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Influence of Steep Time on Polyphenol Content and Antioxidant Capacity of Black, Green, Rooibos, and Herbal Teas

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Abstract: Potential health benefits of tea consumption are often attributed to the antioxidant activity of polyphenols. Whether steep time, often variable in a real-life situation, makes a biological difference in terms of polyphenol content and antioxidant activity is uncertain. The study objective was to characterize eight popular and commercially available teas for total polyphenol content (TPC) and antioxidant capacity in relation to steep time. Dragonwell (DW), Sencha (S), English Breakfast (EB), Golden Monkey (GM), Green Rooibos (GR), Red Rooibos (RR), Chamomile (C), and Peppermint (P) loose leaf teas were individually steeped in water for 1–10 min at 1 min intervals. TPC increased with longer durations of steep time; however, the majority of polyphenols observed after 10 minutes were extracted in the first 5 min regardless of tea type. After 5 min of steeping, differences ($p < 0.05$) in TPC were observed across teas (JS~EB~P > DW > GM~GR~RR > C). Different teas also varied in their ability to inhibit the free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) when normalized for polyphenol concentration (1 μg/mL) and there was no effect due to steep time. Predicted antioxidant capacity of teas also demonstrated significant differences among teas after 5 and 10 min. In conclusion, steep time modulates TPC but not the antioxidative capacity of tea polyphenols.

Keywords: steep time; polyphenol; antioxidant; green tea; black tea; herbal tea; rooibos

1. Introduction

On a global level, tea is the second most commonly consumed beverage and is only surpassed by water. The cultivation and consumption of tea dates back thousands of years, and the health benefits associated with tea consumption have been well documented, including decreased incidence of cancer and cardiovascular diseases [1,2]. Traditionally, only tea produced from the leaves of *Camellia sinensis* can be classified as a "true" tea. A large variety of teas can be produced from this plant, including: black, green, white, yellow, oolong, and pu-erh. The particular type of tea produced depends on how the leaves are processed and their relative levels of oxidation. Despite not being from *Camellia sinensis*, herbal (i.e., Mint, Chamomile) or rooibos teas can have similar health benefits and have been commonly used in traditional medicine [3–5].

The health benefits observed with the consumption of tea are often attributed to the unique profile and abundance of polyphenols in tea, which vary depending on tea type [6]. One proposed mechanism of action is through antioxidant activity. In the human body, free radicals are produced as a result of both normal metabolic function and external sources (smoke, X-rays, pollutants, and chemicals) and tend to accumulate as we age [7]. This accumulation of free radicals often leads to oxidative stress which is capable of modifying cellular structures: membranes, proteins, lipids and DNA [8,9]. Oxidative stress has been linked with the development of many diseases spanning a wide range

of systems, including cancer, cardiovascular disease, Alzheimer's disease, osteoporosis and chronic obstructive pulmonary disease [10–14]. In vivo, polyphenols exhibit antioxidant activity and inhibit free radical production. Human consumption of approximately three cups of black or green tea per day is capable of significantly increasing plasma antioxidant capacity [15]. More specifically, the phenolic groups of polyphenols are capable of accepting electrons to form relatively stable phenoxyl radicals, thereby inhibiting the propagation of oxidative damage [16]. In humans, studies have demonstrated the ability of tea to function as an antioxidant through elevated plasma ferric-reducing antioxidant power (FRAP), total radical antioxidant trapping parameter (TRAP) and oxygen radical absorbing capacity (ORAC) [17–19].

To date, much of the research regarding tea consumption and health outcomes has investigated either green or black teas. Beyond antioxidant effects, both teas consistently show antimutagenic properties, and are thus capable of protecting against mutagens—chemicals linked to the development of a wide array of diseases [20–22]. Recently, studies using female adult ovariectomized (OVX) rats have provided evidence that the consumption of either black or green tea protects against the loss of bone by either an increase in antioxidant capacity or a reduction in oxidative stress [23,24]. More specifically, it was observed that the consumption of green tea led to a higher femur neck bone mineral density (BMD) and the consumption of black tea resulted in a reduced number of active osteoclasts and greater bone strength. Other health benefits associated with tea consumption include weight loss and a reduced risk of cardiovascular disease, potentially due to reduced levels of inflammation [25–28]. In summary, there are a large number of studies that suggest the consumption of tea, and possibly its polyphenols, have favorable effects for health.

Several factors regarding the extraction process have been shown to influence the polyphenol content in tea preparations, including: temperature, type of tea, concentration of tea, and steep time [29,30]. Steep time is commonly controlled in an experimental setting; however, a general consumer is unlikely to monitor the exact amount of time that their tea is steeping but will keep it within the steep times recommended by the brand, usually 5 min or less. Moreover, some experiments use steep times that do not reflect consumer habits. Currently much of the research in regards to steeping time and its influence on polyphenol content has been conducted in vitro and has utilized different alcohols as the solvent. In general, alcohol solvents such as methanol and ethanol are better able to extract polyphenols from tea samples [31]. However, in a real-life scenario, tea is not steeped with alcohol but with water. While existing literature demonstrates a direct relationship between steep time in water and the quantity of polyphenols extracted [32,33], the majority of studies have only investigated a few steep times that represent a long time-frame (i.e., minutes through hours, or beyond the first 5 min that is typically the maximum time used by consumers) or have only examined one or two tea types. For example, one study steeped black tea at 23.2 °C in one of the multiple solvents (methanol, ethanol, DMSF, acetone or water) and determined the polyphenol content and antioxidant capacity after 2, 8, and 18 h. Others investigated the polyphenol content and antioxidant capacity after a greater range of steep times (0, 1, 2, 4, 6, 8, 10, 12, 14 and 20 min) but for only one specific type of black tea (Ceylon) [33].

To more fully characterize the potential health benefits of tea, it is important to first identify how steep time influences the polyphenol content, and in turn, antioxidant activity. We hypothesized that steep time is an important variable to control in experimental studies. Therefore, the aim of this study was to characterize the polyphenol content and antioxidative capacity in relation to steep time in eight different commercially available and popular teas. This included black (English Breakfast (EB) and Golden Monkey (GM)) and green (Dragonwell (DW) and Sencha (S)) teas as well as rooibos (Green (GR) and Red Rooibos (RR)) and herbals (Peppermint (P) and Chamomile (C)). Steep times of 1–10 min were selected to represent realistic durations used by consumers.

2. Materials and Methods

The eight whole-leaf teas (DW, S, GM, EB, GR, RR, P, C) were purchased from local specialty tea shops (Table 1 for sample characteristics). All other chemicals, including Folin-Ciocalteu's reagent and 2, 2-diphenyl-1-picrylhydrazyl (DPPH), were purchased from Sigma Aldrich (Oakville, ON, Canada). Optical density measurements were determined with a BIO-TEK Synergy HT Multi-Detection Microplate Reader (Winooski, VT, USA).

Table 1. Characteristics of whole-leaf tea samples.

Sample Identification	Common Name	Scientific Name (Variety)	Type
EB	English Breakfast	*C. sinensis*	Black Tea
GM	Golden Monkey	*C. sinensis*	Black Tea
DW	Dragonwell	*C. sinensis*	Green Tea
S	Sencha	*C. sinensis*	Green Tea
GR	Green Rooibos	*A. linearis*	Herbal
RR	Red Rooibos	*A. linearis*	Herbal
P	Peppermint	*M. piperita*	Herbal
C	Chamomile	*M. chamomilla*	Herbal

2.1. Extraction of Polyphenols

Tea polyphenols were extracted according to the International Organization of Standardization (ISO 14502-1) with slight modification. Tea samples (200 mg) were steeped with 5 mL of dH_2O for 1–10 min (1 min intervals) at the manufacturer's recommended temperature (79 °C: DW, S, 96 °C: EB, GM, GR, RR, P, C). Following the prescribed steeping time, samples were centrifuged at 200× g and subsequently filtered with a 0.2 µm filter to remove any debris from tea leaves.

2.2. Determination of Total Polyphenol Content

Total polyphenol content (TPC) of tea preparations were determined using Folin-Ciocalteu's method (ISO 14502-1) with gallic acid used as a standard [34]. In brief, tea preparations were diluted to 1:100 using dH_2O. Diluted tea samples (250 µL) were subsequently added to 1.25 mL of Folin-Ciocalteu's reagent (10% v/v in dH_2O). After 5 min, 1 mL of sodium carbonate (7.5% m/v in dH_2O) was added and the resulting mixture was allowed to incubate at room temperature for 1 h. Following the incubation period, the resulting optical density (OD) of each sample was measured in triplicate at 765 nm. TPC is expressed as mg gallic acid equivalents (GAE)/gram of tea.

2.3. Determination of Antioxidant Activity

Following the determination of TPC, the ability of a normalized amount of tea polyphenols (1 µg GAE/mL to scavenge the free radical DPPH was measured [35]. In brief, 50 µL of each tea sample (40 µg GAE/mL) were added to 1.95 mL of 60 µM DPPH (in methanol) giving a final polyphenol concentration of 1 µg GAE/mL. The mixture was then incubated at room temperature in the dark for 1 h. As a control, 50 µL of dH_2O was added to 1.95 mL of 60 µM DPPH. The resulting OD of each sample and control were then measured in triplicate at 517 nm. To further quantify the antioxidant activity of each tea, the percentage of DPPH inhibition was calculated according to Equation (1).

$$\text{DPPH Inhibition } (\%) = \frac{\text{Control} - \text{Sample}}{\text{Sample}} \times 100\% \quad (1)$$

The total antioxidant capacity of each tea was then calculated. The antioxidant activity that was calculated using Equation (1) was used in Equation (2) to calculate the concentration of DPPH that was inhibited per unit of GAE. This value was then converted into mM and multiplied by the TPC that was expressed as GAE (Equation (3)).

$$\text{DPPH Inhibited } (\mu M) = \%\text{DPPH Inhibition} \times 60\ \mu M \quad (2)$$

$$\text{Total Antioxidant Capacity } (mM) = \text{DPPH Inhibited } (mM) \times \text{TPC } (\text{GAE}) \quad (3)$$

2.4. Statistical Analysis

One-way ANOVA was used to assess differences in TPC and predicted total antioxidant capacity. More specifically, the following comparisons were analyzed for each measurement: (1) within a tea type after varying durations of steep time; and (2) among tea types at each steeping duration. For DPPH inhibition, only differences among teas were assessed and this was conducted through the use of a one-way ANOVA. Differences between means were deemed significantly different if $p < 0.05$. If a significant difference was observed in any of the statistical analyses, Tukey's post-hoc test was performed to further identify where the specific differences are located. To further evaluate potential differences within a specific tea type after 5 and 10 min of steeping, *T*-tests were performed to assess TPC and predicted total antioxidant capacity, and considered significant if $p < 0.05$. All statistical analysis was conducted using GraphPad Prism™ V5 (La Jolla, CA, USA). Results are reported as mean ± SEM.

3. Results

3.1. Total Polyphenol Content

Samples from eight different tea types were steeped from 1–10 min, at 1 min intervals, and the resulting TPC was measured. There was a visual increase in TPC with a longer steep time for each tea investigated (Figure 1a). However, it was not a linear relationship as the majority of polyphenols observed after 10 min of brewing were extracted in the first 5 min regardless of tea type (% of total TPC extracted after 5 min: DW = 66.08 ± 6.36, S = 81.94 ± 2.00, EB = 71.75 ± 9.47, GM = 74.41 ± 8.43, GR = 67.78 ± 6.47, RR = 77.52 ± 10.46, P = 83.90 ± 1.85, C = 56.58 ± 14.23). TPC was observed to be significantly greater ($p < 0.05$) after 10 minutes of brewing than it was after 5 min for all tea types (Figure 1b). After 5 min of steeping, there was a significant difference in TPC among the teas (S > EB~P > DW > GM~GR~RR > C, $p < 0.05$). After 10 min of steeping, significant differences were also observed in TPC among teas (EB~S > P~DW > GM~GR > RR > C, $p < 0.05$) (see Table A1 for further detailed statistical analysis).

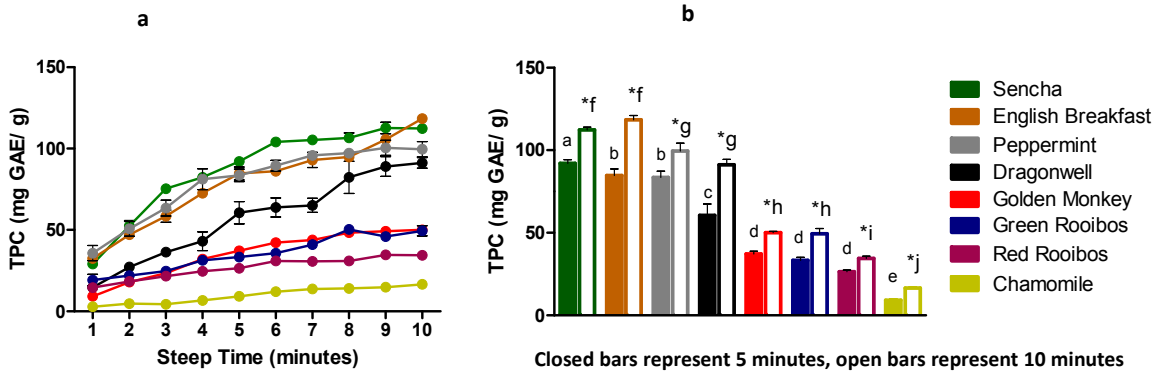

Figure 1. Influence of steep time on TPC of eight different types of tea. (**a**) TPC/g of tea at steep times of 1–10 min; (**b**) Comparison between TPC/g of tea after a 5 (closed bars) and 10 min (open bars) steep time. Error bars are ±SEM, $n = 4$/sample, GAE = Gallic acid equivalents. * indicates a significant difference ($p < 0.05$) between 5 and 10 min within a tea, differing letters indicate a significant difference ($p < 0.05$) in TPC among teas after 5 (**a**–**e**) and 10 min (**f**–**j**).

3.2. Antioxidant Capacity

Following the determination of TPC for each sample, the ability of a normalized amount of polyphenols from each tea sample (1 µg GAE/mL) to inhibit the free radical DPPH was monitored. Steep times of 1–10 min, at 1 min intervals, did not alter the antioxidant capacity for any of the teas (Figure 2a). As a result, all time points shown in Figure 2a were compressed for a specific type of tea, and the mean antioxidant capacity was calculated and compared across teas (Figure 2b). Significant differences ($p < 0.05$) among the antioxidant capacity of different teas were observed: DW > GM~EM~S > P~GR > RR~C (Figure 2b).

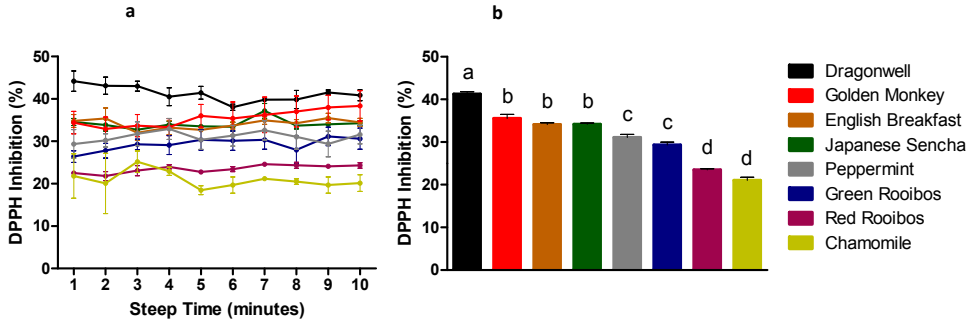

Figure 2. Ability of a normalized amount of tea polyphenols (1 µg/mL) to scavenge and inhibit the free radical DPPH. (**a**) The % inhibition of DPPH by a normalized amount of tea polyphenols (1 µg GAE/mL) with steep times of 1–10 min; (**b**) Comparison between % inhibition of DPPH for all teas when values for steep times from 1–10 min are averaged. Steep time did not influence the overall capacity to scavenge DPPH within a tea. Error bars are ±SEM, $n = 4$/sample. Differing letters indicate a significant difference ($p < 0.05$) in % inhibition of DPPH among teas.

3.3. Predicted Total Antioxidant Capacity

After determination of the TPC and the ability of a normalized amount of polyphenols (1 µg GAE/mL) to function as an antioxidant, the predicted total antioxidant capacity of each sample was calculated. The predicted total antioxidant capacity increased ($p < 0.05$) with the steep time for all teas investigated (Figure 3a). It was also observed that DW, EB, S, and P had a greater ($p < 0.05$) predicted total antioxidant capacity than GM, GR, RR, and C after both 5 and 10 min of steep time (Figure 3b). Also, GM and GR had a greater ($p < 0.05$) predicted antioxidant activity than RR and C (Figure 3b) (see Table A2 for further detailed statistical analysis).

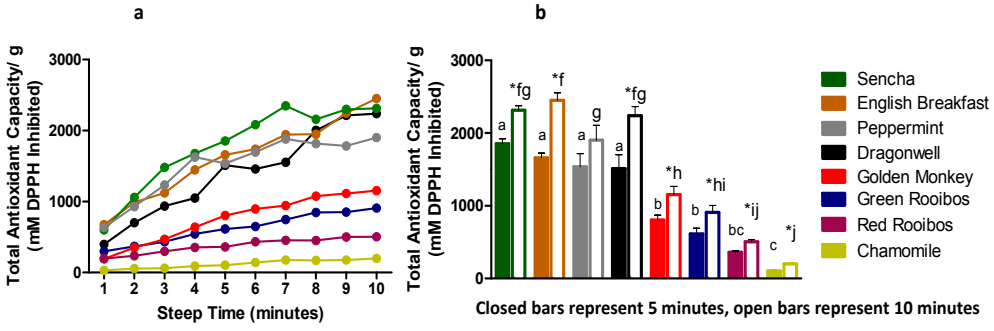

Figure 3. Predicted total antioxidant capacity (per gram) of eight different teas after varying durations of steep time. (**a**) Predicted total antioxidant capacity per gram of tea at steep times of 1–10 min; (**b**) Comparison between predicted total antioxidant capacity/g of tea after a 5 (closed bars) and 10 min (open bars) steep time. Error bars are ±SEM, $n = 4$/sample. * indicates a significant difference ($p < 0.05$) between 5 and 10 min within a tea, differing letters indicate a significant difference ($p < 0.05$) in TPC among teas after 5 (**a**–**e**) and 10 min (**f**–**j**).

4. Discussion

We characterized a wide range of eight different teas for both polyphenol content and antioxidant capacity after each subsequent minute of steeping from the onset of steeping to a final time of 10 min. TPC was influenced by steep time, as the measured TPC after 10 min was significantly greater than what was measured after 5 min. However, it was determined that the increase in TPC with steep time was not linear, as the majority of polyphenols measured at 10 min of steeping were extracted in the first 5 min. The observation that the TPC increased with the longer duration of steep time is supported in the literature by several studies that investigated individual tea types and/or a limited number of time points [36,37]. Past studies have experimentally determined the half time (time to reach 50% extraction of the total polyphenols) to be between 100–150 s for "true teas", which provides evidence as to why commercial tea companies recommend 2–3 min of steeping [38]. Theoretically, if the health benefits that are associated with the consumption of tea are due to the presence of polyphenols, then having a greater quantity would be beneficial and would support the supposition of steeping tea longer to extract more polyphenols. However, when tea leaves are steeped for too long they release tannins, compounds which create a bitter taste and can cause the drink to be unpleasant [39]. One method of receiving more polyphenols without consuming a bitter drink is to selectively choose a tea type that has a greater quantity of polyphenols extracted after similar durations of steeping. Steeping "true" teas (black and green teas) for 5 and 10 min displayed greater polyphenol content than their herbal and rooibos tea counterparts, with the exception of Golden Monkey. We did not directly measure the specific polyphenol profile but future studies should investigate the specific profile of polyphenols from each tea after varying durations of steep time as some polyphenols may have different biological effects (i.e., stronger antioxidants and/or act via another mechanism) or have synergistic effects in the presence of other polyphenols.

As previously mentioned, black and green teas are produced from the leaves of *Camellia sinensis* with the only difference being the level of oxidation of the leaves themselves (black tea is 100% oxidized) [40]. Thus, the differences in TPC among teas could simply be attributed to the plant source as *Camellia sinensis* may have a larger quantity of polyphenols than the plants and herbs that are used to make the herbal and rooibos teas. One discrepancy that is seen with this postulation is the higher levels of polyphenols from Peppermint, a herbal tea, when compared to Golden Monkey, a black tea. However, this could be due to differences in the size of the tea leaves used. The Peppermint leaves used in the current study are much finer than that of Golden Monkey, but not all brands of Peppermint tea may have fine leaves. The size of the tea leaf has been shown to be inversely proportional to the extraction time of polyphenols [41]. Therefore, it is possible that the polyphenols from Peppermint tea were extracted faster and that could be the reason behind the observed discrepancy.

The ability of a normalized amount of tea polyphenols to inhibit the free radical DPPH after each steep time was measured as an indirect way of monitoring if the polyphenol profile of each specific tea changed over time. Our finding that black and green teas had higher antioxidant capacity when compared to the rooibos and herbal teas is in agreement with past studies that have assessed herbal teas in comparison to teas from *Camellia sinensis*. One study determined the antioxidant potency of tropical and temperate herbal teas (peppermint, rosemary, and oregano) after 1 h of steeping to be less than that of teas (green, black, and oolong) coming from *Camellia sinensis* [42]. This finding supports the notion that polyphenols in black and green teas are more potent antioxidants and could lead to greater health benefits. The polyphenol profile of green tea in particular has a large quantity of catechins including epigallocatechin gallate (EGCG) and epigallocatechin (EGC), whereas theaflavins and thearubigins primarily dominate the polyphenol profile of black tea [43]. Despite being chemically different, these compounds have been shown to be equally effective antioxidants [44]. This specific difference in the polyphenol profile between "true" and herbal or rooibos teas could be the direct cause of the differences in the antioxidant capacity between tea types in the current study. Surprisingly, there were no alterations in the measured antioxidant capacity of any of the teas after varying the duration of the steep time. This indirectly suggests that the polyphenol profile of each tea is unaltered by the steeping

time. It is also possible that the alterations could have been too small to detect with the techniques used or that changes in the profile did occur, though they did not affect the antioxidant capacity.

Taking into account both the polyphenol content and the ability of a normalized amount of tea polyphenols to act as antioxidants, a predicted total antioxidant capacity was calculated. The results showed similar comparisons to what was observed when looking at TPC alone; however, there appeared to be a divergence between the "true" teas and herbal or rooibos teas as the steeping time increased. As such, green and black teas not only have a greater quantity of polyphenols but their unique profile also acts as a more potent antioxidant than the polyphenols in herbal teas. However, herbal teas are used in traditional medicine as natural remedies and have been shown to help with many ailments including stomach aches, hypertension, and asthma [45–47]. The usefulness of herbal teas in treating these ailments could be due to the synergistic effects of the polyphenols in their profiles, due to trace elements or other mechanisms.

5. Conclusions

In conclusion, our study demonstrated that "true" teas such as black and green tea have greater quantities of polyphenols and more potent antioxidant capacity than rooibos and herbal teas. This suggests that in terms of health benefits associated with greater antioxidant activity, these teas may be the most effective. We have also established that steep time directly influences TPC. However, longer steep time is not associated with a corresponding higher antioxidant activity. Thus, steep time is a modifiable factor that could potentially affect the ability of tea to improve health outcomes and it should be monitored and controlled for within experiments.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

TPC	Total Polyphenol Content
DW	Dragonwell
S	Sencha
EB	English Breakfast
GM	Golden Monkey
GR	Green Rooibos
RR	Red Rooibos
C	Chamomile
P	Peppermint
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric-Reducing Antioxidant Power
TRAP	Total Radical Antioxidant Trapping Parameter
ORAC	Oxygen Radical Absorbing Capacity
OVX	Ovariectomized
BMD	Bone Mineral Density
OD	Optical Density
GAE	Gallic Acid Equivalents
EGCG	Epigallocatechin Gallate
EGC	Epigallocatechin
dH_2O	Distilled water

Appendix A

Table A1. Measured TPC (in mg GAE/g of tea) of various loose leaf teas after steeping in dH_2O for differing amounts of time. Values are expressed as mean ± SD, n = 4/sample, GAE= Gallic acid equivalents. Differing letters within a row indicate a significant difference ($p < 0.05$) in TPC after varying steep time of a specific tea type, and differing roman numerals in a column indicate a significant difference ($p < 0.05$) in TPC among teas after a specific duration of steeping.

	Steep Time (Minutes)																			
	1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**	
Sencha	29.06±4.39	a I,II	52.42±6.90	b I	75.31±4.88	c I	82.33±1.81	c,d I	92.11±4.37	d,e I	104.06± 5.82	e,f I	105.26±4.09	f I	106.60±5.92	f I	112.65±7.10	f,g I	112.37±3.03	f I
English Breakfast	32.47±3.26	a I,II	47.10±5.78	b I	58.44±7.46	b,c II	72.55±3.85	c,d I	84.65±7.99	d,e I	86.08±5.68	d,e,f II	92.99±9.40	e,f I	94.85±5.94	e,f I	105.59±2.35	f,g I	118.44±5.29	g I
Peppermint	35.71±9.38	a I	50.68±9.07	a,b I	63.45±9.66	b,c I,II	81.21±12.77	c,d I	83.53±7.65	c,d I	89.52±6.59	d II	95.85±4.70	d I	97.16±5.00	d I	100.48±9.25	d I,II	99.59±9.43	d II
Dragonwell	14.75±3.42	a III	27.19±4.34	a,b II	36.28±4.27	a,b,c III	43.01±11.50	b,c,d II	60.55±13.83	c,d,e II	63.80±11.79	d,e,f III	65.09±8.67	d,e,f II	82.32±19.75	e,f,g II	88.96±12.14	f,g II	91.19±6.44	g II
Golden Monkey	9.20±2.49	a III	17.94±2.51	b II	23.49±2.74	b IV	32.11±3.06	c II,III	37.19±3.13	c,d III	42.14±2.19	d,e IV	43.82±4.94	d,e,f III	48.31±2.25	e,f III	49.21±4.68	e,f III	50.11±1.41	f III
Green Rooibos	19.20±7.06	a II,III	21.91±3.28	a,b II	24.64±0.81	a,b,c III,IV	31.26±2.68	b,c,d II,III	33.32±3.67	c,d III	35.72±3.11	d IV	40.97±0.21	d,e III	50.31±2.88	e III	46.00±5.15	e III	49.43±6.29	e III
Red Rooibos	14.60±2.14	a III	18.34±2.14	a,b II	21.56±2.50	b,c IV	24.61±3.44	b,c,d III	26.48±1.84	c,d,e,f III	30.98±3.36	e,f IV	30.72±1.90	d,e,f III	30.92±2.83	d,e,f IV	34.75±1.52	f III	34.41±2.70	f IV
Chamomile	2.83±1.71	a IV	2.62±1.71	a III	4.31±1.58	a,b V	6.70±0.70	a,b,c IV	9.23±1.73	b,c,d IV	12.04±2.83	c,d,e V	13.73±5.03	d,e IV	14.01±0.79	d,e IV	14.81±2.10	d,e IV	16.60±1.68	e V

Table A2. Predicted total antioxidant capacity (per gram of tea) of various loose leaf teas after steeping in dH2O for differing amounts of time. Values are expressed as mean ± SD, $n = 4$/sample. Differing letters within a row indicate a significant difference ($p < 0.05$) in predicted total antioxidant capacity after varying steep time of a specific tea type, and differing roman numerals in a column indicate a significant difference ($p < 0.05$) in predicted total antioxidant capacity among teas after a specific duration of steeping.

	Steep Time (Minutes)																			
	1		**2**		**3**		**4**		**5**		**6**		**7**		**8**		**9**		**10**	
Sencha	601.50±85.06	a II	1057.94±93.54	b I	1480.33±127.67	c I	1675.10±78.64	c,d I	1854.90±132.22	d,e I	2084.07±64.57	e,f I	2349.76±243.34	f I	2157.71±194.38	e,f I	2299.70±136.56	f I	2312.67±125.51	f II
English Breakfast	674.48±42.98	a I	990.00±95.26	b I	1120.43±177.86	b I,II	1445.30±150.38	c I,II	1658.33±133.32	c,d I	1739.75±74.55	c,d I,II	1943.00±141.46	d,e I,II	1948.27±93.75	d,e I	2245.94±105.08	e,f I,II	2451.43±203.14	f I,II
Peppermint	635.72±220.29	a I,II	928.68±251.66	a,b I,II	1232.83±406.34	a,b,c I,II	1625.12±455.93	b,c I	1535.25±361.30	a,b,c I	1697.26±387.90	b,c I,II	1881.27±335.91	c II	1816.39±395.73	b,c I	1782.59±489.83	b,c II	1900.51±413.89	c II
Dragonwell	396.31±127.86	a II,III	700.94±103.27	a II	936.51±118.99	a,b II	1048.78±296.22	a,b II,III	1511.51±380.75	b,c I	1459.79±301.23	b,c II	1554.44±208.09	b,c,d II	2002.76±695.15	c,d I	2214.93±294.72	c,d I,II	2239.11±248.71	d I,II
Golden Monkey	193.78±74.61	a III	350.82±55.46	a,b III	469.78±43.73	a,b,c III	639.90±77.18	b,c,d III,IV	803.98±133.63	c,d,e I	896.20±214.78	d,e III	943.57±193.25	d,e III	1076.97±236.50	e II	1114.22±141.97	e III	1153.90±225.87	e III
Green Rooibos	298.69±89.03	a III	368.80±95.92	a,b III	433.49±43.51	a,b III,IV	543.08±67.68	a,b,c IV,V	612.68±156.65	b,c,d II	649.27±125.34	b,c,d III	746.30±106.98	c,d III,IV	847.11±218.20	c,d II,III	851.84±77.60	c,d III,IV	907.93±193.01	d III,IV
Red Rooibos	197.41±30.63	a III,IV	236.94±23.50	a,b III	299.75±54.93	b,c III,IV	353.33±42.34	c,d IV,V	362.28±31.89	c,d,e II,III	434.92±43.08	d,e,f III,IV	453.60±32.26	e,f IV,V	452.07±52.12	d,e,f II,III	502.31±10.95	f IV,V	503.31±62.68	f IV,V
Chamomile	30.61±9.85	a IV	56.14±28.53	a,b III	61.89±14.57	a,b IV	92.08±12.31	a,b,c V	102.67±25.85	a,b,c III	140.73±40.14	b,c,d IV	175.99±71.36	c,d V	172.44±13.21	c,d III	176.62±54.48	c,d V	198.98±33.66	d V

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