecanoic acid/Stearic acid	5-Alphareductase inhibitor, Cosmetic, Flavor, Hypocholesterolemic	Gopalakrishnan and Vadivel (2011)
9-octadecenoic acid/Oleic acid	Antiinflammatory, Antiandrogenic Cancer preventive, Dermatitigenic Hypocholesterolemic, 5-Alpha reductase inhibitor, Anemiagenic Insectifuge, Flavor	Jananie, Priya, and Vijayalakshm (2001)
9,12-octadecadienoic Acid/Linoleic acid	Hypocholesterolemic, Nematicide Antiarthritic, Hepatoprotective Anti androgenic, Hypocholesterolemic Nematicide, 5-Alpha reductase inhibitor, Antihistaminic, Anticoronary Insectifuge, Antieczemic, Antiacne Anticancerous and diuretic	Mathew (2011), Nehlig, Daval, and Debry (1992)
1H-purine-2,6-dione/Caffein	Prevents: liver cirhossis, Type 2 diabetes, apnea of prematurity, bronchopulmonary dysplasia in premature infants.Secondary metabolic products of caffeine: *paraxanthin* increase lipolysis, *theobromine* is a vasodilator, *theophylline* acts as a muscle relaxant, anti asthmatic.	Kugelman and Durand (2011), Van (2008), Muriel and Arauz (2010)

Table 5. Effect of extraction time on total phenolic content (TPC) and antioxidant activity of two green tea types

Time	Leaf-grade type		Sanding type	
	TPC (GAE/g)	DPPH (% inhibition)	TPC (GAE/g)	DPPH (% inhibition)
20	$120.79^{b} \pm 0.69$	$73.69^{b} \pm 0.31$	$116.59^{c} \pm 1.3$	$72.55^{b} \pm 1.2$
40	$137.59^{a} \pm 1.56$	$78.34^{a} \pm 0.94$	$131.37^{a} \pm 1.2$	$76.4^{a} \pm 0.62$
60	$118.71^{b} \pm 0.48$	$74.13^{b} \pm 1.0$	$127.45^{b} \pm 0.96$	$74.45^{ab} \pm 1.3$

Notes: The values are mean ± SD of three independent determinations.

Means followed by different superscripts within a column are significantly ($p \leq 0.05$) different.

3.3. DPPH free radical scavenging activity

It is well known that the antioxidant activity of plant extracts containing polyphenolic components is due to their capacity to be donors of hydrogen atoms or electrons and to capture the free radicals (Shon, Kim, & Sung, 2003). Free radicals are known to be a major factor in biological damages. In brief, the reduction capacity of DPPH was determined by the decrease in its absorbance at 517 nm, which is reduced by the antioxidant (Duh, 1998). The extent of the reaction depends on the hydrogen-donating ability of the antioxidant (Bondet, Brand-Williams, & Berset, 1997).

The scavenging ability of both extracts of green tea at 20 and 120 min did not vary significantly ($p \leq 0.05$), whereas it was significantly ($p \leq 0.05$) higher at 40-min time of extraction (Table 5). The prolonged extraction time decreases the antioxidant capacity of green tea. This may be due to the thermal degradation of the antioxidant components at higher extraction time. The decrease in antioxidant activity at higher extraction time may also be due to the decrease in polyphenols because polyphenols exhibit antioxidant properties (Baba et al., 2014). The leaf-grade variety showed comparatively better antioxidant activity than sanding.

4. Conclusion

The present study confirmed the presence of various health-benefiting compounds using GC–MS. Leaf grade was found to be richer in stearic acid, palmitic acid, linoleic acid, and myristic acid than sanding. The antioxidant and phenolic content of both tea types were found to be higher at 40-min time of extraction, whereas prolonged extraction time of 120 min decreased the polyphenols and antioxidants from both types of green tea. It was concluded that 20–40 min extraction time is the most suitable for maximum retention of bioactive compounds in green tea.

Acknowledgment
The authors are highly thankful to the Department of Biotechnology, Government of India.

Funding
The authors received no direct funding for this research.

Competing interests
The authors declare no competing interest.

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Citation information
Cite this article as: Effect of extraction time on antioxidants and bioactive volatile components of green tea (*Camellia sinensis*), using GC/MS, Mudasir Ahmad, Waqas N. Baba, Adil Gani, Touseef Ahmed Wani, Asir Gani & F.A. Masoodi, *Cogent Food & Agriculture* (2015), 1: 1106387.

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Cogent Food & Agriculture **(ISSN: 2331-1932) is published by Cogent OA, part of Taylor & Francis Group.**