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Variation in catechin contents in relation to quality of '*Huang Zhi Xiang*' Oolong tea (*Camellia sinensis*) at various growing altitudes and seasons

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ABSTRACT

The variations in the contents of catechins and phenolic compounds in relation to the quality scores (QS) and taste scores (TS) in the '*Huang Zhi Xiang*' Oolong tea grown in different seasons and at different altitudes were determined. The study demonstrated that the contents of (−)-epigallocatechin gallate (EGCG), catechin gallate (CG), and total catechins (TC) in the Oolong teas grown at a high altitude were significantly higher than those grown at a low altitude. Furthermore, the contents of EGCG, CG, and TC in the autumn tea were significantly higher than those from the spring tea grown at a low altitude. However, no significant differences in the contents of EGCG, CG, and TC were observed between the spring and autumn teas grown at a high altitude. In addition, the contents of (−)-epigallocatechin (EGC), (+)-catechin (C), (−)-gallocatechin (GC), and simple catechins (SC) in the spring tea leaves were significantly higher than those in the autumn tea leaves grown at a low altitude, but they were not significantly different from those in the autumn tea leaves grown at a high altitude. The contents of EGCG, CG, and TC in the Oolong teas were positively and significantly correlated to the taste score (TS) and quality score (QS) of the Oolong tea. Thus, the contents of EGCG, CG, and TC could be the important quality parameters of the '*Huang Zhi Xiang*' Oolong tea. This work suggested that the production of the '*Huang Zhi Xiang*' Oolong tea grown at low altitudes should utilize the autumn tea leaves while the production of Oolong tea grown at high altitudes can use both the spring and autumn tea leaves.

1. Introduction

Oolong tea, which is commercially available in China, the United States, Japan and elsewhere, has also been studied for its antioxidant properties (Benzie & Szeto, 1999), its effect on cardiovascular disease (Yang & Koo, 1997) and on obesity (Han, Takaku, Li, Kimura, & Okuda, 1999). As important stimulating compositions of Oolong tea, catechins are one class of polyphenols that have been reported to exhibit anti-cancer, anti-oxidant, anti-inflammatory and anti-bacterial activity (Way et al., 2009).

Oolong tea is a semi-fermented tea that is allowed to oxidize only partially to lock-in the rich flavour that is associated with high quality of Oolong tea. Six catechins and catechin gallates, i.e. (+)-catechin (C), (−)-gallocatechin (GC), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG) have been identified as the major phenolic compounds present in Oolong tea (Lin, Lin, Liang, Lin-Shiau, & Juan, 1998). Among these catechins, EC, EGC, GC, and C were simple catechins (SC), whereas ECG and EGCG are catechin gallates (CG) (Sanderson, 1972; Sanderson & Graham, 1973). The polyphenol profile of Oolong tea is similar to that of green or black tea, but it has a lower content of EGCG, EC, and ECG than that found in green tea because the fresh Oolong tea leaves are subjected to a partial fermentation step before drying (Zuo, Chen, & Deng, 2002). In terms of catechins, the levels of EC, ECG and EGCG were significantly different ($P < 0.05$) in some Taiwan Oolong teas grown in different seasons (spring and summer), but the levels of GC, C, EGC, and total catechins (TC) were not significantly different ($P < 0.05$). Furthermore, among the tea varieties investigated, most catechin levels were similar (Wang, Tsai, Lin, & Ou, 2006). Thus, the altitude of tea planting and seasons of tea picking may influence the composition and level of catechins in Oolong teas during their growth.

The '*Huang Zhi Xiang*' Oolong tea is one of the most popular types of Oolong teas that have been grown in the *Phoenix* Mountain of Chaozhou City in Guangdong province, China for many decades. This tea is made from the '*Huang Zhi Xiang*' tea bush that has been cultivated from the oldest vegetative propagated cultivar (700 years old). The objective of this study was to understand to the effects of different growing conditions (altitude above sea level

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and seasons) on the levels of catechins and their corresponding relationships to '*Huang Zhi Xiang*' Oolong tea quality using the single Oolong tea clone.

2. Materials and methods

2.1. Materials

Tea leaves of the '*Huang Zhi Xiang*' tea bush were harvested from the Phoenix Mountain (latitudes 23°32′N and longitude 116°63′E) at the altitudes of 500 m and 350 m above the mean sea level (AMSL) in April (spring) and September (autumn). The '*Huang Zhi Xiang*' tea bushes chosen for this study were growing on a tea plantation that was planted over 20 years ago under the same soil conditions and were managed under uniform agronomic principles. Three sites in the tea plantation with an area of about 400 m^2 at the altitude of 500 m AMSL and an additional three sites with an area of about 400 m^2 at the altitude of 350 m AMSL were chosen for the experiment. Young shoots with 3–4 leaves and a bud were harvested. The flavanol standards (EGCG, GC, EC and C) were purchased from Sigma–Aldrich (Shanghai, China) while the flavanol standards (EGC and ECG) were obtained from Aladdin-reagent Co., Ltd (Guangzhou, China).

2.2. Manufacture of Oolong tea

The fresh tea leaves picked from the six locations were given a solar withering process for 2 h, followed by indoor withering for 4 h, and then five cycles of hand rolling for 20 min. The final steps consisted of 2 h of fermentation, followed by 5 min of killing, 5 min of kneading, and 5 h of roasting. The Oolong tea manufacturing process was replicated five times. For the codes for the '*Huang Zhi Xiang*' Oolong tea samples describing the different combination of altitudes and seasons used in the study were $S_{500,}$ spring tea sample from an altitude of 500 m AMSL; S_{350}, spring tea sample from an altitude of 350 m AMSL; A_{500}, autumn tea sample from an altitude of 500 m AMSL; and A_{350}, autumn tea sample from an altitude of 350 m AMSL.

2.3. Sensory quality assessment of tea samples

Three grams of tea leaves (dry matter, DM) were infused with 150 ml freshly boiled water for 5 min. The sensory quality of the tea was estimated and scored by professional tea tasters from the South China Botanical Garden of the Chinese Academy of Sciences. The grading system was based on a quality score (QS) of 100, of which 10% was awarded for the tea appearance score, 40% for the tea aroma score, 40% for the tea taste score (TS) and 10% for the infused leaf score (Table 1). This grading system is commonly used to evaluate the Oolong tea quality in China.

2.4. Determination of contents of phenolic compounds (PC)

Total phenolic content was measured by the methods of Roberts (1962) and Harbowy and Balentine (1997), with some modifications. Tea (2 g) was infused at 90 °C in 200 ml distilled water and stirred for 10 min with a magnetic bar. After filtration, the tea solution was made up to a final volume of 250 ml with distilled water. The concentration of tea solution was then measured at 540 nm using a UV-2802 spectrophotometer (Unic, Shanghai, China).

2.5. Catechin determination by high-performance liquid chromatography (HPLC)

The tea infusion was filtered through a filter cartridge (DIS MIC 13HP, Advantec Toyo, Japan) before HPLC analysis on a Pinnacle C18 column (250 × 4.6 mm, 5 μm particle size, Waters, Millford, MA, USA) and a Waters 2478 Dual λ Absorbance Detector (Waters). The mobile phase A was 0.1% phosphoric acid in water while the mobile phase B was 100% acetonitrile. The HPLC running program was as follows: 0–5 min, 10% B and 90% A; 5–25 min, 25% B and 75% A; 25–26 min, 100% B; 26–35 min, 100% B; and 35–45 min, 10% B and 90% A, at a constant flow rate of 1 ml/min. Catechins were identified by comparing their spectral characteristics with those of the standard compounds at 270 nm and then quantified using their peak areas.

2.6. Statistical analysis

All data were analyzed using SAS 8.1 (SAS Institute Inc., Cary, North Carolina, USA). A correlation analysis (CORR) and a variance analysis (ANOVA) were followed by an analysis of least significant difference ($P < 0.05$) among means by the Duncan's multiple range test.

3. Results and discussion

3.1. Variation in catechin contents in Oolong teas based on various growing altitudes

The altitude influenced markedly the catechin profile in the Oolong tea samples. Fig. 1a and b shows the higher contents of EGCG in the spring Oolong teas (S_{500} and S_{350}) grown at a high altitude than those grown at a low altitude, as indicated by the peak areas. However, Fig. 1c and d exhibits no obvious differences in the EGCG contents of autumn Oolong tea samples between the high and low altitudes. Furthermore, the EGCG content in all the Oolong teas studied was the highest among these tea catechins, which makes EGCG the predominated catechin in Oolong tea. Similar results were reported by Katalinic, Milos, Kulisic, and Jukic (2006) who showed high levels of EGCG were found in the Oolong and green teas. As shown in Table 2, for both of the spring and autumn teas, the contents of EGCG, CG and TC in the Oolong teas grown at a high altitude were significantly higher ($P < 0.05$) than those grown at a low altitude. However, for the spring Oolong tea, the contents of GC, EGC and SC from a low altitude were significantly higher ($P < 0.05$) than those from a high altitude. For the spring Oolong tea, the content of (+)-catechin at a low altitude was significantly higher ($P < 0.05$) than that at a high altitude. In contrast, the content of (+)-catechin in the autumn Oolong tea grown at a low altitude was significantly lower ($P < 0.05$) than that grown at a high altitude. The contents of EC and ECG were not significantly

Table 1
Sensory quality score of the '*Huang Zhi xiang*' oolong tea samples assessed by the tea tasters ($n = 3$, mean ± S.D.[a]).

Samples	Appearance score	Aroma score	Taste score (TS)	Infused leaf score	QS
S_{500}	8.2 ± 0.2	31.8 ± 0.8	31.2 ± 0.3	8.3 ± 0.1	79.5 ± 0.8
S_{350}	7.1 ± 0.1	27.0 ± 0.5	27.7 ± 0.8	7.1 ± 0.1	68.8 ± 1.4
A_{500}	7.9 ± 0.1	32.5 ± 0.5	32.1 ± 0.7	8.2 ± 0.1	80.7 ± 0.9
A_{350}	7.3 ± 0.2	31.3 ± 0.6	29.4 ± 1.9	7.5 ± 0.1	75.5 ± 2.7

[a] S.D., standard deviation.

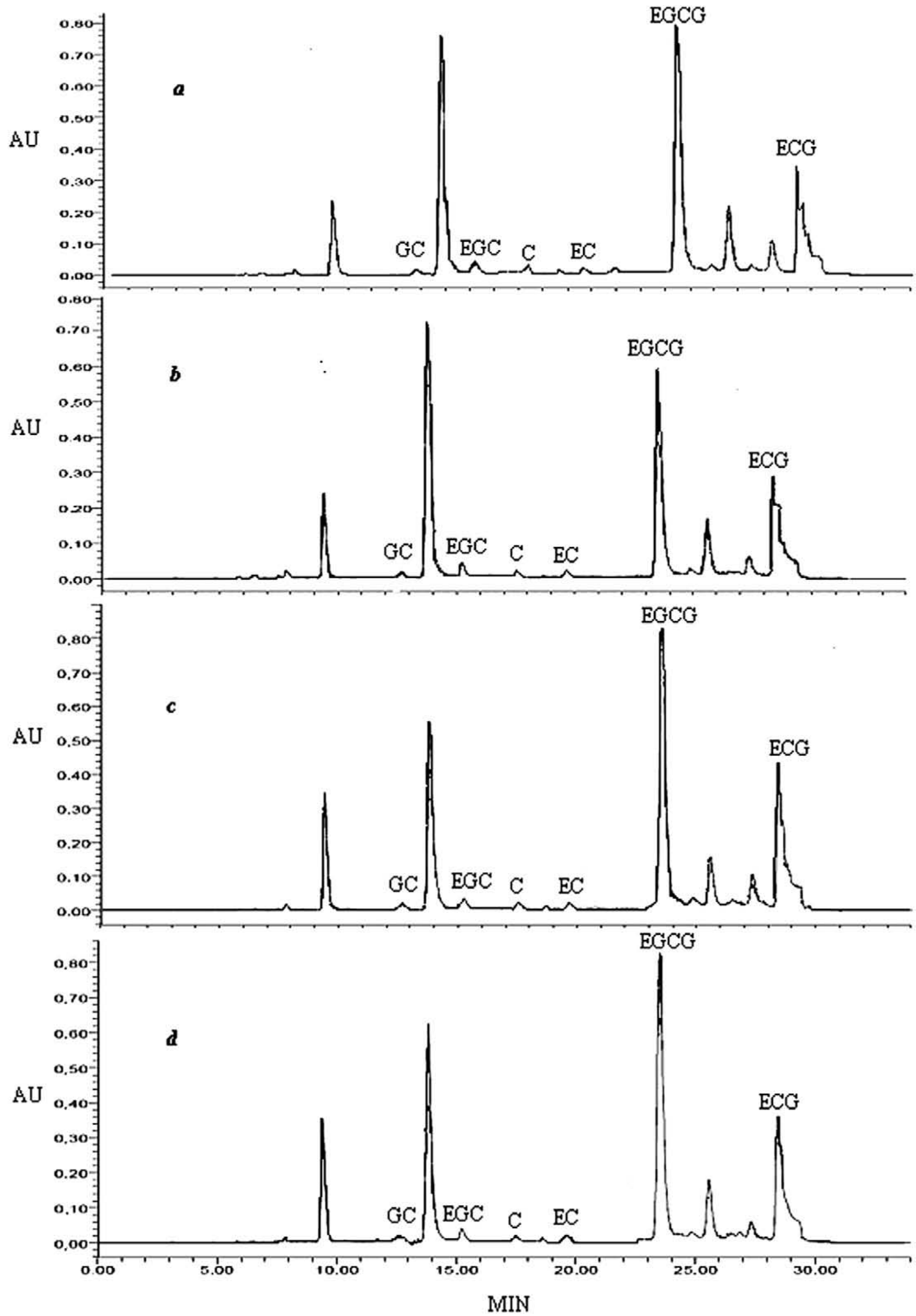

Fig. 1. HPLC profile of catechins in the infusion of the '*Huang Zhi Xiang*' Oolong tea from S_{500} (a), S_{350} (b), A_{500} (c) and A_{350} (d).

changed in the Oolong tea samples that were grown at different altitudes. Therefore, the variation in simple catechin content in Oolong tea grown at different altitudes was different from that of catechin gallates.

Higher air temperatures, less mist and more sunshine time at a low altitude would accelerate the growth of tea leaf (Hilton & Palmer-Jones, 1973). The faster growth of green tea leaf is accompanied by a lower accumulation of secondary metabolites such as TC (Gulati & Ravindranath, 1996; Yao et al., 2005). However, the green tea leaves, which grow faster at a low altitude, accumulated a higher level of EGC in the old leaf (Chen et al., 2003; Yao et al., 2005). The results suggest that the altitude affected the catechins in green tea leaves during growth, which may have influenced their concentrations in the subsequent drying steps.

Table 2
Comparative analyses of contents of catechins and polyphenols of the '*Huang Zhi xiang*' Oolong tea (mg g^{-1} DM) and QS[a].

	Spring Oolong tea		Autumn Oolong tea		LSD ($P < 0.05$)
	Altitude: 500 m	Altitude: 350 m	Altitude: 500 m	Altitude: 350 m	
QS	79.5 ± 0.8a	68.8 ± 1.4c	80.7 ± 0.9a	75.5 ± 2.7b	3.0
GC	8.2 ± 0.5b	10.1 ± 0.9a	8.0 ± 0.1b	8.0 ± 0.7b	1.2
EGC	16.8 ± 2.0b	20.3 ± 2.3a	17.6 ± 1.9ab	16.0 ± 0.1b	3.4
C	5.9 ± 0.1bc	7.9 ± 1.2a	7.0 ± 0.1ab	5.6 ± 0.9c	1.4
EC	4.2 ± 0.6a	5.4 ± 1.2a	5.5 ± 0.6a	4.7 ± 0.6a	1.5
EGCG	102.8 ± 0.7a	76.6 ± 5.6c	103.4 ± 3.1a	90.6 ± 5.5b	8.0
ECG	20.8 ± 3.1b	20.9 ± 3.1b	25.2 ± 1.3ab	25.6 ± 1.0a	4.4
TC	158.3 ± 2.1ab	142.0 ± 11.4c	167.2 ± 2.5a	151.5 ± 5.5bc	12.3
SC	36.1 ± 3.2b	44.0 ± 5.0a	38.6 ± 3.1ab	34.4 ± 1.0b	5.7
CG	124.4 ± 1.3ab	97.4 ± 5.5c	129.6 ± 0.7a	116.9 ± 6.9b	8.4
PC	176.2 ± 3.7c	132.4 ± 4.9d	200.2 ± 9.9b	217.1 ± 7.8a	13.2

[a] The experimental results were analyzed in one-way analysis of variance with Duncan's Multiple Range Test (DMRT) ranking – means ± S.D. within a row followed by the same letter are not significantly different at $P < 0.05$ according to DMRT.

The data from this study suggests that the higher air temperature, reduced mist, and greater sunshine hours at a low altitude favours the accumulation of GC, C, and SC in the green tea leaves. According to Chen et al. (2003), the level of EC in the Oolong tea at a low altitude was higher than that at a high altitude. However, the contents of EC and ECG at a low altitude were not significantly higher than those at a high altitude in this study (Table 2), which may be due to the different varieties and manufacture procedure of Oolong tea.

In regards to investigation into the relationship between tea quality and altitude, Owuor, Obanda, Nyirenda, and Mandala (2008) reported that tea shoots grown at different altitudes caused the difference in the quality of black tea observed at the Tea Research Foundation of Central Africa (TRFCA) and the Tea Research Foundation of Kenya (TRFK). The quality of the black teas grown at a high altitude was better than those grown at a low altitude (Owuor et al., 2008). Because more secondary metabolites such as TC of green tea leaves accumulated at a high altitude than those at a low altitude (Gulati & Ravindranath, 1996; Yao et al., 2005). Similar effects were observed in our research, with the QS (80.1) of the Oolong tea at a high altitude being significantly higher ($P < 0.05$) than that (72.2) at a low altitude (Table 1).

3.2. Variation in catechin contents in Oolong teas based on various growing seasons

Table 2 indicates that there were no significant differences among the contents of EGCG, CG, and TC between the spring and autumn teas grown at a high altitude. At low altitude, however, the contents of EGCG, CG and TC of the autumn tea were significantly higher ($P < 0.05$) than those of the spring tea. Furthermore, there was no significant difference among the contents of EGC, ECG, C, GC, and SC in the Oolong teas between the spring and autumn production at a high altitude, whereas, the contents of EGC, C, GC, and SC from the spring Oolong tea were significantly higher ($P < 0.05$) than those from the autumn Oolong tea at a low altitude. In addition, the contents of ECG were significantly lower ($P < 0.05$) in the spring Oolong tea than in the autumn Oolong tea, but, the content of EC was not affected by seasons or change in altitude.

At a low altitude, the higher temperature in the autumn accelerated the accumulation of EGCG and ECG in the green tea leaves more than that in the spring (Yao et al., 2005). In this study, the contents of EGCG and ECG in the autumn Oolong tea were higher than those in the spring Oolong teas at a low altitude. As CG comprises the majority of the total catechins in Oolong tea (Katalinic et al., 2006), the content of TC in the autumn Oolong tea was also higher than that in the spring Oolong tea at a low altitude. The influence of seasons on tea catechins was also reported on black tea and green tea shoots. The catechin compositions of the commercial black tea changed markedly with tea species, growing season and manufacturing process (Bhatia & Ullah, 1968; Lin, Juan, Chen, Liang, & Lin, 1996). The contents of EC, C, and ECG in the green teas picked in the summer were higher than those in the spring (Yamamoto, Juneja, Chu, & Kim, 1997).

3.3. Interaction of altitude and season on the contents of catechins

Table 3 presents the effects of the interactions of altitude and season on the catechins compositions of Oolong tea. A significant interaction effects between altitude and season on the contents of EGCG, GC, C, EGC, SC, PC, and CG were obtained, but no significant interaction effects were found with EC, ECG and TC ($P < 0.05$). The contents of EGCG and CG in green tea leaves and commercial tea are affected by many factors including altitude and season (Bhatia & Ullah, 1968). Thus, these factors need to be taken into consideration in the future investigations on the contents of catechins in Oolong tea.

3.4. Correlation between the contents of catechins, the taste score (TS) and the QS

As shown in Table 4, the contents of EGCG ($r = 0.981$, $P < 0.05$), CG ($r = 0.972$, $P < 0.05$), and TC ($r = 0.987$, $P < 0.05$) in Oolong tea are significantly correlated to their taste score (TS). As well, the contents of EGCG ($r = 0.995$, $P < 0.01$), CG ($r = 0.996$, $P < 0.01$), and

Table 3
Comparative analyses of contents of catechins and polyphenols of the '*Huang Zhi xiang*' Oolong tea (mg g^{-1} DM) and QS[a].

	Altitude		Season		LSD (P < 0.05)	A × S
	500 m	350 m	Spring	Autumn		
QS	80.1a	72.2b	74.2b	78.1a	2.1	*
GC	8.1b	9.0a	9.1a	8.0b	0.8	*
EGC	17.2a	18.2a	18.5a	16.8a	2.4	*
C	6.5a	6.7a	6.9a	6.3a	1.0	**
EC	4.8a	5.0a	4.8a	5.1a	1.1	n
EGCG	103.1a	83.6b	89.7b	97.0a	5.7	*
ECG	23.0a	23.2a	20.8b	25.4a	3.1	n
TC	162.8a	146.8b	150.2b	159.4a	8.7	n
SC	37.4a	39.2a	40.0a	36.5a	4.5	*
CG	127.0a	107.2b	111.0b	123.2a	6.0	*
PC	188.2a	174.8b	154.3b	208.7a	9.3	**

A × S: interaction of altitude and seasons; n: not significant.
[a] The experimental results were analyzed in two-way analysis of variance with Duncan's Multiple Range Test (DMRT) ranking – means within a row followed by the same letter are not significantly different at $P < 0.05$ according to DMRT.
* Significant at $P < 0.05$ level.
** Significant at $P < 0.01$ level.

Table 4
Linear correlation coefficient (*r*) between chemical constituents, taste and QS of the '*Huang Zhi Xiang*' Oolong tea.

	GC	EGC	C	EC	EGCG	ECG	TC	SC	CG	PC	TS
EGC	0.941										
C	0.804	0.957*									
EC	0.448	0.655	0.808								
EGCG	−0.861	−0.695	−0.522	−0.373							
ECG	−0.637	−0.553	−0.376	0.242	0.304						
TC	−0.793	−0.547	−0.305	−0.049	0.944	0.458					
SC	0.906	0.996**	0.979*	0.708	−0.644	−0.505	−0.473				
CG	−0.920	−0.751	−0.557	−0.299	0.982*	0.477	0.960*	−0.695			
PC	−0.921	−0.897	−0.765	−0.257	0.618	0.862	0.615	−0.865	0.740		
TS	−0.803	−0.582	−0.368	−0.187	0.981*	0.346	0.987*	−0.517	0.972*	0.58	
QS	−0.894	−0.725	−0.539	−0.332	0.995**	0.397	0.958*	−0.671	0.996**	0.68	0.982*

* Significant at $P < 0.05$ level.
** Significant at $P < 0.01$ level.

TC ($r = 0.958$, $P < 0.05$) in the Oolong teas are positively and significantly correlated to their quality score (QS). It appears that the contents of EGCG, CG, and TC could be the important quality parameters of the '*Huang Zhi Xiang*' Oolong tea. Similar results were also reported for black and green teas. Owuor et al. (2006) reported that high levels of flavan-3-ol gallate esters had a dominant role in Kenyan black tea quality while Liang, Ma, Lu, and Wu (2001) exhibited the EGCG was the most important catechin in unfermented green tea.

In this study, no significant correlations between the QS and contents of EC, EGC, ECG, C, GC, SC, and PC were obtained (Table 4). However in another study, the content of EC in the black tea infusion was significantly correlated with QS (Mahanta, Baruah, Owuor, & Murai, 1988). Meanwhile, Liang, Lu, Zhang, Wu, and Wu (2003) exhibited that the levels of GC, EGC, C, EC and ECG of black tea were significantly correlated with the QS.

4. Conclusion

The contents of EGCG, CG and TC in the Oolong tea were positively and significantly correlated to the TS and QS. Thus, the contents of EGCG, CG and TC could be considered to be the important quality parameters for the '*Huang Zhi Xiang*' Oolong tea. The contents of EGCG, CG, and TC in the '*Huang Zhi Xiang*' Oolong teas grown at a high altitude were significantly higher than those grown at a low altitude, whereas the contents of EGCG, CG, and TC in the autumn tea were significantly higher than those in the spring tea at low altitude but no significant differences in the contents of EGCG, CG and TC were observed between the spring and autumn teas grown at a high altitude. It was suggested that the production of the '*Huang Zhi Xiang*' Oolong tea grown at low altitudes should utilize the autumn tea leaves while the production of the Oolong tea grown at high altitudes can use both the spring and autumn tea leaves.

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