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# Simultaneous determination of caffeine and 8 catechins in oolong teas produced in Thailand

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**Abstract**

A simple, rapid and reliable method of high performance liquid chromatography (HPLC) was developed for the simultaneous determination of bioactive compounds in oolong teas. The optimized method consisted of the use of a C18 reversed-phase column, an isocratic elution system (2 ml/min) of water:acetonitrile (87:13) containing 0.05% trifluoroacetic acid and the detection wavelength of 210 nm. The developed system sufficiently separated (-)-gallocatechin (GC), (-)-epigallocatechin (EGC,), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), caffeine (CF), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG) and (-)-catechin gallate (CG) within 12 min elution time at 30°C. This HPLC method had been proved to be appropriate for the identification and quantification of caffeine and 8 catechins, and exhibited good correlation coefficients, detection levels, precision and recovery rates. The developed analytical method was subsequently applied for determination of these bioactive compounds in 17 oolong teas produced in Thailand. Analysis under the optimized conditions revealed that the contents of caffeine and total catechin were 2.25±0.48 and 9.92±1.46 g/100 g DW, respectively. The levels of EGC (2.87±0.75 g/100 g DW) and EGCG (2.60±0.66 g/100 g DW) were the highest among quantified catechins.



## Introduction

Tea (*Camellia sinensis* L.) is the most popular beverage in the world. Its popularity is attributed to its sensory properties and potential health benefits. It is normally produced from the leaves of two varieties of the plant *Camellia sinensis*: var. sinensis and var. assamica. Tea products can be divided into three categories based on the degree of fermentations: green tea (unfermented), oolong tea (partially fermented), and black tea (fully fermented). Green tea is heated to avoid enzymatic oxidation in fermentation process. Oolong tea is semi-fermented to permit a partial level of enzymatic oxidation. Black tea is the most thoroughly oxidized enzymatically. Green tea contains significant quantities of the unoxidized catechins: (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG) and (-)-catechin gallate (CG); the oxidized derivatives of the catechins, theaflavins (TFs) and thearubigins (TRs), are found in fully fermented (black) and semi-fermented (oolong) teas (Khan and Mukhtar, 2007). Catechins are the highest in concentration in green teas, while oolong and black tea has substantially fewer due to its oxidative production.

The consumption of tea has been linked to its beneficial health properties as natural antioxidant due to the presence of catechins, TFs and TRs which has potent antioxidant activity. The antioxidant activity of these polyphenols is indeed thought to account for protective role against such conditions as cardiovascular (Hodgson and Croft, 2010; Deka and Vita, 2011), cancer (Yang *et al*., 2007; Yuan *et al*., 2011), inflammation (Hamer, 2007; Gonzalez de Mejia *et al.*, 2012), poor oral health (Narotzki *et al*., 2012), diabetes (Grant and Dworakowska, 2013) and obesity (Rains *et al*., 2011; Sae-tan *et al.*, 2011). Because the health benefit of polyphenols in teas, many studies have been focused on the determination of polyphenols, mainly in green and black teas (Wright *et al*., 2001; Liang *et al.*, 2003; Sharma *et al*., 2005; Wang *et al.*, 2008; Chen *et al.*, 2009; Alaerts *et al*., 2012). There are a few studies focused on oolong teas (Chen *et al.*, 2010; Wang *et al.*, 2011; Lin *et al*., 2013). This may be because the most tea consumed is black tea (78%) followed by green tea (20%), while oolong tea is consumed only 2% worldwide. The major chemical constituents of oolong tea are polyphenols, caffeine, amino acids, protein, and chlorophyll. Among these chemicals, polyphenols and caffeine are well-studied bioactive components (Wang *et al.*, 2011). The major phenolic compounds identified in oolong tea are (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (-)-epigallocatechin

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gallate(EGCG),(-)-epicatechin(EC),(-)-gallocatechin gallate (GCG), and (-)-epicatechin gallate (ECG). Therefore, the identification and determination of polyphenols and/or caffeine have been focused for the chemical control of oolong tea (Zuo *et al*., 2002; Kilmartin and Hsu, 2003; Dou *et al.*, 2007; Wang *et al.*, 2008; Suteerapataranon *et al*., 2009; Chen *et al*., 2010; Wang *et al.*, 2011; Wu *et al*., 2012).

In Thailand, tea is cultivated in the north part of the country in the provinces of Chiang Rai and Chiang Mai (accounting for 93% of tea production in Thailand). About 30% of the production is commercialized in the domestic market, whereas the remaining 70% is exported. Most Thai people like to drink green and oolong tea. Among 3 types of teas, oolong tea has the highest price in Thai tea market. Normally, it is produced from young green shoots of two Chinese sub-varieties, cv. "oolong no. 12" and cv. "oolong no. 17". Oolong tea processing in Thailand occurs in seven steps: harvesting, outdoor withering, indoor withering, fixation, rolling, drying and packing. First the shoot leaves are harvested and withered in the sun for a short period of time. They are then placed into baskets and shaken, which bruises the leaves. The juices in the leaves are now exposed to the air, which begins the process of fermentation. The leaves are fired, which stops the fermentation process, rolled and then dried. With the same processing, the taste of oolong tea is dependent on the sub-variety. In Thailand, oolong tea produced from oolong no.17 sub-variety is the most popular type due to its flavor, aroma and taste characteristics.

As known that the benefit of drinking tea derived from the chemical compounds, it is necessary to know the polyphenols content in tea consumed. Data based on chemical compositions are complementary indicators of the quality of tea, regarding its biological activities. Due to variability in the compositions of tea catechins and their potential health benefits, it is critical to establish a simple and reliable analytical method for the determination of these compounds. Therefore, the present work was aimed at the investigation of a sensitive, fast, and accurate HPLC method to determine catechins and caffeine in tea and characterization of commercial oolong teas produced in tea manufacturers of Thailand.

## Materials and Methods

*Samples*

A total of 17 oolong teas of major sub-cultivars, *C. sinensis* cv. "oolong no. 17", were collected from factories in Thailand from April to June 2011. The samples were analyzed within 6 months of their production. For each factory, three samples were sampled.

*Chemicals and reagents*

The standards , which include (-)-gallocatechin (GC, ≥ 98%), (-)-epigallocatechin (EGC, ≥ 98%), (+)-catechin (C, ≥ 98%), (-)-epicatechin (EC, ≥ 90%), (-)-epigallocatechin gallate (EGCG, ≥ 95%), caffeine (CF, ≥ 99%), (-)-gallocatechin gallate (GCG, ≥ 98%), (-)-epicatechin gallate (ECG, ≥ 98%) and (-)-catechin gallate (CG, ≥ 98%), were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Acetonitrile, trifluoroacetic acid (TFA) and methanol (HPLC-grade) were purchased from Fluka (Buchs, Switzerland).

*Sample extraction*

Collected tea samples were ground and samples (2 g, weighed to the nearest 0.001g) were extracted with 200 ml of boiling distilled water at a temperature of 95°C. The extraction mixture was constantly stirred with a magnetic stirrer. After 10 min, the extraction mixture was filtered through a filter paper (Whatman No. 4). The residue was washed with distilled water (3x10 ml). The tea solution was cooled to room temperature and adjusted to 250 ml with distilled water. All samples were extracted in triplicate (Khokhar and Magnusdottir 2002; Rusak *et al.*, 2008).

*Determination of moisture content*

Tea samples, 5 g, weighed to the nearest 0.001 g, were placed in a moisture can and heated in an oven at 103±2°C for at least 16 h to constant weight. The percentage of moisture content (%MC) and dry matter (%DM) in the samples were then calculated from the weight differences. All tests were performed in triplicate.

*Optimization of HPLC condition for caffeine and catechins analysis*

The percentage of purity from the certificate was used to prepare the stock standard solution. The reference standards of the target compounds, that are, GC, EGC, C, EC, EGCG, CF, GCG, ECG, CG were accurately weighed and dissolved in methanol to generate a stock concentration of 1,000 µg/ml. The mixed stock standard solution was prepared by mixing 1 ml of each stock standard into a 10-ml volumetric flask and made to volume with distilled water. Working standard solutions were prepared by 1-500 fold dilution of the mixed stock solution and then filtered through a 0.45 µm PTFE filter. HPLC analysis of standards and samples was conducted on

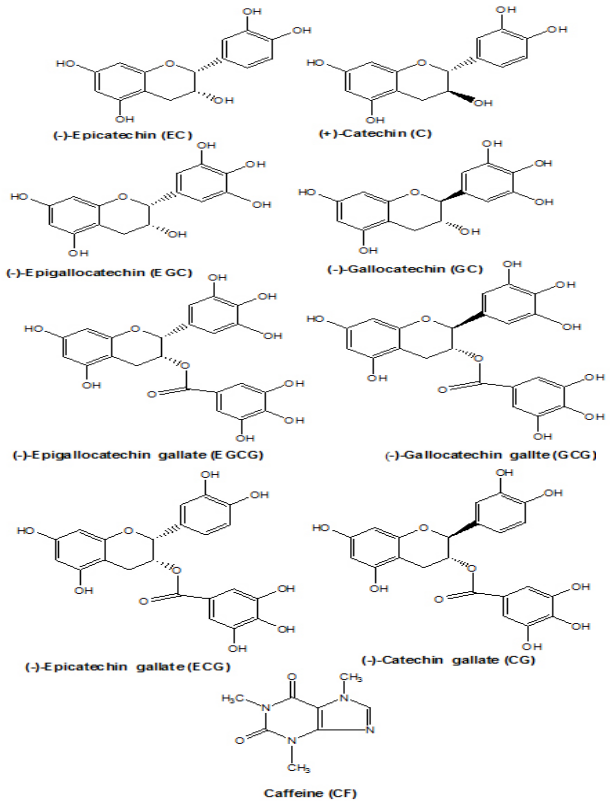


Figure 1. The chemical structures of eight catechins and caffeine

Water 966 high performance liquid chromatography comprising vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment and photo diode array detector. The column used was a Platinum EPS C18 reversed phase, 3 µm (53 x 7 mm), operated at 30°C.The analytical conditions were optimized mainly on the basis of peak resolution, baseline, elution time, mobile phase composition, flow rate, temperature and detection wavelength. Mobile phase eventually optimized was water/acetonitrile (87:13) containing 0.05% (v/v) trifluoroacetic acid (TFA) with the flow rate of 2 ml/min. Absorption wavelength was 210 nm. All injection volumes of samples and standard solutions were 20 µl.

The criteria for the identification of catechins and caffeine were established based on comparisons of the retention time and spectrum in the 190-400 nm range of an unknown compound with HPLC library data of standards. They were quantified using a calibration curve of each standard, together with a formula shown in Figure 1. The results were expressed as g/100 g dry weight (DW).The total catechins (TC) as g/100 g DW was determined by the summation of individual catechins (GC, EGC, C, EC, EGCG, GCG, ECG and CG).

Where

As = Peak area of the analyte in the samples

b = Peak area at the point of interception on y-axis of calibration curve

m = Slope of calibration curve

Vs = Sample extraction volume (ml)

DF = Dilution factor

Ws = Sample weight (g)

%DM = Percentage of dry matter of the samples

*Method validation*

Validation was carried out in compliance with the AOAC Intl. Guidelines for Single Lab. Validation of Chemical Methods for Dietary Supplements and Botanicals (AOAC, 2002). Calibration graphs for the caffeine and 8 catechins were constructed using nine levels of concentration which covered the concentration ranges expected in the tea samples. The characteristics of the calibration curves, including the regression equation, correlation coefficient (r), range of linearity, limit of detection (LOD) and limit of quantitation (LOQ) were determined. The LOD and LOD were based on a signal-to-noise (S/N) ratio as 3:1 and 10:1, respectively. Precision was determined by analyzing known concentrations of the nine standards in seven replicates. The relative standard deviation (RSD) was calculated as a measure of precision. Recoveries were carried out to investigate the accuracy by spiking three concentration levels of the mixed standard solutions to known amounts of the tea samples. The samples were then extracted and analyzed with the described method. The average percentage recoveries were evaluated by calculating the ratio of detected amount versus the added amount.

*Statistical analysis*

Data were expressed as means ± standard deviation. The data were also subjected to analysis of variance (ANOVA) and Duncan's multiple range tests using SPSS 16.0 for Windows. The significance level of $P < 0.05$ was considered significantly different.

## Results and Discussion

*Development of analytical methods*

We developed the optimal conditions for the analysis of the eight catechins (GC, EGC, C, EC, EGCG, GCG, ECG and CG) and caffeine (CF) in tea samples using a rapid and simple isocratic system for HPLC. The analytical conditions were optimized mainly on the basis of peak resolution, baseline, elution time, mobile phase composition, flow rate, temperature and detection wavelength. In most studies, water/methanol/acid, water/acetonitrile/ethyl acetate and water/acetonitrile/acid have been used as mobile phase for catechin analysis (Goto *et al*., 1996; Wang *et al.*, 2000; Zuo *et al*., 2002; Nishitani

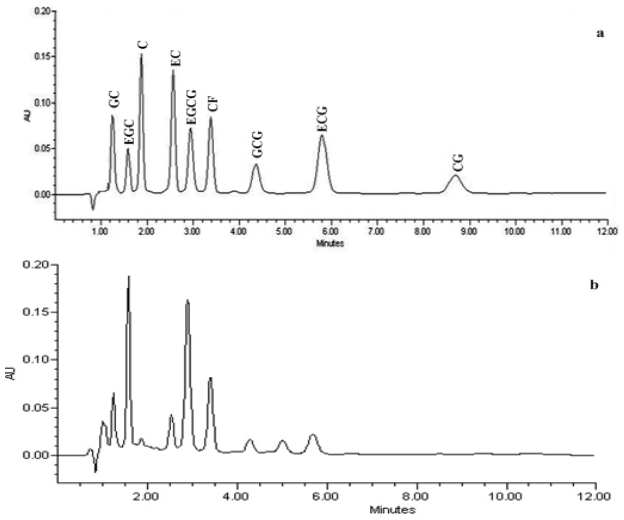


Figure 2. HPLC chromatogram of standards (a), oolong tea produced from *C. sinensis* cv. "oolong no. 17" (b)

and Sagesaka, 2004; El-Shahawi *et al*., 2012). It has been reported that the use of acetonitrile, instead of methanol, as eluent provided a higher UV absorbance and easy detection of trace concentrations of catechins or caffeine at 205 nm instead of 275 nm (Lee and Ong, 2000). The presence of TFA is essential for high resolution and efficient chromatography of catechins in tea (Dalluge *et al*., 1998). In the present study, our investigation has revealed that, the mobile phase of water/acetonitrile (87:13) containing 0.05% (v/v) TFA with the flow rate of 2 ml/min and the temperature of 30°C resulted in a good chromatographic separation. The detection wavelength was selected at 210 nm, at which all of the compounds had adequate absorption and no interference. A representative HPLC profile for the nine standards (GC, EGC, C, EC, EGCG, GCG, ECG, CG and CF) is shown in Figure 2a. It can be seen that a good separation can be achieved within 12 min using the conditions described. With 5 min of post-run for re-equilibration, the column can be brought to the initial conditions ready for the next injection. The elution order was GC, EGC, C, EC, EGCG, CF, GCG, ECG and CG. Retention time of different compounds were, 1.25 min for GC, 1.59 min for EGC, 1.88 min for C, 2.56 min for EC, 2.94 min for EGCG, 3.40 min for CF, 4.36 min for GCG, 5.81 min for ECG and 8.71 min for CG.

*Method validation*

To test the validity of the analytical method, several characteristics of the method, including the regression equation, correlation coefficient (r), range of linearity, limit of detection (LOD), limit of quantitation (LOQ), precision and accuracy were determined. The calculated results are given in Table 1. All the analytes exhibited good linearity (r) over

Table 1. Characteristics and performances of calibration curves

| Compound | Regression equation | *r* | Linear range (µg/ml) | LOD (µg/ml) | LOQ (µg/ml) | RSD (%) | Recovery (%) |
|---|---|---|---|---|---|---|---|
| GC | y = 53.922x - 18.059 | 0.9979 | 0.10-51.90 | 1.34 | 2.70 | 1.12 | 98.2±1.0 |
| EGC | y = 61.859x - 26.513 | 0.9972 | 0.09-43.10 | 0.60 | 2.16 | 0.89 | 96.7±1.2 |
| C | y = 42.701x - 16.261 | 0.9979 | 0.20-100.00 | 0.22 | 0.61 | 0.71 | 99.7±1.8 |
| EC | y = 48.532x - 36.379 | 0.9970 | 0.19-97.00 | 0.21 | 0.60 | 0.78 | 102.4±0.8 |
| EGCG | y = 59.389x - 34.226 | 0.9985 | 0.16-80.00 | 0.49 | 1.31 | 0.81 | 99.1±1.9 |
| CF | y = 32.198x - 8.4054 | 0.9997 | 0.20-100.00 | 0.31 | 1.28 | 0.83 | 99.3±1.8 |
| GCG | y = 50.984x - 28.425 | 0.9965 | 0.11-52.90 | 0.52 | 1.87 | 0.96 | 98.1±1.1 |
| ECG | y = 44.169x - 28.187 | 0.9986 | 0.20-98.00 | 0.47 | 1.60 | 1.05 | 97.3±1.7 |
| CG | y = 44.556x - 15.765 | 0.9989 | 0.09-44.10 | 0.47 | 2.0 | 1.01 | 97.9±1.9 |

the range tested with correlation coefficient from 0.9965 to 0.9997. The LODs ranged from 0.21 µg/ml for EC to 1.34 µg/ml for GC, and the LOQs ranged from 0.60 µg/ml for EC to 2.70 µg/ml for GC. These concentrations are low enough to analyze all eight catechins and caffeine in real tea samples. Precision was determined by the relative standard deviation (RSD) of known concentrations of the nine standards in seven replicates. All RSDs were less than 1.12%. Accuracy was tested by spiking three concentration levels of the mixed standard solutions to known amounts of the tea samples. The recovery rates of all compounds were in the range of 96.7 and 102.4%. To check specificity, we performed peak purity tests (data not shown) by analyzing tea samples using photo-diode array analysis and confirmed that each chromatographic peak of the eight catechins and caffeine was attributable to a single component. The validation of the method compared satisfactorily with the reported methods for the analysis of catechins and caffeine in green tea water extracts (Khokhar *et al*., 1997; Wang *et al*., 2000; Zuo *et al*., 2002; Bonoli *et al.*, 2003; Wang *et al.*, 2003; Nishitani and Sagesaka, 2004). Our validation results showed the method was validity and can be used for determination of caffeine and catechins in teas.

*Determination of caffeine and catechins in oolong tea*

The developed analytical method was subsequently applied to simultaneous determination of GC, EGC, C, EC, EGCG, CF, GCG, ECG and CG in oolong teas. The representative chromatograms of oolong tea produced from *C. sinensis* cv. "oolong no. 17" is shown in Figure 2b. The peaks corresponding to each chemical were well-separated. It can be clearly seen that oolong teas contained CF and 7 catechins (GC, EGC, C, EC, EGCG, GCG and ECG), but CG was not detected in oolong teas produced from oolong no. 17 sub-variety. A typical chromatogram obtained from the oolong tea samples can be regarded as the fingerprint of oolong tea products produced from oolong no. 17 sub-variety cultivated in Thailand.

Results of the CF, TC, GC, EGC, C, EC, EGCG, GCG, ECG and CG are presented in Table 2. Analysis of eight catechins and caffeine, under the optimized conditions, revealed the presence of reasonable

Table 2. The average content of caffeine, total catechins and individual catechins of 17 oolong teas (g/100 g dry weight)

| No. | CF | TC | Individual catechins | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | GC | EGC | C | EC | EGCG | GCG | ECG |
| 1 | 1.77±0.01$^{fg}$ | 11.40±0.18$^{ab}$ | 1.47±0.13$^{abc}$ | 4.06±0.21$^{a}$ | 0.70±0.06$^{efg}$ | 0.99±0.04$^{cde}$ | 3.09±0.20$^{abc}$ | 0.56±0.01$^{fg}$ | 0.57±0.01$^{cde}$ |
| 2 | 2.24±0.01$^{cde}$ | 10.82±0.27$^{abcd}$ | 1.27±0.02$^{cd}$ | 3.45±0.06$^{ab}$ | 0.59±0.06$^{g}$ | 0.84±0.03$^{gh}$ | 3.33±0.08$^{a}$ | 0.82±0.01$^{bcd}$ | 0.56±0.01$^{cde}$ |
| 3 | 1.70± 0.14$^{fg}$ | 10.09±1.11$^{bcde}$ | 1.44±0.03$^{abc}$ | 3.58±0.13$^{ab}$ | 0.74±0.06$^{defg}$ | 1.02±0.04$^{cd}$ | 2.38±0.06$^{def}$ | 0.53±0.05$^{fg}$ | 0.44±0.03$^{ef}$ |
| 4 | 1.61±0.14$^{g}$ | 9.38±0.78$^{cdef}$ | 1.34±0.17$^{bcd}$ | 3.30±0.37$^{abc}$ | 0.73±0.04$^{defg}$ | 1.01±0.06$^{cd}$ | 2.14±0.45$^{f}$ | 0.46±0.12$^{g}$ | 0.42±0.11$^{f}$ |
| 5 | 3.25±0.08$^{a}$ | 10.42±0.01$^{bcde}$ | 1.21±0.01$^{d}$ | 2.20±0.10$^{e}$ | 1.11±0.07$^{a}$ | 1.16±0.01$^{a}$ | 2.96±0.07$^{abcde}$ | 0.88±0.06$^{abc}$ | 0.93±0.65$^{a}$ |
| 6 | 2.32±0.08$^{cd}$ | 11.13±0.84$^{ab}$ | 1.47±0.04$^{abc}$ | 3.20±0.16$^{bcd}$ | 0.67±0.09$^{efg}$ | 0.92±0.04$^{defg}$ | 3.28±0.17$^{ab}$ | 0.99±0.03$^{a}$ | 0.60±0.04$^{cd}$ |
| 7 | 2.07±0.01$^{def}$ | 10.67±0.13$^{abcd}$ | 1.45±0.02$^{abc}$ | 3.39±0.01$^{abc}$ | 0.65±0.05$^{fg}$ | 0.85±0.01$^{fg}$ | 3.03±0.07$^{abcde}$ | 0.76±0.04$^{cde}$ | 0.56±0.01$^{cde}$ |
| 8 | 2.04±0.49$^{def}$ | 6.82±1.84$^{g}$ | 1.61±0.02$^{a}$ | 1.35±0.29$^{f}$ | 0.95±0.04$^{abc}$ | 1.06±0.03$^{bc}$ | 1.37±0.17$^{g}$ | 0.64±0.02$^{ef}$ | 0.54±0.02$^{cdef}$ |
| 9 | 1.88±0.01$^{efg}$ | 7.45±1.05$^{fg}$ | 1.53±0.06$^{ab}$ | 1.55±0.40$^{f}$ | 0.91±0.08$^{bcd}$ | 0.92±0.07$^{defg}$ | 1.34±0.59$^{g}$ | 0.71±0.01$^{de}$ | 0.48±0.06$^{def}$ |
| 10 | 1.99±0.11$^{defg}$ | 9.92±0.42$^{bcde}$ | 1.34±0.11$^{bcd}$ | 3.10±0.06$^{bcd}$ | 0.80±0.05$^{cdef}$ | 0.95±0.08$^{def}$ | 2.51±0.03$^{cdef}$ | 0.74±0.05$^{cde}$ | 0.48±0.05$^{def}$ |
| 11 | 2.28±0.26$^{cd}$ | 10.98±0.13$^{abc}$ | 1.44±0.06$^{abc}$ | 3.22±0.23$^{bcd}$ | 0.87±0.20$^{bcde}$ | 0.94±0.01$^{defg}$ | 3.05±0.14$^{abcd}$ | 0.76±0.12$^{cde}$ | 0.62±0.01$^{c}$ |
| 12 | 1.94±0.07$^{defg}$ | 9.37±0.76$^{cdef}$ | 1.43±0.02$^{abc}$ | 2.72±0.48$^{cde}$ | 0.67±0.11$^{efg}$ | 0.75±0.01$^{h}$ | 2.60±0.38$^{bcdef}$ | 0.76±0.04$^{cde}$ | 0.46±0.03$^{ef}$ |
| 13 | 2.49±0.03$^{bc}$ | 9.20±0.70$^{def}$ | 1.31±0.04$^{cd}$ | 2.53±0.40$^{de}$ | 0.78±0.07$^{cdef}$ | 0.89±0.04$^{efg}$ | 2.35±0.31$^{ef}$ | 0.86±0.02$^{abc}$ | 0.48±0.01$^{def}$ |
| 14 | 2.24±0.03$^{cde}$ | 8.93±1.05$^{ef}$ | 1.45±0.03$^{abc}$ | 2.38±0.56$^{e}$ | 0.80±0.01$^{bcdef}$ | 0.94±0.05$^{defg}$ | 2.12±0.49$^{f}$ | 0.78±0.04$^{cde}$ | 0.47±0.05$^{ef}$ |
| 15 | 3.18±0.05$^{a}$ | 12.35±0.13$^{a}$ | 1.33±0.04$^{bcd}$ | 3.46±0.24$^{ab}$ | 0.96±0.02$^{abc}$ | 1.22±0.01$^{a}$ | 3.62±0.11$^{a}$ | 0.95±0.08$^{ab}$ | 0.82±0.12$^{b}$ |
| 16 | 2.50±0.16$^{bc}$ | 9.27±1.05$^{cdef}$ | 1.22±0.11$^{d}$ | 2.26±0.40$^{e}$ | 0.91±0.05$^{bcd}$ | 0.85±0.01$^{fg}$ | 2.62±0.46$^{bcdef}$ | 0.93±0.06$^{ab}$ | 0.52±0.07$^{cdef}$ |
| 17 | 2.74±0.23$^{b}$ | 10.50±0.01$^{bcde}$ | 1.62±0.16$^{a}$ | 3.10±0.15$^{bcd}$ | 0.99±0.10$^{ab}$ | 1.14±0.09$^{ab}$ | 2.42±0.12$^{cdef}$ | 0.67±0.09$^{ef}$ | 0.56±0.04$^{cde}$ |
| **Minimum** | **1.61** | **6.82** | **1.21** | **1.35** | **0.59** | **0.75** | **1.34** | **0.46** | **0.42** |
| **Maximum** | **3.25** | **12.35** | **1.62** | **4.06** | **1.11** | **1.22** | **3.62** | **0.99** | **0.93** |
| **Mean** | **2.25** | **9.92** | **1.41** | **2.87** | **0.81** | **0.97** | **2.60** | **0.75** | **0.56** |
| **SD** | **0.48** | **1.46** | **0.13** | **0.75** | **0.15** | **0.13** | **0.66** | **0.15** | **0.14** |

Values are expressed as means ± SD (n=3). Different letters in the same column indicate significant difference at p < 0.05.CG was not detected in any sample.

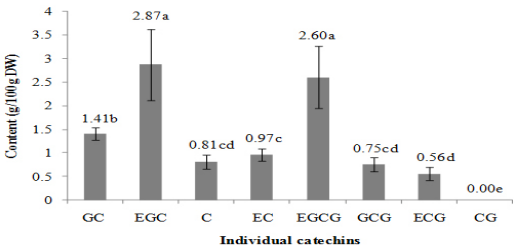


Figure 3. Mean values of individual catechins in oolong green teas. Bars represent the mean±SD of 17 oolong tea samples. Different letters indicate significant differences.

amounts of catechins and caffeine in the investigated oolong tea samples. The caffeine ranged from 1.61 to 3.25 with a mean of 2.25±0.48 g/100 g DW. Our values are in the range of previous work which reported that caffeine content was 1.44-4.03 g/100 g DW (average 2.60, n=287) (Obuchowicz *et al.*, 2011). In these 17 oolong tea samples, total catechins were the large group of constituents, with up to 6.82 to 12.35 with a mean of 9.92±1.46 g/100 g DW. This result is in accordance with mean values of total catechins obtained in previous studies, 6.57-21.38 g/100 g DW (average 11.70, n=358) (Obuchowicz *et al*., 2011), 8.50-20.60 g/100 g DW (average 15.10, n=51) (Engelhardt, 2005) and 2.80-22.80 g/100 g DW (average 10.90, n=18) (Unachukwu *et al*., 2010).

Comparison of the individual catechins of 17 oolong tea products indicated that all samples contain 7 catechins, including GC, EGC, C, EC, EGCG, GCG and ECG. The CG was not detected in any sample. Among quantified catechins, two most abundant catechins were EGC and EGCG (Figure 3). The EGC ranged from 1.35 to 4.06 with an average of 2.87±0.75 g/100 g DW. The EGCG ranged from 1.34 to 3.62 with an average of 2.60±0.66g/100 g DW. The EGC and EGCG had the highest mean value of all catechins quantified, which make EGCG and EGC the predominated catechin in Oolong tea. The average contents of EGC and EGCG were not significantly different among samples analyzed. The remaining catechins, GC (1.41±0.13 g/100 g DW), EC (0.97±0.13 g/100 g DW), C (0.81±0.15 g/100 g DW), GCG (0.75±0.15 g/100 g DW), and ECG (0.56±0.14 g/100 g DW), were found in small amount when compared to EGCG and EGC. The four major catechins present in green and oolong tea were reported as EGCG, EGC, ECG, and EC (McKay and Blumberg, 2002; Peterson *et al.*, 2005). It has been reported recently that the amount of EGCG was the highest, followed by EGC (Katalinic *et al.*, 2006; Wang *et al*., 2011). Our results are in reasonable agreement with these previous reports. However, it should be noted that it is quite impractical to compare the individual catechins in oolong teas with other published works because many factors such as species, cultivating season, plucking standards or methods, horticultural conditions and processing can all influence the catechin contents (Jayasekera *et al.*, 2011; Wei *et al*., 2011) However, it can be stated that the two major catechins in *C. sinensis* oolong teas produced in Thailand were EGCG and EGC. Both catechins could be the important quality parameters of the Thai oolong teas produced from oolong no. 17 sub-variety.

In fact, the variations in the abundance of compounds in teas could be related to the quality of tea grown in different regions having their soil and climatic conditions different, cultivation practices, post-harvesting handling and processing techniques by different manufacturers (Sultana *et al*., 2008; Chen *et al.*, 2010). The results of catechins and caffeine analysis reported here were derived from the analysis of samples collected from tea factories. It is well recognized that uncontrolled variables mentioned above may affect the chemical compositions. However, this investigation provides basic information on important chemical compounds of Thai oolong tea which can be used as a guideline for establishing the chemical standard of Thai tea in the future.

## Conclusion

The developed HPLC method allows rapid and simultaneous determinations of individual catechins and caffeine in oolong tea. Using this analytical method, 8 catechins and caffeine in oolong tea could be determined simultaneously, and the validity of the determinations was also verified. The described method could provide an efficient and comprehensive tool for the quality evaluation of oolong tea in the market. Moreover, the content of caffeine and catechins reported in this study provides basic information on important chemical compounds present in Thai oolong tea, which can be added to the national and regional databases. The data can also be used as a guideline for establishing tea standards and

for further detailed studies.

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