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Seasonal Variations of Phenolic Compounds in Australia-Grown Tea (*Camellia sinensis*)

LIHU YAO,[†,‡] NOLA CAFFIN,[‡] BRUCE D'ARCY,[‡] YUEMING JIANG,*[,†] JOHN SHI,[§] RIANTONG SINGANUSONG,[#] XU LIU,[||] NIVEDITA DATTA,[‡] YUKIO KAKUDA,[⊥] AND YING XU[⊗]

South China Botanic Garden, The Chinese Academy of Sciences, Guangzhou ReYiJu 510650, People's Republic of China; School of Land and Food Sciences, The University of Queensland, Brisbane, Queensland 4072, Australia; Food Research Center, Agriculture and Agri-Food Canada, 93 Stone Road West, Guelph, Ontario N1G 5C9, Canada; Department of Agro-Industry, Faculty of Agriculture, Natural Resources and Environment, Naresuan University, Muang, Phitsanulok 65000, Thailand; Tea Research Institute, Agricultural Academy of China, Hangzhou 310008, People's Republic of China; Department of Food Science, University of Guelph, Guelph, Ontario N1G 2W1, Canada; and College of Food Science, Southwest Agricultural University, Chongqing 400716, People's Republic of China

Seasonal variations of phenolic compounds in fresh tea shoots grown in Australia were studied using an HPLC method. Three principal tea flavanols [epigallocatechin gallate (EGCG), epicatechin gallate (ECG), and epigallocatechin (EGC)] and four grouped phenolics [total catechins (Cs), total catechin gallates (CGs), total flavanols (Fla), and total polyphenols (PPs)] in fresh tea shoots were analyzed and compared during the commercial harvest seasons from April 2000 to May 2001. The levels of EGCG, ECG, and CGs in the fresh tea shoots were higher in the warm months of April 2000 (120.52, 34.50, and 163.75 mg/g, respectively) and May 2000 (128.63, 44.26, and 183.83 mg/g, respectively) and lower during the cool months of July 2000 (91.39, 35.16, and 132.30 mg/g, respectively), August 2000 (91.31, 31.56, and 128.64 mg/g, respectively), and September 2000 (96.12, 33.51, and 136.90 mg/g, respectively). Thereafter, the levels increased throughout the warmer months from October to December 2000 and remained high until May 2001. In the warmer months, the levels of EGCG, ECG, and CGs were in most cases significantly higher ($P < 0.05$) than those in the samples harvested in the cooler months. In contrast, the levels of EGC and Cs were high and consistent in the cooler months and low in the warmer months. The seasonal variations of the individual and grouped catechins were significant ($P < 0.05$) between the cooler and warmer months. This study revealed that EGCG and ECG could be used as quality descriptors for monitoring the seasonal variations of phenolics in Australia-grown tea leaves, and the ratio (EGCG + ECG)/EGC has been suggested as a quality index for measuring the differences in flavanol levels in fresh tea shoots across the growing seasons. Mechanisms that induce seasonal variations in tea shoots may include one or all three of the following environmental conditions: day length, sunlight, and/or temperature, which vary markedly across seasons. Therefore, further studies under controlled conditions such as in a greenhouse may be required to direct correlate flavonoid profiles of green tea leaves with their yields and also to with conditions such as rainfall and humidity.

KEYWORDS: Tea; fresh shoots; seasonal variation; phenolic compounds; HPLC; quality

INTRODUCTION

The phenolic compounds that are present in young tea shoots (also referred to as fresh green leaves, fresh tea shoots, or flushes) are known to be one of the main factors in determining the quality of the resulting tea drink (*1*−*3*). The content of total polyphenols in tea shoots grown in Kenya has been found to correlate significantly with Kenyan plain tea quality parameters (*4*). Clones with low total polyphenol content produce low-quality black teas and vice versa (*5*, *6*). Thus, the total polyphenol levels are important to the quality of black tea, and those levels are affected by their levels occurring in the fresh tea shoots (*3*, *7*).

An early study in central Africa showed that the level of flavanols (catechins and catechin gallates) in the fresh apical

* Author to whom correspondence should be addressed (telephone + 86 20 37252525; fax + 86 20 37252831; e-mail ymjiang@scib.ac.cn).
[†] The Chinese Academy of Sciences.
[‡] The University of Queensland.
[§] Agriculture and Agri-Food Canada.
[#] Naresuan University.
[||] Agricultural Academy of China.
[⊥] University of Guelph.
[⊗] Southwest Agricultural University.

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shoots was highest during the cold season (*8*). Tea shoots plucked during slow growth conditions such as in the winter contained a higher proportion of simple catechins relative to catechin gallates, with epigallocatechin (EGC) being the most significantly affected (*8*). In contrast, in the northern hemisphere, total flavanol content is greatest during the height of the growing season (i.e., summer) and the least at the end of the season (late autumn) (*8*). A direct relationship between the level of EGC in tea shoots and the level of total theaflavins, which are known to be quality indicators, in the resultant black tea has been found (*9*). Thus, the seasonal variations of EGC in green leaves could be a chemical indicator of the seasonal variations in tea quality in central Africa.

Nakagawa and Torri (*1*) reported that EGC, epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) were the main flavanols in tea shoots for both *Camellia sinensis* var. *sinensis* and *C. sinensis* var. *assamica* grown in Japan, with EGCG predominating. EGC showed a higher level in spring than in summer, whereas ECG and EGCG showed higher levels in summer than in spring (*2*). Furthermore, it was reported that the levels of ECG and EGCG were higher in young and tender shoots, whereas EGC was higher in the fully developed shoots (*2*). This variation of leaf flavanol constituents is thought to be the main factor affecting the quality of the resulting tea (*1*, *2*, *5*, *6*, *10*−*12*). Thus, data on EGCG, ECG, and EGC levels in the green shoots of fresh tea grown in Australia could be used as an indicator of the seasonal variations in the quality of the resultant black tea. However, no such data are currently available.

The main black tea polyphenols, theaflavins, impart to black tea the distinct sensory characteristics such as color and taste. The level of total theaflavins of the resultant black tea is highly correlated with the content of EGC in the fresh shoots (*9*). However, this result does not represent the contribution of the other flavanols to the formation of theaflavins, because only 2 of 10 individual theaflavins are formed from EGC (*9*, *13*, *14*). Under field conditions, the phenolic composition of tea shoots varies considerably with seasonal, genetic, and agronomic factors (*14*). In his early work, Roberts (*15*, *16*) found that EGC and EGCG were responsible for the formation of theaflavins. However, Bryce et al. (*17*), Coxon et al. (*18*−*20*), and Sanderson (*21*) found that more than seven flavanols [catechin (C), epicatechin (EC), catechine gallate (CG), ECG, gallocatechin (GC), EGC, and EGCG) and one phenolic acid (gallic acid) in the fresh tea shoots were responsible for the formation of various theaflavins. Therefore, the quality of black tea is dependent in the first instance on the chemical composition, in particular, the flavanols of the harvested shoots, and in the second instance on the way in which they are handled, processed, and stored (*22*).

Australia is one of a few countries that harvests tea year round, including winter (*23*). The aim of this study was to determine the variations of individual and grouped flavonoids, such as EGCG, ECG, EGC, total catechin gallates (CGs), and total polyphenols (PPs), in Australia-grown fresh tea shoots over different production seasons. The results of this study will provide useful information for the tea industry in Australia and other countries in selecting and processing good-quality black tea.

MATERIALS AND METHODS

Tea Samples. Fresh tea shoots (*C. sinensis*), consisting of one apical bud and two adjoining leaves, were hand-plucked from the fields of the tea farm of Glen Allyn Tea Estates, Malanda, North Queensland, Australia. The samples were plucked just before each commercial harvest at a 3-week interval, except during the winter seasons when the harvests were 4−5 weeks apart. These samples were collected from April 2000 to May 2001 for a total of 15 harvests. The weather during the sampling period was typical of the tropical weather for normal commercial black tea production: the cooler months were dry and the warmer months were wet. Data on the average rainfall and air temperatures around the farm from the Bureau of Meteorology are available (*51*). At harvest, one sample of ~200 g of fresh tea shoots was randomly hand-plucked from designated bushes at each sampling location within each selected paddock. After collection, the samples were wrapped in washed calico, packed, and delivered with dry ice by overnight cargo flight to Brisbane and then road transport to the laboratory. All samples were stored in a freezer at −80 °C prior to analysis.

Chemical Solvents. The solvent used for the extraction of tea samples was analytical grade methanol. The solvents used for the HPLC analysis were HPLC grade acetonitrile and acetic acid and Milli-Q (Millipore Australia Pty. Ltd., North Ryde, New South Wales, Australia) deionized water.

Chemical Standards. Caffeic acid, coumarin, *p*-coumaric acid, ECG, EGCG, gallic acid (GA), 3-(*p*-hydroxyphenyl)propionic acid (3PA), kaempferol, and theobromine were obtained from the Chinese Tea Research Institute, Zhejiang, China, and the College of Food Science, Southwest Agricultural University, Chongqing, China. Chlorogenic acid (CA) and EC were purchased from Sigma Chemical Co., St. Louis, MO. C, CG, ECG, EGC, EGCG, gallocatechin (GC), and gallocatechin gallate (GCG) were kindly provided by Food Research Laboratories, Tokyo Food Technology Co. Ltd. (Mitsui Norin Co. Ltd.), Miyabara, Fujieda, Shizuoka, Japan.

Solvent Extraction. The extraction was based on an international standard procedure (*24*) and previous work (*25*, *26*−*32*). The development and optimization of methanol extraction of phenolic compounds from fresh tea shoots was described by Yao et al. (*33*). Briefly, 15−20 g of fresh tea shoots was blended with methanol (180 mL) for 5 min in a blender (MX-T30GP Panasonic Super Blender, Matsushita Electric, Taiwan; 2 L). The resultant mixture was filtered through cotton wool, and the residue was washed thoroughly with methanol (3 × 10 mL). The combined extract was then diluted to 200 mL with methanol. The whole experiment was replicated twice. Each solution was filtered through a 0.45 μm membrane filter prior to HPLC analysis.

Moisture Analysis. The moisture content for tea samples from the field was determined using international standard procedures (*24*, *34*) and AOAC official methods 925.19 and 934.01 (*35*), with the following changes to the vacuum oven conditions: temperature, 75 °C, and pressure, 65 kPa. All of the results reported in this study are on a dry weight basis.

Equipment. A Shimadzu Class-VP HPLC was computer-controlled with upgraded Class-VP 5.03 software and an SCL-10A VP System controller. The accessories of the HPLC system consisted of a Shimadzu GT-104 degasser, an FCV-10AL mixer, two LC-10AD Shimadzu liquid chromatography pumps, an SIL-10a XL autoinjector, a CTO-10A column oven, and an SPD-M10A VP diode array detector.

HPLC Analysis. Tea solutions were analyzed by HPLC using a 5 μm Hypersil ODS S5 250 × 4.6 mm reversed phase column (TheroQuest Hypersil, Runcorn, Cheshire, U.K.). A 10 × 4 mm guard column packed with Exsil ODS 5 μm packing material, contained in a 10GCH-guard cartridge holder (SGE Exsil, Ringwood, Victoria, Australia), was used. The absorbance data were collected from 220 to 600 nm, and the chromatograms were monitored at 280, 310, 340, 380, 450, and 510 nm. The temperature of the column oven was 35 °C. Mobile phase A was 2% aqueous acetic acid, and mobile phase B was 100% acetonitrile. A linear gradient was programmed from 8 to 31% B over 50 min, then to 100% B over 2 min, maintained at 100% B for 3 min, and then returned to 8% B for 10 min. The flow rate was 1.2 mL/min. An autoinjector was used to inject 20 μL of the standard or the test solution into the HPLC system.

Identification and Quantification of Tea Phenolic Compounds. The linear photodiode array (PDA) technology can simultaneously monitor the ultraviolet (UV) and visible (VIS) regions of the test solutions. Identification of tea polyphenols was done using multiwave-

Table 1. Mean Content of Phenolic Compounds in Hand-Plucked Fresh Tea Shoots

	individual and grouped polyphenols[a,b] (mg/g, dry basis)						
harvest date	EGCG	ECG	EGC	Cs	CGs	Fla	PPs
April 12, 2000	120.52gh	34.50abcd	47.65defg	75.25bcd	163.75efg	239.00bc	275.29ab
May9, 2000	128.63h	44.26f	51.79g	80.28def	183.83h	264.11e	304.73c
July 19, 2000	91.39a	35.16abcd	50.65fg	91.96h	132.30ab	224.25ab	264.22a
Aug 23, 2000	91.31a	31.56a	50.58efg	89.86gh	128.64a	218.49a	258.23a
Sept 23, 2000	96.12ab	33.51ab	50.28efg	87.20fgh	136.90ab	224.10ab	260.75a
Oct 23, 2000	100.82bc	34.29abc	52.33g	91.42h	143.17bc	234.60abc	274.64ab
Nov 13, 2000	103.09bcd	33.37ab	47.47defg	82.67efg	144.65bcd	227.32abc	264.61a
Dec 6, 2000	110.97def	36.10bcd	45.83cdef	78.98cde	156.63cdef	235.61abc	274.95ab
Dec 27, 2000	112.93efg	37.89cde	41.99bc	75.83bcde	159.91efg	235.74bc	275.47ab
Jan 15, 2001	113.65efg	37.48cde	45.63cde	82.69efg	161.16efg	243.85cd	286.22bc
Feb 1, 2001	121.41gh	40.62ef	46.45cdef	83.05efg	173.51gh	256.56de	300.14c
Feb 22, 2001	112.14defg	37.33cde	41.79bc	72.34abc	160.19efg	232.53abc	268.69ab
March 19, 2001	108.63cde	37.55cde	35.49a	66.21a	157.03def	223.24ab	262.88a
April 9, 2001	118.38fg	38.04de	39.82ab	71.29ab	167.36fg	238.65bc	277.53ab
May 2, 2001	104.72bcde	37.32cde	43.94bcd	76.61bcde	152.39cde	228.99abc	268.28ab
LSD	9.39	3.71	5.00	7.39	13.68	17.21	19.88

[a] Means in columns followed by a common letter are not significantly different ($P > 0.05$) [e.g., 118.38fg EGCG of April 9, 2001, has no letter in common with 104.72bcde EGCG of May 2, 2001 (or fg vs bcde), so the EGCG levels are significantly different]. [b] For abbreviations refer to the Abbreviations Used paragraph.

length detection and the UV−vis spectra reported in the literature (*28*−*31*, *36*, *37*). The PDA detector was used in the role of a coupled chromatographic−spectroscopic technique to obtain information about the complex tea liquor. The identification of tea compounds in this study was done by comparing the retention time and spectrum of the unknown compound with standards. A detailed procedure (*33*) was used for the identification of these compounds.

The quantification was carried out using the external standard method. Response factors (concentration of standard/peak area of standard) were determined under the same HPLC operating conditions as the samples. The concentration of a compound was calculated as peak area of the compound × response factor. Eight catechins and catechin gallates (C, CG, EC, ECG, EGC, EGCG, GC, and GCG) were quantified using the response factors of their standards, respectively. Gallic acid was also quantified against an authentic standard. For compounds for which no authentic standards were available, a reference standard with similar chromatographic and spectroscopic properties was selected. For example, the response factor of kaempferol at 280 nm was used for the quantification of flavonoid glycosides, such as quercetin glycoside (QG), quercetin 3-glucoside (Q3G), quercetin 3-rhamnosylglucoside (Q3RG), kaempferol glycoside (KG), and kaempferol 3-rhamnosylglucoside (K3RG). Epicatechin digallate (ECDG) and epigallocatechin digallate (EGCDG) were quantified using the response factors of ECG and EGCG, respectively. Isochlorogenic acid, theogallin, and *p*-coumarylquinic acid were quantified against chlorogenic acid, GCG, and caffeic acid at 280 nm, respectively.

Statistical Analysis. Quantitative data from the HPLC analysis were compared using analysis of variance (ANOVA). For variables for which significant F values ($P < 0.05$) were found, Fisher's least significant difference (LSD) was used for comparison of means. In this study, the phenolic compounds were grouped and analyzed as follows. Cs represented all four catechins (C, EC, GC, and EGC); CGs represented all six catechin gallates (CG, ECG, GCG, EGCG, ECDG, and EGCDG); Fla represented all flavanols including four catechins and six catechin gallates; and PPs represented the total phenolic compounds including the 10 flavanols and 5 flavonoid glycosides (QG, Q3G, Q3RG, KG, and K3RG). Additionally, the three principal tea flavanols (ECG, EGC, and EGCG) were analyzed individually.

RESULTS AND DISCUSSION

EGCG. The content of EGCG varied significantly ($P < 0.05$) across the harvest seasons. The EGCG contents in fresh tea shoots harvested during the warm months on April 12 and May 9, 2000, were significantly higher ($P < 0.05$) than in the shoots harvested during the cooler months of July−September 2000 (**Table 1**). The shoots harvested during the cooler months had the lowest EGCG contents. There was a slight rise in EGCG content among the samples harvested from October 2000 to February 2001, which followed the rise in temperatures. From January to May 2001, there were no marked changes in the EGCG levels. These results suggest that the synthesis of EGCG in tea shoots is temperature sensitive or dependent. Similar seasonal effects were reported in northeastern Indian tea (*38*, *39*), in which the EGCG content rose during the warm season and fell during the cold season. In Japan, the EGCG content of green tea was found to be 88.0 mg/g of dry mass in spring and 122.0 mg/g in summer (*40*).

Bockuchava and Skobeleva (*10*) suggested that the increase of EGCG during the summer months could be due to the active synthesis of EGCG in the plant tissues and that this physiological process becomes less active during the cooler months. It has also been suggested that the active synthesis of EGCG may be related to the length of daytime or stronger sunlight during the summer months (*7*). Furthermore, a number of studies have suggested that EGCG could be used as a quality indicator of black tea (*5*, *6*, *41*−*43*). If true, the result of this investigation would indicate that there is potential to produce higher quality black tea during the warmer months in Australia.

ECG. No significant differences ($P > 0.05$) in the ECG contents of fresh tea shoots were observed among the samples harvested during the warmer months from December 2000 to May 2001 (**Table 1**) and from August to November 2000. However, samples harvested between August and November 2000 showed significantly lower ($P < 0.05$) ECG levels than those samples harvested from December 27, 2000, to May 2, 2001, except for the October 23 harves5, which was not significantly different ($P > 0.05$) (**Table 1**). ECG levels significantly peaked ($P < 0.05$) at the May 9, 2000, harvest, similar to the EGCG levels.

The seasonal pattern of ECG in the fresh tea shoots was similar to the EGCG pattern, indicating that ECG may be synthesized according to the same metabolic pathway as EGCG. In addition, lower levels of ECG occurred in the cooler months (July−September 2000), as was the case with EGCG (**Table 1**). These results are in agreement with the findings of Bokuchava and Skobeleva (*10*). The seasonal variation of ECG levels in the *C. sinensis* var. *sinensis* was 28.0 mg/g in the spring and 41.0 mg/g in the summer (*40*), which is slightly lower than

the contents (31.56 mg/g in cooler months and 44.26 mg/g in warm months) found in Australia-grown fresh tea shoots (**Table 1**). This difference may be due to a clonal difference, because Australian tea belongs to *C. sinensis* var. *assamica* (*23*). This clonal difference is supported by the findings of Obanda et al. (*5*, *6*) that showed the level of ECG ranged from 18.3 to 49.1 mg/g in green leaves from different clones. Nevertheless, the seasonal trend is clear from the results of Chu and Juneja (*40*) and from the present study that warmer months favor the synthesis of ECG in the fresh tea shoots.

It has been shown that the biosynthesis of phenolic compounds can be effectively induced by sunlight (*7*). That is why in shaded tea flushes the concentrations of polyphenols are much lower (*44*). On the basis of this information, the differences in ECG and EGCG levels between fresh leaves harvested in cooler and warmer months in Australia may not be just a temperature effect but also a day length and sunlight effect. However, further studies are required to elucidate the induction of the biosynthesis of ECG and EGCG by day length and sunlight exposure correlating to the UV index.

Forrest and Bendall (*45*) demonstrated that ECG and EGCG do not occur in the embryo of tea seeds. They are synthesized during the germination and development of tea shoots. For the catechin-related compounds, including gallated catechins, only catechin and epicatechin (EC) are detectable in the embryo of tea seeds. Catechin gallates are formed during the germination, with ECG predominating. EGCG increases rapidly after the emergence of the plumule and becomes dominant at the time of the leaf development (*45*). However, the results of this Australian study and others in which the level of EGCG in the fresh shoots was 3 times the level of ECG may imply that the synthesis of EGCG is more active and surpasses the synthesis of ECG during the growth of tea shoots. Another possibility is that ECG may be formed by the gallated esterification of EC, whereas EGCG may be formed from EGC during the development of tea shoots (*7*).

It has been suggested that ECG is a quality indicator of black tea (*39*, *41*, *43*). Along with EGCG, the active synthesis of ECG in summer may explain the increase in total flavanols and other polyphenols observed in tea flushes (*10*). Again, as observed for EGCG, the seasonal pattern of ECG formation in Australia-grown fresh tea shoots suggests that tea harvested during the warmer months in Australia may produce better quality black tea.

EGC. The seasonal profiles of EGC content in the fresh tea shoots showed no significant differences ($P > 0.05$) among the samples harvested from April 12 to November 13, 2000, and from December 6, 2000, to February 22, 2001 (**Table 1**). However, significantly higher levels ($P < 0.05$) of EGC content were found in fresh shoots harvested from April to October 2000 (mean = 50.55 mg/g) than in those from January to May 2001 (mean = 42.19 mg/g), with the latter months showing a declination in EGC levels (**Table 1**). EGC showed the highest levels during the cooler months of July−October 2000 and then slowly decreased as the temperature and day length increased into the warmer months of January−April 2001.

The seasonal pattern of EGC in tea flushes was different from those of ECG and EGCG (**Table 1**). Whereas the gallate ECG and EGCG levels tended to decrease during the cooler months and increase during the warmer months, EGC remained constant during the cooler months and then decreased as the temperature increased (November 2000−April 2001). Bokuchava and Skobeleva (*10*) also found that the formation of EGC remains at a higher level during the winter months.

In the northern hemisphere, tea trees are not harvested in the winter, which is different from the production of tea in the southern hemisphere. In Australia, EGC levels in fresh tea shoots are highest and constant during the cooler months as the plant continues to grow. Hilton (*8*) also found that EGC levels in fresh tea shoots harvested in central Africa during the cold seasons were higher than those harvested in the warmer seasons. The lower EGC content in fresh leaves during the summer months may imply that the active biosynthesis of EGCG consumes large amounts of EGC, thus the content of EGCG in fresh leaves is higher in summer, whereas the content of EGC remains low. However, the biosynthesis of EGCG slows during the winter, which allows the accumulation of EGC in the fresh leaves. This is a possible explanation for the higher EGC content in winter than in summer. Further studies are necessary to confirm the relationship between EGC and EGCG in fresh tea shoots.

The level of EGC in tea shoots has been found to positively correlate with the total level of theaflavins in the resulting black tea (*9*, *38*). Thus, EGC could be an important catechin in the determination of black tea quality because theaflavins are regarded as the quality indicator for black tea (*46*). Both ECG (*5*, *6*, *38*, *39*, *43*) and EGCG (*5*, *6*, *43*) were also found to positively correlate with the quality of black tea. In his early studies, Roberts (*15*, *16*, *42*) suggested that EGC and EGCG in tea flushes were important quality indicators of the resulting black tea. However, on the basis of the findings of this study, it is unlikely that the maximum levels of all three flavanols would be attained at the same period of harvest, because opposite seasonal trends occur among EGC, EGCG, and ECG in tea shoots. Therefore, the catechin gallates could be more important than the catechins in determining the quality of the resulting black tea due to fact that the catechin gallates quantitatively override the catechins in the tea shoots.

Cs. The levels of Cs in the tea flushes harvested during the cooler months from July 19 to September 28, 2000, were significantly higher ($P < 0.05$) than those in tea shoots harvested during the warmer months from February 22 to April 9, 2001, which themselves showed no significant differences ($P > 0.05$) from one another (**Table 1**).

Similar to the EGC results, the levels of Cs peaked during the cooler months and then gradually decreased as the temperature rose during the warmer months. This effect would be expected because EGC represents >50% of the Cs. During the period from October 23, 2000, to May 2, 2001, the variation of the levels of Cs in fresh shoot mirrored the variation in the levels of EGC. Thus, the variations in the other catechins (C, EC, and GC) are likely to mirror that of EGC. In conclusion, the seasonal variation of EGC and therefore the total catechins in Australia-grown tea can be described as highest during the cooler months and lowest during the warmer months. These findings are in agreement with those of Hilton (*8*). The similarity between the content of EGC and that of total catechins appears to be reasonable because the winter seasons favor the accumulation of catechins, EGC and EC (*10*), which together are the major catechins in fresh tea shoots.

CGs. The levels of CGs in the fresh tea shoots harvested in the cooler months from July 19 to September 28, 2000, were significantly lower ($P < 0.05$) than those harvested in the warmer months (April 12 and May 9, 2000; December 6, 2000, to May 2m 2001) (**Table 1**). The CGs in fresh tea shoots gradually increased from low levels of cooler months to high levels in the warmer months, which showed a very similar seasonal pattern to that of ECG and, in particular, EGCG (**Table**

1). This is reasonable because EGCG represents ~70% of the CGs in the tea flushes (**Table 1**) and thus quantitatively dominates the seasonal pattern of these grouped gallates. In addition, ECG, representing >20% of the CGs in the fresh tea shoots, also has a seasonal pattern similar to that of EGCG.

As discussed earlier, the biosynthesis of EGCG and ECG is active during the warm months (*10*), which will increase the CGs in the tea flushes harvested during these months. The lower content of CGs in winter may be due to a corresponding less active biosynthesis of ECG and EGCG during cooler weather. In addition, the cooler months of 2000 coincided with lower recorded rainfall compared to the warmer months from November 2000 to April 2001, for which a much higher rainfall was recorded (*47*). Thus, it is possible that the rainfall may contribute to the decrease and/or increase pattern of the CGs. However, more information is required to better relate rainfall to the seasonal variations of individual or total catechin gallates, such as the irrigation and other agronomic management practices conducted during the dry months. Further studies are also recommended to perform greenhouse experiments at controlled growing conditions to compare the influence of light, temperature, and precipitation (rainfall).

Fla. Comprising all the catechins and catechin gallates, they are referred to as flavanols or total flavanols (*8*, *9*, *48*). The levels of Fla in tea flushes showed no significant differences ($P > 0.05$) among the samples harvested from July 19 to September 28, 2000, from October 23, 2000, to January 15, 2001, and from February 22 to May 2, 2001 (**Table 1**). The lowest level of flavanols in the fresh tea shoots occurred during the cooler months from July 19 to September 28, 2000, after which the levels rose as the temperature increased. This trend is similar to that of EGCG and the CGs (**Table 1**) and reveals that catechin gallates, particularly EGCG, dominate the seasonal distribution pattern of flavanols in the tea flushes, because they represent ~65% of the total flavanols.

The contents of the flavanols were not significantly different ($P > 0.05$) among the samples harvested from July 19 to December 6, 2000, suggesting that the differentiation of the flavanols between cooler and warmer months is complex. This is because the seasonal patterns of the catechins were the reverse of that for the catechin gallates. Thus, when those two groups of compounds were added together as the total flavanols, some of the seasonal differences were obscured. The results of this study indicate that total flavanols could represent only a general pattern in tea flushes and do not precisely reveal the true seasonal patterns of individual or grouped catechins or catechin gallates. This study has shown that the variations of the individual compounds ECG, EGC, and EGCG are better at predicting the seasonal variations in fresh tea shoots than the flavanols. This is a significant result because ECG, EGC, and EGCG are considered to be quality indicators for black tea processing. Therefore, the variations of ECG, EGCG, and EGC levels in fresh tea shoots may be used as indicators of the likely variation that may occur in black tea quality.

PPs. In this study, PPs include all of the flavanols, phenolic acids, and flavonoid glycosides. There was no specific overall trend of the PPs in fresh tea shoots (**Table 1**), particularly between the cooler and warmer months, as observed earlier with the other tea components. Again, the higher level of this group of compounds that occurred during the warmer months is due to the level of catechin gallates that overrides the level of catechins in the fresh tea shoots. Thus, the PPs in fresh tea shoots show a seasonal pattern similar to those of Fla, CGs, and EGCG, suggesting that the seasonal variations of the PPs could be measured by using the variations of catechin and catechin gallate concentrations.

Table 2. Content of Minor Phenolic Compounds in Hand-Plucked Fresh Tea Shoots

	content of grouped minor compounds (mg/g, dry basis)		
level	mPPs[a]	mCs[b]	mCGs[c]
low	36.15	27.60	5.75
high	43.58	41.31	11.49
average	39.30	34.26	9.18

[a] Minor phenolic compounds include all of the phenolic compounds measured in fresh tea shoots excluding catechins and catechin gallates. [b] Minor catechins include all of the catechins measured in the tea shoots excluding EGC. [c] Minor catechin gallates include all of the catechin gallates measured in the tea shoots excluding EGCG and ECG.

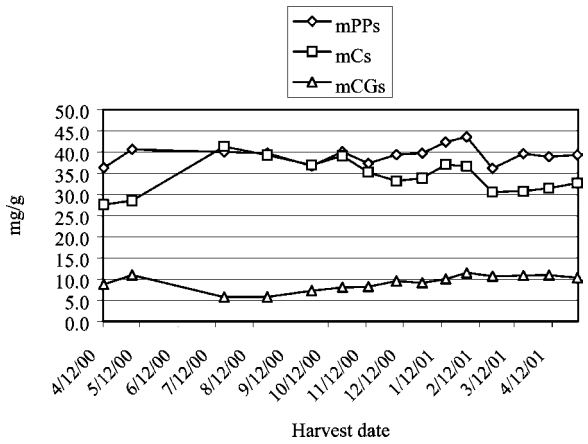

Figure 1. Seasonal variation of minor phenolic compounds in fresh tea shoots: mPPs, minor phenolic compounds include all of the phenolic compounds measured in the tea shoots excluding catechins and catechin gallates; mCs, minor catechins include all of the catechins measured in the tea shoots excluding EGC; mCGs, minor catechin gallates include all of the catechin gallates in the tea shoots excluding EGCG and ECG.

Minor Phenolic Compounds. These compounds were classified into three groups in this study: 1, minor phenolic compounds (mPPs) include kaempferol glycoside, kaempferol 3-rhamnosylglucoside, quercetin glycoside, quercetin 3-glucoside, quercetin 3-rhamnosylglucoside, gallic acid, theogallin, *iso*-chlorogenic acid, *p*-coumaryl quinic acid, chlorogenic acid, 3-(*p*-hydroxyphenyl)propionic acid, and *p*-coumaric acid; 2, minor catechins (mCs) include catechin (C), EC, and GC; and 3, minor catechin gallates (mCGs) include CG, GCG, ECDG, and EGCDG. The mPPs in hand-plucked fresh tea shoots range from 36.15 to 43.58 mg/g during the sampling seasons, with an average of 39.30 mg/g (**Table 2**). The seasonal pattern of this minor group of compounds (**Figure 1**) appears to mirror that of the PPs. The mCGs range from 5.75 to 11.49 mg/g across the seasons, with an average of 9.18 mg/g (**Table 2**). Again, the seasonal pattern of mCGs (**Figure 1**) is similar to that of the total catechin gallates. The mCs range from 27.6 to 41.31 mg/g with an average of 34.26 mg/g, which represents ~45% of the total catechins (**Table 2**). Unlike mCGs and mPPs, the seasonal pattern for mCs (**Figure 1**) is not similar to either the total catechins or EGC. However, the seasonal patterns of the phenolic compounds in the fresh tea shoots are dominated by the principal phenolic compounds, not the minor ones, across the growing seasons.

Ratio between Flavanols. The ratio of EGCG to ECG has been used to compare the development of tea shoots after

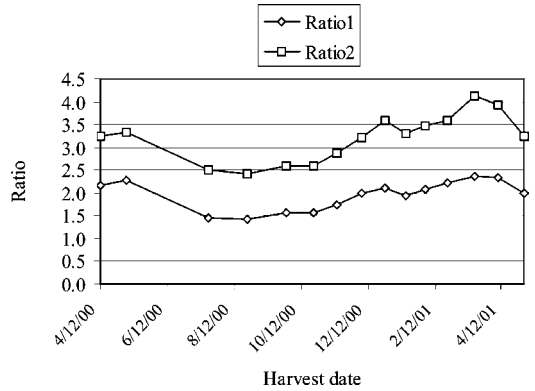

Figure 2. Seasonal variation of the ratios of catechin gallates to catechins in fresh tea shoots [ratio 1 = (EGCG + ECG)/EGC; ratio 2 = CGs/Cs].

germination (*38*, *41*) and clonal selection (*38*). Nakagawa and Torri (*1*, *2*) used EC and EGC as simple flavanols and ECG and EGCG as galloyl flavanols to compare the seasonal variations of tea shoots and found that the proportion for simple flavanols was higher in the spring, whereas the proportion of galloyl flavanols was higher in the summer. In addition, the ratio (EGCG + ECG) × 100/EGC has been used as a quality index for Chinese green teas (*49*, *50*). On many occasions, a number of researchers (*7*, *8*, *14*, *22*, *42*, *45*) have suggested that the flavanol components and their proportions in tea flushes could affect the quality of the resultant black tea. In this study, the seasonal variation in the ratio of CGs to Cs appears to be weather dependent, with harvests in the warmer months showing higher ratios and harvests in the cooler months showing lower ratios (**Figure 2**). In this study, all of the tea plants on the farm were considered to be from the same variety, var. *assamica* (*23*). Therefore, the ratio (CGs/Cs) can be used to monitor seasonal changes in tea shoots grown on the Australian tea farms. However, this ratio is quite complex because of the large number of different flavanols (about 10 principal and minor catechins and catechin gallates). The ratio (EGCG + ECG)/EGC in the fresh tea shoots also shows a seasonal pattern similar to that of the CGs/Cs ratio (**Figure 2**). As expected, the seasonal distribution pattern of the ratio among the individual flavanols mimics the seasonal pattern of the ratio between the two grouped flavanols. Therefore, it is ideal to use the ratio (EGCG + ECG)/EGC rather than the ratio CGs/Cs as a quality index of fresh tea shoots because of its sensitivity to seasonal variations.

ABBREVIATIONS USED

ANOVA, analysis of variance; C, catechin; CA, chlorogenic acid; CG, catechin gallates; CGs, total catechin gallates; Cs, total catechins; EC, epicatechin; ECDG, epicatechin digallate; ECG, epicatechin gallate; EGC, epigallocatechin; EGCDG, epigallocatechin digallate; EGCG, epigallocatechin gallate; Fla, total flavanols; GA, gallic acid; GC, gallocatechin; GCG, gallocatechin gallate; HPLC, high-performance liquid chromatography; K3RG, kaempferol 3-rhamnosylglucoside; KG, kaempferol glycoside; LSD, Fisher's least significant difference; mCGs, minor catechin gallates; mCs, minor catechins; mPPs, minor phenolic compounds; 3PA, 3-(*p*-hydroxyphenyl)propionic acid; PDA, photodiode array; PPs, total polyphenols; Q3G, quercetin 3-glucoside; Q3RG, quercetin 3-rhamnosylglucoside; QG, quercetin glycoside.

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