# White and Green Teas (Camellia sinensis var. sinensis): Variation in Phenolic, Methylxanthine, and Antioxidant Profiles

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# White and Green Teas (*Camellia sinensis* var. *sinensis*): Variation in Phenolic, Methylxanthine, and Antioxidant Profiles

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**Abstract:** Recent investigations have associated white teas with anti-carcinogenic, immune-boosting, and antioxidative properties that may impact human health in a manner comparable to green teas. An in-depth chemical analysis of white tea types was conducted to quantify polyphenols and antioxidant potential of 8 commercially available white teas, and compare them to green tea. Extraction and HPLC protocols were optimized and validated for the quantification of 9 phenolic and 3 methylxanthine compounds to examine inter- and intra-variation in white and green tea types and subtypes. A sampling strategy was devised to assess various subtypes procured from different commercial sources. Variation in antioxidant activity and total phenolic content (TPC) of both tea types was further assessed by the 1-1-diphenyl-2-picrylhydrazyl (DPPH) and Folin–Ciocalteau (F–C) assays, respectively. Total catechin content (TCC) for white teas ranged widely from 14.40 to 369.60 mg/g of dry plant material for water extracts and 47.16 to 163.94 mg/g for methanol extracts. TCC for green teas also ranged more than 10-fold, from 21.38 to 228.20 mg/g of dry plant material for water extracts and 32.23 to 141.24 mg/g for methanol extracts. These findings indicate that statements suggesting a hierarchical order of catechin content among tea types are inconclusive and should be made with attention to a sampling strategy that specifies the tea subtype and its source. Certain white teas have comparable quantities of total catechins to some green teas, but lesser antioxidant capacity, suggesting that white teas have fewer non-catechin antioxidants present.

**Keywords:** antioxidant, *Camellia sinensis*, catechins, green tea, white tea

**Practical Application:** In this investigation white and green teas were extracted in ways that mimic common tea preparation practices, and their chemical profiles were determined using validated analytical chemistry methods. The results suggest certain green and white tea types have comparable levels of catechins with potential health promoting qualities. Specifically, the polyphenolic content of green teas was found to be similar to certain white tea varieties, which makes the latter tea type a potential substitute for people interested in consuming polyphenols for health reasons. Moreover, this study is among the first to demonstrate the effect subtype sampling, source of procurement, cultivation, and processing practices have on the final white tea product, as such analysis has previously been mostly carried out on green teas.

## Introduction

Tea from the young buds and leaves of *Camellia sinensis* (L.) O. Kuntze (Theaceae) is the most widely consumed beverage in the world following water and is valued for its taste, aroma, health benefits, and cultural practices (Khokhar and Magnusdottir 2002). The tea plant is considered native to southwestern China and is cultivated in tropical regions globally (Pettigrew 2004). White, green, oolong, black, and pu'erh teas are the major tea types sourced from leaves and buds of the tea plant and are categorized based on variation in harvesting, processing, and associated degree of oxidation of polyphenols in fresh tea leaves (Pettigrew 2004). These tea types also differ based on the variety of *Camellia sinensis* used in their production. For instance, Chinese and Japanese green teas are made from *Camellia sinensis* var *sinensis* while black teas are made from *Camellia sinensis* var *assamica* (Takeo 1992). Of all these tea types, white teas are less known in western communities but a valued tea in Asia; its flavor is even more accepted than that of green tea in Europe (Almajano and others 2008).

White teas have been reported to possess higher antielastase, anticollagenase, and antioxidative activity than certain green tea, suggesting its ability to promote strong and elastic skin and alleviate inflammation and rheumatoid arthritis, has led to an increased interest in this tea type (Thring and others 2009). White tea lipolytic activity and its ability to inhibit adipogenesis have received particular attention especially in developed countries battling with dramatic increases in obesity and obesity-related diseases (Sõhle and others 2009).

White teas differ from other tea types by being produced from unopened buds, classified as silver needle (bai hao Yinzhen), or

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incorporating an unopened bud and 2 immature leaves, covered in white leaf hairs, at an early stage of chlorophyll formation, as is the case for white peony (bai mudan) (Pettigrew 2004). To distinguish between green and white tea production processes, mature leaves for green tea production are withered, briefly pan-fried, rolled, and dried in the traditional Chinese green tea making process. Some Chinese tea manufacturers steam rather than pan-fry their tea leaves, much like the Japanese style of green tea production (Pettigrew 2004; Hilal and Engelhardt 2007; Ho and others 2009). Both tea heating methods deactivate the polyphenol oxidase enzymes. In some published reports, some white teas, are also steamed during processing to deactivate enzymes. Most white teas use new buds plucked before they are opened then are withered and air dried in the shade, under sunshine, or in a temperature controlled room to remove moisture content (Pettigrew 2004). The dried buds have a curled silvery appearance.

Some studies support that among all tea types, green teas contain the highest amount of catechins, a group of polyphenolic flavan-3-ol monomers and their gallate derivatives (Lin and others 2003). The major catechins include (−)-epicatechin (EC), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), and (−)-epigallocatechin-3-gallate (EGCG). These compounds are primarily responsible for many of the health protective properties associated with tea including antioxidative (Mildner-Szkudlarz and others 2009), antiinflammatory (Cao and others 2007), neuroprotective (Mandel and Youdim 2004), anti-cancer (Yang and others 2002) antimicrobial, and antiatherosclerotic properties (Zhen 2002). However, other reports suggest that catechin and total phenolic content (TPC) cannot be used as a criterion for differentiating between green and white teas (Hilal and Engelhardt 2007). Chinese white teas have also been reported to possess greater antimutagenic properties than premium green teas (Sanatan-Rios and others 2001; Hilal and Engelhardt 2007) and comparable antioxidant effects as green teas in body plasma and some organs (Koutelidakis and others 2009). White teas have also been found to contain higher amounts of caffeine than green teas (Hilal and Engelhardt 2007) which along with other methylxanthines, such as TB and TP, the amino acid theanine, and free sugars, are compounds commonly found in tea.

Given the numerous factors that affect the end tea product for consumption or laboratory analysis including climate, soil, plucking time, as well as processing and preparation methods (Zhen 2002; Lin and others 2003; Pettigrew 2004) and, the growing role of tea in daily food intake in the United States over the past 2 decades (Sultana and others 2008), the inter-variation of beneficial compounds between green and white teas and intra-variation within each tea type should be recognized. The *Camellia sinensis* variety used in tea production also determines the amount of these beneficial compounds where for instance, the *assamica* variety has been reported to possess more than twice the flavanol content of the *sinensis* variety although its use is restricted to the production of black tea due to the bitter flavor that results from its high flavanol content (Takeo 1992). A literature survey on the chemical profile and bioactivity of all tea types reveals a lack of information or agreement on the nature of white teas in comparison to other tea types while the variation in composition and bioactivity between white and green teas and their subtypes remains unclear (Hilal and Engelhardt 2007). The present study develops and validates an efficient tea extraction and chromatographic method for the quantitative analysis of 9 catechins and 3 methylxanthines in white tea. Previous studies on white teas have treated it as a single product by including only 1 white tea subtype in their sampling protocol (Rusak and others 2008; Horzic and others 2009), or by not specifying the white tea subtypes being investigated (Hilal and Engelhardt 2007).

The present study accounts for possible intra-variation of compounds in white tea subtypes by examining 2 subtypes of white tea from 4 commercial sources and comparing these with 5 subtypes of green tea from 5 commercial sources. The phenolic composition and antioxidant properties of white and green teas are further investigated using the Folin–Ciocalteau (F–C) TPC assay and the 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay, respectively.

## Materials and Methods

### Chemicals and reagents

Solvents for high-performance liquid chromatography (HPLC) analysis include trifluoroacetic acid (Fisher Scientific, Fair Lawn, N.J., U.S.A.), HPLC-grade acetonitrile (J.T. Baker, Phillipsburg, N.J., U.S.A.) and water distilled using a Milli-Q system (Millipore Lab., Bedford, Mass., U.S.A.). HPLC-grade methanol (E. Merck, Darmstadt, Germany) was used for sample preparation. Reagent-grade ethanol (Fisher Scientific), ascorbic acid, and 1-1-diphenyl-2-picrylhydrazyl (DPPH, Sigma Chemical Co., St. Louis, Mo., U.S.A.) were used for the DPPH scavenging assay. F–C reagent (2N) and sodium carbonate powder (>99.5% purity) (Sigma Chemical Co.) were used for the TPC assay.

### Standards

Pure standards of gallic acid (GA) (1), (+)-catechin (C) (2), caffeine (CAF) (3), (−)-epigallocatechin 3-gallate (EGCG) (4), and (−)-gallocatechin (GC) (5) were purchased from ChromaDex (Santa Ana, Calif., U.S.A.). (−)-Epicatechin 3-gallate (ECG) (6), (−)-epigallocatechin (EGC) (7), and (−)-catechin 3-gallate (CG) (8) were purchased from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Theobromine (TB) (9), theophylline (TP) (10), (−)-gallocatechin 3-gallate (GCG) (11) and (−)-epicatechin (EC) (12) were obtained from Sigma Chemical Co. Quercetin dihydrate (Sigma Chemical Co.) was used as an internal standard for calibration purposes.

### Plant materials

A total of 8 samples of white teas of 2 major subtypes, white peony (bai mudan) and Yin Zhen silver needle (bai hao Yin Zhen), both from the Fujian Province in China (Pettigrew 2004), and 19 samples of green tea representing 5 subtypes (dragonwell, gunpowder, jasmine pearl, sencha, and gykuro) were obtained from 5 commercial companies—A,B,C,D, and E. Loose leaf tea samples were utilized for all experiments because extraction of compounds from loose leaf teas have been shown to be more effective than bagged teas in quantification of analytes (Rusak and others 2008). All tea sample analysis was carried out in triplicate.

### Sample extraction

Water and aqueous methanol extracts of tea were prepared for quantification of phenolic and methylxanthine composition and bioactivity. Tea water extraction procedures replicated preparation conditions of cosmopolitan tea drinking (Khokhar and Magnusdottir 2002; Rusak and others 2008). Dry tea leaves (1 g) were steeped in 100 mL of deionized water at 95 to 100 °C for 5 min. The resultant tea mixture was filtered under vacuum using Whatman nr 5 filter paper. An aliquot (1.5 mL) of the filtrate was centrifuged at 15000 rpm for 15 min and the supernatant was passed through a 0.45 $\mu$m nylon membrane filter prior to HPLC analysis. The remainder of the filtrate was frozen at −20 °C and

freeze-dried in a lyophilizer to obtain dried water extracts for DPPH and TPC assays.

Alcohol extraction was carried out to mimic industrial and research conditions (Rusak and others 2008). Methanol extracts were obtained using a modification of the procedure previously described (Nuntanakorn and others 2007). Ground dry tea leaves were extracted with 80% aqueous methanol in a ratio of 1 g : 10 mL solvent (w/v) using a sonicator for 30 min. The supernatant was filtered under vacuum using Whatman nr 5 filter paper and the filtrate was centrifuged at 15000 rpm for 15 min and filtered through a 0.45 $\mu$m nylon membrane filter after pre-flushing with the sample prior to HPLC analysis.

### High-performance liquid chromatography (HPLC)

HPLC analysis was performed using a Waters 2695 HPLC (Milford, Mass., U.S.A.) module equipped with a 996 photodiode array detector (PDA) and operated with Empower software. Samples and standards were separated on a Synergi Fusion, 4 $\mu$m, 250 × 4.6 mm ID, C-18 reversed-phase column (Phenomenex, Torrance, Calif., U.S.A.). Column temperature was maintained at 30 °C with a column heater and the autosampler temperature at 4 °C. A gradient system was used for the mobile phase comprising 0.05% (v/v) trifluoroacetic acid in distilled water (A) and in acetonitrile (B), with a flow rate of 1 mL/min and duration of 35 min. The gradient profile employed was as previously described (Dalluge and others 1998) and is as follows: 0 to 25 min, 12% to 21% B; 25 to 30 min, 21% to 25% B. The column was flushed with 100% B for 10 min and re-equilibrated for 5 min to starting conditions. Sample volume injected was 5 $\mu$L for methanol extracts and 40 $\mu$L for water extracts. The UV-vis spectra were recorded from 254 to 400 nm and relevant peaks were detected at 280 nm. Peaks were identified based on characteristic absorbance spectra and retention time.

### Validation of HPLC method

Validation was carried out in compliance with the AOAC Intl. Guidelines for Single Lab. Validation of Chemical Methods for Dietary Supplements and Botanicals (AOAC 2002). The method was validated with respect to selectivity, linearity, recovery, detection, and quantification limits and precision.

Calibration curves were established on 5 to 7 data points for serial dilutions of standards 1 to 12 using 80% methanol, measured at 280 nm and covering a concentration range of 2.23 to 11000 $\mu$g/mL. Recovery studies were conducted by spiking loose leaf tea leaves with 0.985, 1.97, and 3.94 mg/mL concentrations of quercetin dihydrate (3,3′,4′,5,7-pentahydroxyflavone) used as an internal standard.

For intra-day assay, 10 replicate analyses of standards 1, 4 to 12 was carried out and was also performed at 2 different concentrations (0.6 and 1 mg/mL) of the mixture of 10 standards on 2 different days for inter-day precision studies. Precision at each concentration was expressed as % RSD of measured peak areas from mean peak area. Peak resolution ($R_s$) was calculated as the ratio of the difference in retention times ($T_{1\&2}$) between adjacent peaks to the summation of peak bandwidths at half height ($W_{0.5,1\&2}$) according to the formula below (Snyder and others 1997):

$$\mathrm{Rs} = 1.18(T_2 - T_1)/\, W_{0.5,1} + W_{0.5,2}$$

Dilutions of standards 1 to 4, 6, 9, and 10 were analyzed by HPLC to obtain concentrations with peak signal-to-noise ratio of about 3 : 1 (limit of detection [LOD]) and 10 : 1 (LOQ). Results were expressed as detectable or quantifiable concentrations in micrograms per milliliter.

### 1-1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The DPPH radical scavenging assay was performed as previously described (Saito and others 2007b). Dissolved tea water extracts (50 $\mu$L) were mixed with 150 $\mu$L of 400 $\mu$M DPPH solution. The mixtures were incubated for 30 min at 37 °C and absorbance values were measured at 517nm using a Softmax Pro 3.0 microplate reader (Molecular Devices, Sunnyvale, Calif., U.S.A.). Radical scavenging ability of samples was calculated as the percentage of DPPH free radicals inhibited by samples in comparison to radical inhibition in the negative water control used:

$$(\mathrm{ABS}_{\text{negative control}} - \mathrm{ABS}_{\text{sample}}/\mathrm{ABS}_{\text{negative control}}) * 100$$

Gallic acid (0.015625 to 0.25 mg/mL) and ascorbic acid (0.03125 to 0.5 mg/mL) were used as positive controls. Values obtained were plotted against concentration ($\mu$g/mL) of sample dilutions and final results are expressed as $IC_{50}$ values (concentration of samples required to scavenge 50% of DPPH radicals).

### Total phenolic content (TPC) assay

TPC was determined spectrophotometrically using F–C reagent as previously described (Prior and others 2005). To 100 $\mu$L of dilutions of tea water extracts was added 1mL of 10% (v/v) 2N F–C reagent and after incubation at room temperature for 5 min, 1 mL of 10% (m/v) sodium carbonate solution was added to make extracts alkaline. Mixtures were incubated for 90 min at room temperature after which absorbance was measured at 765 nm and results expressed as gallic acid equivalents (GAE) in milligram per gram dry plant material. The concentration of polyphenols in samples was derived from a standard curve of absorbance of gallic acid concentrations ranging from 31.25 to 500 $\mu$g/mL.

### Statistical analysis

Results were analyzed statistically using JMP 7.0 software (SAS) to determine mean values, standard deviations and standard error of means of quantified masses of compounds analyzed by HPLC in triplicates. ANOVA with a significance level of $\alpha = 0.05\%$ was performed to determine the relationship between tea type and total catechins, individual catechins, $IC_{50}$ antioxidant activity, and TPC. Differences with $P \leq 0.05$ were considered significant. Total catechin content (TCC) for water and methanol extracts was resolved by the addition of the amounts of individual catechins (EGCG, ECG, EGC, GCG, CG, C, and EC). Similar calculations have been assumed by previous analytical studies of tea samples (Khokhar and Magnusdottir 2002; Rusak and others 2008). Correlation analysis was carried out for DPPH $IC_{50}$ against TPC, TCC against DPPH $IC_{50,}$ and TCC against TPC to evaluate relationships between both quantities in tea extracts analyzed. Graphs were constructed using JMP 7.

## Results and Discussion

### HPLC validation

The modification of the analytical method separated a mixture of standards of caffeine, gallic acid, and eight catechins (EGC, EC, EGCG, GCG, (+)-C, CG, GC, and ECG) within 22 min. A different column type (Synergi Fusion, Phenomenex, Torrance, Calif., U.S.A.), with similar characteristics as the column (Zorbax

Eclipse XDB-C18, Rockland Technologies Inc./Dupont, Newport, Del., U.S.A.) utilized by Dalluge and his colleagues enabled the separation of 5 more compounds compared to the 7 compounds (6 phenolics and caffeine) obtained by these researchers (Dalluge and others 1998). Figure 1 shows chromatograms of some white and green tea samples used in this investigation.

Separation of standards was achieved by HPLC with a critical band pair value (Rs. = 3.81) calculated as previously described (Sharma and others 2005). Good linearity was observed for all catechins and gallic acid ($r^2 > 0.99$) in the given concentration range except for caffeine whose lower correlation coefficient ($r^2 = 0.9787$) was due to optimization of the linear range to account for higher concentrations observed for caffeine in some tea water extract samples. The LOD and the limit of quantification (LOQ) of 7 standards; GA, C, CAF, EGCG, ECG, TP, and TB were found to be in the ranges of 0.05 to 1 $\mu$g/mL and 0.1 to 5 $\mu$g/mL, respectively.

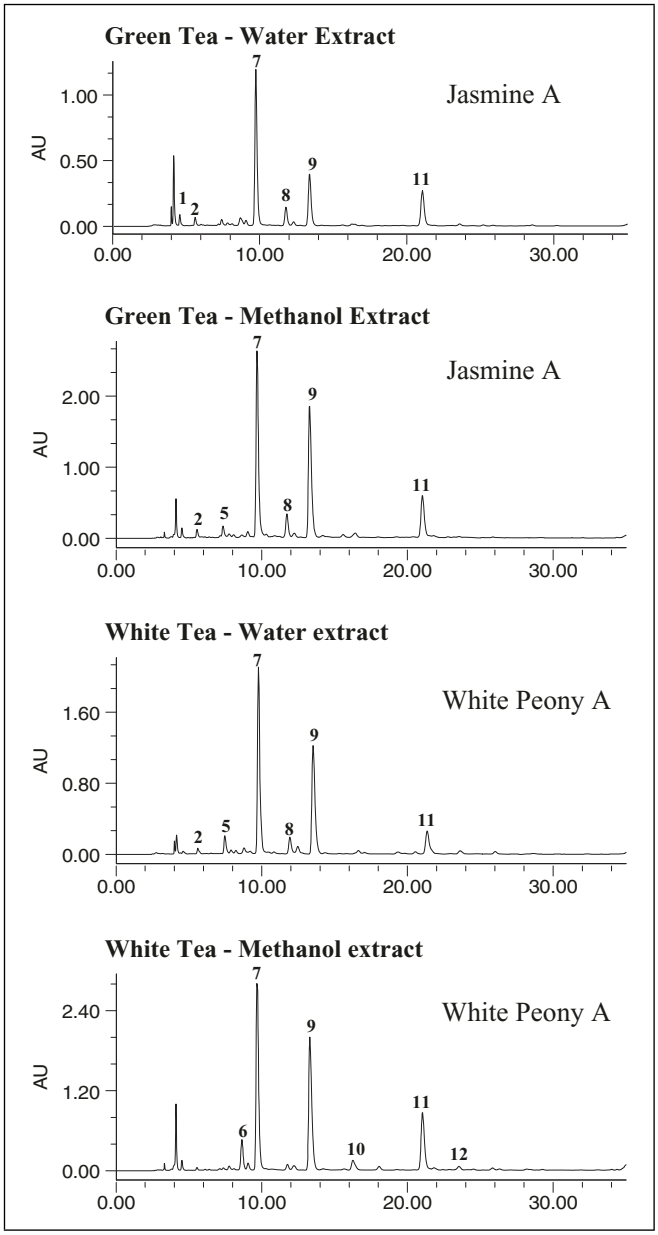


**Figure 1–Sampled chromatograms of some white and green teas analyzed using Synergi Fusion $C_{18}$ reverse phase HPLC column 4 $\mu$m, 250 × 4.6 mm ID by gradient elution as described in Materials and Methods. Detection was carried out with UV at $A_{280}$. Sample source companies are denoted with alphabets A to D. Peak identification and approximate retention times in minutes in parentheses are as follows: 1. GA (4.60 ± 0.28); 2. TB (5.45 ± 0.11); 3. GC (6.28 ± 0.025); 4. TP (6.71 ± 0.134); 5. EGC (7.66 ± 0.69); 6. C (9.64 ± 0.87); 7. CAF (9.80 ± 0.19); 8. EC (12.34 ± 0.74); 9. EGCG (14.30 ± 1.31); 10. GCG (16.92 ± 1.70); 11. ECG (22.42 ± 2.04); and 12. CG (24.69 ± 2.10).**

Intra-day analysis of a standard mixture of 10 compounds containing GA, GCG, CG, EGCG, GC, ECG, EGC, TB, TP, and EC ranged between 0.19% and 0.53% for repeatability precision experiments, while inter-day analysis of the same standard mix in 2 concentration levels (0.6 and 1 mg/mL) over 2 different days yielded % RSD percent ranging from 0.13% to 1% for 0.6 mg/mL concentrations, and 0.1% to 1.07% for 1 mg/mL concentrations. Percent recovery of internal standard, quercetin dihydrate ($r^2 = 0.9996$), using an extraction method modified from the published procedure (Nuntanakorn and others 2007; see Appendix 1) ranged from 64.2% to 93% for green teas and 81.6% to 101.7% for black teas (see Appendix 2).

## Comparing total catechin content (TCC) of white and green teas

TCC results show inter- and intra-variation in tea types and subtypes as well as with the type of extraction method employed. TCC for white tea methanol extracts ranged from 47.16 to 169.94 mg/g and from 32.23 to 141.24 mg/g for green tea methanol extracts (Table 1). These values constituted mean percent weight of dried plant material of 6.77% for green teas and 7.62% for white teas. These results are in accordance with values obtained in previous studies using similar extraction methods and solvents on green teas (Sharma and others 2005). White tea catechin content was higher than green teas with a mean value of 76.15 mg/g dry tea material (Table 1).

TCC for white tea water extracts analyzed ranged more than 25-fold (14.40 to 369.60 mg/g dry plant material; Table 1) and had about an 11-fold range (21.38 to 228.20 mg/g) for green teas. Mean total catechins of water extracts constituted 8.20% of dry loose-leaf white teas and 9.97% for green teas. Fluctuations in retention times and co-elution between TB and GC necessitated an exclusion of these 2 compounds from quantification to avoid systematic errors. TP was not detected in any of the tea samples and was also excluded from calculations as was the case in several other investigations of green teas (Khokhar and Magnusdottir 2002; Sharma and others 2005).

While green tea water extracts had a higher mean total catechin composition of 99.66 mg/g (Table 1), certain subtypes of white tea possessed higher TCC than green tea subtypes. For instance, Company A's white peony possessed a TCC of 369.60 mg/g dry leaf water extract and Company C's silver needle yielded 163.94 mg/g TCC for tea methanol extracts. The highest green tea TCC was observed for company E's green tea water extract with a quantity of 228.20 mg/g dry tea. However, white peony samples from other companies contained significantly lower catechin content, making statements regarding the tea subtype with higher catechin content inconclusive (Table 1).

Rusak and others (2008) have previously reported that green teas have significantly higher amounts of phenolics and flavonoids in the first 5 min of extraction as compared to white teas when both water and aqueous methanol solvents are used for extraction. The lipophilic cell wall of trichomes on white tea buds appear to affect the extraction kinetics of hydrophilic catechins (Rusak and others 2008). Conversely, Hilal and Engelhardt (2007) found

higher mean levels of total catechins in white teas than in green tea samples analyzed. In the present study, higher levels of catechins in some white tea samples and variation among samples of a particular subtype from different commercial sources shows that categorical statements regarding the relative quantities of catechins in green and white teas should be made with considerations to the specific subtype and source of tea analyzed as has been previously noted (Friedman and others 2006). Additionally, in a one-way ANOVA among tea types for TCC, there was no significant difference between green and white teas in their water ($F_{1,79} = 0.72$, $P = 0.4004$) and methanol extracts ($F_{1,79} = 1.38$, $P = 0.2437$) for TCC, further making hierarchical statements of catechin content between both tea types questionable (see Appendixes 3 and 4).

## Comparing levels of individual catechins, caffeine, and gallic acid in white and green teas

Comparison of the quantified catechin and methylxanthine profile of white and green tea methanol and water extracts are provided in Figure 2 and 3 respectively. EGCG had the highest mean value of all catechins quantified in both green and white teas. This compound did not vary significantly in quantity between white and green tea types in their methanol extracts ($P = 0.63$) and water extracts ($P = 0.18$) (see Appendixes 5 and 6).

In the few quantification studies involving both white and green teas, green teas have been reported to be a richer source of phenolics than white teas (Rusak and others 2008; Horzic and others 2009). The limited studies on white tea have mostly based their conclusions on results of analysis of few tea samples and without regard to tea subtype (Hilal and Engelhardt 2007). In the present study, white tea methanol extracts yielded significantly higher mean levels of ECG, C, GCG, and gallic acid ($P < 0.0001$) and relatively higher amounts of EGC and GCG in their water extracts than green teas. White teas also contained higher mean caffeine levels than green teas although the difference was not statistically significant (see Appendixes 5 and 6). Caffeine levels are similar to those published by Saito and others (2007a), using similar extraction solvents. Khokhar and Magnusdottir (2002) reported green teas extracted using aqueous methanol contained caffeine in the range 11 to 20 mg/g, a range covering the amount obtained in this study.

Quantitative analysis of teas reveals strikingly different amounts of most catechins among tea subtypes within the same tea group. For instance, water extracts of certain green teas such as Company D's sencha, Company A's gunpowder and sencha, Company E's green tea, and Company B's gyokuro possessed distinctively high amounts of caffeine, EGCG, and EGC. Most of the white peony subtypes and Company B's silver needle yielded high amounts of EGC and EGCG (see Appendix 7). Methanol extracts of both tea types revealed far less variation among tea subtypes in their catechin and caffeine content. However, certain subtypes as Company C's dragonwell green tea and silver needle white tea, and Company E's jasmine green tea, also possessed distinctively high amounts of EGC, EGCG and ECG (see Appendix 8). These results reveal the individualistic nature of chemical profiles of tea subtypes and make it imperative that further conclusions regarding the comparative amounts of phenolic compounds between green and white teas be made with caution. Khokhar and Magnusdottir (2002) had earlier cited variations in the abundance of compounds in teas as being

**Table 1–TCC (mg/g, dry weight) of green and white tea types and sub-types sourced from 5 major tea companies quantified by HPLC-PDA.**

| Tea company | Tea types and sub-types | TCC[a] – water extracts mass (mg/g dry tea ± SD)[b] | TCC[a] -methanol extracts mass (mg/g dry tea ± SD)[b] |
|---|---|---|---|
| | **Green teas** | | |
| A | Jasmine Pearl | 28.24 ± 0.11 | 51.21 ± 0.23 |
| A | Gunpowder | 80.70 ± 0.08 | 61.05 ± 0.34 |
| C | Gunpowder | 37.73 ± 3.81 | 59.41 ± 0.31 |
| B | Gunpowder | 94.74 ± 0.16 | 32.23 ± 0.34 |
| E | Gunpowder | 188.37 ± 0.44 | 46.21 ± 0.33 |
| A | Gyokuro | 34.03 ± 0.11 | 52.55 ± 0.14 |
| A | Sencha | 166.44 ± 0.61 | 47.54 ± 0.18 |
| C | Sencha Overture | 40.71 ± 5.72 | 74.50 ± 0.67 |
| C | Gyokuro | 78.39 ± 9.34 | 67.47 ± 0.48 |
| C | Dragonwell | 86.82 ± 4.96 | 91.72 ± 7.30 |
| D | Gyokuro | 80.75 ± 16.79 | 57.05 ± 0.31 |
| D | Jasmine Pearls | 21.38 ± 0.21 | 74.24 ± 0.30 |
| D | Sencha | 161.74 ± 2.40 | 73.19 ± 0.66 |
| B | Gyokuro | 179.35 ± 0.20 | 36.90 ± 0.22 |
| B | Dragonwell | 175.01 ± 0.29 | 63.18 ± 0.46 |
| B | Jasmine Green | 142.74 ± 0.19 | 57.16 ± 0.25 |
| E | Jasmine | 149.37 ± 1.97 | 123.42 ± 3.76 |
| E | Green Tea | 228.20 ± 3.32 | 141.24 ± 6.60 |
| | Mean ± SE (mg/g) | 109.71 ± 3.00 | 67.23 ± 1.54 |
| | **White Teas** | | |
| A | Silver Needle | 48.04 ± 0.53 | 76.69 ± 0.59 |
| A | White Peony | 369.60 ± 0.16 | 47.16 ± 0.12 |
| C | Silver Needle | 14.40 ± 4.49 | 163.94 ± 0.55 |
| C | White Peony | 39.27 ± 0.65 | 71.37 ± 0.15 |
| D | Yinzhen Silver Needle | 15.24 ± 0.13 | 49.17 ± 0.10 |
| D | White Peony | 35.50 ± 0.13 | 64.42 ± 0.60 |
| B | Silver Needle | 62.29 ± 0.17 | 72.75 ± 0.66 |
| B | White Peony | 89.64 ± 0.03 | 69.99 ± 0.28 |
| | Mean ± SE (mg/g) | 82.01 ± 12.73 | 76.15 ± 5.61 |

NP = not performed.
[a]TCC is computed by adding means of 7 catechins—EGC, C, EC, EGCG, GCG, ECG, CG.
[b]Mean and standard deviation (SD) of triplicate HPLC injections.

dependent on sample tea subtypes and the different subtypes result from different tea processing protocols, horticultural practices, and geographic settings (Pettigrew 2004; Sultana and others 2008).

Teas from 5 different companies were used in this investigation, and similar tea subtypes possessed varied quantities of phenolics and caffeine depending on the tea company source. For instance, TCC of water extracts of white peony white tea subtype produced by 3 different companies; A, B, and D are 369.6, 89.64, and 35.5 mg/g, respectively, although their catechin content yield in methanol extracts did not vary greatly. Gunpowder green tea subtype from Companies B and E yielded 94.74 and 188.37 mg/g in tea water extracts, and 32.23 and 46.21 mg/g, respectively in their methanol extracts (Table 1). Variation in phytochemical composition among samples of a particular tea subtype from different commercial sources may be the result of storage conditions and storage duration (Friedman and others 2008). Some manufactures follow protocols to ensure freshness of their tea products while other manufacturers store their tea products for extended periods with less attention to optimal storage conditions; the latter practice likely resulting in degradation of phytochemical composition (Friedman and others 2008).

Results from this study support that solvent, extraction protocols, and the source of tea procurement yields variations in the relative amount of compounds in teas. As such, it is suggested that investigations into the chemical profile of teas should follow sampling protocols that include tea samples from various sources and manufacturers and be accompanied with tea subtype specifications, a practice already being adopted by some researchers (Saito and others 2007b; Lin and others 2008).

## Phenolic content and antioxidant activity of white and green teas

White teas yielded mean DPPH $IC_{50}$ values of 36.07 $\mu$g/mL while green teas exhibited significantly ($P = 0.0002$) higher antioxidant activity with $IC_{50}$ values of 23.26 $\mu$g/mL (Figure 4). Gallic and ascorbic acids were used as positive controls resolving scavenging activity with mean $IC_{50}$ values of 3.68 $\mu$g/mL ($r^2 = 0.9943$) and 11.56 $\mu$g/mL ($r^2 = 0.9998$) respectively. Comparable DPPH results from investigations by Saito and others (2007b) on green teas have $IC_{50}$ values ranging from 8.33 to 16.10 $\mu$g/mL, values slightly lower than obtained in our investigation; however, the tea extraction methods differ. Manian and

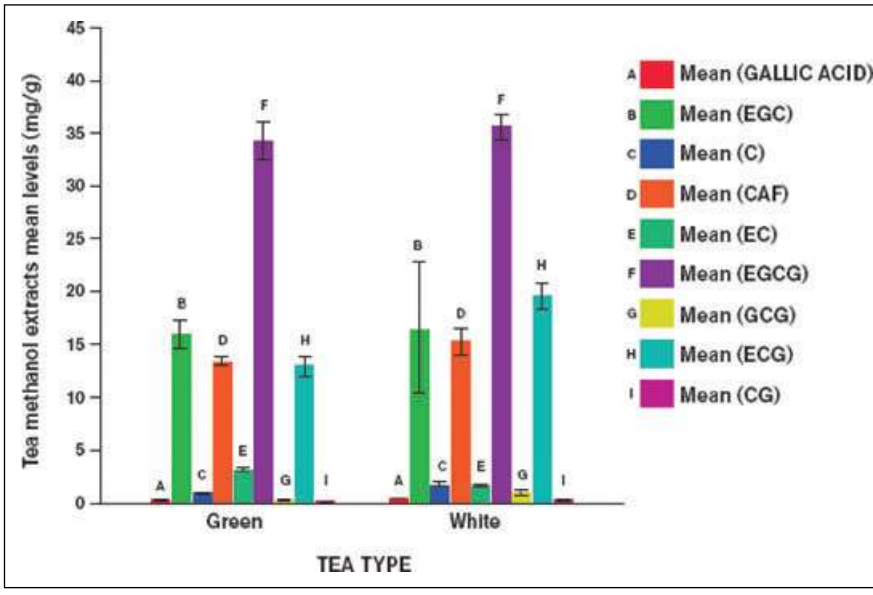


Figure 2–Quantified compound profile of tea methanol extracts for green and white tea types showing mean values (mg/g). Error bars represent 1 standard error from the mean (SEM) values of triplicate injections of sample solutions analyzed by HPLC. Tea sample sizes: $N = 19$ (green tea) and 8 (white tea).

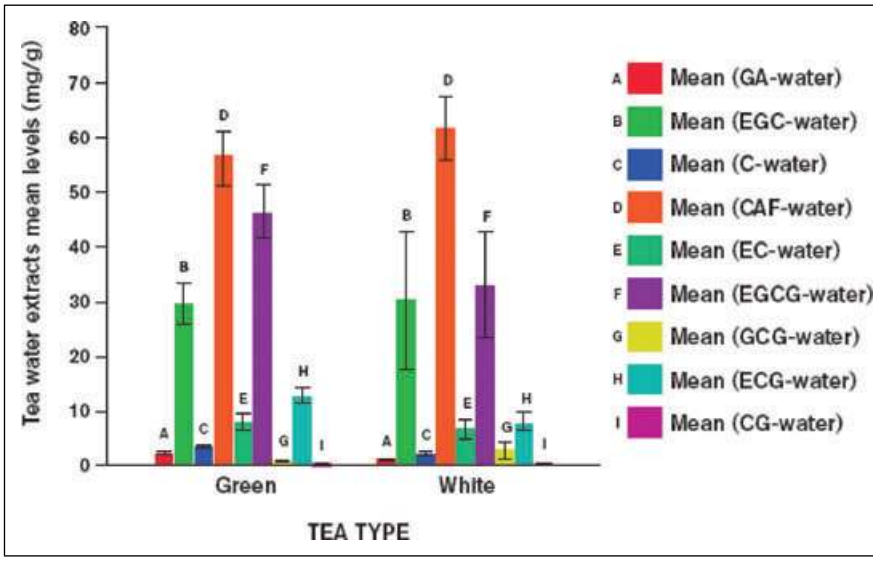


Figure 3–Quantified compound profile of tea water extracts for green and white tea types showing mean values (mg/g). Error bars represent 1 standard error from the mean (SEM) values of triplicate injections of sample solutions analyzed by HPLC. Tea sample sizes: $N = 19$ (green tea) and 8 (white tea).

others (2008) observed DPPH $IC_{50}$ values for green tea extracts of 19.50 $\mu$g/mL with a slightly different extraction method. Green teas also possessed significantly ($P < 0.0001$) higher mean TPC (7.72 mg GAE/g dry tea) than white teas (3.42 mg GAE/g dry tea) (Figure 5). Green tea TPC values range from 1.17 to 18.59 mg GAE/g while the range for white tea TPC was 0.96 to 5.62 mg GAE/g. Rusak and others (2008) reported white tea phenolic content values in the range of 0.4 to 2.1 mg GAE/g although the acid hydrolysis procedure and the varying solvents employed in their sample extraction may account for differences with results obtained in this investigation, a trend also observed in their green tea results (0.8 to 2.4 mg GAE/g). Khokhar and Magnusdottir (2002) reported higher phenolic content levels for green tea (65.8 to 106.2 mg/g) using similar leaf extraction methods as in the present study, but the different values may stem from variations in the F–C assay protocols (Prior and others 2005).

Previous researchers have attributed the DPPH scavenging activity of tea principally to the presence of catechins, especially EGCG (Nanjo and others 1999). This study reveals that although white and green tea subtypes have comparable TCC and EGCG levels, green tea samples possessed significantly higher antioxidant activity. This is likely due to the presence of additional antioxidant compounds such as glycosylated flavonols, proanthocyanidins, and phenolic acids and their derivatives (Lin and others 2008) in green tea as evident by higher TPC levels. According to Lin and others (2008), teas consisting of younger buds and leaves harvested in the early-leaf growing stage (white teas) contained lower levels of these additional phenols than more mature leaves used in tea production (green teas). Further, the researchers reported that hot water infusions of white teas, prepared in a similar way as in this study, would not contain acylated flavonol glycosides, which may result in lower TPC levels and antioxidant activity. Horzic and others (2009) also asserts that the antioxidant capacity of tea is not determined by one or few phytochemical compounds in botanicals, but is widely distributed among a range of phenolics including catechins.

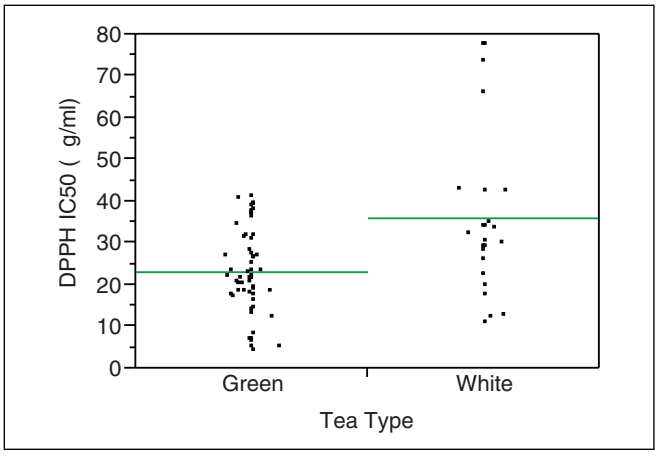


**Figure 4–One-way ANOVA for DPPH $IC_{50}$ among tea types extracted using 100 °C water for 5 min ($F_{1,79} = 15.21$, $P = 0.0002$). Number of samples ($N$) = 19 and 8 for green and white tea samples, respectively. Bars depict mean $IC_{50}$ values expressed in microgram per milliliter.**

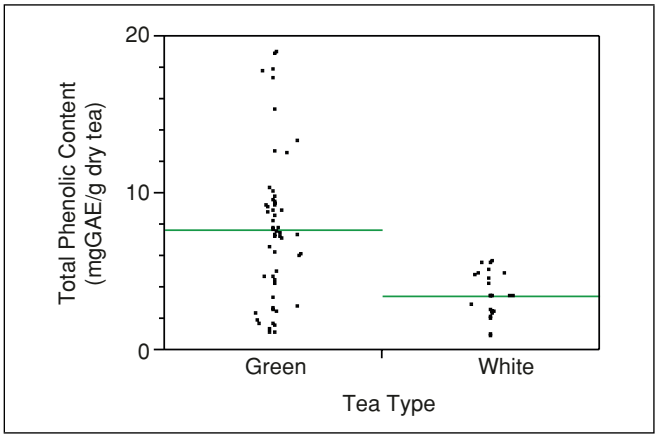


**Figure 5–One-way ANOVA for TPC among tea types extracted using 100 °C water for 5 min ($F_{1,79} = 19.49$, $P < 0.0001$). Number of samples ($N$) = 19 and 8 for green and white tea samples, respectively. Bars depict mean phenolic content values expressed in milligram GAE per gram dry tea.**

To confirm the phenol–redox assay relationship, a multivariate correlation analysis between DPPH $IC_{50}$ values and TPC ($r = -0.3058$) of white tea water extracts in this study demonstrates the increase in antioxidant activity (lower $IC_{50}$ values) of the tea samples as their total phenolic strength increases. However, very low positive correlation obtained between TCC of white tea extracts and their DPPH $IC_{50}$ values ($r = 0.0093$) and TPC ($r = 0.1080$) shows that catechins are not the only compounds responsible for the redox reactions measured in both assays. In contrast, TPC of green tea water extracts was positively correlated with the TCC ($r = 0.6217$) and negatively correlated with DPPH $IC_{50}$ values ($r = -0.4143$). TCC of green tea water extracts contributed to the radical scavenging abilities of the tea type ($r = -0.4845$). Correlation values obtained for green teas are similar to results by Saito and others (2007b) (TCC against DPPH; $r = -0.628$), although sample extraction systems differ slightly.

## Conclusions

This study provides evidence of the dependability of relative amounts of compounds in tea types on factors involved in the cultivation, processing, handling, and packaging of teas that lead to the commercially available subtypes (Khokhar and Magnusdottir 2002). Present findings support that order ranking of tea types for abundance of constituent compounds such as phenolics or methylxanthines would be valid for comparative purposes only if cultivation and tea processing practices can be controlled. For validity purposes, comparisons widely asserted in many publications of the relative amount of phenolic compounds to be in the order of green > oolong > black > white (Lin and others 1998; Lin and others 2003; Rusak and others 2008) or the order black > oolong > green > white (Khokhar and Magnusdottir 2002; Lin and others 2003) with regards to caffeine content, should be based on a sampling protocol inclusive of a range of subtypes procured from various commercial sources. Certain white tea subtypes such as white peony contained high amounts of catechins and caffeine that varied depending on extraction protocols and commercial source. These subtypes generate interest in other white tea types such as the silver-tipped Sri Lankan cultivars (Pettigrew 2004), in possible comparative yields obtainable when white tea cultivars are analyzed along with other tea types, or if white tea sampling sizes are increased.

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## Appendix

(1) Table of calibration curves correlation coefficients, limits of detection (LOD) and quantification (LOQ), selectivity, repeatability, and intermediate precision results of standards.

(2) Data of recovery experiments by Internal Standard Method.

(3) One way analysis of variance for total catechins extracted using water solvent, by tea type.

(4) One-way ANOVA for total catechins extracted using methanol by tea type.

(5) Mean distribution and ANOVA results for significant difference among tea types extracted using aqueous methanol.

(6) Mean distribution and ANOVA results for significant difference among tea types extracted using water.

(7) Masses of quantified compounds (mg/g, dry weight) in green and white tea types and sub-types obtained using water at 100 °C and quantified by HPLC-PDA.

(8) Masses of quantified compounds (mg/g, dry weight) in green and white tea types and sub-types obtained using aqueous methanol and quantified by HPLC-PDA.