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ORIGINAL PAPER

Seasonal climate effects on flavanols and purine alkaloids of tea (*Camellia sinensis* L.)

L. Y. Wang · K. Wei · Y. W. Jiang · H. Cheng · J. Zhou · W. He · C. C. Zhang

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Abstract The seasonal climate changes and their effects on flavanol and purine alkaloid contents of two leaves and a bud of tea were evaluated among 3 cultivars grown in Hangzhou, China. The results showed (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) increased, but (+)-catechin (C) decreased with increasing daily average temperature (DAT). Long period of precipitation (PRE) led to the declines of EGC, EC, ECG, EGCG and their total content (TC) but increased C content. Furthermore, relative humidity (RH) significantly affected EGC, TC and caffeine. Therefore, DAT, PRE and RH should not be ignored in tea production. The order of climate dependence according to the regression models was C > EGCG > TC > EGC > EC > caffeine > ECG > theobromine, indicating seasonal climate had greater effects on flavanols than purine alkaloids.

Keywords Seasonal climate effect · Flavanol · Purine alkaloid · *Camellia sinensis*

Abbreviations

C	(+)-catechin
DAT	Daily average temperature
EC	(−)-Epicatechin
ECG	(−)-Epicatechin gallate
EGC	(−)-Epigallocatechin
EGCG	(−)-Epigallocatechin gallate
PRE	Precipitation
RH	Relative humidity
SH	Sunshine hours
TC	Total content of flavanol

L. Y. Wang and K. Wei contributed equally to this work.

L. Y. Wang · K. Wei · Y. W. Jiang (✉) · H. Cheng (✉) · J. Zhou · W. He · C. C. Zhang
National Center for Tea Improvement, Tea Research Institute Chinese Academy of Agricultural Sciences (TRICAAS), 9 Meiling South Road, Hangzhou 310008, Zhejiang, People's Republic of China
e-mail: jiangyw@mail.tricaas.com

H. Cheng
e-mail: chenghao@mail.tricaas.com

Introduction

Tea (*Camellia sinensis*) is the most popular beverage in the world in terms of consumption. Consumer preference for tea is related not only to its rich flavor and taste but also to its many pharmacologic effects, like suppressing tumor cell growth, reducing cardiovascular disease and anti-obesity [1, 2]. Flavanols and purine alkaloids, which take account for 15–25% dry tea leaves [3], are the main constituents corresponding to these effects [4]. There are five major flavanols in tea, including (−)-epigallocatechin (EGC), (−)-epicatechin (EC), (+)-catechin (C), (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG) [5]. Among them, the gallate ester type catechins (EGCG and ECG) are more astringent in taste [6] and effective in inhibiting tumor cell growth [7]. Nevertheless, they considerably reduce saliva lubricity as compared with free catechins (EGC, EC and C) [8]. Therefore, the composition of flavanols would largely affect both sensory quality and health benefits of tea. Meanwhile, the contents of purine alkaloids, mainly caffeine and theobromine, are also important quality parameters [9]. Caffeine gives a bitter taste to humans and is a central nervous system stimulant [10]. Both caffeine and theobromine are associated with diuretic responses and speculated to increase the solubility of the flavoring substances [11–13].

On the other hand, the quality and value of tea are strongly affected by considerable climatic changes throughout the year. Early flush tea, generally valued for high flavor and liquor characteristics, undergoes quality decline with progress in season [14]. It was also reported that tea plucked during slow growth conditions contained a lower proportion of gallate ester–type catechins to free catechins [15]. The seasonal effect on tea quality has long been known [14, 15]. However, to what extent each climate factors affects tea quality still remains elusive. An understanding of the climate effect on flavanols and purine alkaloids is important for both quality management and consumer-oriented production.

In this study, we evaluated the dynamic changes in flavanol and purine alkaloid contents in three tea cultivars during the growth period in 2009. Their relationships with climate factors were also studied. The aim of this study was to determine the seasonal climate effects on flavanols and purine alkaloids in tea.

Materials and methods

Materials

Fresh material (two leaves and a bud) of three tea cultivars (ZC108, ZC302 and LJ 43) grown in experimental farm of Tea Research Institute of Chinese Academy of Agricultural Sciences was collected for 11 batches (7 April, 14 April, 23 April, 6 July, 27 July, 4 August, 12 August, 19 August, 1 September, 11 September and 24 September 2009) with three replications. The two leaves and a bud stage for tea plants generally lasts 6–10 days, which is easily affected by weather condition. We chose these plunking dates in accordance with local tea growth and to make sure samples from each batch were newly grown but not the materials from last batch. As the spring tea plucking was stop and tea plants were pruned in the end of April, we did not collect samples from April 24 to July 5, 2009. All the samples were fixed by microwave oven (power 1.0 kW, 2 min) immediately after being plucked and then dried with hot air at 60 °C. Samples were stored in airtight containers in the dark before they were analyzed.

Chemicals and standards

The reference substances C, EC, EGC, ECG, EGCG, caffeine and theobromine were purchased from Sigma Chemical Company (St. Louis, MO, USA). The other substances were obtained from Huadong Medicine Co., Ltd (Hangzhou, China).

Flavanol and purine alkaloid analysis on HPLC

Tea sample (0.2 g) was extracted with 5 mL 70% methanol at 70 °C for 10 min with intermittent shaking (10 s on vortex mixer). The sample was then centrifuged at 1,400*g* for 10 min under room temperature. The supernatant was taken into a 10-mL volumetric flask, and the extraction steps were repeated to reach the final volume of 10 mL. The extracts were filtered through a 0.5-mm Millipore filter before the injection was made. The extract is stable for 24 h when stored at 4 °C. Tea infusions were analyzed for catechins and purine alkaloids according to the method modified from BS ISO 14502-2:2005 [16]. An Agilent 1100 series HPLC (Agilent Technology, San Diego, CA, USA) using a reversed-phase (RP) C18 column (5.0 μm, 250 mm × 4.6 mm) fitted with a C18 guard column was utilized for analysis. The column was eluted at 35 °C with a binary gradient of 100% solution A [9% acetonitrile, 2% acetic acid, containing EDTA (20 mg mL^{-1})] for 10 min, 68% solution A and 32% of solution B [80% acetonitrile, 2% acetic acid, containing EDTA (20 mg mL^{-1})] for 10 min at a flow rate of 1.0 mL min^{-1}. The eluent was monitored at 278 nm. The sample injection volume was 20 μL. The signals were verified by using UV spectra (diode array detector) and comparisons of the retention time with reference compounds. Figure 1 indicates the chromatogram of standards and a tea sample. Quantification was carried out using the relative response factor concept of BS ISO 14502-2:2005 [16]. Three replications were taken for each measurement.

Climate factor measurements

The data of climate factors, including the daily average temperature (DAT), relative humidity (RH), precipitation (PRE) and sunshine hours (SH), were downloaded from China meteorological data sharing service system (http://www.cdc.cma.gov.cn) dated from March 15, 2009, to September 30, 2009. The average values of each index over 2 weeks before tea sample collection were calculated (Table 1) and utilized for correlation and linear regression analysis.

Statistical analyses

The results were analyzed by analysis of variance and calculation of correlations and regression with SPSS 16.0 statistical software. Differences between means were evaluated using the Duncan's multiple range test. Correlation analysis and multiple linear regression models were used to test the relationship between climate factors, and flavanol and purine alkaloid contents in tea.

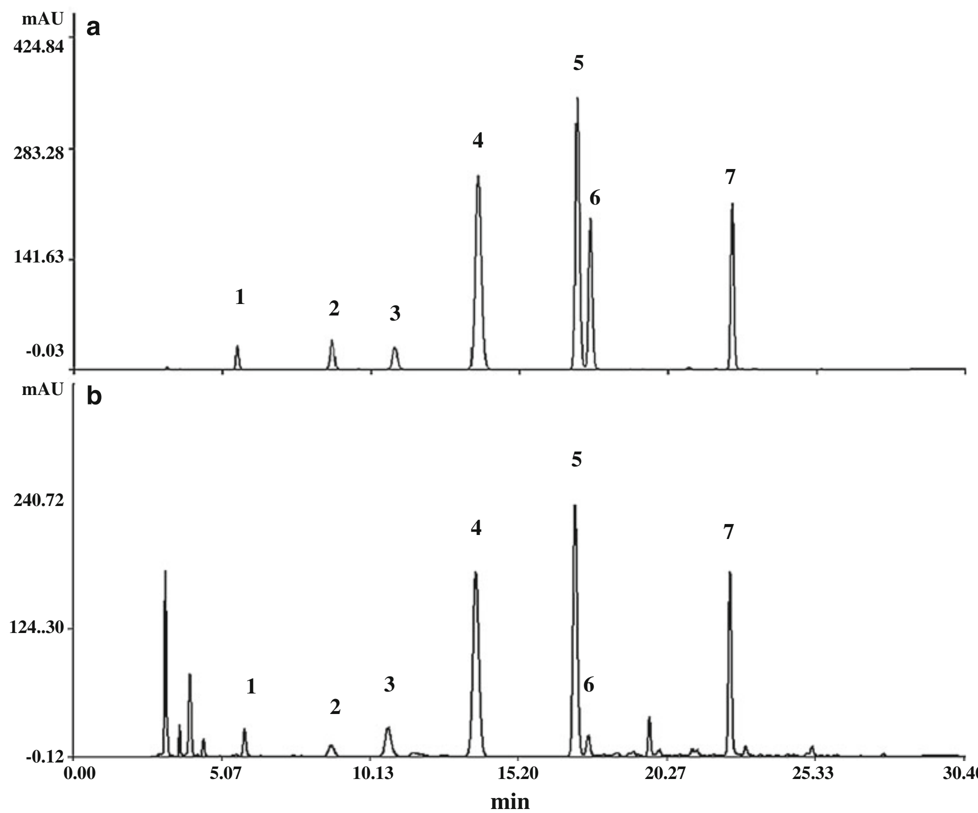

Fig. 1 Chromatogram of standards and a typical tea sample. **a** Chromatogram of standards; **b** Chromatogram of a typical tea sample. *1* theobromine; *2* (−)-epigallocatechin (EGC); *3* (+)-catechin (C); *4* caffeine; *5* (−)-epigallocatechin gallate (EGCG); *6* (−)-epicatechin (EC); *7* (−)-epicatechin gallate (ECG)

Table 1 Average value of climate factors over 2 weeks before tea sample collection

Date of sample collection	DAT (°C)	PRE (mm)	SH (h)	RH (%)
7 April	12.7	46.6	4.33	66.9
14 April	15.4	30.9	5.58	66.3
23 April	19.4	36.2	6.01	71.4
6 July	27.8	65.9	4.96	71.8
27 July	30.9	91.1	7.14	63.9
4 August	26.8	140.3	2.04	83.6
12 August	27.6	124.7	2.31	84.0
19 August	28.4	102.8	4.11	80.8
1 September	28.6	16.9	4.09	74.0
11 September	26.7	1.4	5.18	71.9
24 September	24.8	46.4	3.30	78.2

Results

The climate factor changes during the growing season of tea

The climate factor changes, including DAT, RH, SH and PRE, during the growing seasons of tea in 2009 are presented in Fig. 2. The tendency curves for DAT, RH and SH were derived from calculating the average data of 7 days (Fig. 2a–c). As shown in Fig. 2a, the general trend of DAT is increasing from March to mid-July, undergoes a sharp decline during late July and then decreases gradually with slight fluctuation. In terms of SH and RH, sharp fluctuations were observed during the growth period of tea (Fig. 2b, c). Figure 2d depicts the distribution of PRE through the experimental period. A long period of rain occurred in late July and early August, which was accompanied with the rapid decrease in DAT and SH and upward of RH. The correlation analysis revealed highly significant correlations between PRE and other three climate factors (data not shown), which suggests that heavy PRE would seriously affect the whole climate condition.

The dynamic changes in flavanols and purine alkaloids in tea

The dynamic changes in five major flavanols and their total content (TC) during the experimental period are given in Fig. 3. In general, the EGC, EC and EGCG contents ascended from spring to early summer (samples harvested on 7 April, 14 April, 23 April and 6 July, respectively), dropped rapidly from late July to early August and then recovered to relatively high level until the end of the experiment, irrespective of cultivars. Rapid and significant decreases in early August were also observed in ECG and TC. By contrast, C showed an opposite tendency as

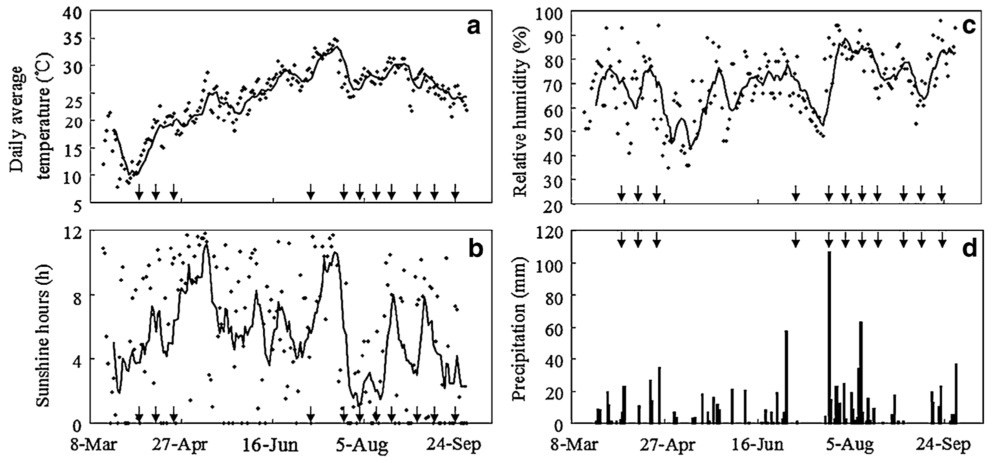

Fig. 2 Climate changes during the experimental period. **a** Daily average temperature; **b** Sunshine hours; **c** Relative humidity; **d** Precipitation. *Arrows* represent the pluck dates

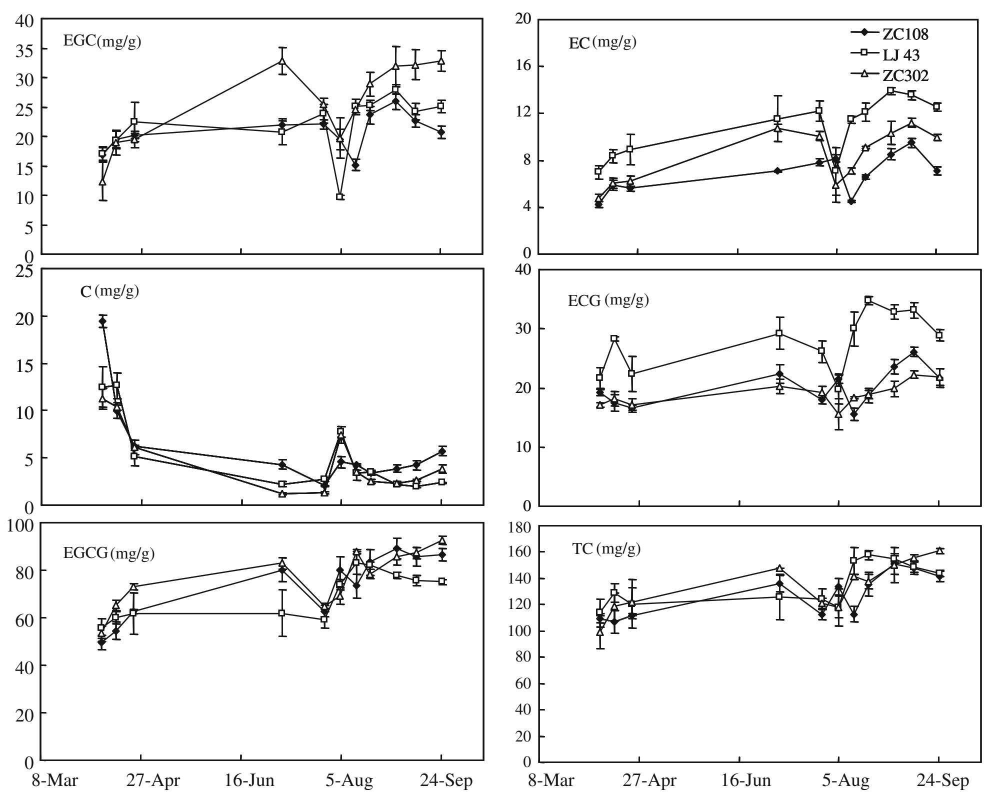

Fig. 3 Catechin components and total catechin changes during the experimental period

compared with other flavanols, with relatively high contents in early April and early August. The dramatic increase in C content and decline of other flavanol contents from late July to early August might be correlated with the long period of rain during that time (Figs. 2, 3).

In addition, Fig. 4 exhibits the seasonal changes in purine alkaloids. The caffeine content, which accounted for more than 80% of total purine alkaloid contents, varied from 25.4 to 40.5 mg g^{-1} with slight fluctuation through time. Meanwhile, three cultivars displayed slight differences in seasonal variation in caffeine contents. By contrast, significantly higher theobromine contents in 'ZC302' were identified than those in other two cultivars, except for samples collected on 7 April and 12 August (Fig. 4). The theobromine contents of 'LJ43' and 'ZC108' varied from 0.7–3.4 mg g^{-1} to 0.4–2.1 mg g^{-1}, respectively, which was quite low in comparison with caffeine and flavanol contents.

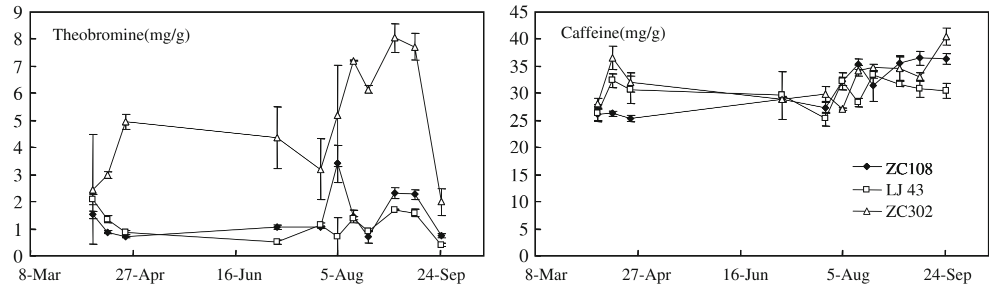

Fig. 4 Purine alkaloid changes during the experimental period

Table 2 Correlation between climate parameters and catechin contents

	EGC	EC	C	EGCG	ECG	TC
DAT	0.508**	0.540**	−0.875**	0.636**	0.246	0.558**
PRE	−0.257	−0.199	−0.134	0.054	−0.228	−0.177
SH	0.118	0.122	−0.027	−0.457**	0.012	−0.264
RH	−0.002	−0.041	−0.204	0.575**	0.008	0.347*

* Significant at $P < 0.05$

** Significant at $P < 0.01$

The relationship between flavanol, purine alkaloid contents and preharvest climate factors

Correlation and linear regression analysis were utilized to study the relationship between flavanol, purine alkaloid contents and preharvest climate factors, which are essential for leaf development (Tables 2, 3, 4, 5). DAT was significantly correlated with all flavanols, except ECG (Table 2). Moreover, the highest correlation coefficient in this study was identified between DAT and C (−0.875), indicating DAT is the most important climate factor influencing both the total and composition of flavanols. Furthermore, RH was significantly and positively correlated with EGCG and TC, while SH was negatively correlated with EGCG, which suggests conditions with high humidity and that short sunlight exposure time might favor the formation of EGCG (Table 2).

No significant correlation was identified between PRE and flavanols (Table 2). However, the effect of PRE on flavanols could be seen in the regression models (Table 3). The effect of PRE on flavanols is presumably to be indirect and mainly via the complicated changes in climate. Wide variations exist in the seasonal dependence of flavanols, which ranged from a slight climate effect (ECG $r^2 = 0.198$), over a moderate climate influence (EGC $r^2 = 0.490$, EC $r^2 = 0.477$), up to a strongly distinctive climate effect (C $r^2 = 0.809$, EGCG $r^2 = 0.782$, TC $r^2 = 0.701$) (Table 3). Besides, DAT and PRE are the major climate factors present in all the regression models of flavanols, suggesting their influences should not be ignored in tea production.

The coefficients of correlation between purine alkaloid contents and climate factors are given in Table 4. There was no significant correlation between theobromine and climate factors, which was consistent with the result of regression analysis (Table 5). This led to the assumption that the synthesis of theobromine might be none or less affected by climate factors. RH was the only climate factor significantly correlated with caffeine (Table 4). Simultaneously, the regression analysis showed the RH and PRE

Table 3 Catechin components and total catechin contents of tea as a function of climate parameters ($n = 33$)

Dependent variable	Regression equation[a]	Multiple coefficient of determination
EGC (mg g^{-1})	$y = 10.95 + 0.65X_1 - 0.65X_2$	0.490**
EC (mg g^{-1})	$y = 2.60 + 0.32X_1 - 0.29X_2$	0.477**
C (mg g^{-1})	$y = 20.16 - 0.66X_1 + 0.21X_2$	0.809**
EGCG (mg g^{-1})	$y = -4.79 - 1.27X_1 + 1.36X_4 - 1.68X_2$	0.782**
ECG (mg g^{-1})	$y = 18.72 + 0.35X_1 - 0.55X_2 - 0.52X_3 + 0.01X_4$	0.198
TC (mg g^{-1})	$y = -11.08 + 1.90X_1 - 3.03X_2 + 1.57X_4$	0.701**

** Significant at $P < 0.01$

[a] X_1 = daily average temperature; X_2 = precipitation; X_3 = sunshine hours; X_4 = relative humidity

Table 4 Correlation between purine alkaloid contents and climate factors

	Theobromine	Caffeine
DAT	0.155	0.244
PRE	−0.007	−0.130
SH	−0.130	−0.330
RH	0.144	0.359*

* Significant at $P < 0.05$

Table 5 Purine alkaloid contents of tea as a function of climate parameters ($n = 33$)

Dependent variable	Regression equation[a]	Multiple coefficient of determination
Theobromine (mg g^{-1})	$y = 0.14 + 0.07X_1 - 0.09X_2 - 0.17X_3 + 0.03X_4$	0.056
Caffeine (mg g^{-1})	$y = 5.10 + 0.39X_4 - 0.46X_2$	0.309**

** Significant at $P < 0.01$

[a] X_1 = daily average temperature; X_2 = precipitation; X_3 = sunshine hours; X_4 = relative humidity

dependence of caffeine was moderate, with regression coefficient of 0.309 (Table 5), indicating RH should also be taken into account in the production process.

Discussion

Tea and fruits are generally considered as the most important sources of flavanols for human beings [17]. Accordingly, the variations of flavanols have been widely studied in the past few years [15, 18–20]. It was found EC and EGC are the precursors of ECG and EGCG, and the variations of flavanols could be affected by species [21], climates [22], altitude [23] and agronomic factors, such as fertilizer application [24] and pruning [25]. Among them, the seasonal climate effect might be the most important one [18–20, 22]. Yao et al. [19] studied the seasonal variations of flavanols in Australia-grown tea and found higher levels of EGCG and ECG, but lower levels of EGC and TC in warm months [19]. Whereas Chen et al. [23] demonstrated that the contents of EGCG and TC in the autumn tea were significantly higher, the contents of ECG and C were significantly lower than those from the spring tea [23]. In this study, DAT was the key climate factor significantly correlated with all flavanols, except ECG (Table 2). Moreover, the highest correlation coefficient in this study was identified between DAT and C, which implies temperature is essential for both the total and individual flavanol biosynthesis.

The effect of precipitation on flavanols is generally ignored, as it is quite difficult to determine [19]. In this experiment, a long period of rain occurred in summer, which offered a good opportunity to study its effect on the biosynthesis of flavanols (Fig. 2d). Significant rise in C content and declines of EGC, EC, ECG, EGCG and TC contents were observed during the heavy rain period (Fig. 3), suggesting the effect of PRE should also be taken into consideration in tea production. The effect of PRE on flavanols might be via a complicated change in climate, especially sunlight strength and length. It is considered that flavanols are mainly synthesized in plants for the protection of UV rays in sunlight [26]. Zheng et al. [27] found UV-B irradiation stimulated the accumulation of flavanols in tea plant [27]. The long period of PRE would significantly reduce the strength and length of sunlight, including UV rays. Therefore, we hypothesized the effect of PRE on the biosynthesis of flavanols is mainly due to its effect on sunlight strength and length.

In terms of flavanol components, there were different seasonal dependences, which varied from a low climate effect (ECG), over a moderate climate influence (EGC, EC), up to a high climate effect (C, EGCG, TC) (Table 3). As EGCG and TC are the most important quality parameters of tea [20], DAT, RH and PRE, which explained 78.2 and 70.1% of the variability of EGCG and TC (Table 3), should be taken into account in tea production. Furthermore, the dynamic change in C content was opposite with other flavanols (Fig. 3). Synthesis of C was reported to be catalyzed from leucocyanidin by leucocyanidin reductase [28]. Meanwhile, Wellmann et al. [29] found that anthocyanidin synthase could convert C to other products [29], indicating C might be involved in several biosynthetic pathways as an intermediate. Nevertheless, the exact relationship among flavanols is still largely unknown. The results of our study suggest C might take part in the conversion to other flavanols.

On the other hand, the seasonal climate effect on purine alkaloids was relatively slight as compared with those on flavanols (Tables 4, 5). Caffeine is the major component of purine alkaloids. Recently, the major biosynthetic route of caffeine is considered to involve four steps: xanthosine-7-methyxanthosine-7-methylxanthine-theobromine-caffeine [30]. Both theobromine and caffeine were found only in young tea leaves [31]. Suzuki et al. [32] reported the production and accumulation of purine alkaloids were associated with seasonal changes [32]. While Koshiishi et al. [33] revealed sunlight did not significantly influence caffeine biosynthesis [33]. Unfortunately, to date, the exact climate effects on purine alkaloid contents in tea have not been reported. From the present results, it could be found that high RH stimulated the synthesis of caffeine (Table 4), although the correlation coefficient is not high (0.357). Besides, no significant correlation was identified between other climate factors and purine alkaloids. Lee et al. [34] found caffeine content was higher in tea under high temperature, long-time sun exposure and high rainfall conditions, which might also be associated with RH changes [34]. The exact effect of RH on caffeine biosynthesis pathway needs further study to clarify.

In conclusion, this study demonstrated that there were significantly seasonal climate effects on both the total and composition of flavanols. High DAT and low PRE favored the synthesis of EGC, EC, ECG and EGCG but decreased the content of C (Table 3), whereas relatively low climate influence on purine alkaloids was found and RH was the only climate factor significantly correlated with caffeine. Therefore, the preharvest DAT, PRE and RH should be taken into account in tea production.

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