JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Effect of Growing and Drying Conditions on the Phenolic Composition of Mate Teas (*Ilex paraguariensis*)

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Mate tea (*Ilex paraguariensis*) has been used for centuries and is widely consumed in Brazil, Argentina, Paraguay, and Uruguay. The aim of the present study was to determine how growing and drying conditions affect the phenolic concentration and antioxidant capacity of 15 Mate teas from forest or plantation cultivations, dried either with hot air or wood smoke. The total polyphenol concentration determined with Folin−Ciocalteu ranged from 100.3 ± 5.5 to 179.7 ± 3.6 mg equiv chlorogenic acid/g dry leaves. The antioxidant capacity according to the oxygen radical absorbance capacity assay ranged from 1.5 ± 0.3 to 4.1 ± 0.1 mmol Trolox equiv/g dry leaves. Ten phenolic compounds were identified and correlated with antioxidant capacity ($R^2 = 0.80$). Principle component analysis and multivariate linear regression were conducted to assess the effect of growing and drying conditions. Sun-exposed (plantation grown) Mate teas exhibited higher levels of all polyphenols as compared to shaded (forest grown) Mate teas ($P < 0.05$). Lower rainfall, temperature, and drying conditions had varying effects on the phenolics. On average, plantation grown Mate teas had a greater concentration of phenolics than forest grown teas. Thus, plantation grown Mate teas represent better potential sources for their commercial extraction.

KEYWORDS: Mate; polyphenols; *Ilex paraguariensis*; growing; drying

INTRODUCTION

Yerba Mate tea (Mate, MT), an herbal tea beverage widely consumed in Southern Latin American countries (Brazil, Argentina, Paraguay, and Uruguay), is gaining rapid penetration into world markets, including the United States. It is made from an infusion of the dried leaves and stems of *Ilex paraguariensis*, a plant in the Aquifoliaceae family (*1, 2*). In Latin America, Mate is often drinken out of a dried gourd using a metal straw called a "bombilla". The dry leaves (DL) (about 50 g) are packed into the gourd, and hot water is poured over them, which is then repeated multiple times, with as much as 0.5 to 1 L of water. In the United States, however, MT leaves are commercially packed in individual tea bags (1−2 g) or as a concentrate for use as an ingredient in food or dietary supplements industries.

Mate is grown in two distinct manners: Mate plantations and harvested from natural forests. The most productive and popular of these methods are the Mate plantations, where Mate is planted in large open areas where it can be manually or mechanically harvested. This method is the most efficient and cost effective. Natural forest harvest consists of harvesting Mate from the surrounding rainforest, and it is the most inconsistent for quality and quantity, although some believe it yields the most natural product. Both methods can be used to produce organic products. Forest grown Mate is widely found in Brazil and to some extent in Paraguay. Plantation cultivation is the major method of production in Argentina and to a large extent in Paraguay (*3*).

Not only is Mate grown differently depending on location, but it is also processed using a variety of methods. Mate leaves are first blanched after harvest to inactivate degradation enzymes, such as polyphenol oxidase, to halt fermentation. This process involves a rotating metal cylinder in close proximity to an open flame and is known as sapeco. The leaves are then further dried to reduce their moisture content in different types of driers, usually continuous rotary or belt driers, or the traditional discontinuous bed drier, called a barbaqua. The process traditionally is done using a wood fire. This wood-drying process is known to give MT characteristic sensory attributes preferable to some Mate consumers. However, newer methods have been developed that use either filtered smoke or air drying, in an effort to reduce the exposure of the tea to smoke. The dry product or canchada is then seasoned for up to a year in cedar, metal, or cement chambers (*4*).

It is known in plant chemistry that growing conditions play a role in the production of phytochemicals of the plant (*5*). It

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10.1021/jf801748s CCC: $40.75
Published on Web 08/30/2008

Table 1. Polyphenol Concentration of MTs from Different Origins and Drying Conditions[a]

Mate	country	location	drying	mg IT/g DL	mg equiv CHA/g IT	mg equiv CHA/g DL
I	Argentina	forest	wood	269.0 ± 14.7 d,e,f	373.1 ± 20.2 d,e	100.3 ± 5.5 j
II	Argentina	plantation	air	271.4 ± 10.1 d,e,f	380.5 ± 16.7 d,e	103.3 ± 7.0 h,i,j
III	Argentina	plantation	wood	271.8 ± 10.5 d,e,f	505.3 ± 29.5 a	137.3 ± 7.0 d
IV	Paraguay	forest	wood	304.1 ± 1.1 c,d	353.4 ± 22.6 e	107.5 ± 6.7 g,h,i,j
V	Paraguay	forest	air	238.4 ± 15.3 f	484.2 ± 32.6 a	115.8 ± 13.4 f,g,h,i
VI	Paraguay	plantation	wood	261.0 ± 5.5 e,f	393.4 ± 26.8 c,d	102.7 ± 7.7 i,j
VII	Paraguay	plantation	air	333.3 ± 5.2 b,c	505.1 ± 17.4 a	168.3 ± 4.1 a,b
VIII	Argentina	plantation	wood	329.6 ± 15.7 b,c	372.6 ± 7.5 d,e	122.8 ± 4.4 e,f
IX	Argentina	forest	wood	306.5 ± 3.9 c,d	346.2 ± 12.2 e	106.1 ± 3.0 h,i,j
X	Brazil	plantation	air	298.2 ± 6.2 c,d,e	403.8 ± 10.4 b,c,d	120.4 ± 4.7 e,f,g
XI	Brazil	forest	air	358.2 ± 16.8 b	431.5 ± 10.7 b,c	154.6 ± 9.0 c
XII	Paraguay	plantation	wood	361.7 ± 3.9 b	434.3 ± 12.5 b	157.1 ± 4.8 b,c
XIII	Paraguay	forest	wood	325.4 ± 3.9 b,c	405.1 ± 22.9 b,c,d	131.8 ± 8.6 d,e
XIV	Paraguay	plantation	wood	437.6 ± 0.3 a	410.7 ± 8.3 b,c,d	179.7 ± 3.6 a
XV	Paraguay	forest	wood	331.1 ± 1.0 b,c	351.7 ± 11.5 e	116.4 ± 3.6 f,g,h

[a] Values represent averages ± standard deviations. Different letters in a column indicate statistical differences, $p < 0.05$. Abbreviations: IT, instant tea; CHA, chlorogenic acid.

has also been shown that adverse growing conditions, such as damage due to insects and other pests, decrease nutrients. Pests also can elicitate phenolic compounds that could have some benefits to human health such as chlorogenic and isochlorogenic acids, which are believed to act as antimicrobials (*6*).

Even excess ultraviolet light can cause the production of compounds designed to protect the plant (*5*). Often, these compounds are phenolics, and many have been shown to possess antioxidant and other properties in other living systems (*7*).

The present study aimed to determine the effect of growing and drying conditions on the phytochemical composition of several MTs grown either on plantations or in the forest and dried using either traditional wood-drying or modern air-drying methods. The results of this study will contribute to improve the quality of MT for commercial production.

MATERIALS AND METHODS

Plant Material. Fifteen different MT products were acquired directly from producers in Argentina, Paraguay, and Brazil from March, 2007, to January, 2008. Each production facility provided representative samples from forest-grown and plantation-grown Mate, with each growing location having tea processed using air and wood drying. **Table 1** presents the codes assigned to each MT (i.e., I, II,..., XV) and their growing and processing conditions.

Chemicals. High-performance liquid chromatography (HPLC) grade water and fluorescein [3′,6′-dihydroxyspiro (isobenzofuran-1[3*H*],9′[9*H*]-xanthen)-3-one] were purchased from Fisher Scientific (Hanover Park, IL). Theophylline (99%) was purchased from Fluka (Milwaukee, WI). Rutin (95%), theobromine (99%), chlorogenic acid (CHA) (95%), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Folin−Ciocalteu's phenol reagent (2 N), HPLC grade methanol, and formic acid (>95%) were purchased from Sigma Chemical Co. (St. Louis, MO). AAPH [2,2′-azobis (2-amidinopropane) dihydrochloride] and caffeine (99%) were purchased from Aldrich (Milwaukee, WI).

Preparation of MTs. The MT brewing process was carried out following the protocol described by Chandra and Gonzalez de Mejia (*8*). Briefly, dried leaves were kept in plastic bags at 4 °C. Tea extracts were prepared from 2.7 g of DL that were soaked in 250 mL of boiling water (98 °C) for 10 min. The mixture was cooled to room temperature before filtration using Whatman-1 filter paper, and the filtrate was lyophilized in a Labconco FreeZone 6 L freeze-dry system (Kansas City, MO). The freeze-dried teas (IT) were kept at −20 °C in plastic tubes, sealed with parafilm, and protected from light. All teas were prepared in duplicate preparations and analyzed in triplicate.

Total Polyphenol Concentration (TPC). The total phenolic concentration was measured by the Folin−Ciocalteu method, adapted to a microassay, from the method described by Chandra and Gonzalez de Mejia (*8*). Briefly, samples were prepared from dry tea powder by dissolving in water and filtering with a 0.2 μM syringe filter. To a 96 well flat bottom plate (Fisher 12-565-501), 50 μL of 1 N Folin−Ciocalteu's phenol reagent was added, and then, 50 μL of either sample, standard, or blank was added; this mixture was allowed to stand for 5 min before the addition of 100 μL of 20% Na_2CO_3. The solution was then allowed to stand for 10 min before reading at 690 nm in an ELX 808_{IU} microplate reader spectrophotometer (Bio-Tek, Winooski, VT). CHA was used as a standard with final concentrations of 2.5, 5, 7.5, 10, 12.5, 15, and 17.5 μg/mL. Results were expressed as either mg equiv CHA per g instant tea (IT) (mg equiv CHA/g IT) or as mg equiv CHA/g DL, using the standard curve $y = 0.0087x - 0.0348$, $R^2 = 0.99$.

Identification and Quantification of Phenolic Compounds. Identification of phenolic compounds was conducted with HPLC adapted from Chandra and Gonzalez de Mejia (*8*). Analysis was conducted using a 1050 Hewlett-Packard (Palo Alto, CA) gradient liquid chromatograph, equipped with a 1050 HP autosampler, a 1050 HP gradient pump, a 1050 HP photodiode array detector (PDA), and helium sparge. A C_{18} RP guard column and a C_{18} RP Phenomenex Prodigy ODS column (250 mm × 4.6 mm × 5 μm) were used. The column temperature was kept at ambient temperature, and the flow rate was 0.9 mL/min and was performed with a solvent gradient. The solvent gradient consisted of solvent A (water/methanol/formic acid, 79.7/20/0.3) and B (methanol/formic acid, 99.7/0.3) mixed, starting with 0% B, linearly increasing to 52% B in 50 min, increasing to 80% B in 5 min, and held at 80% B for 3 min, then a linear decrease to 0% B in 5 min and held at 0% B for 5 min. The PDA detector was set to read from 195 to 450 nm with outputs at 260, 280, and 330 nm. Peaks were compared to retention times of standards to determine identity.

The compound concentration was calculated using standard curves of commercially available standards. Compounds were quantified, and their respective standard curves were as follows: caffeine ($y = 3 \times 10^6x + 17296$, $R^2 = 0.999$), CHA ($y = 1 \times 10^6x + 82582$, $R^2 = 0.98$), rutin ($y = 857094x - 3781$, $R^2 = 0.999$), and theobromine ($y = 1 \times 10^6x - 4237$, $R^2 = 0.999$). No commercial standard was available for dicaffeoylquinic acid.

HPLC-MS was conducted to confirm the identities of peaks. The LCQ Deca XP mass spectrometer (Thermo Finnigan Corp., San Jose, CA), MS version 1.3 SRI electrospray ionization (ESI) in positive and negative mode (*m*/*z* 150−2000), version 1.2, and autosampler version 1.2 were used. The same column, solvent, gradient, and flow rate were used as in HPLC analysis; the injection volume was 10 μL. Data analysis was conducted with Xcalibur software. Identification was based on molecular weight, retention times, UV−VIS spectra, and comparison with the literature.

Antioxidant Capacity. The oxygen radical absorbance capacity (ORAC) assay was adapted from published methods (*9, 10*). Briefly, 18.81 mg of fluorescein was dissolved in 50 mL of 75 μM phosphate buffer, pH 7.4. Next, 0.109 g of AAPH was dissolved in 10 mL of 75 μM phosphate buffer, pH 7.4. In triplicate, 20 μL of tea sample (0.5 μg/mL final concentration), Trolox standard (6-hydroxy-2,5,7,8-tet-

ramethylchroman-2-carboxylic acid) dissolved in 75 μM phosphate buffer, pH 7.4 (1−8 μM final concentration), or 75 μM phosphate buffer, pH 7.4, blank was added to a 96 well black-walled plate (Fisher #07-200-590), followed by 120 μL of fluorescein (70 nM final concentration), and was incubated with a cover for 15 min at 37 °C. After incubation, 60 μL of AAPH (12 mM final concentration) was added and read immediately in the fluorescent plate reader, FLx800tbi (Bio-Tek, Winooski, VT), at 37 °C, sensitivity 60, and was read every 2 min for 120 min with excitation at 485 nm and emission at 528 nm. Fluorescein reacted with free radicals generated by AAPH yielding a nonfluorescent product. The loss of fluorescence was measured over time, and the area under the curve was calculated using the following equation:

$$\mathrm{AUC} = 1 + f_1/f_0 + f_i/f_0 + \ldots + (f_n/f_0)^1$$

$$\text{net AUC} = \mathrm{AUC}_{\text{sample}} - \mathrm{AUC}_{\text{blank}}$$

where AUC is the area under the curve, f_1 is the fluorescence of the first reading (2 min), f_0 is the fluorescence of the reading at time zero, and f_n is n fluorescence readings. Results were expressed as μM Trolox equiv (TE) based on the standard curve for trolox $y = 2.77x + 0.31$, $R^2 = 0.99$.

Statistical Analysis. All statistical methods were conducted using SAS 9.1.3 software (SAS Institute Inc., Cary, NC). The statistical difference was tested using the proc glm procedure in SAS followed by posthoc analysis using Tukey's test. The α was 0.05. Principle component analysis (PCA) was conducted and used a Varimax rotation, the most popular rotation, to simplify interpretation by associating each variable with one or as few as possible components. This rotation searches for a linear combination of the components to maximize the variance of the variable loadings. Utilizing the principle components (PCs), multivariate linear regression was conducted. Linear regression uses mathematical modeling to model the parameters of the data to the independent variables.

$$y = \beta_0 + \beta_1 x_1 + \ldots \beta_n x_n + \varepsilon$$

where y = the dependent variable, β_0 = the intercept, β_1 = the parameter estimate for variable 1, x = the independent variable, βn = the parameter for n variables, and ϵ = the observational error. For nominal variables such as growing conditions, dummy variables were created for the regression.

This method allowed for the determination of how much the variables affected the data and their effects. Pearson's correlation analysis was then conducted to determine the correlation that the significant variable had on the data.

RESULTS AND DISCUSSION

TPC. MT (VIII) prepared with 10 g DL/L represented traditional tea bag American style preparations and yielded 2.0 mg equiv CHA/mL and 200.7 mg equiv CHA/g DL. MT (VIII) prepared using 30 g DL/L showed the highest level of extraction, 5.9 mg equiv CHA/mL and 197.8 mg equiv CHA/g DL. Thus, 30 g DL/L had a 3-fold increase in concentration per mL with roughly the same extraction rate. However, as the amount of solids in solution increased, the extraction efficiency decreased by 25%, from approximately 200 mg equiv CHA/g DL (30 g DL/L) to 150 mg equiv CHA/g DL (50 g DL/L), respectively. These data can facilitate the commercial optimization of extracted polyphenols by using an appropriate amount of DL per volume of water.

TPC in MTs from Different Origins and Drying Conditions. Table 1 presents the amount of IT extracted from 2.7 g of tea leaves in 250 mL of water and TPC for the tea preparations expressed as mg equiv CHA/g IT and mg equiv CHA/g DL. The concentration of polyphenols in the 15 MT studied ranged from 100.3 ± 5.5 to 179.7 ± 3.6 mg equiv CHA/g DL. The concentrations of TPC in teas used in this investigation were higher than previously reported (77.6 ± 2.7 to 81.2 ± 4.0 mg equiv gallic acid/g DL) (*11*). The higher concentrations in this study may be attributed to the use of CHA as the standard for quantification, known to be in high quantities in MT, while Bravo et al. (*11*) used gallic acid. Gallic acid is a flavonoid, and because MT contains little flavonoids, the previous TPC of MT may have been underestimated. Bastos et al. (*12*) reported TPC by Folin−Ciocalteu at roughly 7 mg equiv CHA/mL, from 5 g/100 mL; this is in agreement with the concentration, 7.5 ± 0.01 mg equiv CHA/mL, found in fresh tea prepared with 50 g DL /L in this study.

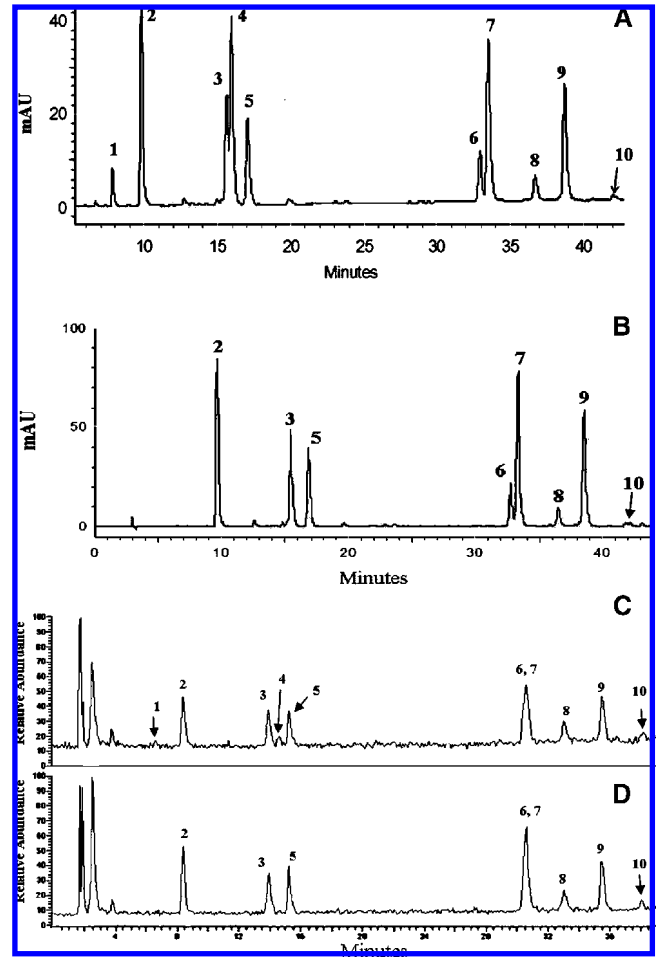

Figure 1. HPLC chromatogram and LC-MS profile of MT. (**A**) 280 nm, (**B**) 330 nm, (**C**) positive ion, and (**D**) negative ion. Peaks: 1, theobromine; 2, neo-CHA; 3, CHA; 4, caffeine; 5, crypto-CHA; 6, 3,4-dicaffeoylquinic acid; 7, 3,5-dicaffeoylquinic acid; 8, rutin; 9, 4,5-dicaffeoylquinic acid; and 10, luteolin diglycoside.

Characterization and Quantification of Polyphenols. Figure 1 presents the HPLC and LC-MS profiles of MT and the 10 major phenolic compounds identified. These identities were confirmed by comparing retention times, absorbances at 280 (**Figure 1A**) and 330 nm (**Figure 1B**), and elution patterns with the literature (*13*). It should be noted that no distinguishable differences in the number of peaks at 260 and 280 nm could be identified; only a lower absorbance at 260 nm was identified; thus, 260 nm was not used for further identification. Caffeine and theobromine were only detected at 280 nm (**Figure 1A**) but not at 330 nm (**Figure 1B**), allowing for the differentiation between the CHA peak and the caffeine peak, since they eluted nearly simultaneously. To further confirm the identity of these compounds, LC-MS was conducted, utilizing the same protocol as HPLC. Using positive ion mode (**Figure 1C**) and negative ion mode (**Figure 1D**) ESI, compounds were identified based

Table 2. Phenolic Compounds Identified in All MTs by LC-MS

peak	RT (min)	λ (nm)	$[M - H]^+$	$[M - H]^-$	fragment ions[a]	compound
1	6.8	280	181	N/A	none	theobromine (3,7-dimethylxanthine)
2	8.6	280, 360	355	353	163^+, 372^+, 179^-, 191^-, 707^-	neo-CHA (5-caffeoylquinic acid, CQA)
3	14.1	280, 360	355	353	163^+, 372^+, 179^-, 191^-, 707^-	CHA (3-CQA)
4	14.7	280	195	N/A	none	caffeine (1,3,7-trimethylxanthine)
5	15.4	280, 360	355	353	163^+, 372^+, 179^-, 191^-, 707^-	crypto-CHA (4-CQA)
6	30.5	280, 360	517	515	163^+, 354^+, 1050^+, 179^-, 191^-, 353^-, 1031^-	dicaffeoylquinic acid (3,4-dicaffeoylquinic acid)
7	30.6	280, 360	517	515	163^+, 354^+, 1050^+, 179^-, 191^-, 353^-, 1031^-	dicaffeoylquinic acid (3,5-dicaffeoylquinic acid)
8	33.0	280, 360	611	610	300^-, 722^-	rutin (quercetin-3-rhamnoglucoside)
9	35.4	280, 360	517	515	163^+, 354^+, 1050^+, 179^-, 191^-, 353^-, 1031^-	dicaffeoylquinic acid (4,5-dicaffeoylquinic acid)
10	37.8	280, 360	595	593	287^+, 284^-	luteolin diglycoside

[a] + and − indicate ion mode. Abbreviations: RT, retention time.

on retention times, major ions, and fragment ions. **Table 2** lists the 10 peaks identified, their retention times (RT), wavelengths (λ), major ions, fragment ions, and compound names.

Peak 1 with a retention time of 6.8 min at 280 nm but not at 360 nm, characteristic of methylxanthine compounds, showed a $[M - H]^+$ ion in the ESI-MS positive mode at *m/z* 181; the positive ion mode adds one proton to the mass of the compound; therefore, the true *m/z* is 180, positively identifying this compound as theobromine.

Peaks 2, 3, and 5, at 8.6, 14.1, and 15.4 min, respectively, showed similar ion patterns in both positive and negative modes and absorbed at both 280 and 360 nm. Their $[M - H]^+$ and $[M - H]^-$ were *m/z* 355 and *m/z* 353, indicating a molecular weight of 354, corresponding to CHA (caffeic acid esterified to quinic acid). To further confirm this, fragment ions at *m/z* 179 and 191 in negative mode would correspond to caffeic acid and deprotonated quinic acid, respectively. A fragment ion at *m/z* 372 in positive ESI is indicative of the CHA molecule plus water and at *m/z* 163 in positive ESI would be a dehydrated caffeic acid. The final fragment ion associated with CHA was 707 in the negative ESI, corresponding to a dimer of CHA. This evidence presents three peaks as isomers of CHA. Identification of these compounds as neo-CHA, 5-CQA, CHA, 3-CQA, crypto-CHA, and 4-CQA was obtained from the literature (*11, 14*). Peak 4 eluted at 14.7 min but only at 280 nm, again characteristic of methylxanthine compounds. It generated a $[M - H]^+$ ion of *m/z* 195. Thus, on the basis of its retention pattern in comparison with pure standards and a molecular weight of 194, this compound was positively identified as caffeine. Peaks 6, 7, and 9 eluted at 30.5, 30.6, and 35.5 min, respectively, in HPLC; however, in LC-MS, peaks 6 and 7 eluted simultaneously; thus, peaks 6/7 and 9 showed similar ion patterns (**Table 2**). Each generated an $[M - H]^+$ and $[M - H]^-$ of *m/z* 517 and 515, indicating a molecular weight of 516, which is two caffeic acids esterified to a quinic acid, providing the identity of these compounds as isomers of dicaffeoylquinic acid. Fragment ions were similar to CHA, with *m/z* 179, 191, and 163. A fragment ion of *m/z* 354 in positive mode and *m/z* 353 in negative mode corresponded to that of CHA due to a loss of a dehydrated caffeic acid (*m/z* 179) from the dicaffeoylquinic. Ions at *m/z* 1050 and 1031, in negative and positive modes, respectively, would correspond to dimers of the dicaffeoylquinic acids with *m/z* 1050 being the addition of water. According to the literature, peaks 6, 7, and 9 were likely 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and 4,5- dicaffeoylquinic acid, respectively (*11*). Peak 8 eluted at 33 min and absorbed at 280 and 360 nm. It generated a $[M - H]^+$ and $[M - H]^-$ ion at *m/z* 611 and 610, respectively. The ion corresponds to the molecular weight of rutin, 610.5. Rutin is a flavonoid glycoside composed of quercetin bound to a sugar (quercetin-3-rhamnoglucoside); the fragment ion of *m/z* 300 in the negative mode corresponds to quercetin. This identification was again compared with Bravo et al. (*11*) and with pure standards.

The final peak identified in these teas was peak 10, which eluted at 37.8 min and again absorbed at 280 and 360 nm. It generated $[M - H]^+$ and $[M - H]^-$ ion at *m/z* 595 and 593, respectively; a fragment ion at 287 in positive and 284 in negative indicate the loss of a glucosyl residue leaving luteolin (molecular weight 286). There was no standard available for this compound; thus, its identification as luteolin diglycoside was based solely in the literature. As a result, the precise conformation is not known (*13*).

Rutin and luteolin diglycoside were the only two flavonoids identified in MTs. Two methylxanthines, caffeine and theobromine, were found, although theophylline was not, as well as six caffeoyl derivatives, three isomers of CHA, and three dicaffeoylquinic acid isomers. The compounds found in this study correlated with those found in previous literature (*15*). **Figure 2** presents the chemical structures of compounds identified in MT. Bravo et al. (*11*) identified several other flavonols, including kaempferol glycosides, which were not found in this study. However, they used in their study organic solvent extracts, which may have facilitated the extraction of much less water soluble compounds than would be found in traditional Mate infusions, such as those tested in this study. Other methods such as capillary electrophoresis have successfully been used in the separation of organic acids with high efficiency and sensitivity (*16*).

In comparison to green tea (GT), MT possesses no catechin compounds and very little flavonols. Catechins are the predominant polyphenols in GT and have been shown to contribute to the high antioxidant capacity (*8*). MT on the other hand was shown to contain high levels of caffeoyl derivatives. Both GT and MT have been shown to contain flavonols, although GT has much more diversity, quercetin, kaempferol, and myricetin. While those in MT, such as rutin, are present to a much lesser extent. Even though MT does not contain catechins, it has been shown to have a high antioxidant capacity and to be hypocholesterolemic and hepatoprotective, to be a central nervous system stimulant and diuretic, and to benefit the cardiovascular system (*17*).

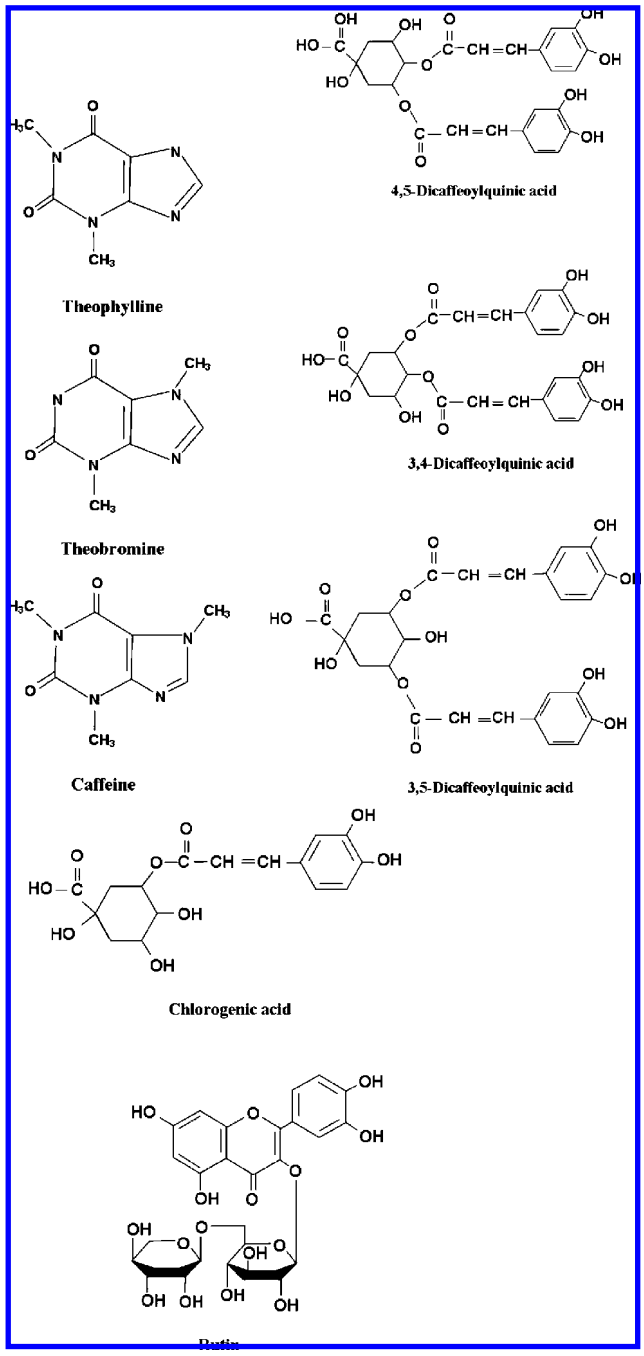

Figure 2. Chemical structures of the identified compounds in MT.

Specific Polyphenols in MTs. From the 10 phenolic compounds identified with HPLC and LC-MS, six were able to be quantified with pure standards, theobromine, caffeine, rutin, and the three CHA isomers. Because all three CHA isomers have the same molecular weight, they were quantified against pure CHA. **Table 3** lists the concentration of each individual quantified phenolic compound and total CHA, as the sum of all three isomers.

While there have been several studies on the identification of phenolic compounds in MT, there are not comprehensive studies on the total quantification of these substances. For the two methylxanthine compounds found in MT, theobromine ranged from 1.5 ± 0.1 (XIII) to 7.6 ± 0.3 (XIV) mg/g DL ($P < 0.05$) and caffeine from 3.9 ± 0.2 (XI) to 16.7 ± 0.9 (XIV) mg/g DL ($P < 0.05$). Tea XIV from Paraguay plantation wood-dried showed the highest concentration of both caffeine and theobromine. The three CHA isomers identified in the teas each showed varying levels across all teas ($P < 0.05$). Neo-CHA ranged from 33.0 ± 0.7 (VIII) to 6.9 ± 0.7 (XV) mg/g DL, CHA from 24.9 ± 0.7 (VIII) to 4.8 ± 1.1 (XIII) mg/g DL, and crypto-CHA from 41.3 ± 2.3 (XIV) to 4.6 ± 0.4 (XI) mg/g DL. The total CHA of all three isomers combined was 80.5 ± 5.3 (XIV) to 20.9 ± 2.2 (XIII) mg/g DL. Again, tea XIV showed the highest combined total of all CHA. As with the methylxanthines, the lowest level of combined CHA was a forest grown product. The final individual quantified phenolic compound was rutin, and again, tea XIV was the highest with 11.5 ± 1.1 mg/g DL, and the lowest was a forest grown product (V) ($P < 0.05$).

Overall, tea XIV, a plantation grown product, showed the highest level of nearly all polyphenols, except neo-CHA and CHA. This tea also showed the highest TPC, 179.7 ± 3.6 mg equiv CHA/g DL, and the lowest was tea I from an Argentinian forest wood-dried, 100.3 ± 5.5 mg equiv CHA/g DL (**Table 1**). It should be noted however, that other polyphenols that were present in MT, specifically dicaffeoylquinic acids, would contribute highly to the TPC assay but were not quantified in these teas. Thus, teas showing a low level of total CHA but a high level of TPC had higher levels of dicaffeoylquinic acids based on HPLC results. For example, tea XIII had the lowest total CHA, 20.9 ± 2.2 mg/g DL, but had a modest TPC value of 131.9 ± 8.6 mg equiv CHA/g DL.

PCA. MT is an agricultural product, and as such, it is influenced by countless variables during its cultivation. These variables include geographical location, rainfall, temperature, elevation, sun exposure, soil composition, and many more. Recent evidence has shown that the macro- and micronutrient concentrations are affected by whether the plant is grown in shade or exposed to full sun as well as fertilizer conditions; shaded plants had a higher level of minerals, and the cultivation and age of leaves were major influences on these compounds (*18*). Despite this evidence, it should be mentioned that current studies have focused on one or two variables without consideration for the interactions. Not only do the growing conditions affect the properties of the teas, but the drying characteristics can play a role (*19*).

PCA is a powerful statistical tool for determining the effects that variables have on a set of data. This study used PCA to assess the effects of six variables on phenolic concentration such as growing location (plantation or forest), average annual rainfall (in.), average annual temperature (°C), elevation (m), country of origin, and drying (wood or air-dried) conditions. These six variables were determined to be reasonable criteria that would likely have influence on the growth of the plant and thus possibly affect the level of polyphenols.

It is generally accepted in PCA analysis that an eigenvalue greater than 1 indicates a significant effect on a component. Only five PCs had Eigenvalues greater than 1; thus, these were the only factors retained. The five PCs accounted for a total of 85.4% of the total variance in the data. The independent variables growing location, elevation, temperature, rainfall, drying process, and country had significant effects on the data, while the phenolic concentrations themselves did not influence each other.

The level of significance (load) that a PC had on a variable can be noted by looking at the loading plots of the Eigenvectors in **Figure 3**. These plots show the load that each set of data places on the PC. The further the data is along the PC axis indicates a higher load and thus more of an effect by that PC on the data. PC1 corresponds to the growing location, plantation or forest grown, explaining 36% of the variance. PC1 has all of the data loaded on the middle to upper half of the +1 axis,

Table 3. Concentrations of Theobromine, Caffeine, Rutin, CHA, and CHA Isomers in MTs[a]

	mg/g DL						
Mate	theobromine	caffeine	rutin	neo-CHA (5-CQA)	CHA (3-CQA)	crypto-CHA (4-CQA)	total CHA[b]
I	2.9 e	11.7 b,c,d	3.3 g,h	21.4 c,d	16.6 b,c	10.2 e,f,g	48.1 c,d
II	4.5 c	7.5 f	6.0 c,d,e	16.1 e,f	12.2 e,f,g	9.8 f,g	38.1 e,f
III	7.0 b	8.4 e,f	6.5 c	18.4 d,e	10.3 f,g,h	9.4 g	38.0 e,f
IV	2.5 e,f	8.0 e,f	5.6 c,d,e,f	18.8 c,d,e	14.7 b,c,d,e	10.1 f,g	43.6 d,e
V	3.8 d	10.8 c,d,e	2.6 h	13.2 f,g	10.0 g,h,i	9.0 g	32.2 f
VI	3.6 d	8.3 e,f	5.4 c,d,e,f	16.8 e	11.3 e,f,g,h	8.8 g	36.9 e,f
VII	7.1 a,b	12.4 b,c	8.8 b	21.9 c	13.7 d,e,f,g	11.6 d,e,f	47.2 c,d
VIII	4.1 c,d	8.2 e,f	8.0 b	33.0 a	24.9 a	17.0 c	74.8 a
IX	2.5 e,f	10.5 c,d,e,f	4.4 f,g	26.6 b	15.6 b,c,d	12.2 d	54.4 b,c
X	7.3 a,b	16.6 a	4.3 f,g	31.4 a	13.9 c,d,e,f	12.2 d,e	57.3 b
XI	2.1 f	3.9 g	4.6 e,f,g	12.0 g	6.6 i,j	4.6 h	23.0 g
XII	2.7 e	14.6 a,b	6.3 c,d	32.5 a	17.8 b	13.0 d	32.3 b
XIII	1.5 g	8.9 d,e,f	5.2 c,d,e,f	11.0 g	4.8 j	5.2 h	20.9 g
XIV	7.6 a	16.7 a	11.5 a	17.6 e	21.6 a	41.3 a	80.5 a
XV	2.4 e,f	12.1 b,c	4.9 d,e,f	6.9 h	8.1 h,I,j	20.4 b	35.5 f

[a] Values represent averages, $n = 3$. Different letters indicate statistical difference, $P < 0.05$. [b] Total of neo-CHA, CHA, and crypto-CHA.

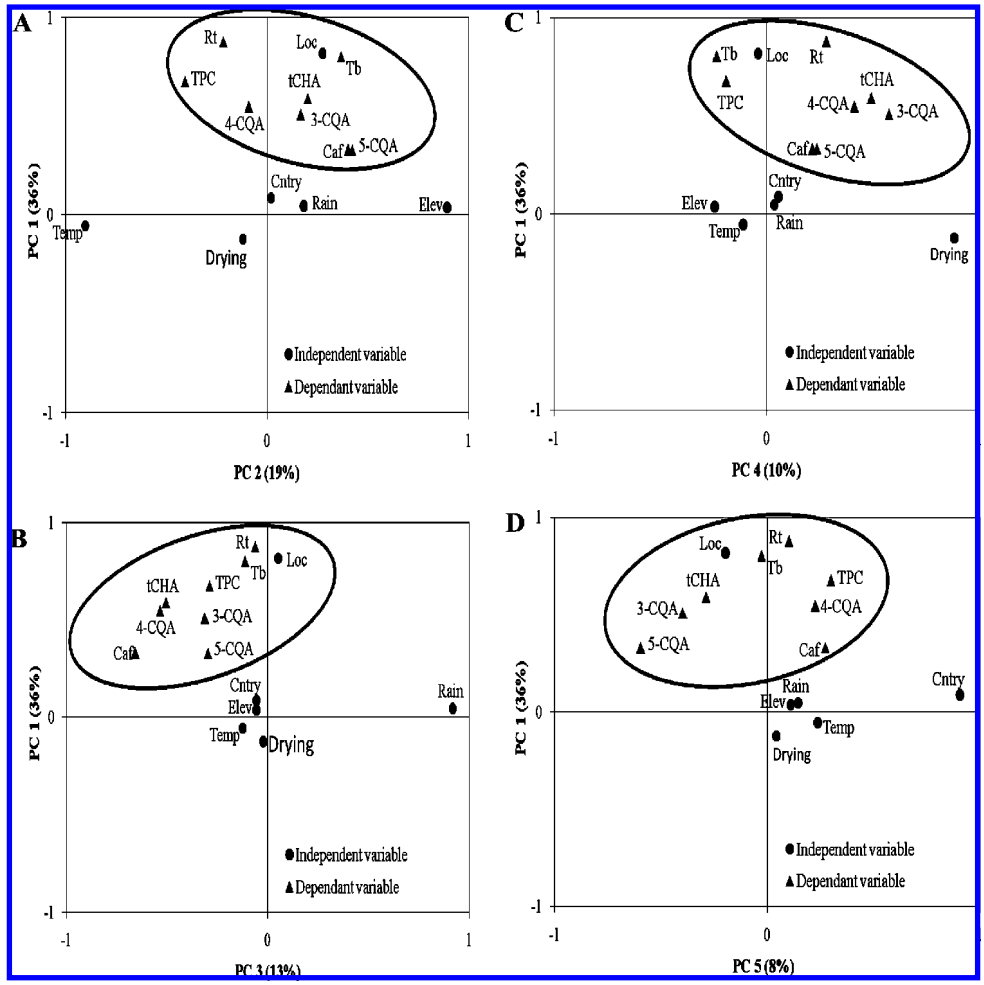

Figure 3. PCA of phenolic compounds in MT. Loadings plots for each component and its respective percentage of variance. Abbreviations: tCHA, total CHA; TPC, total polyphenols determined by Folin−Ciocalteu; Temp, temperature; Loc, location; Rt, rutin; Tb, theobromine; Caf, caffeine, Cntry, country; and Elev, elevation.

indicating that this PC has a large effect on all of the data, as indicated by the ellipses on all of the plots. In **Figure 3A**, two variables, temperature and elevation, appear on the horizontal axis of PC2, explaining 19% of the variance. They had an absolute eigenvector greater than 0.30, −0.90 and 0.89, respectively. Temperature and elevation are inversely related; as elevation increased, temperature decreased. This is not surprising, and because of this collinearity, elevation and temperature were combined as PC2. Thus, temperature was the variable used in further analysis. The data were weighted more heavily on the positive end of the temperature/elevation axis, indicating that many of the variables have a negative association with the variable temperature. For PC3, -4, and -5, they had only one variable associated with their axis; all others revolved around the center (**Figure 3B−D**). PC3, eigenvector 0.92, accounted for 13% of the variance, and the data were strongly

loaded to the negative side of the *x*-axis, while the variable rain was on the +1. This means that phenolic compounds were negatively associated with rainfall. PC4, eigenvector 0.88, accounted for 10% of the variance with drying condition being on the +1 *x*-axis. The variables were loaded toward the positive end of the axis. Finally, PC5 eigenvector 0.91 accounted for only 8% of the variance with country of origin being on the +1. The phenolics were distributed on both the negative and the positive of the axis.

PCA analysis demonstrated that independent variables were able to explain 85% of the variance in the data. The PCs generated were then used in multivariate linear regression to determine the specific effect that each variable (growing location, average annual rainfall, average annual temperature, and drying condition) had on the phenolic concentration, independent of one another.

Multivariate Linear Regression. For this analysis, because the variables of growing location, drying condition, and country of origin were categorical variables, they were converted to dummy variables. PCs were assigned a parameter (β_n) for linear regression: growing location (β_1), drying conditions (β_2), average annual rainfall (β_3), average temperature (β_4), and country of origin (Brazil, β_5, and Paraguay, β_6) with the slope coefficient (β_0) representing Argentina. The regression equation for these variables was as follows:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4 + \beta_5 x_5 + \beta_6 x_6$$

Collinearity analysis was then run on this regression to ensure that none of the independent variables were linearly correlated with one another. A proportion of variance that is high for a variable with an index value greater than 20 presented a high rate of collinearity. Brazil and Paraguay had index values of 25.0 and 56.3, respectively. Brazil had a high proportion of variation with rainfall, 0.51, and Paraguay was highly correlated with temperature, 0.72, and rainfall, 0.48. Because of this multicollinearity, the variable of country, β_5 and β_6, was dropped from the multivariate equation; this also removed Argentina as the intercept value. The new equation generated was then as follows:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_4 x_4$$

where β_0 is the intercept, β_1 is the growing location, β_2 is the drying condition, β_3 is the average annual rainfall, and β_4 is the average temperature. Collinearity diagnostics of the new equation showed no collinearity of variables between each other. **Table 4** presents the linear regression estimates for each set of data. Growing location had a significant effect on all polyphenols and TPC ($P < 0.05$). The drying condition (process) showed a significant effect on theobromine, rutin, 3-CQA, 4-CQA, and total CHA. Average annual rainfall for the growing region showed an effect on the levels of caffeine and all three CQAs. Finally, average annual temperature affected theobromine, caffeine, 5-CQA, rutin, total CHA, and TPC. Other than growing location, temperature was the only parameter to affect TPC, even though the other parameters had a wide-ranging effect on the other polyphenols, suggesting that other polyphenols, such as dicaffeoylquinic acids, likely have a great impact on TPC.

The data showed that different conditions like rainfall and temperature play different roles in the level of phenolics produced in MT. To determine the extent of the effect on these phenolic compounds, Pearson's correlation (R^2) was conducted on the linear regression data (**Table 4**). Because of the fact that these results were gathered on agricultural data, this study determined a correlation of 0.7−1 for a strong effect, 0.3−0.7 for a moderate effect, and less than 0.3 for a very minimal effect. Rain was negatively associated with all of the variables. For caffeine, rain had a moderate negative association with concentration (−0.40); this means that as the amount of rainfall increased, the level of polyphenols decreased; also, as temperature increased, TPC value increased.

Table 4. Linear Regression Parameter Estimates and Statistics[a]

variable	parameter estimate	*t* value	Pr > |t|	Pearson R^2
	theobromine			
intercept	11.22	4.21	<.0001	
location	2.59	8.78	<.0001	**0.72**
process	−1.05	−3.52	0.0007	−0.29
rain	−0.03	−1.54	0.1279	−0.02
temp (°C)	−0.26	−3	0.0036	**−0.39**
	5-CQA			
intercept	80.39	6.73	<.0001	
location	5.82	4.4	<.0001	**0.49**
process	1.28	0.96	0.3417	0.08
rain	−0.39	−3.91	0.0002	−0.23
temp (°C)	−1.86	−4.79	<.0001	**−0.46**
	3-CQA			
intercept	28.89	3.09	0.0027	
location	4.66	4.51	<.0001	**0.44**
process	3.56	3.4	0.001	0.28
rain	−0.16	−2.09	0.0396	−0.18
temp (°C)	−0.47	−1.56	0.123	−0.24
	4-CQA			
intercept	27.69	1.98	0.051	
location	6.21	4.01	0.0001	0.30
process	5.17	3.3	0.0014	0.30
rain	−0.52	−4.53	<.0001	**−0.45**
temp (°C)	0.49	1.07	0.2872	0.08
	caffeine			
intercept	39.38	6.65	<.0001	
location	1.32	2.01	0.0473	0.28
process	0.68	1.03	0.3055	0.12
rain	−0.25	−5.1	<.0001	**−0.40**
temp (°C)	−0.67	−3.49	0.0008	−0.29
	rutin			
intercept	−7.26	−2.25	0.0271	
location	3.32	9.28	<.0001	**0.60**
process	1.18	3.27	0.0015	0.18
rain	0.01	0.28	0.7813	−0.07
temp (°C)	0.46	4.37	<.0001	0.12
	Tot CHA[b]			
intercept	136.97	5.65	<.0001	
location	16.69	6.22	<.0001	**0.51**
process	10.01	3.69	0.0004	0.27
rain	−1.07	−5.34	<.0001	**−0.39**
temp (°C)	−1.85	−2.34	0.0216	−0.25
	TPC[c]			
intercept	−42.27	−1.1	0.2724	
location	26.50	6.25	<.0001	**0.34**
process	−4.49	−1.05	0.2971	−0.12
rain	−0.49	−1.54	0.1281	−0.24
temp (°C)	8.63	6.93	<.0001	**0.45**

[a] A P value < 0.05 indicates a significant effect in the model. Abbreviations: Tot CHA, total CHA; TPC, total polyphenols; Temp, temperature. A positive Pearson correlation coefficient (R^2) indicates a positive linear relationship, and a negative slope indicates a negative linear relationship. Bold values indicate a correlation greater than 0.3, signifying a moderate to strong effect. [b] Total of neo-CHA, CHA, and crypto-CHA. [c] Determined with Folin−Ciocalteau.

Figure 4 presents the relationship of the dummy variables for growing location, forest and plantation, and drying condition, air-dried and wood-dried, for some of the phenolic compounds. For caffeine (**Figure 4A**), theobromine (**Figure 4B**), total CHA (**Figure 4C**), and TPC (**Figure 4D**), the slope rises from forest to plantation. This means that the plantation grown MT exposed to full sun had higher levels of phenolic compounds than the

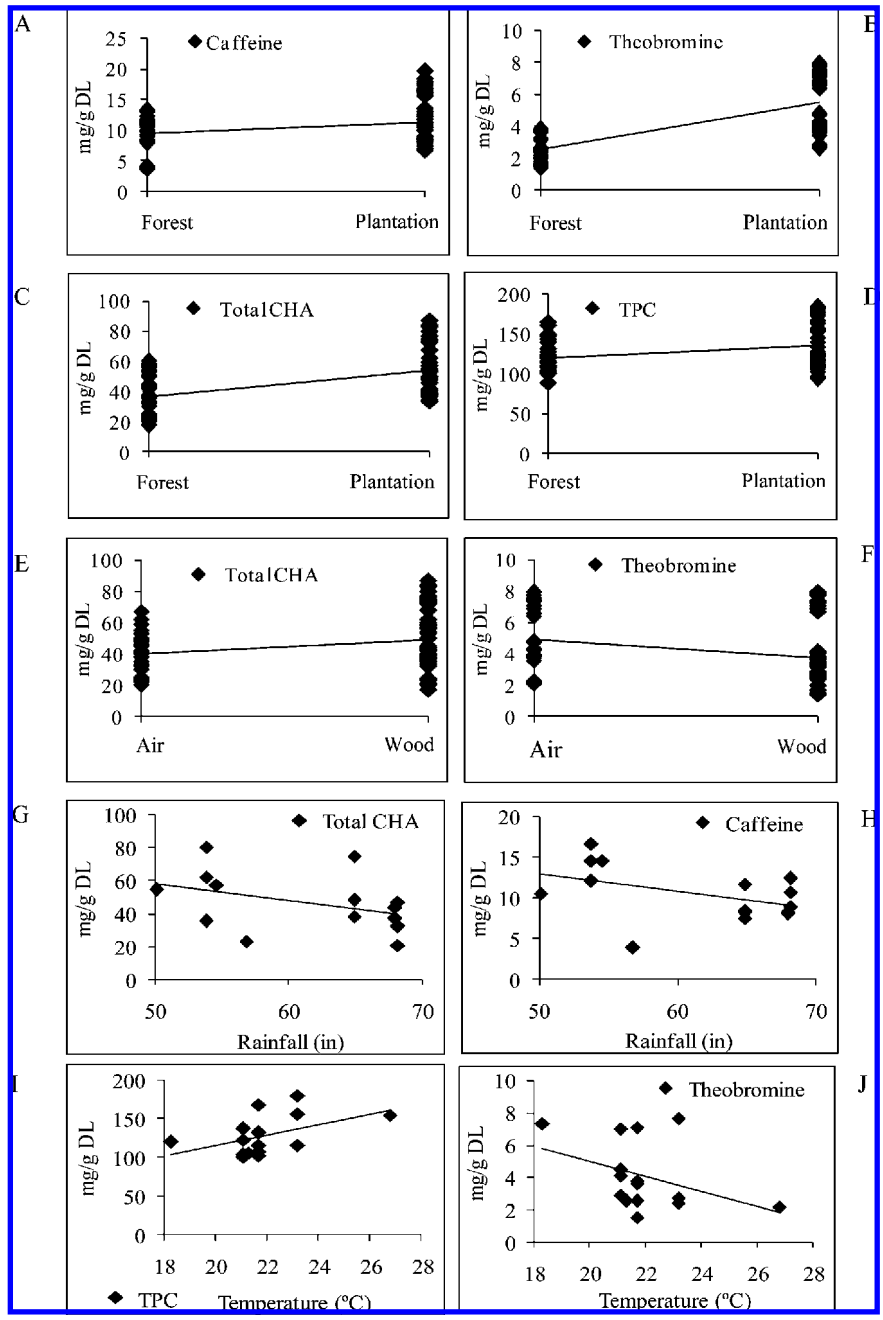

Figure 4. Correlation of phenolic compounds based on growing and drying conditions. (**A**) Caffeine vs growing conditions, (**B**) theobromine vs growing conditions, (**C**) total CHA vs growing conditions, (**D**) TPC vs growing conditions, (**E**) total CHA vs drying conditions, (**F**) theobromine vs drying conditions, (**G**) total CHA vs rainfall (in.), (**H**) caffeine vs rainfall (in.), (**I**) TPC vs temperature (°C), and (**J**) theobromine vs temperature (°C).

forest grown. Theobromine and the powerful antioxidant CHA were more greatly affected. **Figure 4E** shows that wood-dried MTs were higher in total CHA as compared to air-dried MTs, while air-dried products were higher in theobromine than wood-dried products (**Figure 4F**). **Figure 4G–H** shows that decreasing rainfall increased the concentration of phenolic compounds, including caffeine. Temperature increased TPC (**Figure 4I**); however, theobromine increased with decreasing temperature (**Figure 4J**), again, perhaps because of the fact that TPC contains compounds not individually quantified.

Several phenolic compounds are produced by plants in response to environmental stimuli. Generally, it is to protect the plant from environmental factors such as stress, pests, and sun (*5*). By growing in open full sun plantations, the plants produced higher levels of these compounds as compared to those grown in the protected environment under the shaded canopy of the forest (*20*). When exposed to full sun, the plants are exposed to a much greater concentration of UV light. The light that is not absorbed by the leaves to produce energy is then able to generate free radicals and induce cellular damage. To protect against this, the plant produces antioxidant compounds; thus, those exposed to full sun have higher level of CHAs, which have been shown to be potent antioxidants.

Figure 5 demonstrates that the concentrations of all phenolic compounds were significantly ($P < 0.05$) greater in the plantation grown MTs as compared to forest grown MTs. Increasing concentrations of antioxidant phenolics and stimulant compounds will benefit the commercialization of MT.

Antioxidant Capacity. This study utilized the ORAC assay as it has been shown to closely mimic in vivo reactions and can be readily compared to other foods (*21*). Measuring the loss of fluorescence over time, the ORAC assay conducted on

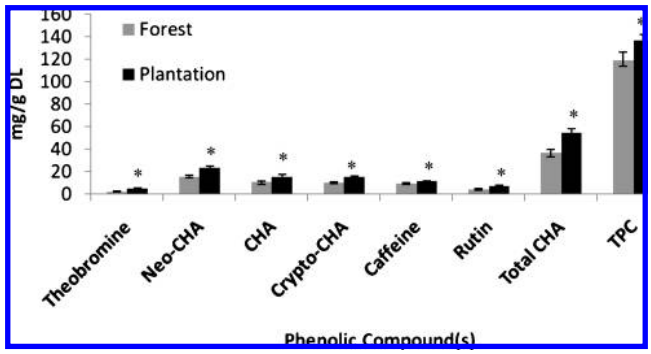

Figure 5. Concentration of phenolic compounds in forest and plantation grown MTs. *Indicates a statistical difference ($\alpha < 0.05$).

Table 5. Antioxidant Capacity of MTs by ORAC Assay[a]

Mate	mmol Trolox equiv/g IT	mmol Trolox equiv/g DL
I	5.9 ± 0.2 b	1.6 ± 0.1 c
II	6.2 ± 0.3 b	1.6 ± 0.04 c
III	6.7 ± 0.5 a,b	1.8 ± 0.2 b,c
IV	6.0 ± 0.9 b	1.6 ± 0.3 c
V	5.5 ± 0.1 b	1.7 ± 0.03 c
VI	6.2 ± 1.7 a,b	1.5 ± 0.3 b
VII	6.5 ± 0.5 b	2.2 ± 0.2 b,c
VIII	5.8 ± 0.1 b	1.9 ± 0.06 b,c
IX	5.3 ± 0.1 b	1.6 ± 0.04 c
X	6.5 ± 0.8 b	1.9 ± 0.2 b,c
XI	6.5 ± 0.1 b	2.6 ± 0.2 c
XII	7.2 ± 0.6 a	2.6 ± 0.1 c
XIII	6.6 ± 0.3 a,b	2.2 ± 0.1 b,c
XIV	9.3 ± 0.3 a	4.1 ± 0.1 a
XV	5.2 ± 1.2 b	1.7 ± 0.4 c

[a] Values represent the averages ± standard deviations. Different letters indicate a statistical difference ($P < 0.05$).

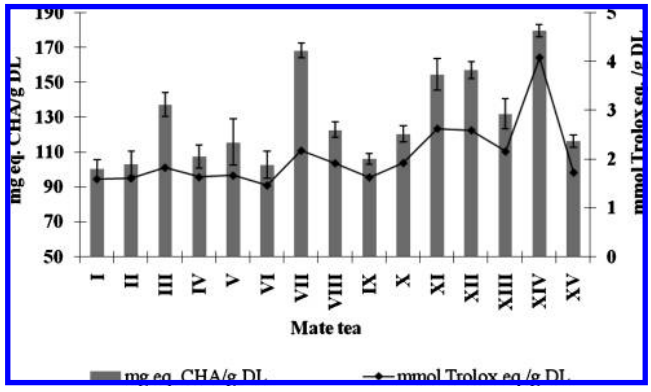

Figure 6. Relationship of TPC and antioxidant capacity for all 15 MTs grown under different conditions.

MT showed a wide range capacity to trap free radicals. **Table 5** presents the antioxidant capacity for the 15 MT as mmol TE/g IT and mmol TE/g DL. Although there was a wide range of antioxidant capacities between the teas, there was a positive correlation with TPC ($R^2 = 0.8$). **Figure 6** presents the relationship of polyphenols concentration to antioxidant capacity for all 15 MTs, showing that antioxidant capacity follows the trend of polyphenol concentration.

Using the ferric reducing antioxidant power (FRAP) assay, a high correlation between the polyphenol concentration and the antioxidant capacity was found for MT ($R^2 = 0.92$) (*11*). It was also noted that when normalized to this polyphenol concentration, MT showed a higher antioxidant capacity than both GT and red wine (*8, 22*). Chandra and Gonzalez de Mejia (*8*) reported ORAC values of MT as 1.2 ± 0.06 mmol TE/g DL; this is slightly lower than the lowest value of the MT studied here, 1.5 ± 0.3 mmol TE/g DL. This suggests that MTs have a wide range of polyphenols concentrations; however, they did not report the TPC, and they used an ORAC method that has been shown to be susceptible to contaminate interference and bleaching; this may have contributed to the lower readings (*21*). They also reported GT as having a value of 1.3 ± 0.06 mmol TE/ g DL, and even considering the lower value of their assay, it can be said that the high antioxidant capacity of MT XIV (4.1 ± 0.1 mmol TE/g DL) suggests that some MT products have a substantially higher antioxidant capacity than GT. It has also been shown that MT has a significantly higher antioxidant capacity than black tea using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, 61 to 29%, respectively (*23*).

The antioxidant capacity of MT in this study ranged from 1.5 ± 0.3 to 4.1 ± 10. mmol Trolox equiv/g DL ($P < 0.05$). Thus, these results showed a strong correlation with polyphenol concentration, confirming that greater polyphenol concentrations in MT yield greater antioxidant capacity. MT proved to be a good candidate for polyphenol extraction and potential incorporation into other foods to improve antioxidant levels. Because of the fact that antioxidant capacity is directly related to TPC, the antioxidant capacity is also affected by growing conditions.

In summary, data from the multivariate linear regression and correlation analysis made it clear that growing and drying conditions had an impact on phenolic concentrations in MT. Plantation grown products exposed to full sun showed far greater values in phenolic compounds than forest grown products that were at least partially shaded. As this study showed, polyphenol concentrations, antioxidant capacities, and caffeine were higher in plantation grown MT, making these products more suitable for higher extraction rates for commercial applications.

ACKNOWLEDGMENT

Special thanks to Guayaki SRP, Inc., and Aviva Ltd. for providing MT and to Divya Chona for technical assistance.

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Received for review June 6, 2008. Revised manuscript received July 29, 2008. Accepted August 1, 2008.

JF801748S