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Total Polyphenol Content and Antioxidant Capacity of Tea Bags: Comparison of Black, Green, Red Rooibos, Chamomile and Peppermint over Different Steep Times

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Abstract: Globally, traditional and herbal teas are a prominent dietary source of polyphenols, and represent a class of bioactive molecules that are closely associated with a variety of health benefits. Most consumers prepare tea using tea bags, although there is little information about whether this production step alters the content of the final product. The study purpose was to investigate the effect of steep time and tea type on the polyphenol content and predicted antioxidant capacity of commercially available tea bag products, including Green, Orange Pekoe, Red Roiboos, Peppermint, and Chamomile. Total polyphenol content (TPC), antioxidant capacity (1,1-diphenyl-2-picrylhydrazyl inhibition), and total predicted antioxidant capacity were measured in aliquots sampled every minute for 10 min. Polyphenols were extracted into solution in a nonlinear fashion, with ~80–90% of the TPC appearing within 5 min of tea bag immersion. Moreover, a significant range in TPC values was observed between products, with true teas containing at least two-fold greater polyphenol content than the herbal varieties. Our results are consistent with previous work using loose-leaf tea products and demonstrates that tea bag products are an effective source of polyphenols that may offer health benefits relating to their constituent antioxidant activity.

Keywords: tea; polyphenol; antioxidant; steep time; *Camellia sinensis*; *Aspalathus linearis*; *Mentha piperita*; *Matricaria chamomilla*

1. Introduction

Tea, particularly green and black, is increasingly being studied for its potential role in the prevention and attenuation of a wide variety of diseases [1–6]. Given that the term tea is often used erroneously to also refer to rooibos and herbal beverages that do not originate from *Camellia sinensis*, it is important to acknowledge that these beverages are most accurately referred to as tisanes while recognizing that both tea and tisanes all have polyphenols, a common component that is believed to mediate health benefits. However, the polyphenol profile is markedly different among teas and tisanes and this may in part explain the diverse potential biological activities that includes antiosteoporotic, antioxidant, antiatherosclerotic, antiallergic antifibrotic, hypolipidemic, hypocholesterolemia, anti-obesity, antiviral, antimutagenic, antimicrobial, anticarcinogenic, antidiabetic, and neuroprotective effects [1–8].

Rooibos, green or red, is derived from *Aspalathus linearis*, with red rooibos being the oxidized form, while common herbal teas such as peppermint and chamomile are from *Mentha piperita* and *Matricaria chamomilla*, respectively [7–11]. There is a strong interest in rooibos as it does not contain caffeine and many herbal tisanes are also caffeine-free, whereas a single cup of green or black tea from the *Camellia sinensis* plant contains approximately 30–50 mg of caffeine per serving while a serving of brewed coffee contains 135 mg [12]. To put this in perspective, Health Canada's recommendations suggest no adverse effects in adults with caffeine intakes of up to 400 mg [12].

In studying the health benefits of tea consumption in the laboratory setting, it is important to understand how steep time alters total polyphenol content (TPC) and antioxidant capacity, and to also prepare tea used in laboratory experiments in a similar manner as a consumer. Two considerations include the steep time as well as whether loose tea or a commercial tea bag is used. The effect of steep time on polyphenol content and antioxidant activity in eight commercially available and popular loose teas has been previously reported by our group [13]. Steep time was studied from 1 to 10 min at 1-min intervals, thus mimicking steep times often used by consumers. This study showed that over 50% of polyphenols were released in the initial 5 min of steep time and that TPC increased in a nonlinear relationship for all tea types tested [13]. Interestingly, longer steep time was not associated with greater antioxidant capacity (measured as the percent inhibition of the free radical DPPH) when the polyphenol content was standardized, but tea types did differ in their antioxidant capacity. Specifically, the green and black teas resulted in greater DPPH inhibition compared to rooibos (green or red) or herbals (peppermint or chamomile). However, when differences in TPC were considered, one but not both black teas studied and both green teas as well as peppermint had a higher predicted total antioxidant capacity than the rooibos (red, green) and chamomile tisanes. The finding of peppermint having a higher antioxidant capacity may have been due to smaller leaf size, and thus increased surface area that may have resulted in the higher TPC with longer steep time. Importantly, the aforementioned study investigated samples from loose-leaf teas only; however, it is unclear if these findings represent the steep time-polyphenol concentration relationship that might exist when bagged teas are brewed under similar conditions.

Others have reported that particle size and use of packaging in a tea bag modulates polyphenol content [14,15]. In a study of black tea, tea from tea bags showed a significantly higher level of polyphenols than loosely-packed brands after a 5-min steep time [15]. Moreover, for bagged tea, TPC was significantly lower after a 1 min versus a longer steep time of 3, 4 or 5 min [15]. Tea in tea bags is widely available and in recent years has become an increasingly common choice for consumers. For example, in the UK, the UK Tea and Infusions Association reports an increase in use of teabags has increased from 5% in 1960 to 96% in 2007 [16].

The present study further develops the experimental paradigm from our previous study in which we showed that steep time altered TPC in a variety of loose-leaf teas and tisanes. Given the widespread and increasingly prevalent usage of tea bags by consumers, the objective of this study was to determine how steep time altered TPC and antioxidant activity of commercially-available tea bags that contained black and green teas, as well as red rooibos and popular herbals (chamomile, peppermint).

2. Materials and Methods

The methods used in the current experiments have been described in detail previously [13], and only minor changes have been made to the apparatus to allow the steeping of tea prepared from tea bags, which requires a larger vessel than that required for loose-leaf products. Tea samples consisted of Orange Pekoe (OP) or Green (G) teas, or Red Rooibos (RR), Peppermint (P), or Chamomile (C): all products were purchased from a local supplier and prepared according to the manufacturers recommendations (see Tables 1 and A3 for tea product details). All experiments for each tea were performed from a single batch of product. Tea samples were steeped using a tea mass to distilled water ratio of 0.04 g/mL in a specially prepared glassware apparatus with a Liebig condenser (Thermo Fisher Scientific, Burlington, ON, Canada) to prevent loss of volume by vaporization (see Figure 1). Samples

(100 μL) were extracted at 1-min intervals for 10 min, after which they were microfiltered (0.2 μm) and immediately analyzed for polyphenol content. The boiling vessel used in the present study was larger than that used for our previous work with loose leaf teas [13], and therefore rather than independent tea batches steeped to each time point, we utilized a repeated-measures design where small aliquots were removed from the 500 mL vessel for each of the time points to reduce inter-batch variability in the study design.

Table 1. Origin and description of teabag samples.

Common Name	Scientific Name (Variety)	Tea Type	Steep Temperature (°C)	Tea Mass Per Bag (g)
Orange Pekoe	*Camellia sinensis*	Black Tea	96°C	3.07 ± 0.01
Green Tea	*Camellia sinensis*	Green Tea	79°C	2.07 ± 0.02
Red Roiboos	*Aspalathus linearis*	Red Rooibos	96°C	2.08 ± 0.01
Chamomile	*Matricaria chamomilla*	Herbal	96°C	1.21 ± 0.01
Peppermint	*Mentha piperita*	Herbal	96°C	1.94 ± 0.01

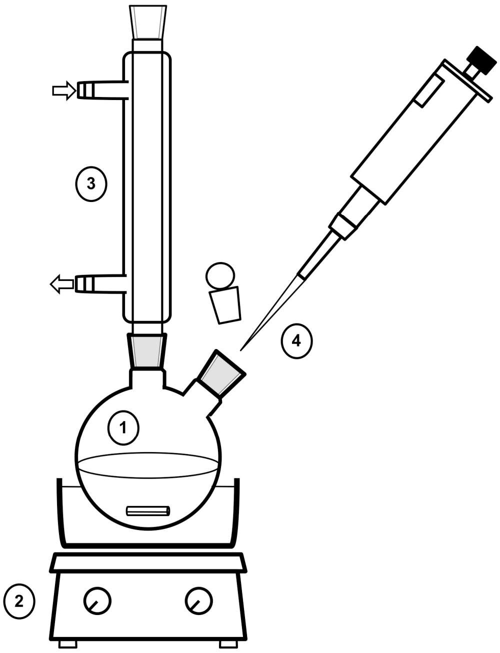

Figure 1. Experimental apparatus for aqueous extraction of tea polyphenols. Tea bags were added to a glass boiling flask, with a 500 mL capacity, that contained distilled water that was preheated to the required temperature (1); constant temperature of the mineral oil bath and continuous agitation via magnetic stir bar was maintained for all experiments (2); loss of water by evaporation was prevented using a Liebig condenser (3); tea samples (100 μL) were removed by pipette once per minute during each period of steeping (4).

2.1. Polyphenol Determination

Polyphenols were extracted using a modified Folin–Ciocalteu's method with a Gallic acid standard, as previously described [13,17] and in accordance with the International Organization of Standardization (ISO 14502-1). Using distilled water, tea samples were prepared using dilutions of 1:100. 1.25 mL of Folin-Ciocalteu's reagent (10% v/v in distilled water) was added to 250 μL of the diluted tea samples. The solution was allowed to stand for 5 min before the addition of 1 mL of sodium bicarbonate. After the addition of sodium bicarbonate, the mixture was incubated for 1 h at room temperature. Triplicates of each sample at 200 μL were measured for optical density at 765 nm following time of incubation. The TPC of the teas were expressed in gallic acid equivalents (GAE) per gram of tea.

2.2. Antioxidant Scavenging Ability Determination

To determine the ability of a tea to scavenge free radicals, a normalized amount of polyphenols (1 µg GAE/mL) was procured from the TPC determination (detailed methods have been reported previously) [13]. The teas were measured on their ability to scavenge the free radical DPPH. To summarize, 50 µL of each sample (in a dilution of 40 µg GAE/mL) were combined with 1.95 mL of 60 µM DPPH (in methanol) obtaining a 1 µg GAE/mL polyphenol concentration. In addition, 50 µL of distilled water in 1.95 mL of 60 µM DPPH was used to function as a control. This solution was incubated for the duration of one hour in darkness at room temperature. The optical density of each sample and control was then measured at 517 nm in triplicate post-incubation period. The percentage of DPPH was then calculated according to the following equation to further quantify antioxidant activity:

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

Total Antioxidant capacity was then calculated for each tea. The antioxidant activity calculated in Equation (1) was utilized to calculate the concentration of DPPH inhibited per gallic acid equivalents (GAE) unit in Equation (2). The obtained value was then permuted into mM and multiplied by TPC content expressed as GAE in Equation (3):

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

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

2.3. Rate of Polyphenol Appearance

Polyphenol data from the current study and loose leaf tea data from McAlpine and Ward [13], collected under similar conditions and with identical methods, were used to calculate the rate at which polyphenols appeared into the steeping flask. The first derivative of the polyphenol-time function was calculated for each sample, where the slope of the function during each minute represents the rate at which polyphenols were extracted from the tea leaves (in mg GAE/[g·min]). The greatest time period of polyphenol appearance for each sample, regardless of timing (i.e., 0–10 min), is presented in Table 2 as the maximum rate of polyphenol appearance. Conversely, the average rate of polyphenols extraction over the initial 5 min of steeping was also calculated for each sample.

Table 2. Rate of polyphenol appearance from tea bags and loose-leaf tea.

Common Name	Scientific Name (Variety)	Sample Type	Rate of Polyphenol Appearance (mg GAE/(g·min))	
			Maximum Value (0–5 min)	Avg. (0–5 min)
Orange Pekoe (Black)	*C. Sinensis*	Tea bag	26.1 ± 3.0	8.7 ± 0.6
Green	*C. Sinensis*	Tea bag	23.5 ± 1.9	10.7 ± 0.5
Peppermint	*M. Piperita*	Tea bag	11.2 ± 1.1	4.1 ± 0.3
Red Rooibos	*A. Linearis*	Tea bag	9.6 ± 1.5	3.8 ± 0.2
Chamomile	*M. Chamomilla*	Tea bag	3.0 ± 0.2	1.1 ± 0.1
Dragonwell (Green)	*C. Sinensis*	Loose leaf	27.7 ± 5.5	9.8 ± 0.8
Sencha (Green)	*C. Sinensis*	Loose leaf	27.6 ± 3.3	15.0 ± 0.4
English Breakfast (Black)	*C. Sinensis*	Loose leaf	19.6 ± 1.4	10.7 ± 0.4
Golden Monkey (Black)	*C. Sinensis*	Loose leaf	11.5 ± 0.4	6.6 ± 0.4
Peppermint	*M. Piperita*	Loose leaf	22.0 ± 3.9	10.8 ± 0.9
Green Roiboos	*A. Linearis*	Loose leaf	10.5 ± 0.8	3.3 ± 1.0
Red Roiboos	*A. Linearis*	Loose leaf	8.6 ± 0.7	3.3 ± 0.6
Chamomile	*M. Chamomilla*	Loose leaf	5.0 ± 0.5	1.8 ± 0.2

Note: Current *Tea Bag* data is compared to *Loose leaf* data calculated with permission from the TPC values of McAlpine & Ward (2016).

2.4. Statistical Analyses

To evaluate differences in each outcome variable (TPC, predicted total antioxidant capacity) with respect to steep time, a one-way repeated-measures ANOVA was conducted for each tea type (see Tables A1 and A2), and differences between means was evaluated with the Bonferroni adjustment (differences shown using lowercase letters). Within each time point (i.e., 1–10 min), a one-way ANOVA was utilized to make inter-tea comparisons (also shown in Tables A1 and A2), with specific post hoc testing between means using Tukey's test (differences shown using Roman numerals).

For summary data comparing the 5 to 10 min value for each dependent variable (see Figures 2–4, Panel B), a two-way paired sample *t*-test was used to evaluate significant differences between means. For these data, differences between tea types at each time point (5 and 10 min) were independently analyzed using a one-way ANOVA with Tukey's post-hoc test.

For all tests, a significant difference between means was identified if $p < 0.05$. All statistical analyses were performed using SPSS (IBM Corporation, New York, NY, USA). All data are shown as mean ± standard error of the mean.

3. Results

3.1. Total Polyphenol Content

Our selection of five different types of teabags were tested for TPC at 1 min intervals during a steep time of 1–10 min. Progressive increases in TPC for each tea type were visually observed. Our measurements demonstrated that the majority of polyphenols were released after 5 min of steep time, as evidenced by the nonlinear relationship shown in Figure 2a. (% of total TPC extracted after 5 min: OP = 97.72 ± 1.64, G = 88.20 ± 2.56, RR = 94.06 ± 2.63, C = 77.79 ± 7.05, P = 96.46 ± 3.14). Importantly, with the exception of Green tea, the polyphenol content of samples was not significantly higher at the 10-min time point (see Figure 2b). Notable differences in TPC among tea types were observed across all time points; with statistical comparisons of values at the 5 and 10 min point shown in Figure 2b (5 min: OP ~G > RR ~P > C, $p < 0.05$; 10 min: G > OP > RR ~P > C, $p < 0.05$).

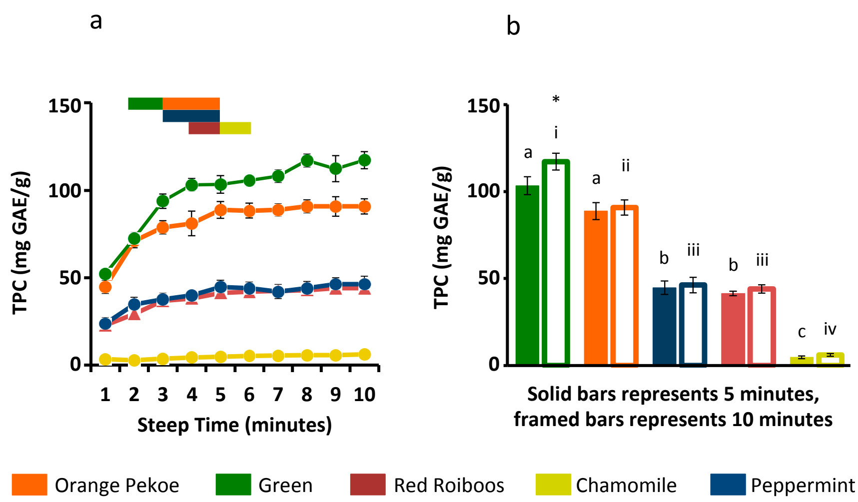

Figure 2. The effect of steep time on TPC of five different types of teabags. (**a**) measured TPC/g at steep times of 1–10 min. Coloured bars indicate the manufacturers suggested steep time for each tea type; (**b**) The TPC/g of tea after a steep time of 5 min (solid coloured bars) compared to 10 min (framed bars). $n = 6$/sample (excluding time point 1 min for chamomile which had $n = 3$/sample), error bars are ±SEM, GAE = Gallic acid equivalents. * represents a significant difference between 5 and 10 min for Green tea ($p < 0.05$). Between tea comparisons were made separately for 5 min (letters) and 10 min (Roman numerals) data ($p < 0.05$). Specifically, TPC at 5 min significantly differs among tea types that have different letters while TPC at 10 min significantly differs among tea types that have different Roman numerals.

3.2. Antioxidant Capacity

Inhibition of the free radical DPPH was measured following the normalization of each sample to its polyphenol content (1 µg GAE/mL). These data are presented in Figure 3a across all steep times, and no time-dependent relationship was observed. A mean value for each tea was calculated by collapsing all time points to estimate overall antioxidant capacity (Figure 3b). When further investigated, significant differences ($p < 0.05$) in DPPH inhibition were observed between tea types: P > G > OP > RR ~C (Figure 3b).

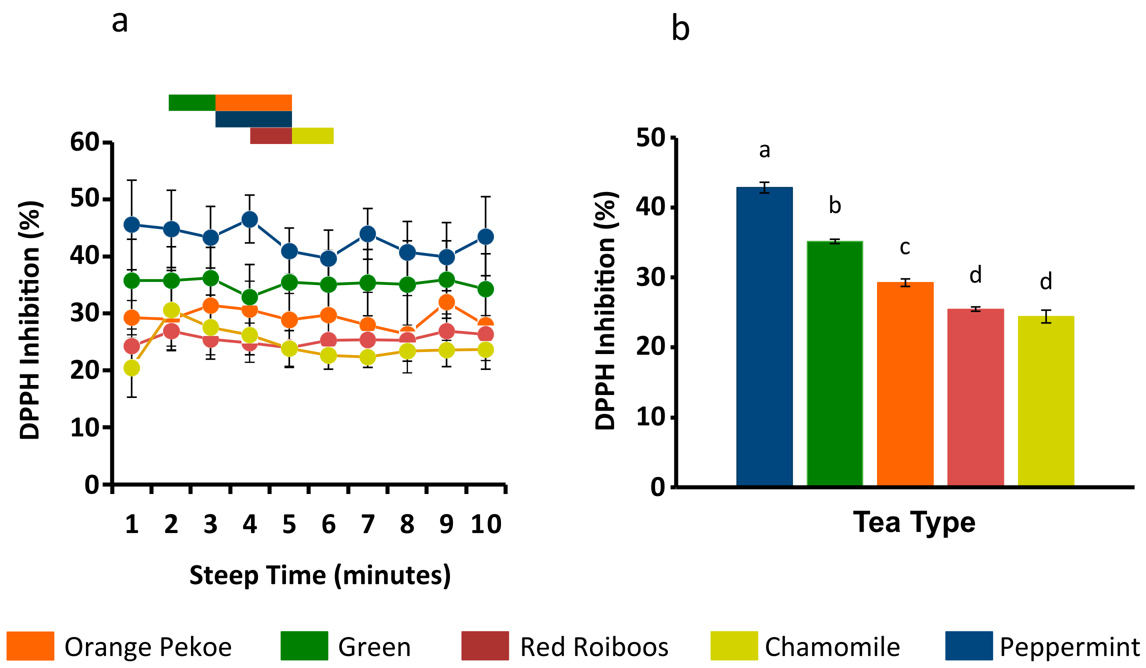

Figure 3. Antioxidant scavenging ability of teas: Orange Pekoe, Green tea, Red Rooibos, Chamomile, and Peppermint over a 10 min steep time window with minutely extractions. (**a**) normalized amount of tea polyphenols (1 µg GAE/mL) and % of DPPH inhibited through various steep times. Coloured bars indicate the manufacturers suggested steep time for each tea type; (**b**) DPPH inhibition in all tea types with steep times of 1–10 min averaged. A significant difference ($p < 0.05$) in percent inhibition of DPPH between tea types is indicated by differing letters.

3.3. Predicted Total Antioxidant Capacity

Predicted total antioxidant capacity was calculated after determining TPC and the antioxidant capacity of each sample after normalizing for polyphenol content (1 µg GAE/mL). A progressive increase in predicted total antioxidant capacity with steep time was observed for all tea types other than Chamomile (Figure 4a). After both the 5 and 10 min steep time points, the inter-tea differences in predicted total antioxidant capacity were as follows: G > OP > P ~RR > C ($p < 0.05$) (see Table A2 for complete analysis at all time points).

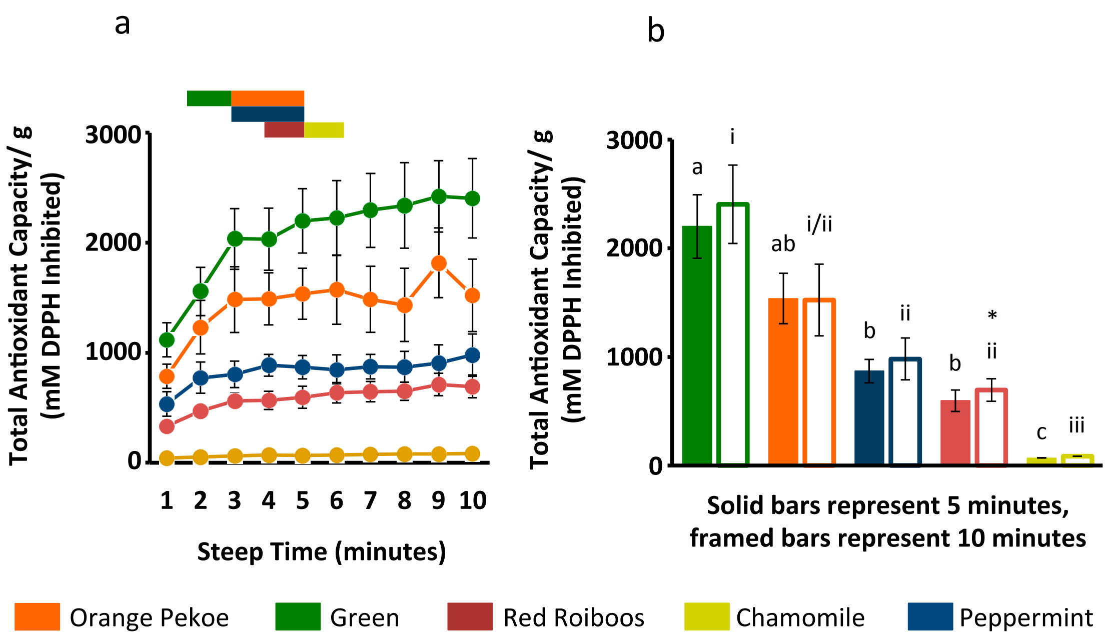

Figure 4. Predicted total antioxidant capacity (per gram) of five different teabag types after multiple points of steep time. (**a**) predicted total antioxidant capacity per gram of tea for a steep time duration of 1–10 min. Coloured bars indicate the manufacturers suggested steep time for each tea type; (**b**) predicted total antioxidant capacity/g of tea after a steep time of 5 min (solid coloured bars) compared to after 10 min (framed bars). * Represents a significant difference in total antioxidant capacity between 5 and 10 min for Chamomile ($p < 0.05$). Between tea comparisons were made separately for 5 min (letters) and 10 min (Roman numerals) data ($p < 0.05$). Specifically, total antioxidant capacity at 5 min significantly differs among tea types that have different letters while total antioxidant capacity at 10 min significantly differs among tea types that have different Roman numerals.

4. Discussion

Our investigation has demonstrated that most (~80–90%) of the polyphenols in tea bags are released in the initial 5 min of steeping, and, importantly, that an additional 5 min of steep time did not generally translate into significantly greater levels. This trend was observed across tea types other than Green tea at this time point, which roughly coincides with the manufacturers recommended instructions for preparation (exception: Green tea, 2–3 min).

Importantly, we observed that the absolute polyphenol content and prediction of total antioxidant capacity was dependent on the type of tea product, with tea samples from *Camellia sinensis* (G, OP) nearly twofold larger than herbal varieties (P, RR, and C). That both black and green teas originate from the same plant, *Camellia sinensis*, whereas the tisanes are derived from completely different plants (Red Rooibos from *Aspalathus linearis*, Peppermint from *Mentha piperita* and Chamomile from *Matricaria chamomilla*) [7,13] likely explains the higher TPC of these teas. In addition to plant type, oxidation level of the plant leaves has been shown to influence the different polyphenol profiles for a tea or tisane [18], which may explain the observed differences in antioxidant capacity between Green and Orange Pekoe teas. Specifically, black tea undergoes 100% oxidation [7], while oxidation is minimized in green tea and the results are a very different polyphenol profile [18]. Different polyphenols have a diverse range of antioxidant activity and thus a tea with a relatively lower TPC may have higher antioxidant capacity for this reason. The antioxidant capacity measured by DPPH inhibition showed Peppermint had the highest capacity, with Green, Orange Pekoe, Red Rooibos, and Chamomile following in close succession.

Although polyphenol content profiles vary between tea types due to the extent of oxidation, similar potential antioxidant activity was observed. For example, green tea is minimally oxidized and contains high levels of catechins (flavanols and flavanol gallates). In comparison, black tea is fully oxidized and through enzymatic processes, catechin content declines and complex flavanols such as theaflavins and thearubgins are formed [19]. Catechins and theaflavins have been shown to have similar antioxidant potential [20], and our finding that Green and Orange Pekoe tea had similar potential antioxidant activity are in agreement with this earlier study.

Of note was that the TPC and antioxidant capacity measured in the present study were similar to findings from our previous study in which we investigated effects of steep time on TPC and antioxidant capacity of loose leaf tea in both true teas and tisanes [13]. The exception was for Peppermint. In particular, the relative order of TPC between differing tea types remained similar with "true teas" (Orange Pekoe and Green) having greater TPC than tisanes in both studies regardless of tea form (loose leaf or bagged). In terms of antioxidant capacity, similarities were seen between the two studies with "true teas" having relatively high antioxidant capacities when compared to their tisane counterparts; however, one specific difference to note is that Peppermint tea had the highest antioxidant capacity when testing across bagged tea, but this was not seen in loose leaf form. Similar results were observed between the two studies for predicted total antioxidant capacity (a composite value incorporating both TPC and antioxidant capacity) with "true teas" having larger values than tisanes. Thus, despite likely differences in tea sourcing (origin), production, storage, and transportation between the products used in these studies, we suggest that the health related qualities of teas prepared from bags are not inferior or radically different from loose leaf preparations of the same variety. From a broader perspective, our current work also emphasizes that, despite being supplied in different forms (bagged or loose leaf) and having a different source of origin that may markedly alter the composition, similar trends are apparent when comparing across differing types of teas or tisanes. In addition to source of origin, which includes variables such as plant variety and growing conditions, other factors including various steps in the preparation and manufacturing methods used may independently affect the health-related characteristics of a tea infusion [14]. For example, in a study comparing green tea infusions, it was reported that the form of the tea (i.e., loose vs. bagged) was a significant factor in the extraction of catechins [21]. Thus, discretion should be used when interpretation of findings and comparisons are made among independent commercially available products.

Few studies have directly investigated the relationship between loose leaf and tea bags with the same biochemical approaches; therefore, our work offers additional evidence to help develop and/or challenge the research question, particularly with respect to methodological approaches. A previous published study [15] investigated the characteristics of black tea steeped from bags versus loosely-packed tea products, reporting and comparing tea polyphenol content and antioxidant capacity for twice the length of steep time (10 versus 5 min). However, we observed that, in general, steeping tea bags for 10 min, and thus beyond the manufacturers recommended steep time, does not seem to provide an additional benefit in the composition of the product (see Figure 2). Interestingly, the report that polyphenol content was higher in bagged versus loose leaf tea preparations [15] is contrary to our current findings, in which we compare our current study with recently performed experiments in our lab [13]. Of note is that methodological factors such as water temperature for tea preparation are not similar between these studies, which may account for these differences (96 °C and 80 °C were the temperatures for black teas prepared in [13] and in [15], respectively).

Tea type influences its antioxidant capacity based on the amount of processing of the tea. The more processed the tea the lower the antioxidant capacity. For example, the processing of black tea for teabag format undergoes the 'crush-tear-curl' (CTC) method via machine, compared to loose leaf black tea, which undergoes 'orthodox rollers' method. The CTC method used for bagged tea has been shown to result in a lower catechin content and antioxidant capacity of black tea [22]. This observation is in agreement with the present study; teabags had lower TPC than was measured in our previous study that investigated loose tea. Antioxidant capacity has been shown to be affected by the storage method of the teas [23]. Specifically, this study demonstrated that storage type correlated to greater antioxidant capacity based on the amount of oxygen permeability of the packaging [23]. In the present study, teabags were stored in standard retail paper based boxes leaving them more susceptible to oxygen permeability, compared with the air-tight tins used to store loose leaf tea in our previous study [13].

In subsequent analysis of our TPC data, we calculated the maximal rate at which polyphenols were released into solution following immersion of the tea bags (see Table 2). Green and Orange Pekoe teas demonstrated a much higher maximal rate of polyphenol appearance in comparison to the herbal tea varieties. Moreover, when values from the current study were compared to values calculated from the loose-leaf TPC data previously collected by our group [13], it was not apparent that the processing of intact tea leaves into tea bags resulted in any consistent effect on the rate at which polyphenols are released during steeping.

Within studies that investigate biological effects of tea, it is ideal for investigators to report the characteristics of the tea studied and record the conditions (steep time, TPC, predicted total antioxidant capacity) of a study to allow for comparison with other studies, and also allow other researchers to replicate findings. In summary, steep time alters TPC but not predicted total antioxidant activity of commercially-available tea bags that contained black or green tea or red rooibos or popular herbals (chamomile, peppermint).

5. Conclusions

There is growing interest in bioactive components of foods and beverages that may confer health benefits with regular consumption, and among these are a class of organic water-soluble molecules known as polyphenols. In this study, we have investigated the extraction and potential health-promoting qualities of polyphenols sampled from a variety of tea products as they would be consumed habitually by millions of individuals across the globe every day.

We conclude that when prepared according to the manufacturers instructions using boiled water, most of the polyphenols are released from prepared tea bags within 5 min of immersion, and that prolonging the steeping time twofold does not seem to translate into additional extraction. The current findings are similar to previous work from our group that studied tea samples prepared from loose leaf products, although it remains unclear whether factors specific to the product preparation may influence the rate at which polyphenols are initially released (i.e., particle/leaf size).

Although many tea products and beverages are consumed with taste as a primary consideration, the current study suggests that when products are prepared with particular health outcomes in mind, the significant differences in polyphenol content that exist between tea types should be considered.

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

Abbreviations

TPC	Total Polyphenol Content
GAE	Gallic Acid Equivalents
OP	Orange Pekoe Tea
G	Green Tea
RR	Red Roiboos
C	Chamomile
P	Peppermint
DPPH	2,2-Diphenyl-1-picrylhydrazyl

Appendix A

Table A1. TPC (in mg GAE/g of tea) measured in black, green, red rooibos, chamomile and peppermint tea bags steeped in distilled water for varying amounts of time. Values are expressed as mean ± standard deviation, n = 6/sample (* denotes groups with n = 4), GAE = Gallic acid equivalents. Columns classified by differing Roman numerals indicate a significant difference ($p < 0.05$) in TPC between tea types at a specific steep time. Rows classified by differing letters indicate a significant difference ($p < 0.05$) in TPC between time points of a specific tea type. Values below reliable detection using our method are listed as BD (below detection).

Tea Type	Steep Time (Minutes)																			
	1		2		3		4		5		6		7		8		9		10	
Orange Pekoe	44.76 ± 9.02	a / I	70.86 ± 9.15	b / I	78.75 ± 9.20	b / I	81.03 ± 17.21	b / I	88.72 ± 11.74	b / I	88.38 ± 10.51	b / I	88.75 ± 8.54	b / I	90.81 ± 9.03	b / I	94.64 ± 13.67	b / I	90.79 ± 10.64	b / I
Green	52.13 ± 6.43	a / I	72.50 ± 7.85	b / I	93.72 ± 10.20	c / II	103.20 ± 8.69	c,d / II	103.32 ± 12.55	c,d / I	105.71 ± 6.74	c,d / II	108.00 ± 8.53	c,d / II	117.01 ± 8.12	d,e / II	112.30 ± 15.06	c,e,f / II	117.15 ± 11.97	d,f / II
Red Rooibos	22.83 ± 2.69	a / II	29.20 ± 6.41	a,b / II	36.78 ± 2.53	b,c / III	38.11 ± 3.25	c / III	41.38 ± 3.21	c / II	41.97 ± 3.97	c / III	42.48 ± 4.39	c / III	42.89 ± 3.86	c / III	44.11 ± 5.30	c / III	43.99 ± 5.75	c / III
Chamomile	BD	-	2.77 ± 0.41 *	a / III	3.63 ± 1.01 *	a,b / IV	4.47 ± 1.02 *	a,b / IV	4.72 ± 1.16	a,b / III	5.37 ± 1.04	b / IV	5.53 ± 0.84	b / IV	5.76 ± 1.22	b / IV	5.64 ± 1.56	b / IV	6.07 ± 1.54	b / IV
Peppermint	23.63 ± 8.36	a / II	34.71 ± 9.92	a,b / II	37.62 ± 8.31	a,b / III	39.92 ± 7.96	a,b / III	44.69 ± 9.51	b / II	44.11 ± 7.92	b / III	42.08 ± 10.00	b / III	44.11 ± 9.29	b / III	46.30 ± 8.96	b / III	46.32 ± 10.97	b / III

Table A2. Predicted total antioxidant capacity (per gram of tea) measured in black, green, red rooibos, chamomile and peppermint tea bags steeped in distilled water for varying amounts of time. Values are expressed as mean ± SD, n = 6/sample (* denotes groups with n = 4), GAE = Gallic acid equivalents. Columns classified by differing Roman numerals indicate a significant difference ($p < 0.05$) in predicted total antioxidant capacity between tea types at a specific steep time. Rows classified by differing letters indicate a significant difference ($p < 0.05$) in predicted total antioxidant capacity between time points of a specific tea type. Values below reliable detection using our method are listed as BD.

Tea Type	Steep Time (Minutes)																			
	1		2		3		4		5		6		7		8		9		10	
Orange Pekoe	786.86 ± 268.40	a / I,II	1226.44 ± 595.59	a / I,II	1493.20 ± 733.72	a / I,II	1456.95 ± 580.48	a / I,II	1516.37 ± 569.41	a / I,II	1538.12 ± 774.06	a / I	1481.41 ± 735.92	a / I	1416.85 ± 814.64	a / I	1707.24 ± 706.84	a / I	1521.25 ± 806.29	a / I
Green	1062.79 ± 379.03	a / I	1540.69 ± 537.71	a / I	2019.63 ± 677.58	a / I	1983.51 ± 690.83	a / I	2131.39 ± 717.33	a / I	2118.26 ± 836.52	a / I	2256.48 ± 825.91	a / I	2286.86 ± 867.80	a / I	2326.20 ± 796.35	a / I	2340.84 ± 883.53	a / I
Red Rooibos	331.49 ± 100.52	a / III	468.40 ± 148.78	a / III,	566.38 ± 163.51	a / III	572.24 ± 205.42	a / III,IV	607.06 ± 245.83	a / III,IV	646.16 ± 232.59	a / I	653.93 ± 227.86	a / I	658.66 ± 205.66	a / I	724.70 ± 252.40	a / I	705.44 ± 257.85	a / I
Chamomile	BD	—	50.04 ± 12.22 *	a / III	55.40 ± 11.43 *	a / III	66.66 ± 14.03 *	a / III	67.20 ± 23.09	a / III	73.99 ± 25.84	a / I	75.31 ± 21.39	a / I	81.06 ± 22.21	a / I	77.89 ± 23.24	a / I	83.32 ± 26.07	a / I
Peppermint	474.09 ± 180.73	a / II,III	719.54 ± 261.11	a / II,III	777.00 ± 262.46	a / II,III	872.87 ± 257.26	a / II,IV	842.67 ± 208.84	a / II,IV	835.58 ± 340.64	a / I	853.72 ± 298.23	a / I	833.02 ± 297.24	a / I	874.21 ± 369.64	a / I	910.32 ± 279.31	a / I

Table A3. Tea product information.

Tea Type	Product Name	Notes	Manufacturer & Supplier Information
OP	Red Rose	Blended black tea leaves	Imported by Unilever Canada, Toronto, ON, Canada. M4W 3R2
G	Green Tea	Blend of Chinese & Japanese green tea	Processed in Canada by Loblaws, Inc. Toronto, ON, Canada. M4T 2S8
RR	Red Roiboos	Fermented (red) roiboos tea leaves	Processed in Canada by Loblaws, Inc. Toronto, ON, Canada. M4T 2S8
C	Chamomile	-	Blended and packed in Canada by Loblaws, Inc. Toronto, ON, Canada. M4T 2S8
P	Pure Peppermint	-	Blended and packed by R. Twining & Company Limited, London, WC2R 1AP, UK

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