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Research Article

Comparison of the Volatile Compounds among Different Production Regions of Green Tea using Simultaneous Distillation Extraction Coupled with Gas Chromatography-mass Spectrometry

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Abstract: Chinese green teas are mainly made of the leaves of the plant *Camellia sinensis* and mainly distributed in provinces of Zhejiang, Anhui, Henan and Jiangsu, etc. Because of having same tea varieties and processing technology, different origins of green teas are very similar in appearance. Whereas different production areas and climatic conditions between them may have cause different quality characters, such as aroma and taste. So it is very necessary to study the different regions of green teas and explore environment and geographical factors to volatile components influence. In this study, the aroma components of four typical green teas: Xihulongjing, Xinyangmaojian, Lu'anguapian and Biluochun, from Zhejiang, Henan, Anhui and Jiangsu in China, were extracted by Simultaneous Distillation Extraction (SDE) and identified by Gas Chromatography-Mass Spectrometry (GC-MS), then compared the similarities and differences between them on the aroma components was made. The result showed that 61 aroma constituents were indentified in four green teas, mainly including alcohols, hydrocarbons and ketones compounds. Through contrast and comparison of results, we find that these different origins of green teas have some differences, but also share some similarities based on the volatile components. The difference of place of origin and elevation will grow different tea plants and will have different volatile components due to the environment.

Keywords: Aroma components, GC-MS, green tea, production regions, SDE

INTRODUCTION

Green tea is a non-fermented tea that is mainly consumed in many Asian countries especially in Japan and China, the common process of green tea production consists of four stages, namely, withering, panfiring, rolling and drying (Wang *et al*., 2011a, 2014b). In China, green tea mainly made by the plant of *Camellia sinensis* var. *sinensis* and mainly distributed in four regions of Zhejiang, Anhui, Henan and Jiangsu Province (Zhu *et al*., 2012). Green tea due to its multiple health-promoting effects as well as its special flavour and taste, has attracted more and more attention worldwide as a "hot topic" in the recent field of tea science (Ko *et al*., 2011; Muller and Pfaffl, 2012).

The major quality attributes of tea are appearance, aroma, color and taste of tea infusion, consumer acceptability of tea depends mostly upon its aroma and taste (Chaturvedula and Prakash, 2011; Qin *et al*., 2013). The quality and health efficiency of teas from different geographical origins are various because of the difference of their climatic conditions, soil, growth altitude and horticultural practices, plucking season, sorting of leaves, processing and storage (Ye *et al*., 2012). The attribute of tea according to its geographical origin is recognized and appreciated by the consumers. Recently, there are a small amount of literatures have distinguished between different regions of the teas using chemical methods, such as Lv *et al*. (2014b) used chemo metrics methods and volatile components to discriminate dark samples from Yunnan and Hunan Province; Wang *et al*. (2011b) used HPLC fingerprint and combined with seven bioactive components to discriminate oolong tea samples from different geographical origins; Kovács *et al*. (2010) distinguish five Sri Lanka black teas from plantations of different geographical origins used electronic nose and electronic tongue. However, it is very little to study of different producing area of green tea by its chemical composition. Therefore, it is very important to investigate different producing area of green tea, because it is crucial to quality control.

Aroma plays an important role in determining the quality of green tea (Lee *et al*., 2013; Yang *et al*., 2013), the extraction method for aroma components from tea samples is a critical procedure for the

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characterization of aroma components (Gu *et al*., 2009). Due to the main advantages of simplifying experimental procedures, relatively low cost, saving organic solvents and high uptake, Steam Distillation Extraction (SDE) has been widely used for analyzing the volatile components of various foods, with satisfactory result (Chen *et al*., 2011; Du *et al*., 2014; Watkins *et al*., 2012).

The aim of this study is comparing the similarity and difference of aroma components to determine the geographical origin of green tea, exploring different geographical factors which influence aroma components and analysis the effect of these factors on green tea aroma. Green tea from four different production regions (Zhejiang, Henan, Anhui and Jiangsu province) in China were analyzed by the SDE coupled with Gas Chromatography-Mass Spectrometry (GC-MS) method.

MATERIALS AND METHODS

Tea samples: In this study, four green tea samples collected from four different green tea producing areas (Zhejiang, Henan, Anhui and Jiangsu province) in China, there were Xihulonging, Xinyangmaojian, Lu'anguapian and Biluochun green tea. All tea samples were produced in 2013 year and the quality level is grade one. Samples were stored in a cool and dry place.

Extraction of volatile compounds: The preparation of tea extract and chromatographic conditions for analysising of volatile compounds were developed and validated according to the method the same as previously described (Lv *et al*., 2014a, c). Briefly, 20 g of ground tea powder was weighed and extracted with 400 mL boiling water by the SDE with 40 mL dichloroethane as the solvent. The dichloroethane was dehydrated with 5 g anhydrous sodium sulphate and then concentrated to 1 mL under a purified nitrogen stream.

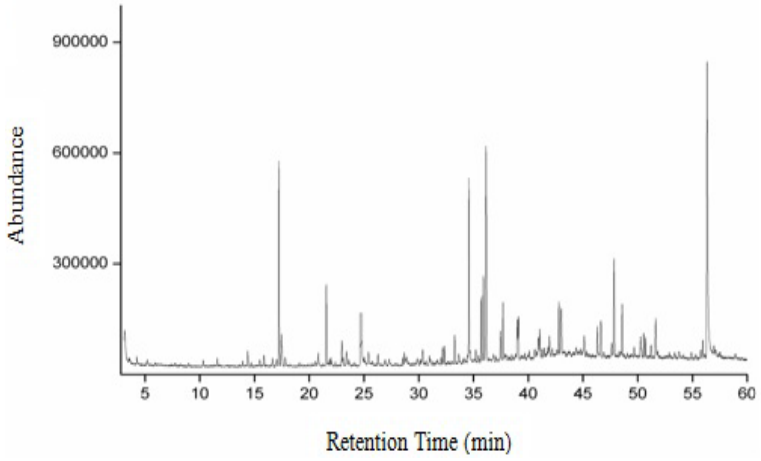
GC-MS analysis: The volatile compounds in the extract were analyzed by Agilent 7890B GC system, coupled with 5977A mass selective detector (Agilent Technologies, Palo Alto, CA, USA). An Agilent HP-5MS capillary column (30 m ×0.25 mm inner diameter, 0.25 μm film thickness) was equipped and the helium (percentage purity >99.999%) flow rate was 1 mL/min. The injector temperature was 250°C and one microlitre was in jected with the injection port orerated at a split ratio of 1:5. The GC oven temperature was held at 50°C for 5 min and programmed at 3°C/min from 50 to 210°C, this temperature was held for 3 min and then increased to 230°C at 15°C/min. The mass spectrometer was operated in an electron-impact mode of 70 eV with a source temperature of 230°C and a quadrupole set of 150°C, the mass scan range was 30-500 Atomic Mass Units (AMU) and solvent delay time was 3.0 min.

Data analysis: Identifications of the peaks were made by searching National Institute of Standards and Technology (NIST) 08.L MS library (a match quality of 85% minimum was used as a criterion). The relative percentages of the detected peaks were obtained by peak-area normalization and all relative response factors being taken as one.

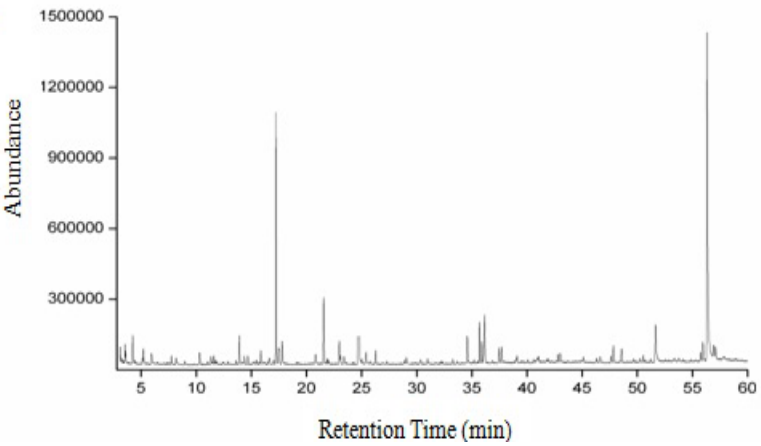
RESULTS AND DISCUSSION

Analysis of volatile compounds in four green tea samples.

In Total Ion Chromatogram (TIC) of tested green tea samples, the identification of peaks was carried out by National Institute of Standards and Technology (NIST) searching. A total of 61 aroma compounds were identified in four green teas, the TICs were presented in Fig. 1 and the information of these compounds was shown in Table 1.



(A)



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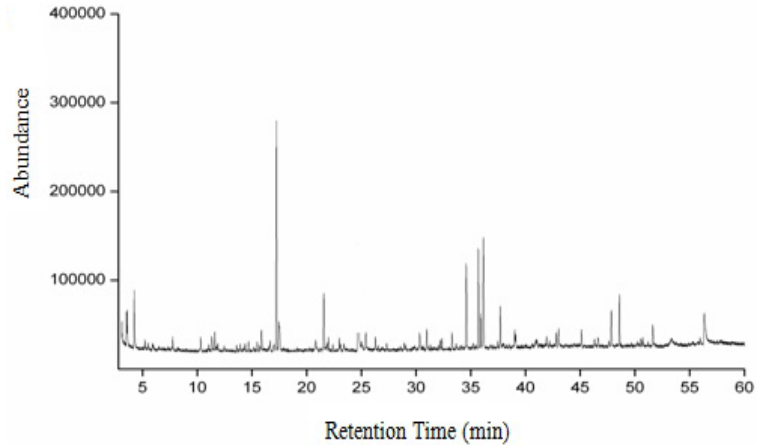

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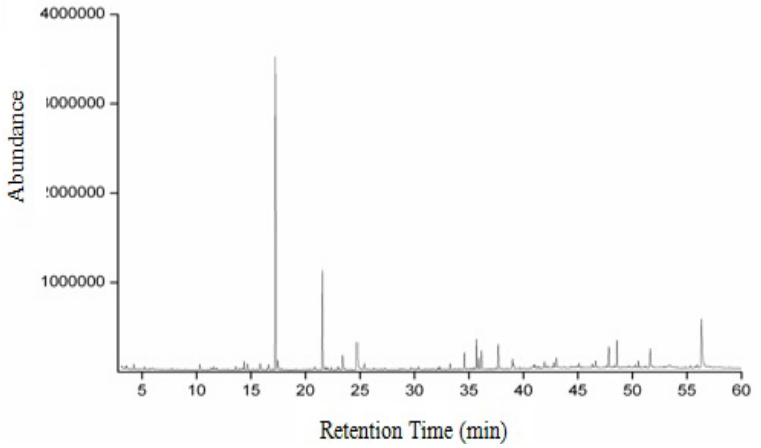

(D)

Fig. 1: The GC-MS TICs of aroma components in the four green teas (A) xihulongjing, (B) xinyangmaojian, (C) lu'anguapian, (D) biluochun

A total of 46 aroma compounds were identified in Xihulongjing green tea and jointly represented of 92.91% of the total extracts. These identified compounds mainly including phytol (21.09%), dihydroactinidiolide (10.61%), linalool (8.25%), β-ionone (7.86%), fitone (4.27%), α-terpineol (3.46%),

Table 1: GC-MS analysis results of aroma components in the four kinds of green tea samples

No.	Retention time (min)	Compound	Matching degree (%)	Relative percentage content (%)			
				Xihu longJing	Xinyang maojian	Lu'an guapian	Biluo chun
1	3.538	1-pentanol	86	-[a]	0.83	1.89	0.24
2	3.603	cis-2-penten-1-ol	90	-	0.51	2.07	-
3	4.238	Hexanal	90	0.36	1.41	3.26	0.60
4	5.206	α-furfural	87	-	1.06	0.88	0.39
5	5.948	3-furfuryl alcohol	83	-	1.12	-	-
6	7.752	Heptaldehyde	92	-	0.58	0.95	-
7	8.173	2-acetyl furan	90	-	0.52	-	-
8	10.316	Benzaldehyde	95	0.30	0.98	1.26	0.81
9	11.604	2, 3-octandione	88	0.37	0.56	1.39	0.46
10	11.877	β-pinene	90	-	-	-	0.31
11	13.604	D-limonene	94	-	-	-	0.51
12	13.907	Enzyl alcohol	97	-	2.03	0.56	0.18
13	14.382	Benzeneacetaldehyde	85	0.69	0.58	0.67	1.09
14	14.690	3, 7-dimethyl-1, 3, 6-octatriene	87	-	0.68	1.05	0.87
15	15.254	2-acetyl pyrrole	91	-	0.22	-	-
16	15.480	Acetophenone	85	0.26	0.48	-	0.15
17	15.856	Linalool oxide I	90	0.55	0.99	1.71	0.86
18	16.643	Linalool oxide II	90	0.55	0.48	-	1.09
19	17.237	Linalool	97	8.25	15.30	17.71	35.86
20	17.462	3, 7-dimethylocta-1, 5, 7-trien-3-ol	78	1.55	1.03	2.12	1.79
21	17.807	Phenethyl alcohol	93	0.48	1.87	-	0.21
22	20.572	Linalool oxide III	86	0.24	-	-	-
23	20.828	Linalool oxide IV	88	0.75	0.69	1.22	0.35
24	21.564	α-terpineol	86	3.46	4.37	4.83	11.70
25	21.860	Styralyl acetate	97	-	-	-	0.30
26	21.991	Safranal	91	0.33	-	-	-
27	22.982	2, 3-dihydrobenzofuran	83	1.18	1.69	1.22	-
28	23.415	Nerol	95	0.85	0.82	-	2.35
29	25.427	Nonanoic acid	85	-	-	-	0.98
30	26.282	Indole	93	0.48	0.93	1.23	0.22
31	28.900	1, 1, 6-trimethyl-1, 2-dihydronaphthalene	90	0.34	-	-	-
32	29.072	Geranic acid	90	-	0.51	-	-
33	30.324	Caproicacidhexneylester	86	-	-	1.86	-
34	30.983	Jasmone	95	-	-	1.64	-
35	30.372	Beta-Damascenone	93	0.97	-	-	0.42
36	32.194	α-ionone	97	0.79	0.23	1.02	0.32
37	32.360	4-(2, 6, 6-trimethyl-1, 3-cyclohexadien-1-yl)-3-buten-2-one	90	0.88	0.20	0.91	0.41
38	33.286	Geranylacetone	95	1.23	0.34	1.45	0.80
39	34.586	β-ionone	96	7.86	1.84	6.46	2.19
40	35.203	n-pentadecane	95	0.50	0.29	0.61	0.19
41	36.153	Dihydroactinidiolide	90	10.61	4.33	10.03	2.47
42	37.476	5-isopropyl-2-methyl -5-cyclohexen-1-one	80	1.38	1.02	0.71	0.16
43	37.696	Nerolidol	87	2.50	1.11	3.37	2.96
44	39.013	Cedrol	90	1.72	0.28	1.53	1.26
45	39.108	1-hexadecene	94	1.82	0.50	1.08	0.54
46	41.067	α-cadinol	91	1.42	0.45	0.52	0.55
47	41.939	2, 2', 5, 5'-tetramethylbiphenyl	80	0.92	-	0.86	0.76
48	42.800	n-heptadecane	97	2.30	0.55	1.49	0.65
49	43.020	2, 6, 10, 14-tetramethylpentadecane	96	2.80	0.80	2.55	1.72
50	45.121	Anthracene	91	0.86	0.41	1.92	0.62
51	46.320	Octadecane	90	1.21	0.25	0.58	0.44
52	46.646	2, 6, 10, 14-tetramethyl hexadecanedioic	96	2.60	0.70	0.46	1.46
53	47.845	Fitone	96	4.27	1.20	3.26	2.60
54	48.587	Unknown	X	2.36	0.96	4.34	3.37
55	50.267	Farnesylacetone	90	0.93	0.26	-	0.29
56	50.546	Methyl hexadecanoate	98	1.06	0.50	0.58	0.93
57	51.216	Isophytol	90	0.63	0.17	-	-
58	51.673	Hexadecanoic acid	99	-	4.76	-	-
59	55.769	Methyl linoleate	95	0.51	0.66	-	-
60	55.965	Methyl linolenate	95	1.06	1.41	-	0.42
61	56.327	Phytol	91	21.09	35.08	5.76	11.78

[a]: Not detected

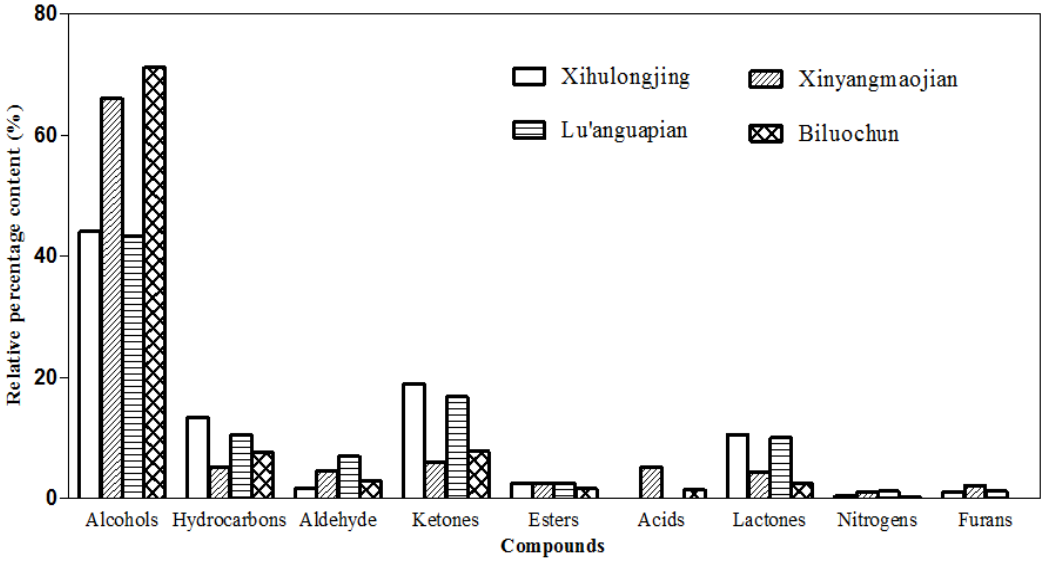

Fig. 2: Comparison of aroma components in four kinds of green tea

2, 6, 10, 14-tetramethyl pentadecane (2.80%), 2, 6, 10, 14-tetramethyl hexadecanedioic (2.60%), nerolidol (2.50%) and heptadecane (2.30%), etc.

A total of 51 aroma compounds were identified in Xinyangmaojian green tea and jointly represented of 97.58% of the total extracts. These identified compounds mainly including phytol (35.08%), linalool (15.30%), hexadecanoic acid (4.76%), α-terpineol (4.37%), dihydroactinidiolide (4.33%), enzyl alcohol (2.03%), phenethyl alcohol (1.87%) and β-ionone (1.84%), etc.

A total of 41 aroma compounds were identified in Lu'anguapian green tea and jointly represented of 92.67% of the total extracts. These identified compounds mainly including linalool (17.71%), dihydroactinidiolide (10.03%), β-ionone (6.46%), phytol (5.76%), α-terpineol (4.83%), nerolidol (3.37%), hexanal (3.26%) and fitone (3.26%), etc.

A total of 47 aroma compounds were identified in Lu'anguapian and jointly represented of 95.26% of the total extracts. These identified compounds mainly including linalool (35.86%), phytol (11.78%), α-terpineol (11.70%), nerolidol (2.96%), fitone (2.60%), dihydroactinidiolide (2.47%), nerol (2.35%) and β-ionone (2.19%), etc.

Analysis of chemical differences of aroma compounds in four green teas

The aroma compounds identified by GC-MS in four green tea samples covered nine categories of compounds in the classification of organic chemistry. These compounds included alcohols, hydrocarbons, esters, ketones, aldehyde, acids, lactones, nitrogens and furans, etc.

The aroma compounds comparison result in four different green teas was shown in Fig. 2. It can be seen that there were great differences in the content of aroma components among four green teas. The rusults showed that alcohols existed at the highest concentrations in the four tested green tea samples and were detected 71.18, 66.01, 44.04 and 43.29%, respectively. The total contents of ketones in Xihulongjing (18.94%) and Lu'anguapian (16.84%) were similar and greater than those in Biluochun (7.80%) and Xinyangmaojian (6.13%). Between the tested green tea samples, the contents of hydrocarbons were the highest in Xihulongjing, followed by that in Lu'anguapian and Biluochun and much lower in Xinyangmaojian. Total lactones contents were higher in Xihulongjing (10.61%) and Lu'anguapian (10.03%), while much lower in Xinyangmaojian (4.33%) and Biluochun (2.47%). The content of aldehyde, esters, acids, nitrogens and furans compounds were relatively lower in all the tested samples. In addtion, acids not detected in Xihulongjing and Lu'anguapian, furans not detected in Biluochun green tea.

From the above result we can see that there are some differences of four different origins of green teas, although they have the same tea varieties and processing technology. The differences between them may relate the difference of natural environment, cultivation conditions and other exogenous induction factors (Wang *et al*., 2008, 2014a). In this study, we just to make a basic research about four different origins green teas, some of the similarities and differences were reflected. However, this is just a tentative study and the small sample size limits to some extent the generalization of the findings made in the study. In the follow-up study, we will increase the number of the samples, combined with a variety of models, such as Principal Component Analysis (PCA), Cluster Analysis (CA), Linear Discriminant Analysis (LDA) and Soft Independent Modeling of Class

Analogy (SIMCA), to establish the classification of green teas from different areas in China. Meanwhile, we will explore the influence of different geographical factors on green tea aroma components and provides the theory basis of quality evaluation of green tea.

CONCLUSION

In this study, SDE coupled with GC-MS was developed for determination of the aroma components of four green teas from different producing regions. The results of this study show that 61 aroma compounds were indentified, among phytol, linalool, α-terpineol, β-ionone, dihydroactinidiolide, nerolidol and fitone were major volatile compounds in all of the four green teas. While the aroma content in different tested teas varied quite widely, these differences may relate the difference of natural environment, cultivation conditions and other exogenous induction factors of the tested green teas. And the potential of the developed method for other kind of teas will be investigated in future research.

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