

Report

Chemical constituents of Oolong tea produced in Thailand and their correlation with infusion colour

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Abstract: Constituents expected to influence the colour of tea infusion, namely total polyphenols, total catechins, eight individual catechins, theaflavins and chlorophylls were determined in 28 samples of Oolong tea manufactured in Thailand. The colours attributes (L^* , a^* , b^* , hue angle and absorbance at 420 nm) of the infusions were measured and the correlation between the constituents and infusion colours was determined. The correlation analysis indicates that theaflavins and chlorophylls strongly correlate with infusion colours but total polyphenols and total catechins do not. Theaflavins correlate negatively with L^* and positively with a^* and b^* . Chlorophylls correlate positively with the L^* but negatively with a^* , b^* and hue angle. Chlorophylls and theaflavins were found to be the most important constituents contributing to the colour of Oolong tea manufactured in Thailand.

Keywords: chemical constituents of tea, tea infusion colour, Oolong tea, Thailand

INTRODUCTION

Tea (*Camellia sinensis* L.) is one of the most consumed beverages across the world. It can be categorised based on the degree of fermentation: green tea (unfermented), Oolong tea (partially fermented) and black tea (fully fermented). Green tea is heated to avoid enzymatic oxidation in a fermentation process. Oolong tea is semi-fermented to permit a level of partial enzymatic oxidation. Black tea is the most thoroughly oxidised enzymatically. Green tea has been regarded as a rich source of catechins, which include (-)-gallocatechin (GC), (-)-epigallocatechin (EGC), (+)-catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin gallate (EGCG), (-)-gallocatechin gallate (GCG), (-)-epicatechin gallate (ECG) and (-)-catechin gallate (CG) [1]. These compounds account for the colour, aroma and taste of green tea. During fermentation, catechins undergo oxidation and polymerisation, resulting in the formation of two groups of colour compounds: theaflavins (TF) and thearubigins (TR). The former group is golden-yellow while the latter group is reddish-brown. These

two groups of compounds impart the colour characteristics for Oolong and black tea infusions [2, 3]. The consumption of tea has been linked to its health benefits due to the presence of catechins, TF and TR. Because of this, many studies have been focused on the determination of polyphenols in green and black teas [4-7] and in Oolong teas [8-10].

The quality of tea is normally assessed by a tea taster. Tea tasting focuses on the appearance of the leaf, the aroma, both before and after the leaves are infused, the flavour and the colour of the infusion. Among these, the infusion colour is one of the most important attributes that is affected by chemical compounds in different kinds of tea. Green tea infusion generally contains no TF or TR and the desired colour of green tea is greenish or yellowish green [11]. The green colour is mainly due to the chlorophyll content. Since chlorophylls are not water soluble, the greenness of the tea infusion may come from other related coloured components such as chlorophyllides, the water-soluble green pigments derived from chlorophylls through the catalysis of chlorophyllase [12]. The red-brown colour is the main shade of black tea infusion, resulting mainly from TR which are formed in the fermentation process [13]. Oolong tea infusion is generally a dark greenish colour in the lightly fermented type and a yellowish-brown colour in the moderately to heavily fermented type. The colour-determining compounds in Oolong tea are comprised of several components such as chlorophylls and their degradation products, catechins and small amounts of TF and TR, depending on the degree of fermentation [11].

Compounds affecting the colour of tea infusion have been investigated in many studies. Borah and Bhuyan [14] indicated that the colour and colour change should be measured to assess tea quality during the process of fermentation. Liang et al. [15] showed that chemical compounds and colour differences in black tea infusions were correlated significantly with the sensory quality assessed by tea tasters. Wang et al. [16] reported that chlorophyll is released from tea leaves during steeping and it could contribute to the greenness of tea. Among the flavonoids (catechins and flavonols) detected in green tea infusions, quercetin was shown to be the most important compound contributing to the greenness of green tea infusions [16]. Kim et al. [17] reported that the green tea infusion colour was affected by heat during tea processing. The colour indicator L^* decreased while indicators a^* and b^* increased with an increase in heating temperature. This result was also related to the oxidation of catechins and degradation of chlorophyll under hot condition.

The quality of tea is influenced by many factors such as cultivar, harvest season, age of plant, climate, environmental condition and processing condition [18-20]. The colour of tea infusions can vary, leading to colour differences between different producers and different samples of tea. In Thailand tea is cultivated in the northern part of the country in the provinces of Chiang Rai and Chiang Mai (accounting for 93% of tea production in Thailand). Among the 3 types of tea, 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'. The chemical constituents affecting the shade of infusion colour of Oolong tea manufactured in Thailand have not been determined so far. The purpose of the present study is to determine some chemical constituents of Oolong tea produced in Thailand and explore their relationship with the infusion colour of the tea.

MATERIALS AND METHODS

Tea Samples, Chemicals and Reagents

Twenty-eight Oolong tea samples were collected from tea factories in Chiang Rai province. All Oolong teas were produced from *Camellia sinensis* var. *sinensis*.

Folin-Ciocalteu's phenol reagent, gallic acid, acetonitrile, acetone, trifluoroacetic acid, ethanol and methanol (HPLC grade) were purchased from Fluka (Switzerland). Anhydrous sodium carbonate was purchased from Merck (Germany). Isobutyl methyl ketone, Flavognost reagent (diphenylboric acid 2-aminoethyl ester) and 4-methyl-2-pentanone were purchased from Sigma-Aldrich (Switzerland). All chemical standards (GC, EGC, C, EC, EGCG, caffeine, GCG, ECG and CG) were purchased from Sigma-Aldrich (USA).

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 $103\pm 2^\circ\text{C}$ for at least 16 hr to constant weight. The percentage of moisture content and dry matter (%DM) in the samples were then calculated from the weight difference [21].

Sample Extraction

Each ground tea sample (~2 g, weighed to the nearest 0.001g) was extracted with distilled water (200 mL) at 95°C . The extraction mixture was constantly stirred with a magnetic stirrer. After 10 min., the extraction mixture was filtered through Whatman No. 4 filter paper. 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.

Determination of Total Polyphenol

The total polyphenol (TP) content was determined by spectrophotometry with gallic acid as standard [22]. The tea solution was diluted 50-fold with distilled water. To a 1.0 mL sample of the diluted solution, 5.0 mL of Folin-Ciocalteu's reagent (10% v/v) and then 4.0 mL sodium carbonate (7.5% w/v) were added. The mixture was mixed and left to stand at room temperature for 60 min. before the absorbance at 765 nm was measured. The concentration of polyphenols in the sample was derived from a standard curve of gallic acid (10-100 $\mu\text{g/mL}$). The TP was expressed as gallic acid equivalents (GAE) in g/100 g dry weight.

Determination of Individual Catechins and Total Catechins

Individual catechins and total catechin (TC) content were determined by ISO method [23] as modified by Theppakorn and Wongsakul [24]. The individual standard solutions of GC, EGC, C, EC, EGCG, GCG, ECG and CG were prepared by dissolution in methanol to generate a stock concentration of 1,000 $\mu\text{g/mL}$. The mixed stock standard solution was prepared by mixing an equal volume of each stock standard. Working standard solutions (0.2-100 $\mu\text{g/mL}$) were prepared by diluting and filtering the mixed stock solution through a 0.45- μm PTFE filter. The HPLC analysis of standards and samples was conducted on a Water 966 high performance liquid chromatograph with a vacuum degasser, quaternary pump, auto-sampler, thermostatted column compartment and photo diode array detector. The column used was a Platinum EPS C18 reverse phase, 3 μm (53x7mm), and operated at 30°C . The mobile phase (flow rate = 2 mL/min.) was water/acetonitrile (87:13)

containing 0.05% (v/v) trifluoroacetic acid. The absorption wavelength was 210 nm and the injection volume was 20 μ L. Individual catechins were identified by comparing their retention times and UV spectra in the 190-400 nm range with the standards and quantified using a caffeine calibration curve, together with the consensus relative response factors with respect to caffeine as shown below. The total TC content was obtained by summation of individual catechins.

$$\text{Individual catechin content (g/100g DW)} = \frac{(A_s - b) \times \text{RRF}_{\text{std}}}{m} \times \frac{V_s \times \text{DF}}{W_s \times 10,000} \times \frac{100}{\% \text{DM}}$$

where

- DW = dry weight;
 A_s = peak area of individual catechin in sample;
 b = peak area at point of interception on y-axis of caffeine calibration curve;
 RRF_{std} = relative response factor of individual catechin with respect to caffeine;
 m = slope of caffeine calibration curve;
 V_s = sample extraction volume (mL);
 DF = dilution factor;
 W_s = sample weight (g);
 %DM = percentage of dry matter of sample.

Determination of Chlorophylls

The content of chlorophylls was determined as follows. About 0.2 g of the ground tea sample was extracted by vortex-mixing with 50 mL of 80% (v/v) acetone for 2 min. and filtered. The extracted solution was analysed spectrophotometrically at the wavelength of 663 nm (for chlorophyll a) and 645 nm (for chlorophyll b). The contents of chlorophyll a, chlorophyll b and total chlorophyll were calculated according to the formula [25]:

$$\text{Chlorophyll a } (C_a, \text{ mg/g}) = \frac{(12.7A_{663} - 2.95A_{645}) \times 50}{W \times 1000}$$

$$\text{Chlorophyll b } (C_b, \text{ mg/g}) = \frac{(22.9A_{645} - 4.67A_{663}) \times 50}{W \times 1000}$$

$$\text{Chlorophyll total } (C_t, \text{ mg/g}) = C_a + C_b$$

(W = weight (g) of tea sample; A_{663} and A_{645} = absorbance at 663 and 645 nm respectively)

Determination of TF

The TF content was determined by the Flavognost method [26]. A tea infusion was made with 375 mL of boiling water and 9 g of tea. After shaking for 10 min., the infusion was filtered through rough cotton wool and allowed to cool to room temperature, and then 10 mL were pipetted into 10 mL of isobutyl methyl ketone. The mixture was shaken for 10 min. and allowed to stand until the layers separated. Two mL of the upper layer, followed by 4 mL of ethanol and 2 mL of Flavognost reagent (2 g of diphenylboric acid-2-aminoethyl ester dissolved in 100 mL of ethanol) were pipetted into a test tube. The contents were mixed and the colour was allowed to develop for 15 min. The absorbance at 625 nm was read against the isobutyl methyl ketone /ethanol (1:1 v/v) blank and used for the following formula:

$$\text{TF content } (\mu\text{mol/g}) = \frac{A_{625} \times 47.9 \times 100}{\% \text{DM}}$$

Colour Analysis of Tea Infusion

By spectrophotometer

A ground tea sample (2 g) was extracted with boiling water (100 mL) for 5 min. and then filtered through Whatman No.1 filter paper. The absorbance of the filtrate at 420 nm was measured with a spectrophotometer with distilled water as blank [25].

By colorimeter

A tea sample (5 g) was brewed with hot water (95°C, 200 mL) for 5 min. and filtered. The CIE $L^*a^*b^*$ (CIELAB) of the tea infusion was measured with a Minolta CR400 colorimeter using standard cuvettes after a proper calibration. Each colour value (L^* , a^* , b^* and hue angle) was measured in triplicate.

Data Analysis

All tests were carried out in triplicate and mean \pm SD values are presented. The chemical compositions were correlated with the colour parameters of tea infusions. A linear regressive analysis was carried out using SPSS 16.0 for Windows.

RESULTS AND DISCUSSION

Chemical Constituents

Table 1 shows the chemical constituents of interest of 28 Oolong teas produced in Thailand. The TP content, varying between 10.97-18.01 g GAE/100g DW, is in the range of that found in green tea [27, 28]. Catechins are the main polyphenolic compounds in fresh tea leaves and in general the total catechin content in green tea products is significantly higher than that in partially or fully fermented teas [29]. Catechins are colourless and water soluble and contribute to the bitterness and astringency of green tea [11]. My results indicate that catechins are also the major polyphenolic constituents of Oolong teas (approximately 70% of TP). I found that all Oolong tea samples contained GC, EGC, C, EC, EGCG, GCG and ECG but no CG, EGC (3.38%) and EGCG (2.85%) being the 2 predominant catechins based on the mean content. These findings are similar to those of Kerio et al. [30].

Due to the partial fermentation in Oolong tea processing, catechins are partially oxidised, mainly to TF, which are a group of major pigments that give a yellow-orange colour in fermented tea. The TF content in Oolong teas in Table 1 varies between 0.50-1.60 μ moles/g. In black teas it varies between 14.75-26.38 μ moles/g [31] and 9.84-20.70 μ mole/g [32]. However, many studies have shown that fermentation time, temperature and tea variety strongly affect the TF content in black tea [2, 33, 34]. Another important colouring matter in Oolong tea is the chlorophyll, which gives a green or yellowish-green hue to the tea infusion. From Table 1, the chlorophyll a content (0.42 \pm 0.13 mg/g) is higher than that of chlorophyll b (0.22 \pm 0.08 mg/g). Chlorophyll a is dark green and chlorophyll b is yellowish-green in colour.

There is no specific fermentation stage during Oolong tea manufacture in Thailand. The partial oxidation of tea polyphenols takes place during the withering and leaf handling stages, in which the tea shoots are partially bruised by mechanical handling, leading to an enzymatic oxidation of tea polyphenols. The major purpose of the withering and leaf handling is to eliminate moisture and promote the enzymatic hydrolysis of glycoside aroma precursors, which releases volatile flavour compounds without accelerating the oxidation of tea polyphenols. As can be seen from the results of

TC and TF, Oolong teas produced in Thailand are slightly fermented, undergoing a restricted level of enzymatic oxidation.

Table 1. Some chemical constituents of 28 samples of Oolong tea produced in Thailand

No.	TP (GAE, g/100g)	TC (g/100g)	Individual catechin content (g/100g)								TF (μ mol/g)	Chlorophyll (mg/g)		
			GC	EGC	C	EC	EGCG	GCG	ECG	CG		a	b	Total
1	16.29±0.87	9.70	1.18	3.77	0.59	1.03	2.38	0.37	0.38	nd	0.79±0.04	0.32±0.03	0.14±0.19	0.47±0.02
2	15.43±0.77	9.36	1.19	3.92	0.40	0.76	2.12	0.42	0.55	nd	0.59±0.02	0.55±0.07	0.27±0.03	0.82±0.01
3	18.01±0.01	10.34	1.34	3.53	0.60	1.00	2.68	0.72	0.47	nd	0.53±0.01	0.54±0.05	0.25±0.06	0.78±0.01
4	15.90±0.17	11.67	1.29	3.79	0.86	1.09	3.41	0.61	0.62	nd	0.50±0.01	0.46±0.04	0.26±0.05	0.72±0.02
5	15.39±0.40	9.38	1.13	3.29	0.53	0.84	2.75	0.38	0.46	nd	0.54±0.02	0.52±0.04	0.27±0.03	0.79±0.01
6	12.44±0.45	9.59	1.18	3.26	0.61	0.92	2.63	0.53	0.46	nd	0.60±0.04	0.51±0.04	0.26±0.02	0.77±0.01
7	16.17±0.84	11.58	1.68	4.15	0.72	1.20	2.71	0.61	0.51	nd	0.61±0.01	0.48±0.03	0.26±0.06	0.75±0.02
8	14.86±0.33	13.27	1.80	5.33	0.76	1.30	2.97	0.65	0.46	nd	0.54±0.01	0.59±0.02	0.34±0.10	0.94±0.01
9	15.47±0.60	10.60	1.44	3.52	0.71	1.18	2.66	0.49	0.60	nd	0.51±0.01	0.59±0.09	0.34±0.08	0.93±0.05
10	14.62±0.87	9.28	1.21	4.57	0.60	1.11	1.31	0.37	0.11	nd	0.79±0.01	0.39±0.06	0.19±0.09	0.58±0.03
11	15.03±0.27	10.61	1.32	3.18	4.59	0.62	0.90	nd	nd	nd	0.65±0.01	0.38±0.08	0.17±0.06	0.55±0.02
12	11.75±0.01	9.60	1.15	3.12	0.60	0.88	2.80	0.56	0.49	nd	0.52±0.01	0.45±0.13	0.23±0.05	0.68±0.02
13	11.19±0.51	9.94	1.19	3.22	0.68	0.94	2.77	0.60	0.54	nd	0.65±0.03	0.50±0.01	0.24±0.07	0.74±0.01
14	13.89±0.05	11.97	1.19	3.66	0.76	1.21	3.71	0.65	0.79	nd	0.58±0.02	0.40±0.02	0.20±0.03	0.61±0.01
15	15.36±0.02	6.66	0.93	1.75	0.61	0.67	1.91	0.41	0.38	nd	0.63±0.02	0.52±0.06	0.26±0.01	0.79±0.01
16	10.97±0.35	9.37	1.20	3.09	0.67	0.90	2.61	0.45	0.45	nd	0.63±0.01	0.59±0.06	0.32±0.01	0.91±0.01
17	14.85±0.28	7.95	1.28	2.37	0.66	0.85	2.04	0.34	0.41	nd	0.68±0.02	0.30±0.03	0.16±0.04	0.46±0.01
18	15.05±0.18	8.26	1.51	5.18	0.53	1.04	nd	nd	nd	nd	0.61±0.01	0.65±0.05	0.34±0.04	0.99±0.02
19	11.05±0.39	9.32	1.18	3.01	0.67	0.93	2.53	0.56	0.44	nd	0.54±0.01	0.61±0.02	0.33±0.01	0.94±0.01
20	15.04±0.45	5.01	0.24	0.66	1.46	1.48	nd	nd	1.17	nd	1.22±0.02	0.25±0.01	0.12±0.01	0.38±0.01
21	16.08±0.04	10.50	1.02	2.82	0.71	1.11	3.38	0.53	0.93	nd	1.06±0.04	0.17±0.01	0.06±0.06	0.22±0.04
22	14.83±0.05	10.85	1.32	3.11	0.75	1.07	3.53	0.31	0.76	nd	0.59±0.01	0.34±0.01	0.22±0.06	0.56±0.01
23	16.29±0.24	13.31	1.11	4.11	0.75	1.29	4.55	0.50	1.00	nd	1.60±0.01	0.31±0.04	0.12±0.06	0.43±0.06
24	12.92±0.52	12.23	0.80	2.77	0.63	0.95	5.27	0.79	1.02	nd	0.59±0.04	0.33±0.02	0.09±0.03	0.41±0.01
25	17.64±0.70	9.65	1.04	5.23	0.57	1.08	1.21	0.47	0.05	nd	0.69±0.02	0.29±0.05	0.09±0.01	0.38±0.01
26	16.73±0.30	12.25	0.87	2.93	0.69	1.00	4.94	0.82	1.00	nd	0.94±0.01	0.31±0.04	0.13±0.02	0.44±0.01
27	16.02±0.07	11.39	0.99	3.18	0.65	0.96	4.17	0.78	0.66	nd	0.83±0.01	0.21±0.02	0.25±0.02	0.46±0.01
28	17.64±0.46	7.55	0.77	2.15	0.64	0.91	2.21	0.41	0.46	nd	0.78±0.03	0.25±0.06	0.25±0.03	0.36±0.01
Min.	10.97	5.01	0.24	0.66	0.40	0.62	0.90	0.31	0.05	nd	0.50	0.17	0.06	0.22
Max.	18.01	13.31	1.80	5.33	4.59	1.48	5.27	0.82	1.17	nd	1.60	0.65	0.34	0.99
Mean	14.89	10.04	1.16	3.38	0.82	1.01	2.85	0.53	0.58	nd	0.71	0.42	0.22	0.64
SD	1.92	1.89	0.29	1.01	0.76	0.19	1.07	0.15	0.27	nd	0.24	0.13	0.08	0.21

Note: Values are expressed as means \pm standard deviation (SD) from triplicate analysis; TP= total polyphenols; TC= total catechins; GC= (-)-gallocatechin; EGC= (-)-epigallocatechin; C= (+)-catechin; EC= (-)-epicatechin; EGCG= (-)-epigallocatechin gallate; GCG= (-)-gallocatechin gallate; ECG= (-)-epicatechin gallate; CG= (-)-catechin gallate; TF= total theaflavins; nd= not detected.

Infusion Colour

Table 2 shows values of L*, a*, b*, hue angle and A₄₂₀ of the liquor of Oolong tea samples. The L* value represents the degree of lightness: the higher it is, the lighter is the colour. The a* value indicates redness when positive and greenness when negative, while the b* value reflects yellowness when positive and blueness when negative. Generally the infusion colour of Oolong tea varies from green to greenish yellow to golden yellow, depending on the fermentation time. The hue angle is often used to express the green colour [35]. The increase in hue angle corresponds to an increase in greenness and a reduction in yellowness. The absorbance at 420 nm is used in China to assess the colour quality of tea: a high absorbance value is preferred for a good quality.

Table 2. L*, a*, b*, hue angle and absorbance at 420 nm of infusions of Oolong tea samples

No.	L*	a*	b*	Hue angle	A ₄₂₀
1	35.41±0.08	0.24±0.02	2.83±0.04	85.11±0.40	0.18±0.03
2	29.75±1.38	0.46±0.04	4.52±0.39	84.19±0.57	0.19±0.04
3	33.91±0.31	0.11±0.08	2.83±0.34	87.70±1.45	0.19±0.16
4	26.92±0.55	1.01±0.07	5.96±0.44	80.30±1.13	0.19±0.02
5	24.17±0.42	1.29±0.09	8.65±0.54	81.50±0.69	0.21±0.01
6	26.04±0.40	1.45±0.11	9.34±1.05	81.13±1.11	0.31±0.01
7	34.20±0.23	0.05±0.09	3.63±0.18	89.35±1.40	0.24±0.02
8	34.53±0.10	-0.09±0.04	1.62±0.21	93.38±1.33	0.14±0.01
9	33.90±0.33	-0.08±0.05	3.84±0.35	91.22±0.84	0.29±0.04
10	34.47±0.49	0.03±0.09	2.23±0.13	89.22±2.22	0.17±0.01
11	34.25±0.04	-0.01±0.10	3.17±0.32	90.22±1.95	0.24±0.03
12	34.27±0.10	-0.11±0.06	2.81±0.12	92.19±1.22	0.20±0.02
13	35.00±0.18	-0.01±0.07	2.43±0.05	90.31±1.69	0.17±0.01
14	34.81±0.01	0.05±0.05	3.18±0.04	89.07±0.87	0.22±0.01
15	35.48±0.02	-0.09±0.09	3.64±0.16	91.31±1.32	0.2±0.01
16	35.32±0.10	-0.04±0.04	3.79±0.11	90.58±0.64	0.21±0.01
17	35.85±0.09	0.17±0.14	4.33±0.23	87.81±1.80	0.28±0.04
18	36.56±0.13	-0.21±0.03	3.42±0.09	93.44±0.63	0.25±0.02
19	36.36±0.12	-0.14±0.10	3.18±0.09	92.54±1.81	0.23±0.01
20	23.96±2.24	1.14±0.45	13.09±4.17	85.14±0.57	0.26±0.06
21	16.19±1.76	2.77±0.64	21.72±1.67	82.81±1.11	0.24±0.01
22	18.59±1.93	1.36±0.34	13.16±3.71	84.06±0.39	0.14±0.03
23	18.51±2.23	1.81±0.32	16.94±1.37	83.93±0.60	0.20±0.01
24	20.78±0.23	1.68±0.14	11.45±0.42	80.51±0.32	0.15±0.03
25	22.94±0.52	1.68±0.06	10.03±0.22	81.66±0.55	0.19±0.03
26	27.10±0.79	0.92±0.09	7.13±0.25	82.63±0.70	0.16±0.01
27	28.62±0.18	0.89±0.06	6.35±0.25	82.09±0.26	0.19±0.01
28	29.30±0.77	0.82±0.11	6.99±0.69	83.32±0.75	0.26±0.03
Min.	16.19	-0.21	1.62	80.30	0.14
Max.	36.56	2.77	21.72	93.44	0.31
Mean	29.90	0.61	6.51	86.67	0.21
SD	6.19	0.77	4.86	4.27	0.04

Note: Values are expressed as means ± SD (n=3).

Correlation between Chemical Constituents and Infusion Colour

Correlation analysis of the 28 Oolong teas revealed that their TP content did not correlate well with L*, a*, b* or A₄₂₀ values but was significantly related with the hue angle (r = 0.391) (Table 3). In Thai Oolong tea catechins comprise approximately 70% of the TP content and about 10% of the dry weight (Table 1). Correlation analysis showed that the TC content did not correlate with any measured colour parameters. Correlation of the most abundant catechin (EGC) also gives a similar result. This may be because all catechins appear to be colourless in water (as observed with the aqueous solutions of authentic compounds), hence their negligible influence the infusion colour. However, some individual catechins (GC, EGC and EGCG) are fairly related to some measured colour parameters. Although the oxidation products of catechins have been reported to increase the redness and yellowness of tea infusions [16], their influence on the colour of freshly brewed Oolong tea infusion seems to be minimal when compared to other compounds.

The correlation analysis in this study shows that the TF content negatively correlates with L* (r = -0.505) and positively correlates with a* (r = 0.508) and b* (r = 0.644). However, it does not correlate with hue angle and A₄₂₀. Interestingly, chlorophylls, the green pigments in tea leaf, strongly correlate with the infusion colour. Chlorophyll a, b and total positively correlate with L* (r = 0.581, 0.584 and 0.603) but negatively correlate with a* (r = -0.636, -0.647 and -0.663), b* (r = -0.602, -0.622 and -0.632) and hue angle (r = -0.612, -0.562 and -0.614). As can be clearly seen from Table 3, TF and chlorophylls show significant correlation with the infusion colour. These findings indicate that TF and chlorophylls may be the most important compounds affecting the infusion colour of Thai Oolong tea.

Table 3. Linear correlation coefficients between some constituents and infusion colour of Oolong tea samples

Constituent	Parameters of infusion colour				
	L*	a*	b*	Hue angle	A ₄₂₀
Total polyphenols (TP)	-0.273	0.293	0.224	0.391*	-0.141
Total catechins (TC)	-0.211	0.17	0.079	-0.145	0.204
GC	0.433*	-0.452*	-0.464*	0.479**	-0.097
EGC	0.15	-0.147	-0.253	0.168	-0.024
C	0.079	-0.112	0.057	0.145	0.018
EC	-0.27	0.22	0.135	-0.036	0.008
EGCG	-0.387*	0.364	0.278	-0.401*	0.274
GCG	-0.09	0.13	-0.031	-0.236	0.079
ECG	-0.579**	0.514**	-0.592	-0.422*	0.189
CG	-	-	-	-	-
Total theaflavins (TF)	-0.505**	0.508**	0.644**	-0.3	0.173
Chlorophyll a	0.581**	-0.636**	-0.602**	-0.612**	-0.099
Chlorophyll b	0.584**	-0.647**	-0.622**	-0.562**	-0.167
Total chlorophylls	0.603**	-0.663**	-0.632**	-0.614**	-0.129

* Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

CONCLUSIONS

This study provides the relationship between the relevant constituents and the infusion colour of Oolong teas produced in Thailand. The results indicate that they seem to undergo only a mild fermentation and their infusion colour is determined mainly by the chlorophylls and theaflavins formed by partial fermentation during the tea processing.

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REFERENCES

1. M. G. Ferruzzi and R. J. Green, "Analysis of catechins from milk-tea beverages by enzyme assisted extraction followed by high performance liquid chromatography", *Food Chem.*, **2006**, 99, 484-491.
2. T. Muthumani and R. S. S. Kumar, "Influence of fermentation time on the development of compounds responsible for quality in black tea", *Food Chem.*, **2007**, 101, 98-102.
3. Y. Kim, K. L. Goodner, J. D. Park, J. Choi and S. T. Talcott, "Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation", *Food Chem.*, **2011**, 129, 1331-1342.
4. Y. Liang, J. Lu, L. Zhang, S. Wu and Y. Wu, "Estimation of black tea quality by analysis of chemical composition and colour difference of tea infusions", *Food Chem.*, **2003**, 80, 283-290.
5. V. Sharma, A. Gulati, S. D. Ravindranath and V. Kumar, "A simple and convenient method for analysis of tea biochemicals by reverse phase HPLC", *J. Food Compos. Anal.*, **2005**, 18, 583-594.
6. D. Wang, J. Lu, A. Miao, Z. Xie and D. Yang, "HPLC-DAD-ESI-MS/MS analysis of polyphenols and purine alkaloids in leaves of 22 tea cultivars in China", *J. Food Compos. Anal.*, **2008**, 21, 361-369.
7. G. Alaerts, J. Van Erps, S. Pieters, M. Dumarey, A. M. van Nederkassel, M. Goodarzi, J. Smeyers-Verbeke and Y. V. Heyden, "Similarity analyses of chromatographic fingerprints as tools for identification and quality control of green tea", *J. Chromatogr. B*, **2012**, 910, 61-70.
8. Y. Chen, Y. Jiang, J. Duan, J. Shi, S. Xue and Y. Kakuda, "Variation in catechin contents in relation to quality of 'Huang Zhi Xiang' Oolong tea (*Camellia sinensis*) at various growing altitudes and seasons", *Food Chem.*, **2010**, 119, 648-652.
9. Y. Wang, Q. Li, Q. Wang, Y. Li, J. Ling, L. Liu, X. Chen and K. Bi, "Simultaneous determination of seven bioactive components in Oolong tea *Camellia sinensis*: Quality control by chemical composition and HPLC fingerprints", *J. Agric. Food Chem.*, **2012**, 60, 256-260.
10. K. Wang, F. Liu, Z. Liu, J. Huang, Z. Xu, Y. Li, J. Chen, Y. Gong and X. Yang, "Analysis of chemical components in Oolong tea in relation to perceived quality", *Int. J. Food Sci. Technol.*, **2010**, 45, 913-920.
11. V. S. P. Chaturvedula and I. Prakash, "The aroma, taste, color and bioactive constituents of tea", *J. Med. Plants Res.*, **2011**, 5, 2110-2124.
12. N. Ogura, M. Ueno, M. Matsunaga, T. Sato and H. Nakagawa, "Chlorophyllase activities and color changes of fresh green leaves on blanching", *Nippon Nogeik. Kaishi*, **1987**, 61, 451-456 (in Japanese).

13. E. A. H. Roberts and R. F. Smith, "The phenolic substances of manufactured tea. IX.—The spectrophotometric evaluation of tea liquors", *J. Sci. Food Agric.*, **1963**, 14, 689-700.
14. S. Borah and M. Bhuyan, "Non-destructive testing of tea fermentation using image processing", *Brit. Inst. J. Nondestr. Test.*, **2003**, 45, 55-58.
15. Y. Liang, J. Lu, L. Zhang, S. Wu and Y. Wu, "Estimation of tea quality by infusion colour difference analysis", *J. Sci. Food Agric.*, **2005**, 85, 286-292.
16. L. F. Wang, S. C. Park, J. O. Chung, J. H. Baik and S. K. Park, "The compounds contributing to the greenness of green tea", *J. Food Sci.*, **2004**, 69, S301-S305.
17. E. S. Kim, Y. R. Liang, J. Jin, Q. F. Sun, J. L. Lu, Y. Y. Du and C. Lin, "Impact of heating on chemical compositions of green tea liquor", *Food Chem.*, **2007**, 103, 1263-1267.
18. Y. S. Lin, Y. J. Tsai, J. S. Tsay and J. K. Lin, "Factors affecting the levels of tea polyphenols and caffeine in tea leaves", *J. Agric. Food Chem.*, **2003**, 51, 1864-1873.
19. R. Cooper, D. J. Morré and D. M. Morré, "Medicinal benefits of green tea: Part I. Review of non-cancer health benefits", *J. Altern. Complement. Med.*, **2005**, 11, 521-528.
20. P. O. Owuor, M. Obanda, H. E. Nyirenda and W. L. Mandala, "Influence of region of production on clonal black tea chemical characteristics", *Food Chem.*, **2008**, 108, 263-271.
21. ISO 1573:1980, "Tea—Determination of loss in mass at 103 degrees C", International Organization for Standardization, Geneva, **1980**.
22. ISO 14502-1:2005, "Determination of substances characteristic of green and black tea—Part 1: Content of total polyphenols in tea—Colorimetric method using Folin—Ciocalteu reagent", International Organization for Standardization, Geneva, **2005**.
23. ISO 14502-2:2005, "Determination of substances characteristic of green and black tea—Part 2: Content of catechins in green tea—Method using high-performance liquid chromatography", International Organization for Standardization, Geneva, **2005**.
24. T. Theppakorn and S. Wongsakul, "Optimization and validation of the HPLC-base method for the analysis of gallic acid, caffeine and 5 catechins in green tea", *Naresuen Univ. J.*, **2012**, 20, 1-11.
25. Tea Institute of Chinese Academy of Agricultural Sciences, "Experiment Handbook of Tea Tree Physiology and Tea Leaves Biochemistry", Press of Chinese Agriculture, Beijing, **1983**.
26. P. J. Hilton and R. Palmer-Jones, "Relationship between the flavanol composition of fresh tea shoots and the theaflavin content of manufactured tea", *J. Sci. Food Agric.*, **1973**, 24, 813-818.
27. K. M. M. Alam, M. S. Uddin, M. A. M. Chowdhury and M. A. Motalib, "Qualitative evaluation of ten major marketed brands of tea in Bangladesh", *Plant Arch.*, **2011**, 11, 173-177.
28. S. Jayasekera, A. L. Molan, M. Garg and P. J. Moughan, "Variation in antioxidant potential and total polyphenol content of fresh and fully-fermented Sri Lankan tea", *Food Chem.*, **2011**, 125, 536-541.
29. S. M. Karori, F. N. Wachira, J. K. Wanyoko and R. M. Ngure, "Antioxidant capacity of different types of tea products", *Afr. J. Biotechnol.*, **2007**, 6, 2287-2296.
30. L. C. Kerio, F. N. Wachira, J. K. Wanyoko and M. K. Rotich, "Total polyphenols, catechin profiles and antioxidant activity of tea products from purple leaf coloured tea cultivars", *Food Chem.*, **2013**, 136, 1405-1413.
31. P. O. Owuor, M. Obanda, H. E. Nyirenda, N. I. K. Mphangwe, L. P. Wright and Z. Apostolides, "The relationship between some chemical parameters and sensory evaluations for

plain black tea (*Camellia sinensis*) produced in Kenya and comparison with similar teas from Malawi and South Africa”, *Food Chem.*, **2006**, 97, 644-653.

32. M. Obanda, P. O. Owuor, R. Mang'oka and M. M. Kavoi, “Changes in thearubigin fractions and theaflavin levels due to variations in processing conditions and their influence on black tea liquor brightness and total colour”, *Food Chem.*, **2004**, 85, 163-173.
33. M. Obanda, P. O. Owuor and R. Mang'oka, “Changes in the chemical and sensory quality parameters of black tea due to variations of fermentation time and temperature”, *Food Chem.*, **2001**, 75, 395-404.
34. P. O. Owuor and M. Obanda, “Comparative responses in plain black tea quality parameters of different tea clones to fermentation temperature and duration”, *Food Chem.*, **2001**, 72, 319-327.
35. M. H. Lau, J. Tang and B. G. Swanson, “Kinetics of textural and color changes in green asparagus during thermal treatments”, *J. Food Eng.*, **2000**, 45, 231-236.