Extraction and Chemical Characterization of Essential Oils from leaves of *Camellia sinensis*

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บทคัดย่อ

องค์ประกอบที่มีบทบาทเกี่ยวกับความหอมในชาที่ปลูกในเมืองไทยถูกสกัดด้วยเทคนิคการกลั่นด้วยไอน้ำแบบต่อเนื่อง หลังจากนั้นพิสูจน์เอกลักษณ์องค์ประกอบที่มีบทบาทเกี่ยวกับความหอมเหล่านั้นด้วยเทคนิคแก๊สโครมาโทกราฟี-แมสสเปกโทรเมตรี พบองค์ประกอบที่มีบทบาทเกี่ยวกับความหอมอย่างน้อย 54 องค์ประกอบ ซึ่งคิดเป็น 76-51-83.32% ขององค์ประกอบที่มีบทบาทเกี่ยวกับความหอมทั้งหมด จากการทดลองพบว่าองค์ประกอบ hotrienol geraniol และ linalool เป็นองค์ประกอบที่มีบทบาทเกี่ยวกับความหอมหลักในชาเขียวอูหลง นอกจากนี้พบว่า linalool geraniol และ α-terpineol เป็นองค์ประกอบที่มีบทบาทเกี่ยวกับความหอมในชาเขียว องค์ประกอบที่มีบทบาทเกี่ยวกับความหอมหลักในชาอูหลงก้านอ่อน (No. 17) ได้แก่ linalool indole และ *cis*-jasmone ในขณะที่องค์ประกอบที่มีบทบาทเกี่ยวกับความหอมหลักในชาอูหลงเบอร์ 12 ได้แก่ *trans*-nerolidol *cis*-jasmone และ geraniol ส่วนชาสี่ฤดูมีองค์ประกอบที่มีบทบาทเกี่ยวกับความหอมหลักคือ indole geraniol และ *cis*-jasmone จากผลการทดลองพบว่าการเปลี่ยนแปลงคุณภาพและปริมาณขององค์ประกอบที่มีบทบาทเกี่ยวกับความหอมในชาเกี่ยวข้องกับกระบวนการหมักชา โดยพบว่าองค์ประกอบที่มีบทบาทเกี่ยวกับความหอมมีจำนวนเพิ่มขึ้นเมื่อหมักชาด้วยกระบวนการหมักชาแบบกึ่งหมัก และจากการศึกษาฤทธิ์การต้านอนุมูลอิสระของสารสกัดชา พบว่าสารสกัดชามีฤทธิ์การต้านอนุมูลอิสระสูงจะมีปริมาณสารฟีนอลสูงตามไปด้วย ซึ่งจากการทดลองพบว่าชาเขียวมีฤทธิ์การต้านอนุมูลอิสระสูงที่สุด รองลงมาคือ ชาสี่ฤดู ชาอูหลงก้านอ่อน (No. 17) ชาพันธุ์ชาเขียวอูหลงและชาอูหลงเบอร์ 12 ตามลำดับ

Abstract

The volatile flavor components of different teas growing in Thailand were extracted using the simultaneous distillation and extraction (SDE) technique. These volatiles were investigated by GC-MS. At least 54 components representing 76.51-83.32% of all samples were identified. Hotrienol, geraniol and linalool were found to be the major components in Green Oolong tea. Green Assam tea contained linalool, geraniol and α-terpineol as the key flavor constituents. Oolong tea No. 17 was dominated by linalool, indole and *cis*-jasmone while the major flavor volatiles of Oolong tea No. 12 were *trans*-nerolidol, *cis*-jasmone and geraniol. Indole, geraniol and *cis*-jasmone were detected as the main constituents in Four Season tea. Change of quality and quantity of volatile flavor components was related to fermentation methods that increased volatiles were illustrated by the semi-fermented tea processing method. Green Assam tea infusion extract was evaluated to have the strongest antioxidant activities with the highest amount of phenol content followed by Four Season tea, Oolong tea No. 17, Oolong tea No. 12 and Green Oolong tea, respectively.

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ABBREVIATIONS AND SYMBOLS

SDE	=	simultaneous distillation and extraction
EGCG	=	(-)-epigallocatechin gallate
EGC	=	(-)-epigallocatechin
C_8	=	octane
C_{22}	=	docosane
DPPH	=	2,2-diphenyl-1-picrylhydrazyl
mL	=	milliliter
h	=	hour
°C	=	degree celsius
v/v	=	volume/volume
GC-MS	=	Gas chromatography-Mass Spectrometry
m	=	meter
mm	=	millimeter
min	=	minute
eV	=	electron volt
m/z	=	mass/charge
UV/vis	=	UV/visible
µg	=	microgramme
IC_{50}	=	50% Inhibition concentration
w/w	=	weight/weight

CHAPTER 1
INTRODUCTION

1.1 Introduction and literature reviews

Tea (*Camellia sinensis*) is and has been one of the most popular drinks in the world for over 4000 years. More than 3 million hectares of the world has been used for planting tea (Ravichandran and Parthiban, 1997). Nowadays, tea is used in pharmaceutical and industrial applications (Falé et al., 2009; Almajano et al., 2008; Ravichandran, 2002). Tea is manufactured in many areas over the world such as Japan, Taiwan, India as well as Thailand. Tea processing methods are classified by the degree of fermentation. Green tea is produced by a non-fermentation tea process while Oolong and red tea are established from partial fermentation. A completely fermented process is applied to black tea. The taste and flavor of tea is controlled by key chemical components which are volatile terpenes, caffeine, organic acids and polyphenols (Borse et al., 2002). Tea volatiles can be divided into two groups comprising non-terpenoid and tepenoid components. Non-terpenoids found in tea are hexenols providing fresh green flavor (Rawat et al., 2007) and products of lipid degradation (Mahata et al., 1993; Robinson and Owuor, 1992; Ganeshan and Ramasamy, 1996). The detected terpenoid components in tea are monoterpene alcohols, including linalool and geraniol, which impart a sweet floral aroma (Robinson and Owuor, 1992; Sanderson and Graham, 1973; Takeo, 1981). The quality and variation of volatile flavor components in tea is due to different environmental and ecological conditions and the tea processing method. Polyphenols are key non-volatile components which also play important role in tea. This component provides important beneficial effects of tea in term of the antioxidant activity and free radical scavenging ability (Frei and Higdon, 2003). Tea contains predominantly polyphenols such as catechins, monomeric flavonols, and (-)-epigallocatechin gallate (EGCG) which are detected in fresh tea leaves at a high level (Almajano et al., 2008; Rietveld and Wiseman, 2003; Chattopadhyay et al., 2004; Lau et al., 2002). The fermentation process also affect to polyphenols in tea. Flavanols, flavandiols and

phenolic acids like gallic acid, cumaric acid and caffeic acid are predominant in non-fermented green tea while some polyphenols are oxidized, degraded and polymerized enzymatically to theaflavins and thearubigens in black tea during the full fermentation tea processing method (Liebert et al., 1999; Weisburger, 1996). Katalinic and co-workers (2006) reported that tea variety and the content of EGCG controlled the antioxidant effectiveness. High content of EGCG and (-)-epigallocatechin (EGC) was detected in non-fermented and semi-fermented tea e.g. green and Oolong tea. Conversely, the content of both components were much lower in black tea.

1.2 Scope of study

The purpose of this present study was to investigate the fingerprint of volatile flavor components of Thai teas obtained from Mae Salong Mountain, Chiang Rai province, in the northern part of Thailand which is the most important area for planting tea in Thailand. The different tea infusions extracts were evaluated for their antioxidant activity and phenolic content.

1.3 Expected output

Value knowledge consisting aroma chemical constituents and antioxidant activity of tea obtained from Mae Salong Mountain, Chiang Rai province, in the northern part of Thailand as well as international publication.

CHAPTER 2
MATERIALS AND METHODS

2.1 Tea samples

Five tea samples manufactured under Thai Tea Suwirun Partnership, Mae Salong Mountain, Chiang Rai, Thailand were used in this study. These five different tea samples with different qualities consisted of non-fermentation tea (Green Oolong tea and Green Assam tea) as well as semi-fermentation tea (Chin Shin Oolong tea, Chin Hsuan Oolong tea and Four Season tea). Each sample lacked any additional manipulation and was kept under a temperature of 5 °C before being subjected to simultaneous distillation-extraction (SDE). All solvents were of analytical grade, dichloromethane, anhydrous sodium sulfate, mixtures of C_8 to C_{22} *n*-alkanes and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were purchased from Merck (Darmstadt, Germany).

2.2 Analysis of volatile flavor constituents

2.2.1 Extraction

The extraction was carried out in a modified Likens-Nickerson SDE apparatus for 5 h. Two hundred grams of tea samples and 200 mL of distilled water were added to a 500 mL round-bottom flask. Dichloromethane (150 mL) was added to another 250 mL round-bottom flask. Both flasks were connected to the apparatus, and more dichloromethane and distilled water were added into the central arm of the apparatus. The flask containing the dichloromethane was heated by using a water bath at 50 °C, while the flask containing the tea leaves and distilled water was heated by using a paraffin oil bath at 200 °C. After extraction, the distillate in a conical flask was concentrated using a vacuum rotary evaporator and then all sample essential oils were stored in the dark at 4 °C. All tea essential oil samples obtained were diluted to 1:10 v/v with dichloromethane prior to injection into the GC-MS instruments.

2.2.2 Gas chromatography-Mass Spectrometry (GC-MS)

The volatile flavor constituents of each sample were analyzed using an HP model 6890 gas chromatograph equipped with an HP-5MS (5% phenyl-polymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 60 °C and then increased by 2 °C/min to 250 °C. The injector and detector temperatures were 250 and 280 °C, respectively. Purified helium was used as the carrier gas at a flow rate 1 mL/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Identification of the volatile components was performed by comparison of their Kovát retention indices, relative to C_8-C_{22} *n*-alkanes, and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275, NIST 98 databases and Adams 1989 with corresponding data of volatile flavor components in tea (Bastos et al., 2006, Rawat et al., 2007, Borse et al, 2002, Wang et al., 2008, Ravichandran & Parthiban, 1997, Kumazawa & Masuda, 2002).

2.3 Antioxidant activity

2.3.1 Preparation of tea extract

Each extract was prepared by adding 100 mL of boiling distilled water to 10 grams of tea sample. Stirring using a magnetic stirrer was employed during the brewing process. After ten minutes, the tea infusion was filtered under vacuum and cooled to room temperature. The obtained tea extract was concentrated using a vacuum rotary evaporator and dried to constant weight in an oven at 105 °C.

2.3.2 DPPH radical scavenging assay

The effect of tea extracts on the content of 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) were evaluated by a spectrophotometric method based on the reduction of a

methanol solution of DPPH according to the modified method of Blois (1958). One milliliter of various concentrations of the each sample in methanol was added to 1 mL of a 0.003% methanol solution of DPPH and the reaction mixture was shaken vigorously. The tubes were allowed to stand at room temperature (27 °C) for 30 min. Each reaction mixture was then placed in the cuvette holder of the Perkin Elmer-Lamda 25 UV/vis spectrophotometer and monitored at 517 nm against a blank which used methanol as the baseline correction. The scavenging ability was calculated as follows: Scavenging ability (%) = 100 × [Absorbance of control – Absorbance of sample/Absorbance of control]. The antioxidant activity of all tea extracts was expressed as IC_{50} which was defined as the concentration (in μg/mL) of tea extract required to inhibit the formation of DPPH radicals by 50%. The experiment was carried out in triplicate and the results are the mean values.

2.4 Determination of total phenolic contents

Total phenolic content of tea extracts obtained from different samples was determined using the Folin–Ciocalteu reagent according to the modified method of Singleton and Rossi (1965) using gallic acid as standard. The tea solution (0.2 mL) was mixed with 1.0 mL of Folin–Ciocalteu reagent, 1.0 mL of an aqueous solution of 7% Na_2CO_3 and 5.0 mL of distillated water, respectively. Then, the mixture was vortexed vigorously. The reaction mixtures were allowed to stand for 30 min before absorbance at 765 nm was measured. The same procedure was also applied to the standard solutions of gallic acid. The calibration equation for gallic acid was $y = 0.00515x - 0.00400$ ($R^2 = 0.999$) where y is the absorbance and x is the concentration of gallic acid in mg/mL.

CHAPTER 3
RESULTS AND DISCUSSION

3.1Volatile flavor compounds analysis

The low percentage yield (0.01-0.03% w/w) of tea essential oils was obtained from all tea samples extracted by SDE technique. Results of the fingerprint in terms of volatile flavor compounds of all tea essential oils analyzed by GC-MS and their quantities determined with the reference to internal standard in different tea samples are summarized in Table 1. Sixty-three volatiles were identified representing 81.37% of the essential oil obtained from Green Oolong tea. Monoterpene alcohols of hotrienol (15.27%), geraniol (10.88%), linalool (10.71%) and α-terpineol (5.78%) were found to be the major components. Small amounts were coumaran (3.14%), nerol (1.82%) and indole (1.74%) were also detected. Fifty-four components representing 83.32% in Green Assam tea essential oil were identified. Linalool (15.61%), geraniol (14.22%), α-terpineol (11.27%) and nerol (5.26%) were found to be the key flavor constituents in Green Assam tea while benzyl ethanoate (3.73%), phytol (3.37%) and *trans*-nerolidol (2.41%) were minor components. A total of 60 constituents representing 81.85% of the Chin Shin Oolong tea essential oil were demonstrated. The dominant components were linalool (14.33%), indole (13.86%), *cis*-jasmone (12.15%) and *trans*-nerolidol (7.35%). They were accompanied by the small amounts of hotrienol, α-terpineol, methyl jasmonate and acetophenone. Chin Hsuan Oolong tea yielded 68 identified volatile compounds representing 76.51% with the dominant components of *trans*-nerolidol (17.84%), *cis*-jasmone (8.26%), geraniol (6.26%), hotrienol (4.81%), linalool (3.61%) and *trans*-linalyl oxide (pyranoid) (3.34%). Seventy-four constituents were identified, accounting for 78.89% in the Four Season tea oil. The principal components detected were indole (10.12%), geraniol (7.13%) and *cis*-jasmone (7%). Other components such as methyl jasmonate (3.72%), α-terpineol (3.23%) and hotrienol (3.22%) were detected in lower amounts. It was found that the fermentation method can cause a change of volatile flavor components in term of quality and quantity related to the study of Wang et al.

(2008). As the results, *trans*-2-hexenol having a grassy and greenish aroma which is a lipid degradation product and impacts an inferior quality to tea was found in all samples. Higher amounts of *trans*-2-hexenol was detected in non-fermented tea including Green Assam tea (0.27%) and Green Oolong tea (0.22%) whereas other teas produced by semi-fermentation tea processing showed significantly lower amounts (0.04-0.08%). This result was different from the study of Borse et al. (2002) who reported that *trans*-2-hexenol only appeared significantly in heavily fermented teas. The process of steaming or pan-firing at high temperature in non-fermented tea method may bring about higher appearance of the lipid degradation product. As indicated that Green Oolong tea contained a higher number of volatile flavor components than Green Assam tea samples. Seven constituents, heptanoic acid, nerol oxide, isoborneol, γ-terpineol, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, nonanoic acid and *trans*-β-damascone were present in only Green Oolong tea whist ascabiol was absent resulting from the different variety among tea samples. A look at the volatile flavor constituents of Oolong tea upon different processing methods of non-fermentation and semi-fermentation tea processing obtained from Green Oolong tea and Chin Hsuan Oolong tea, respectively, increased volatiles were illustrated on the semi-fermented tea process. Ten components were formed in the semi-fermented tea process. These consisted palmitic acid (5.24%), methyl jasmonate (1.72%), methyl 9,12,15-octadecatrienoate (0.92%), methyl linoleate (0.33%), bicyclo[3.3.0]oct-1(2)-en-3-one (0.27%), methyl palmitate (0.25%), 6,10,14-trimethyl-2-pentadecanone (0.23%), *cis*-2-(1-pentenyl)furan (0.22%), geranyl linalyl ester (0.22%), 2(3H)-furanone (0.16%) and geranyl phenylacetate (0.05%). These additional components may be produced by the reaction between enzymes and polyphenolic compounds during the semi-fermentation tea processing method. Except, heptanoic acid, methyl salicylate, methylanthranilate, *trans*-α-ionone, 2-benzofuranmethanol and 4-[1,1-dimethylethyl]-methyl-benzeneethanal disappeared which may be resulted from the rapid vitalization and degradation of these compounds. In addition, Shin Hsuan Oolong tea formed by the semi-fermented tea processing method contained lower amounts (~2 times) of caffeine than those obtained from Green Oolong tea with non-fermentation tea

processing. In the present study, the volatile flavor characteristic in various teas was found with different degree of fermentation. Also, as the flavor intensity varied between individual volatile flavor constituents, the flavor index characterizes only an arbitrary value (Dixon and Hammond, 1984). Hotrienol having green fresh flower aroma reported by Hashimoto et al. (2000) was the key important compound that can be relied on for distinguishing teas obtained from non-fermentation tea processing. As noticed, Green Oolong tea contained much higher level of hotrienol than those obtained in Green Assam tea. On the other hand, linalool and geraniol could not utilize for discriminating non-fermentation teas due to the high level of both compounds contained in all samples. To differentiate semi-fermented tea from non-fermented tea, the content of *cis*-jasmone, *trans*-nerolidol and indole increased dramatically while the green fresh aroma of hotrienol decreased rapidly. It can be considered that the high quality of sweet floral volatile flavor constituents were achieved significantly in semi-fermented tea especially Four Season tea and Chin Hsuan Oolong tea.

Table 1 Volatile flavor compounds of all tea essential oils[a]

Volatile flavor compound	*KI*[b]	1	2	3	4	5
Dihydro-2-methyl-3(2H)-Furanone	805			0.023		
N-Ethylpyrrole	809			0.053		
Furfural	827			0.039		
trans-2-Hexenol	846	0.347	0.521	0.013	0.188	0.159
2-Heptanone	908			0.012		
2-Acetylfuran	910			0.016		0.057
2,5-Dimethylpyrazine	913			0.019		0.208
2-Ethylpyrazine	926			0.020		0.130
Benzaldehyde	966	0.153	0.280	0.001	0.074	0.188
Heptanoic acid	986	0.002	0.647		0.030	0.242
2-Ethyl-5-methyl-Pyrazine	1006					0.490
Benzyl alcohol	1037	0.300	0.389		0.287	0.634
Benzeneacetaldehyde	1042	0.095	0.243	0.045	0.188	0.239
1-Ethyl-2-formyl pyrrole	1050	0.407	1.461	0.230	0.708	1.408
2(3H)-Furanone	1053			0.077	0.353	
Acetophenone	1069	0.745	0.380	0.510	0.244	1.089
Linalool oxide (trans) [furanoid]	1073	1.833	1.649	0.331	1.383	0.978
2-Ethyl-3,5-dimethylpyrazine	1078					0.424
Heptanoic acid	1083	0.188				
Linalool oxide (cis) [furanoid]	1090	1.241	1.671	0.290	1.383	0.700
3,5-Octadien-2-one	1097	0.351	0.389		0.122	0.300
Linalool	1104	15.131	27.203	4.454	8.077	7.179
Hotrienol	1108	21.569	2.568	1.722	10.765	5.062

Table 1 (continued)

Volatile flavor compound	*KI*[b]	1	2	3	4	5
2,6-Dimethyl-Cyclohexanol	1114	0.682	1.061		0.316	0.860
Phenylethyl Alcohol	1118	0.376	0.545		0.425	0.733
alpha-Isophorone	1124	0.179	0.488	0.024	0.182	0.232
Bicyclo[3.3.0]oct-1(2)-en-3-one	1126				0.610	
Benzyl nitrile	1142	0.553	0.380	0.467	0.034	
Nerol oxide	1153	0.497		0.060	0.248	
Isoborneol	1161	0.178			0.003	
Benzyl ethanoate	1164	0.812	6.506	0.076	0.360	
cis-linalyl oxide (pyranoid)	1174	1.195	0.406		1.164	0.593
trans-linalyl oxide (pyranoid)	1178	1.767	1.999		7.479	2.990
lavandulol	1183	1.199	0.335		1.320	0.843
para-Cymen-8-ol	1191	0.593	0.414		0.337	0.418
Methyl salicylate	1195	1.433	0.691	0.500		0.696
alpha-Terpineol	1199	8.165	19.634	1.456	4.095	5.068
gamma-Terpineol	1201	2.123	0.079		1.018	0.005
cis-2-(1-Pentenyl)furan	1207			0.087	0.500	
2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	1212	0.261			0.002	0.244
Nerol	1227	2.571	9.169		0.005	1.842
Coumaran	1233	4.442	1.693	0.255	0.433	2.145
Geraniol	1253	15.377	24.781	0.391	13.993	11.204
Nonanoic acid	1279	1.324			0.013	
2-Pentyl-cyclopent-2-en-1-one	1291	0.934			0.937	
Indole	1299	2.452	1.629	4.309	14.921	15.907

Table 1 (continued)

Volatile flavor compound	*KI*[b]	1	2	3	4	5
para-Vinylguaiacol	1313	0.845	2.075	0.175	0.315	1.451
Methylanthranilate	1343	0.053	0.716			0.251
Geranic acid	1357	1.077	0.799		0.367	1.155
cis-beta-Damascenone	1362	0.251	0.406	0.054	1.310	0.700
trans-beta-Damascenone	1379	0.491	2.564		5.724	2.002
cis-3-Hexenylhexanoate	1381			0.129		
Hexyl hexoate	1388			0.029		
cis-Jasmone	1393	1.186	0.966	3.731	18.477	11.001
Methyl eugenol	1401	0.182	0.814	0.053	0.376	0.493
trans-beta-Damascone	1408	0.184		0.090	0.037	0.328
Megastigmatrienone	1411	0.392	0.770	0.032	0.848	0.629
trans-alpha-Ionone	1422	0.506	0.986			0.524
2-Benzofuranmethanol	1425	0.241	0.653			0.663
4-[1,1-Dimethylethyl]-.alpha.-methyl-benzeneethanal	1428	0.639	1.830			0.623
Coumarin	1436			0.121		0.579
trans-isoeugenol	1450	0.391	1.212	0.158	0.681	0.988
trans-beta-Ionone	1478	2.404	3.253	0.110	1.792	2.707
beta-Ionone epoxide	1482	1.215	2.201	0.035	0.528	1.814
6-(Pent-2'-enyl)-tetrahydropyran-2-one	1491			0.763		4.018
Butylated hydroxytoluene	1503	0.170	0.680	0.024	0.009	0.155
Viridiflorene	1507			0.139		
2,4-Bis(1,1-dimethylethyl)phenol	1512	0.941	5.272	0.074	0.226	0.332
(R)- 5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	1527	1.082	1.650		0.168	0.852

Table 1 (continued)

Volatile flavor compound	*KI*[b]	1	2	3	4	5
alpha-Agarofuran	1545	0.512	1.049	0.104	1.069	0.590
cis-Hexahydro-7a-methyl-1-indanone	1560	1.514	1.111	0.253	1.011	2.728
trans-Nerolidol	1562	2.173	4.195	2.286	39.906	10.510
Megastigmatrienone	1584			0.126		0.390
Longiborneol	1598	0.391	1.178		0.645	0.392
Helifolen-12-al D	1614			0.097		
1-Epicubenol	1627	0.523	1.315		0.586	0.364
alpha-Cadinol	1656	1.535	0.997	0.112	1.415	1.379
Methyl jasmonate	1661			0.585	3.841	5.846
Methyl epijasmonate	1668			0.062		0.477
3Z-Butylidene phthalide	1670	0.860			0.078	0.551
10-nor-Calamenen-10-one	1691	0.378		0.075	0.590	0.283
Cadina-1(10),6,8-triene	1722	0.114			0.666	0.272
Benzyl benzoate	1748			0.033		1.047
Ascabiol	1768		1.057			
Caffeine	1836	3.061	3.097	0.341	1.932	1.749
6,10,14-Trimethyl-2-pentadecanone	1841				0.509	
Benzyl salicylate	1870			0.018		0.478
Geranyl phenylacetate	1909			0.022	0.111	0.003
Methyl palmitate	1926				0.553	0.003
Palmitic acid	1971				5.239	0.093
Geranyl linalyl ester	1924			0.011	0.498	0.078
Methyl linoleate	1994			0.027	0.744	0.625
Methyl 9,12,15-octadecatrienoate	2000			0.083	2.063	0.111
Phytol	2112	1.739	5.875	0.014	6.652	1.363

[a]As ratio of peak area to that of internal standard

[b]*KI* kovats index on DB-5MS

1: Green Oolong tea

2: Green Assam tea

3: Chin Shin Oolong tea

4: Chin Hsuan Oolong tea

5: Four Season tea

3.2 Antioxidant activity

According to various chemical compositions of tea extracts, the antioxidants properties may consider to be different. Antioxidant activities of the all tea extracts were tested by the DPPH radical scavenging. The effect of antioxidant on DPPH radical scavenging was considered due to their hydrogen donating ability or radical scavenging activity. The violet color is disappeared when a DPPH solution is mixed with the substances in the essential oil solution that can donate a hydrogen atom that rise to the reduced form diphenypicrylhydrazine (non radical). The scavenging abilities of tea extracts on DPPH radical are depicted in Fig. 1. Also, IC_{50} values and total phenolic content of different tea infusions extracts are shown in Table 2. Similar results on DPPH radical were obtained in all samples. The appearance of antioxidant activities related to the phenol content of the extracts. The strong antioxidant activity property of all extracts in this study was evaluated by comparing their IC_{50} values. The strongest antioxidant activities were demonstrated in Green Assam tea and Four Season tea. The weakest antioxidant activities were shown in Chin Hsuan Oolong tea, Green Oolong tea and Chin Shin Oolong tea, respectively, although the phenolic content of Green Oolong tea infusion extract is slight higher than that obtained from Chin Shin Oolong tea extract. The similar antioxidant activities of Oolong tea can be attributed in both of non-fermentation and semi-fermentation tea processing as can be seen the IC_{50} value of Green Oolong tea and Chin Hsuan Oolong tea as 2.27 and 2.33 μg/mL, respectively. Antioxidant and some phenol components may not be destroyed and degraded during the

semi-fermentation tea processing. This result was in contrast to the study of Porto et al. (2000) who suggested that antioxidant efficiency increasing of some phenol compounds was observed as a consequence of slight oxidative stress during processing of semi-fermented Oolong tea manufacturing. Although semi-fermented tea processing is used to produce Oolong tea, the using of different high temperatures in some parts during processing may cause to change these components in tea samples. From the results above it can be said that Four Season tea was evaluated to be the important tea having the best quality of aroma and antioxidant ability.

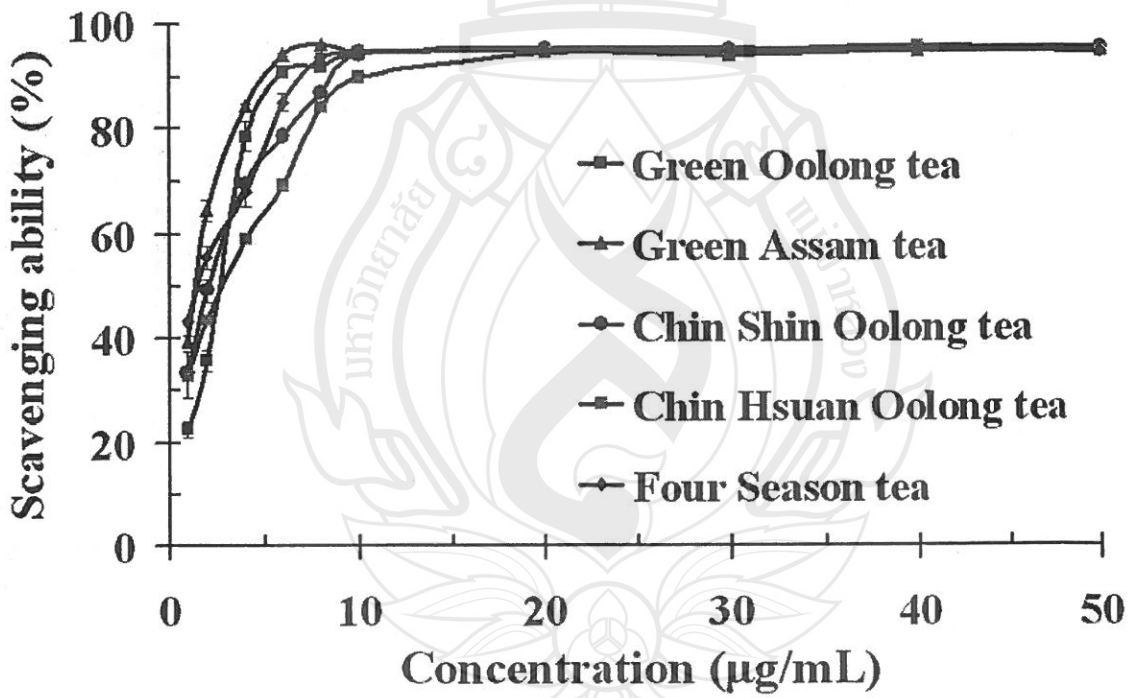

Fig. 1 The scavenging ability of tea extracts on DPPH radical. Data are represent averages ± standard deviations for triplicate experiments

Table 2 Antioxidant activities and total phenolic content of different tea infusions extracts[a]

Extract	DPPH (IC50 μg/mL)	Total phenolic content at concentration of 1 mg/mL
Green Oolong tea	2.27 ± 0.02	118.67 ± 0.74
Green Assam tea	1.31 ± 0.04	155.93 ± 3.64
Chin Shin Oolong tea	1.95 ± 0.03	110.87 ± 6.03
Chin Hsuan Oolong tea	2.33 ± 0.14	109.53 ± 1.16
Four Season tea	1.48 ± 0.06	128.27 ± 0.57

[a] Values represent averages ± standard deviations for triplicate experiments.

CHAPTER 4
CONCLUSION

Every tea has its own volatile flavor components characteristic according to the different origin and genotype breeding. In addition, the fermentation tea processing leads to significant change of volatile flavor components in tea. Higher volatiles were detected in Chin Hsuan Oolong tea obtained from semi-fermentation tea processing than Green Oolong tea obtained from non-fermentation tea processing. Components that provide the greenish and lipid oxidation (rancid) odor were much reduced whist the jasmine floral aroma was increased rapidly during semi-fermentation tea processing. The study on the antioxidant activity of all tea infusions extracts showed that all tea infusions extracts have a similar antioxidant power due to the components response for the antioxidant activity are not destroyed or degraded during fermentation in tea processing.

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PUBLICATION

1. Fingerprint of volatile flavor constituents and antioxidant activities of teas from Thailand (submitted in Food Chemistry journal impact factor 2.8) by **P. Pripdeevech** and T. Machan

Fingerprint of volatile flavor constituents and antioxidant activities of teas from Thailand

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Abstract

The volatile flavor components of different teas growing in Thailand were extracted using the simultaneous distillation and extraction (SDE) technique. These volatiles were investigated by GC-MS. At least 54 components representing 76.51-83.32% of all samples were identified. Hotrienol, geraniol and linalool were found to be the major components in Green Oolong tea. Green Assam tea contained linalool, geraniol and α-terpineol as the key flavor constituents. Chin Shin Oolong tea was dominated by linalool, indole and *cis*-jasmone while the major flavor volatiles of Chin Hsuan Oolong tea were *trans*-nerolidol, *cis*-jasmone and geraniol. Indole, geraniol and *cis*-jasmone were detected as the main constituents in Four Season tea. Change of quality and quantity of volatile flavor components was related to fermentation methods that increased volatiles were illustrated by the semi-fermented tea processing method. Green Assam tea infusion extract was evaluated to have the strongest antioxidant activities with the highest amount of phenol content followed by Four Season tea, Chin Shin Oolong tea, Chin Hsuan Oolong tea and Green Oolong tea, respectively.

Keywords: *Camellia sinensis*, Flavor volatiles, GC-MS, Antioxidant activity, Phenolic content, Tea, Simultaneous distillation and extraction (SDE)

1. Introduction

Tea (*Camellia sinensis*) is and has been one of the most popular drinks in the world for over 4000 years. More than 3 million hectares of the world has been used for planting tea (Ravichandran and Parthiban, 1997). Nowadays, tea is used in pharmaceutical and industrial applications (Falé et al., 2009; Almajano et al., 2008; Ravichandran, 2002). Tea is manufactured in many areas over the world such as Japan, Taiwan, India as well as Thailand. Tea processing methods are classified by the degree of fermentation. Green tea is produced by a non-fermentation tea process while Oolong and red tea are established from partial fermentation. A completely fermented process is applied to black tea. The taste and flavor of tea is controlled by key chemical components which are volatile terpenes, caffeine, organic acids and polyphenols (Borse et al., 2002). Tea volatiles can be divided into two groups comprising non-terpenoid and tepenoid components. Non-terpenoids found in tea are hexenols providing fresh green flavor (Rawat et al., 2007) and products of lipid degradation (Mahata et al., 1993; Robinson and Owuor, 1992; Ganeshan and Ramasamy, 1996). The detected terpenoid components in tea are monoterpene alcohols, including linalool and geraniol, which impart a sweet floral aroma (Robinson and Owuor, 1992; Sanderson and Graham, 1973; Takeo, 1981). The quality and variation of volatile flavor components in tea is due to different environmental and ecological conditions and the tea processing method. Polyphenols are key non-volatile components which also play important role in tea. This component provides important beneficial effects of tea in term of the antioxidant activity and free radical scavenging ability (Frei and Higdon, 2003). Tea contains predominantly polyphenols such as catechins, monomeric flavonols, and (-)-epigallocatechin gallate (EGCG) which are detected in fresh tea leaves at a high level (Almajano et al., 2008; Rietveld and Wiseman, 2003; Chattopadhyay et al., 2004; Lau et al., 2002). The fermentation process also affect to polyphenols in tea. Flavanols, flavandiols and phenolic acids like gallic acid, cumaric acid and caffeic acid are predominant in non-fermented green tea while some polyphenols are oxidized, degraded and polymerized enzymatically to theaflavins and thearubigens in black tea during the full fermentation tea processing method (Liebert et al., 1999; Weisburger, 1996). Katalinic and co-workers (2006) reported that tea variety and the content of EGCG controlled the antioxidant effectiveness. High content of EGCG and (-)-epigallocatechin (EGC) was detected in non-fermented and semi-fermented tea e.g. green and Oolong tea. Conversely, the content of both components were much lower in black tea.

The purpose of this present study was to investigate the fingerprint of volatile flavor components of Thai teas obtained from Mae Salong Mountain, Chiang Rai province, in the northern part of Thailand which is the most important area for planting tea in Thailand. The different tea infusions extracts were evaluated for their antioxidant activity and phenolic content.

2. Materials and Methods

2.1 Tea samples

Five tea samples manufactured under Thai Tea Suwirun Partnership, Mae Salong Mountain, Chiang Rai, Thailand were used in this study. These five different tea samples

with different qualities consisted of non-fermentation tea (Green Oolong tea and Green Assam tea) as well as semi-fermentation tea (Chin Shin Oolong tea, Chin Hsuan Oolong tea and Four Season tea). Each sample lacked any additional manipulation and was kept under a temperature of 5 °C before being subjected to simultaneous distillation-extraction (SDE). All solvents were of analytical grade, dichloromethane, anhydrous sodium sulfate, mixtures of C_8 to C_{22} *n*-alkanes and 2,2-diphenyl-1-picrylhydrazyl (DPPH) and gallic acid were purchased from Merck (Darmstadt, Germany).

2.2 Analysis of volatile flavor constituents

2.2.1 Extraction

The extraction was carried out in a modified Likens-Nickerson SDE apparatus for 5 h. Two hundred grams of tea samples and 200 mL of distilled water were added to a 500 mL round-bottom flask. Dichloromethane (150 mL) was added to another 250 mL round-bottom flask. Both flasks were connected to the apparatus, and more dichloromethane and distilled water were added into the central arm of the apparatus. The flask containing the dichloromethane was heated by using a water bath at 50 °C, while the flask containing the tea leaves and distilled water was heated by using a paraffin oil bath at 200 °C. After extraction, the distillate in a conical flask was concentrated using a vacuum rotary evaporator and then all sample essential oils were stored in the dark at 4 °C. All tea essential oil samples obtained were diluted to 1:10 v/v with dichloromethane prior to injection into the GC-MS instruments.

2.2.2 Gas chromatography-Mass Spectrometry (GC-MS)

The volatile flavor constituents of each sample were analyzed using an HP model 6890 gas chromatograph equipped with an HP-5MS (5% phenyl-polymethylsiloxane) capillary column (30 m × 0.25 mm i.d., film thickness 0.25 μm; Agilent Technologies, USA) interfaced to an HP model 5973 mass-selective detector. The oven temperature was initially held at 60 °C and then increased by 2 °C/min to 250 °C. The injector and detector temperatures were 250 and 280 °C, respectively. Purified helium was used as the carrier gas at a flow rate 1 mL/min. EI mass spectra were collected at 70 eV ionization voltages over the range of *m/z* 29-300. The electron multiplier voltage was 1150 V. The ion source and quadrupole temperatures were set at 230 °C and 150 °C, respectively. Identification of the volatile components was performed by comparison of their Kovát retention indices, relative to C_8-C_{22} *n*-alkanes, and comparison of the mass spectra of individual components with the reference mass spectra in the Wiley 275, NIST 98 databases and Adams 1989 with corresponding data of volatile flavor components in tea (Bastos et al., 2006, Rawat et al., 2007, Borse et al, 2002, Wang et al., 2008, Ravichandran & Parthiban, 1997, Kumazawa & Masuda, 2002).

2.3 Antioxidant activity

2.3.1 Preparation of tea extract

Each extract was prepared by adding 100 mL of boiling distilled water to 10 grams of tea sample. Stirring using a magnetic stirrer was employed during the brewing process. After ten minutes, the tea infusion was filtered under vacuum and cooled to

room temperature. The obtained tea extract was concentrated using a vacuum rotary evaporator and dried to constant weight in an oven at 105 °C.

2.3.2 DPPH radical scavenging assay

The effect of tea extracts on the content of 2,2-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) were evaluated by a spectrophotometric method based on the reduction of a methanol solution of DPPH according to the modified method of Blois (1958). One milliliter of various concentrations of the each sample in methanol was added to 1 mL of a 0.003% methanol solution of DPPH and the reaction mixture was shaken vigorously. The tubes were allowed to stand at room temperature (27 °C) for 30 min. Each reaction mixture was then placed in the cuvette holder of the Perkin Elmer-Lamda 25 UV/vis spectrophotometer and monitored at 517 nm against a blank which used methanol as the baseline correction. The scavenging ability was calculated as follows: Scavenging ability (%) = 100 × [Absorbance of control – Absorbance of sample/Absorbance of control]. The antioxidant activity of all tea extracts was expressed as IC_{50} which was defined as the concentration (in µg/mL) of tea extract required to inhibit the formation of DPPH radicals by 50%. The experiment was carried out in triplicate and the results are the mean values.

2.4 Determination of total phenolic contents

Total phenolic content of tea extracts obtained from different samples was determined using the Folin–Ciocalteu reagent according to the modified method of Singleton and Rossi (1965) using gallic acid as standard. The tea solution (0.2 mL) was mixed with 1.0 mL of Folin–Ciocalteu reagent, 1.0 mL of an aqueous solution of 7% Na_2CO_3 and 5.0 mL of distillated water, respectively. Then, the mixture was vortexed vigorously. The reaction mixtures were allowed to stand for 30 min before absorbance at 765 nm was measured. The same procedure was also applied to the standard solutions of gallic acid. The calibration equation for gallic acid was $y = 0.00515x - 0.00400$ ($R^2 = 0.999$) where y is the absorbance and x is the concentration of gallic acid in mg/mL.

3. Results and Discussion

3.1Volatile flavor compounds analysis

The low percentage yield (0.01-0.03% w/w) of tea essential oils was obtained from all tea samples extracted by SDE technique. Results of the fingerprint in terms of volatile flavor compounds of all tea essential oils analyzed by GC-MS and their quantities determined with the reference to internal standard in different tea samples are summarized in Table 1. Sixty-three volatiles were identified representing 81.37% of the essential oil obtained from Green Oolong tea. Monoterpene alcohols of hotrienol (15.27%), geraniol (10.88%), linalool (10.71%) and α-terpineol (5.78%) were found to be the major components. Small amounts were coumaran (3.14%), nerol (1.82%) and indole (1.74%) were also detected. Fifty-four components representing 83.32% in Green Assam tea essential oil were identified. Linalool (15.61%), geraniol (14.22%), α-terpineol (11.27%) and nerol (5.26%) were found to be the key flavor constituents in Green Assam tea while benzyl ethanoate (3.73%), phytol (3.37%) and *trans*-nerolidol (2.41%) were minor components. A total of 60 constituents representing 81.85% of the

Chin Shin Oolong tea essential oil were demonstrated. The dominant components were linalool (14.33%), indole (13.86%), *cis*-jasmone (12.15%) and *trans*-nerolidol (7.35%). They were accompanied by the small amounts of hotrienol, α-terpineol, methyl jasmonate and acetophenone. Chin Hsuan Oolong tea yielded 68 identified volatile compounds representing 76.51% with the dominant components of *trans*-nerolidol (17.84%), *cis*-jasmone (8.26%), geraniol (6.26%), hotrienol (4.81%), linalool (3.61%) and *trans*-linalyl oxide (pyranoid) (3.34%). Seventy-four constituents were identified, accounting for 78.89% in the Four Season tea oil. The principal components detected were indole (10.12%), geraniol (7.13%) and *cis*-jasmone (7%). Other components such as methyl jasmonate (3.72%), α-terpineol (3.23%) and hotrienol (3.22%) were detected in lower amounts. It was found that the fermentation method can cause a change of volatile flavor components in term of quality and quantity related to the study of Wang et al. (2008). As the results, *trans*-2-hexenol having a grassy and greenish aroma which is a lipid degradation product and impacts an inferior quality to tea was found in all samples. Higher amounts of *trans*-2-hexenol was detected in non-fermented tea including Green Assam tea (0.27%) and Green Oolong tea (0.22%) whereas other teas produced by semi-fermentation tea processing showed significantly lower amounts (0.04-0.08%). This result was different from the study of Borse et al. (2002) who reported that *trans*-2-hexenol only appeared significantly in heavily fermented teas. The process of steaming or pan-firing at high temperature in non-fermented tea method may bring about higher appearance of the lipid degradation product. As indicated that Green Oolong tea contained a higher number of volatile flavor components than Green Assam tea samples. Seven constituents, heptanoic acid, nerol oxide, isoborneol, γ-terpineol, 2,6,6-trimethyl-1-cyclohexene-1-carboxaldehyde, nonanoic acid and *trans*-β-damascone were present in only Green Oolong tea whist ascabiol was absent resulting from the different variety among tea samples. A look at the volatile flavor constituents of Oolong tea upon different processing methods of non-fermentation and semi-fermentation tea processing obtained from Green Oolong tea and Chin Hsuan Oolong tea, respectively, increased volatiles were illustrated on the semi-fermented tea process. Ten components were formed in the semi-fermented tea process. These consisted palmitic acid (5.24%), methyl jasmonate (1.72%), methyl 9,12,15-octadecatrienoate (0.92%), methyl linoleate (0.33%), bicyclo[3.3.0]oct-1(2)-en-3-one (0.27%), methyl palmitate (0.25%), 6,10,14-trimethyl-2-pentadecanone (0.23%), *cis*-2-(1-pentenyl)furan (0.22%), geranyl linalyl ester (0.22%), 2(3H)-furanone (0.16%) and geranyl phenylacetate (0.05%). These additional components may be produced by the reaction between enzymes and polyphenolic compounds during the semi-fermentation tea processing method. Except, heptanoic acid, methyl salicylate, methylanthranilate, *trans*-α-ionone, 2-benzofuranmethanol and 4-[1,1-dimethylethyl]-methyl-benzeneethanal disappeared which may be resulted from the rapid vitalization and degradation of these compounds. In addition, Shin Hsuan Oolong tea formed by the semi-fermented tea processing method contained lower amounts (~2 times) of caffeine than those obtained from Green Oolong tea with non-fermentation tea processing. In the present study, the volatile flavor characteristic in various teas was found with different degree of fermentation. Also, as the flavor intensity varied between individual volatile flavor constituents, the flavor index characterizes only an arbitrary

value (Dixon and Hammond, 1984). Hotrienol having green fresh flower aroma reported by Hashimoto et al. (2000) was the key important compound that can be relied on for distinguishing teas obtained from non-fermentation tea processing. As noticed, Green Oolong tea contained much higher level of hotrienol than those obtained in Green Assam tea. On the other hand, linalool and geraniol could not utilize for discriminating non-fermentation teas due to the high level of both compounds contained in all samples. To differentiate semi-fermented tea from non-fermented tea, the content of *cis*-jasmone, *trans*-nerolidol and indole increased dramatically while the green fresh aroma of hotrienol decreased rapidly. It can be considered that the high quality of sweet floral volatile flavor constituents were achieved significantly in semi-fermented tea especially Four Season tea and Chin Hsuan Oolong tea.

3.2 Antioxidant activity

According to various chemical compositions of tea extracts, the antioxidants properties may consider to be different. Antioxidant activities of the all tea extracts were tested by the DPPH radical scavenging. The effect of antioxidant on DPPH radical scavenging was considered due to their hydrogen donating ability or radical scavenging activity. The violet color is disappeared when a DPPH solution is mixed with the substances in the essential oil solution that can donate a hydrogen atom that rise to the reduced form diphenypicrylhydrazine (non radical). The scavenging abilities of tea extracts on DPPH radical are depicted in Fig. 1. Also, IC_{50} values and total phenolic content of different tea infusions extracts are shown in Table 2. Similar results on DPPH radical were obtained in all samples. The appearance of antioxidant activities related to the phenol content of the extracts. The strong antioxidant activity property of all extracts in this study was evaluated by comparing their IC_{50} values. The strongest antioxidant activities were demonstrated in Green Assam tea and Four Season tea. The weakest antioxidant activities were shown in Chin Hsuan Oolong tea, Green Oolong tea and Chin Shin Oolong tea, respectively, although the phenolic content of Green Oolong tea infusion extract is slight higher than that obtained from Chin Shin Oolong tea extract. The similar antioxidant activities of Oolong tea can be attributed in both of non-fermentation and semi-fermentation tea processing as can be seen the IC_{50} value of Green Oolong tea and Chin Hsuan Oolong tea as 2.27 and 2.33 μg/mL, respectively. Antioxidant and some phenol components may not be destroyed and degraded during the semi-fermentation tea processing. This result was in contrast to the study of Porto et al. (2000) who suggested that antioxidant efficiency increasing of some phenol compounds was observed as a consequence of slight oxidative stress during processing of semi-fermented Oolong tea manufacturing. Although semi-fermented tea processing is used to produce Oolong tea, the using of different high temperatures in some parts during processing may cause to change these components in tea samples. From the results above it can be said that Four Season tea was evaluated to be the important tea having the best quality of aroma and antioxidant ability.

4. Conclusion

Every tea has its own volatile flavor components characteristic according to the different origin and genotype breeding. In addition, the fermentation tea processing leads to significant change of volatile flavor components in tea. Higher volatiles were detected in Chin Hsuan Oolong tea obtained from semi-fermentation tea processing than Green Oolong tea obtained from non-fermentation tea processing. Components that provide the greenish and lipid oxidation (rancid) odor were much reduced whist the jasmine floral aroma was increased rapidly during semi-fermentation tea processing. The study on the antioxidant activity of all tea infusions extracts showed that all tea infusions extracts have a similar antioxidant power due to the components response for the antioxidant activity are not destroyed or degraded during fermentation in tea processing.

5. Acknowledgements

Great appreciation is given to the division of research service and Scientific and Technological Instrument Center (STIC), Mae Fah Luang University for funding and GC-MS instrument.

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Table 1 Volatile flavor compounds of all tea essential oils[a]

Volatile flavor compound	*KI*[b]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Dihydro-2-methyl-3(2H)-Furanone	805			0.023		
N-Ethylpyrrole	809			0.053		
Furfural	827			0.039		
trans-2-Hexenol	846	0.347	0.521	0.013	0.188	0.159
2-Heptanone	908			0.012		
2-Acetylfuran	910			0.016		0.057
2,5-Dimethylpyrazine	913			0.019		0.208
2-Ethylpyrazine	926			0.020		0.130
Benzaldehyde	966	0.153	0.280	0.001	0.074	0.188
Heptanoic acid	986	0.002	0.647		0.030	0.242
2-Ethyl-5-methyl- Pyrazine	1006					0.490
Benzyl alcohol	1037	0.300	0.389		0.287	0.634
Benzeneacetaldehyde	1042	0.095	0.243	0.045	0.188	0.239
1-Ethyl-2-formyl pyrrole	1050	0.407	1.461	0.230	0.708	1.408
2(3H)-Furanone	1053			0.077	0.353	
Acetophenone	1069	0.745	0.380	0.510	0.244	1.089
Linalool oxide (trans) [furanoid]	1073	1.833	1.649	0.331	1.383	0.978
2-Ethyl-3,5-dimethylpyrazine	1078					0.424
Heptanoic acid	1083	0.188				
Linalool oxide (cis) [furanoid]	1090	1.241	1.671	0.290	1.383	0.700
3,5-Octadien-2-one	1097	0.351	0.389		0.122	0.300
Linalool	1104	15.131	27.203	4.454	8.077	7.179
Hotrienol	1108	21.569	2.568	1.722	10.765	5.062
2,6-Dimethyl- Cyclohexanol	1114	0.682	1.061		0.316	0.860
Phenylethyl Alcohol	1118	0.376	0.545		0.425	0.733
alpha-Isophorone	1124	0.179	0.488	0.024	0.182	0.232

Table 1 (continued)

Volatile flavor compound	*KI*[b]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Bicyclo[3.3.0]oct-1(2)-en-3-one	1126				0.610	
Benzyl nitrile	1142	0.553	0.380	0.467	0.034	
Nerol oxide	1153	0.497		0.060	0.248	
Isoborneol	1161	0.178			0.003	
Benzyl ethanoate	1164	0.812	6.506	0.076	0.360	
cis-linalyl oxide (pyranoid)	1174	1.195	0.406		1.164	0.593
trans-linalyl oxide (pyranoid)	1178	1.767	1.999		7.479	2.990
lavandulol	1183	1.199	0.335		1.320	0.843
para-Cymen-8-ol	1191	0.593	0.414		0.337	0.418
Methyl salicylate	1195	1.433	0.691	0.500		0.696
alpha-Terpineol	1199	8.165	19.634	1.456	4.095	5.068
gamma-Terpineol	1201	2.123	0.079		1.018	0.005
cis-2-(1-Pentenyl)furan	1207			0.087	0.500	
2,6,6-Trimethyl-1-cyclohexene-1-carboxaldehyde	1212	0.261			0.002	0.244
Nerol	1227	2.571	9.169		0.005	1.842
Coumaran	1233	4.442	1.693	0.255	0.433	2.145
Geraniol	1253	15.377	24.781	0.391	13.993	11.204
Nonanoic acid	1279	1.324			0.013	
2-Pentyl-cyclopent-2-en-1-one	1291	0.934			0.937	
Indole	1299	2.452	1.629	4.309	14.921	15.907
para-Vinylguaiacol	1313	0.845	2.075	0.175	0.315	1.451
Methylanthranilate	1343	0.053	0.716			0.251
Geranic acid	1357	1.077	0.799		0.367	1.155
cis-beta-Damascenone	1362	0.251	0.406	0.054	1.310	0.700
trans-beta-Damascenone	1379	0.491	2.564		5.724	2.002

Table 1 (continued)

Volatile flavor compound	*KI*[b]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
cis-3-Hexenylhexanoate	1381			0.129		
Hexyl hexoate	1388			0.029		
cis-Jasmone	1393	1.186	0.966	3.731	18.477	11.001
Methyl eugenol	1401	0.182	0.814	0.053	0.376	0.493
trans-beta-Damascone	1408	0.184		0.090	0.037	0.328
Megastigmatrienone	1411	0.392	0.770	0.032	0.848	0.629
trans-alpha-Ionone	1422	0.506	0.986			0.524
2-Benzofuranmethanol	1425	0.241	0.653			0.663
4-[1,1-Dimethylethyl]-.alpha.-methyl-benzeneethanal	1428	0.639	1.830			0.623
Coumarin	1436			0.121		0.579
trans-isoeugenol	1450	0.391	1.212	0.158	0.681	0.988
trans-beta-Ionone	1478	2.404	3.253	0.110	1.792	2.707
beta-Ionone epoxide	1482	1.215	2.201	0.035	0.528	1.814
6-(Pent-2'-enyl)-tetrahydropyran-2-one	1491			0.763		4.018
Butylated hydroxytoluene	1503	0.170	0.680	0.024	0.009	0.155
Viridiflorene	1507			0.139		
2,4-Bis(1,1-dimethylethyl)phenol	1512	0.941	5.272	0.074	0.226	0.332
(R)- 5,6,7,7a-Tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone	1527	1.082	1.650		0.168	0.852
alpha-Agarofuran	1545	0.512	1.049	0.104	1.069	0.590
cis-Hexahydro-7a-methyl-1-indanone	1560	1.514	1.111	0.253	1.011	2.728
trans-Nerolidol	1562	2.173	4.195	2.286	39.906	10.510
Megastigmatrienone	1584			0.126		0.390
Longiborneol	1598	0.391	1.178		0.645	0.392
Helifolen-12-al D	1614			0.097		
1-Epicubenol	1627	0.523	1.315		0.586	0.364

Table 1 (continued)

Volatile flavor compound	*KI*[b]	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
alpha-Cadinol	1656	1.535	0.997	0.112	1.415	1.379
Methyl jasmonate	1661			0.585	3.841	5.846
Methyl epijasmonate	1668			0.062		0.477
3Z-Butylidene phthalide	1670	0.860			0.078	0.551
10-nor-Calamenen-10-one	1691	0.378		0.075	0.590	0.283
Cadina-1(10),6,8-triene	1722	0.114			0.666	0.272
Benzyl benzoate	1748			0.033		1.047
Ascabiol	1768		1.057			
Caffeine	1836	3.061	3.097	0.341	1.932	1.749
6,10,14-Trimethyl-2-pentadecanone	1841				0.509	
Benzyl salicylate	1870			0.018		0.478
Geranyl phenylacetate	1909			0.022	0.111	0.003
Methyl palmitate	1926				0.553	0.003
Palmitic acid	1971				5.239	0.093
Geranyl linalyl ester	1924			0.011	0.498	0.078
Methyl linoleate	1994			0.027	0.744	0.625
Methyl 9,12,15-octadecatrienoate	2000			0.083	2.063	0.111
Phytol	2112	1.739	5.875	0.014	6.652	1.363

[a]As ratio of peak area to that of internal standard
[b]*KI* kovats index on DB-5MS
Sample 1: Green Oolong tea
Sample 2: Green Assam tea
Sample 3: Chin Shin Oolong tea
Sample 4: Chin Hsuan Oolong tea
Sample 5: Four Season tea

Table 2 Antioxidant activities and total phenolic content of different tea infusions extracts[a]

Extract	DPPH (IC50 µg/mL)	Total phenolic content at concentration of 1 mg/mL
Green Oolong tea	2.27 ± 0.02	118.67 ± 0.74
Green Assam tea	1.31 ± 0.04	155.93 ± 3.64
Chin Shin Oolong tea	1.95 ± 0.03	110.87 ± 6.03
Chin Hsuan Oolong tea	2.33 ± 0.14	109.53 ± 1.16
Four Season tea	1.48 ± 0.06	128.27 ± 0.57

[a] Values represent averages ± standard deviations for triplicate experiments.

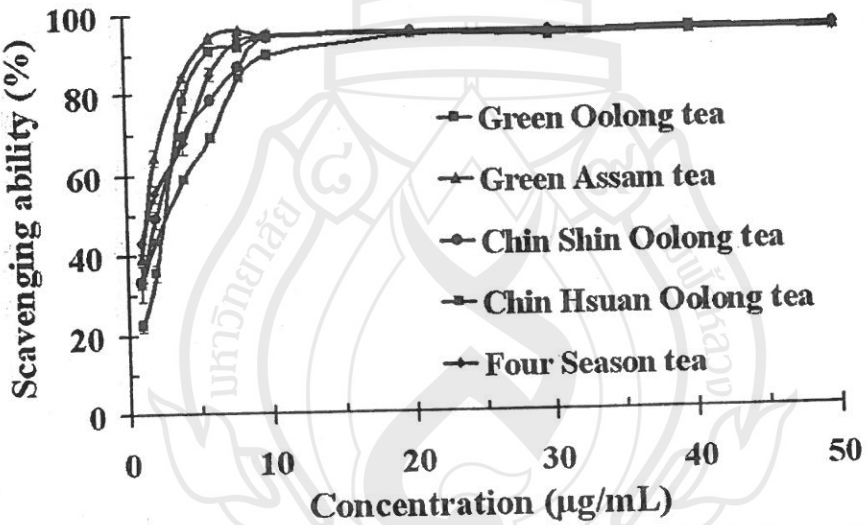

Fig. 1 The scavenging ability of tea extracts on DPPH radical. Data are represent averages ± standard deviations for triplicate experiments