Articles

2017

Polyphenolic Content and Antioxidant Capacity of White, Green, Black, and Herbal Teas: a Kinetic Study

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Recommended Citation

FOOD

RESEARCH

Polyphenolic content and antioxidant capacity of white, green, black, and herbal teas: a kinetic study

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Abstract

Camellia sinensis teas, and tisanes derived from herbs or fruit, are rich in polyphenolic, antioxidant compounds. This study compared the total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), DPPH radical scavenging capacity, and caffeine content of teas (black, green, white, chamomile, and mixed berry/hibiscus) over a range of infusion times (0.5–10 mins) at 90°C. Green, followed by black tea, respectively, had the greatest TPC (557.58 and 499.19μg GAE/g), TFC (367.84 and 325.18μg QE/g), FRAP (887.38 and 209.38μg TE/g), and DPPH radical scavenging capacity (1233.03 and 866.39μg AAE/g). Caffeine content per cup (200mL) in black, green, and white tea was 63, 51, and 49mg respectively. Changes in the phenolic content and antioxidant capacity of teas were modelled using zero, first, and fractional-conversion-first-order (FCFO) kinetic models. Results fitted a FCFO kinetic model, providing useful data for maximum phytochemical preservation in the optimisation of industrial and domestic processing. As a dietary comparison, green, black, and white tea were found to have a greater phenolic content and antioxidant capacity than fresh orange and apple juice. It can be concluded that green and black teas are significant sources of dietary phenolic antioxidants.

1. Introduction

Tea is consumed more than any other beverage worldwide (Ho *et al.,* 2008). It is a hot water infusion of the dried, young leaves and/or buds of the evergreen *Camellia sinensis* plant. Infusions made from other plants such as herbs, berries, or flowers are known as tisanes (Chu and Juneja, 1997; Saberi, 2010). In 2012, 4.884 million tonnes of tea was produced and consumed globally. Per capita, Turkey is the greatest consumer of tea globally, where 3.157kg was consumed per person in 2011; followed by Ireland, where 2.191kg/per capita was consumed (Caballero *et al.*, 2015). Epidemiological studies strongly suggest that regular consumption of plant polyphenols, such as those found in tea and tisanes, can protect against the development of disorders caused by free radical damage, including cardiovascular disease, cancer, diabetes, osteoporosis, and neurodegenerative diseases (Jung and Ellis, 2001; Duthie, 2007; Pandey and Rizvi, 2009; Dai and Mumper, 2010). There are numerous phytochemicals in teas produced from the *C.*

sinensis plant, but polyphenols and the sub-division of flavonoids are considered most important in terms of health benefits due to their ability to act as antioxidants by donating electrons or hydrogen protons to reactive oxygen or nitrogen species. Chamomile and berry/hibiscus tisanes also contain polyphenols that are considered beneficial to health, but at significantly lower levels than *C. sinensis* derived teas. The principle polyphenols in chamomile are chrysin, luteolin, and coumarins (Igile *et al.*, 1994; Yang *et al.*, 2008). The berry/hibiscus tisane used in this study contains procyanidins, rutin, anthocyanins, resveratrol, β-carotene, ellagic acid, and the xanthophyll, lutein (Petronilho *et al.*, 2012; Guimarães *et al.*, 2013; Juneja *et al.*, 2013).

Water temperatures and infusion times used for tea brewing vary greatly, and have a significant effect on the extraction yield of phytochemicals such as polyphenolic compounds; and methylxanthines – for example, caffeine, theophylline, and theobromine. Domestic

temperatures generally range from 65-95°C, with green and white teas commonly brewed at lower temperatures than black varieties. Some studies have shown that increased polyphenolic content of black, green, and white *C. sinensis* teas can be achieved with brewing times in excess of ten minutes (Langley-Evans, 2000; Astill *et al.*, 2001; Komes *et al.*, 2010; Imran *et al.*, 2013). However, this is primarily for extraction of compounds in industrial applications, since astringency and bitterness also increase with time, affecting organoleptic properties of the beverage (Brown, 2014). Loose leaf versus bagged, or powdered tea, and the material from which the bag is made can influence the rate of phytochemical extraction. Variables such as cultivar, ontogenetic factors, geographic location, processing conditions, storage, and particle size, or grade, of tea leaves also influence the phytochemical composition of the beverage (Astill *et al.*, 2001; Samaniego-Sánchez *et al.*, 2011; Brown, 2014; Lee *et al.*, 2015). Kinetic modelling of antioxidant behaviour in food and beverage matrices has provided useful data for the optimisation of phytochemical extraction (Rao *et al.*, 2014). An understanding of the kinetics of phenolic and antioxidant degradation in tea as a function of infusion time may be used to optimise industrial and domestic brewing processes for maximum phytochemical preservation.

Some studies have evaluated the kinetics of solid-liquid extraction of antioxidants in green or black tea (Anissi *et al.*, 2014; Ahmad *et al.*, 2015; Fernando and Soysa, 2015), however there is a void in the literature on kinetic data regarding white and herbal teas in comparison to green and black varieties. The present study focused on quantification and comparison of the total phenolic content, total flavonoid content, caffeine content, ferric reducing antioxidant power, and DPPH radical scavenging capacity of a range of commercial teas (black, white, green, chamomile, and mixed berry/hibiscus) over a range of infusion times (0.5–10 mins), using an average domestic brewing temperature (90°C). As a dietary comparison, the antioxidant and phenolic content of each tea was also compared to that of fresh orange and apple juice. In addition, a kinetic study was carried out over a range of infusion times to determine the rate at which maximum antioxidants are lixiviated from each tea. Based on the findings, an optimum infusion time for antioxidant-rich tea is recommended.

2. Materials and methods

2.1 Samples

Black, green, chamomile, and berry/hibiscus teas were prepared from *Barry's Tea* brand, while white tea samples were prepared from *Qi* brand tea bags. All samples were purchased from local supermarkets in Dublin during September 2013. All chemicals were purchased from Sigma-Aldrich, Ireland unless otherwise indicated.

2.2 Sample preparation

After an initial study, a brewing time of 0.5 to 10 minutes was used to infuse each tea type at a mean, domestic brewing temperature of 90°C. Boiled, potable tap water (100°C, 200 mL) (GH Zeal Ltd L0132 glass thermometer, U.K.) was transferred to a pre-heated ceramic cup. The infusion water was found to have a mean temperature of 90°C after transfer to the cup. One tea bag (mean dry leaf mass ~2.0 g) was added and stirred (360° twice) with a glass rod. After 0.5 mins, a stainless steel, manual tea bag squeezer (Kitchen Craft *leXpress*, U.K.) was used to squeeze (3 sec) and discard the bag. The brewed tea was chilled on ice and stored, away from light, at 4°C (Lec AC150, U.K.). The process was repeated with a fresh tea bag in quadruplicate for each infusion time.

2.3 Determination of total polyphenolic content (TPC)

TPC was carried out according to existing protocols in the laboratory as described by Rajauria *et al.* (2013) using the Folin Ciocalteu assay. In a 96-well microtiter plate, 2% sodium carbonate (2 mL) was added to each tea sample (100 μL). After 2 mins, 50% Folin Ciocalteu reagent (100 μL) was added. After 30 mins incubation at 25°C, protected from light, absorbances were measured at 720 nm using a UV-Vis spectrophotometric microplate reader (BioTek PowerWave, Gen5 Data Analysis, USA). Results were expressed in gallic acid equivalents per gram of dry tea (GAE/g) extrapolated from a calibration curve of gallic acid (0-500 μg/mL).

2.4 Determination of total flavonoid content (TFC)

The aluminium chloride assay described by Jaiswal *et al.* (2012) was used to quantify the TFC of each tea. Briefly, each tea sample (250 μL), ddH$_2$O (1.25 mL) and 5% sodium nitrite (75 μL) were added. After 6 mins, 10% aluminium chloride solution (150 μL) was added. Sodium hydroxide (1M 0.5 mL; Fluka, Ireland) and ddH$_2$O (575 μL) was added to bring the total volume to 2.5 mL. A blank was prepared in the same manner, using ddH$_2$O (250 μL). Absorbances were read at 510 nm. Results were expressed in quercetin equivalents per gram (QE/g) of dry tea, through a calibration curve of quercetin (0-100 μg/mL).

2.5 Determination of ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power assay (Benzie and Strain, 1996) was used to determine antioxidant capacity. FRAP reagent was prepared (300mM sodium acetate buffer, pH 3.6, 20 mM ferric chloride hexahydrate ($FeCl_3$•$6H_2O$), and 10 mM 2,4,6-Tris(2-pyridyl) s-triazine, Fluka, Ireland; in 40 mM HCl, Fisher-Scientific, Ireland, in a ratio of 10:1:1, v/v/v) and incubated in a water bath at 37°C for 5 mins. In a microtiter plate, FRAP reagent (100 μL) was added to each tea sample (50 μL). A blank was prepared in the same manner, using ddH₂O (50 μL) in place of the tea sample. After 10 mins incubation at 25°C, the absorbance was measured at 593nm. Results were expressed in Trolox equivalents per gram (TE/g) of dry tea, extrapolated from a calibration curve of Trolox (0-25 μg/mL).

2.6 Determination of 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity

The antioxidant capacity of each tea was determined using the DPPH radical scavenging capacity assay according to Blois (1958). DPPH radical solution (165 μM, in methanol) or ddH₂O was added to each tea. After 30 mins incubation in darkness at 30°C, absorbances were measured at 517 nm. DPPH radical scavenging capacity was calculated as follows:

% scavenging capacity = [(Abs Control) – (Abs tea)/ (Abs Control)]*100

DPPH radical scavenging capacity was expressed in ascorbic acid equivalents per gram of dry tea (AAE/g), extrapolated from a calibration curve of L-ascorbic acid (0-12 μg/mL).

2.7 Determination of caffeine content

Reverse phase high performance liquid chromatography was used to quantify caffeine (Alliance-Waters e2695 Separations Module, USA), using a C18 reverse phase column (Waters Atlantis T3, 250 x 4.6mm x mm, 5 μm particle size, USA), and a UV photodiode array detector (Waters 2998, USA). Tea samples were syringe-filtered (Millex Durapore PVDF, 0.22 μm pore) into HPLC vials. Two mobile phases were used in a gradient programme for 70 mins at 25°C: sodium acetate (2mM) with acetic acid (6%); and acetonitrile (100%; Fluka, Ireland). Mobile phases were filtered (Merck Millipore HVLP 0.45 μm filter, Germany) and sonicated (Branson Ultrasonic 5510, USA). Injection volume was 20 μL, with a flow rate of 1 mL/min. Analyses were carried out in quadruplicate. Detection was performed at 280 nm. The concentration of caffeine in the tea samples was extrapolated from a calibration curve of caffeine (25 -200 μg/mL).

2.8 Kinetic study

The kinetics of tea phenolic content, flavonoid content and antioxidant capacity were described by fitting a zero order (Equation 1), first-order (Equation 2), and fractional conversion first-order (FCFO) (Equation 3) to the experimental data.

$$A = A_0 \pm kt \tag{1}$$

$$A = A_0 * \exp^{\pm kt} \tag{2}$$

$$A = A_{eq} + (A_0 - A_{eq})/(\exp^{\pm kt}) \tag{3}$$

Where A is the parameter to be estimated, the sub index 0 indicates the initial value of the parameter (absorbance value of tea at 0.5 mins), t is the infusion time, and k is the rate constant at temperature t. The sub index, eq, indicates equilibrium value (final absorbance value of tea at 10 mins). For the parameter estimation, the individual measured concentrations were used instead of mean values of quadruplicate experiments, thus taking into account variability within the samples. The coefficient of determination (R^2) and mean square error (MSE) were used as criteria for adequacy of fit.

2.9 Statistical analysis

Statgraphics Centurion XVII was used for kinetic modelling and statistical analyses, with differences considered significant where $P \leq 0.05$. All experiments were conducted in quadruplicate ($n = 4$) and replicated at least twice. Results were calculated as mean values ± standard deviation. Fisher's Least Significant Difference (LSD) procedure was used to discriminate amongst the means of the variables.

3. Results and discussion

All polyphenolic content and antioxidant capacity values are presented for teas after 5 mins infusion, rather than after the maximum infusion time (10 mins). Five minutes was determined to be the optimum brewing time, as no significant (P > 0.05) increase was found in the phytochemical content between 5 and 10 mins for all tea varieties investigated in this study. Figure 1 shows the comparative TPC, TFC, FRAP, and DPPH radical scavenging capacity of black, green, white, chamomile, and berry teas after 5 mins infusion.
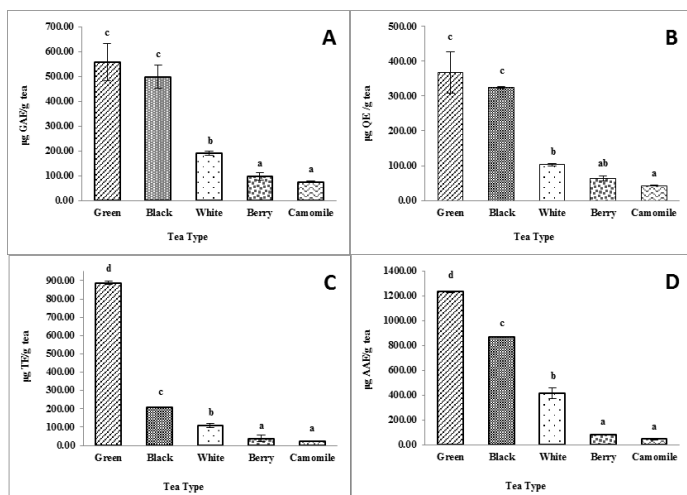
Figure 1. Comparative TPC (A), TFC (B), FRAP (C), and DPPH radical scavenging capacity (D) of black, green, white, chamomile, and berry teas after 5 min infusion. Values are the mean of four replicates for each tea type ± standard deviation. Letters denote least significant difference between columns (*P* ≤ 0.05)

3.1 Total polyphenolic content

After five minutes infusion, green tea had the highest total phenolic content of 557.58 ± 74.98; followed by black 499.19 ± 46.56; white 190.24 ± 7.73; berry 98.86 ± 14.72; and chamomile 75.31 ± 3.65 µg GAE/g tea. No published results are available for the TPC, or any phytochemical content, of the specific *Barry's* and *Qi* brands of teas used in this study. Reported TPC results for similar teas prepared under comparable brewing conditions vary widely. Adjusting for differences in infusion volume and time, Unachukwu *et al.* (2010) quantified a TPC of 1.17mg GAE/g in green tea; and 0.96mg GAE/g in white tea. Yoo *et al.* (2008) found a TPC of 0.746 mg GAE/g in black tea, and 0.84 mg GAE/g in chamomile tea. Achoribo *et al.* (2012) reported 0.65 mg GAE/g for a hibiscus-based tea. As expected from published values, green tea was found to have the highest level of total phenolics (Komes *et al.*, 2010; Anissi *et al.*, 2014), followed by black tea; while the chamomile, berry, and white teas were significantly lower (P ≤ 0.05). White tea, although from the same *C. sinensis* plant as black and green varieties, may have a lower TPC due to the fact that oxidation by polyphenol oxidase is prevented in white tea processing (Astorino *et al.*, 2012). Therefore, the phenolic monomers in the fresh leaves are not complexed into polyphenols such as catechins in green tea, or thearubigins in black tea. Rusak *et al.* (2008) reported that the extraction of total phenolics from white tea leaves occurred at a significantly slower rate than that of other teas. Their study found that although the highest TPC of both white and green teas was established after 30 mins extraction, green tea

reached a significantly higher TPC, particularly flavonoids, within the first 5 mins of extraction. The filamentous trichomes covering the buds from which white tea is prepared to have a lipophilic cuticle surrounding their cell walls (Rusak *et al.*, 2008) which may affect the migration kinetic of hydrophilic catechins during water-infusion.

3.2 Total flavonoid content

After 5 mins infusion, green tea had the highest total flavonoid content of 367.84 ± 59.61 µg QE/g tea. Adjusting for differences in infusion volume and time, published results for comparable TFC experiments have been reported as 1640 µg QE/g for green tea and 1480 µg QE/g for black tea (Oh *et al.,* 2013). A mean TPC of 183,000 µg QE/g was reported in a range of chamomile teas by Haghi *et al.* (2013) and 87.17 µg QE/g in a hibiscus-based tea (Achoribo *et al.,* 2012). No results have been reported in quercetin equivalents for white tea, however, Carloni *et al.* (2013) quantified a TFC of 3900 µg catechin equivalents/g in white tea using the same methodology as the present study. The total flavonoid content of *C. sinensis* and herbal teas was lower than most published values. However, a direct comparison may not be equivalent due to the varying geographic locations, harvesting time, and environmental conditions from which each study selected samples, and the impact this has on phytochemical content.

3.3 Ferric reducing antioxidant power

After 5 mins infusion, green tea the highest ferric reducing power of 887.38 ± 8.71, followed by black 209.38 ± 1.86; white 110.43 ± 11.85; berry 37.96 ± 17.42; and chamomile 24.57 ± 1.26 µg TE/g tea. Reported FRAP results for similar teas prepared under comparable brewing conditions vary. Allowing for differences in mass of dry tea and infusion time, Rusak *et al.* (2008) reported 1.05 mmol TE/L (approximately equivalent to 5250 µg TE/g) in green tea. Prior and Cao (1999) quantified 8.31 µmol TE/mL (approximately equivalent to 2077 µg TE/g) in black tea. Andlauer and Héritier (2011) found 0.484 mg TE/g in white tea. Pellegrini *et al.* (2003) reported 1.26 mmol TE/L in chamomile tea (approximately equivalent to 630 µg TE/g); and 312 µmol Trolox equivalents/100mL (approximately equivalent to 124µg TE/g) was reported for a hibiscus-based tea (Sáyago-Ayerdi *et al.*, 2007). As expected, green tea had the highest ferric reducing antioxidant power, followed by black, white, berry and chamomile teas. A similar study using FRAP analysis of green and black tea bags infused at 90°C for time periods ranging from 0.25 to 15 minutes, found that green tea

had an antioxidant capacity almost three times higher than that of black tea (Langley-Evans, 2000). This is due to the changes that occur in black tea during processing, catalysed by polyphenol oxidase. The flavanols in the (green) tea leaves are converted to oxyproducts in black tea, such as thearubigins and theaflavins, resulting in a loss of antioxidant capacity (Benzie and Szeto, 1999).

3.4 2,2-diphenyl-1-picrylhydrazyl radical scavenging capacity

The same trend observed in TPC, TFC, and FRAP occurred in DPPH radical scavenging capacity, which was greatest in green tea (1233.03 ± 7.88 µg AAE/g); followed by black 866.39 ± 2.97; white 414.03 ± 44.22; berry 82.89 ± 6.13; and lowest in chamomile (47.44 ± 1.04 µg AAE/g). Reported DPPH radical scavenging capacity for similar teas prepared under comparable brewing conditions vary widely. Allowing for differences in mass of dry tea and infusion time, Oh *et al.* (2013) found green tea to have a DPPH radical scavenging capacity of 12,390 µg AAE/g and 9890 µg AAE/g in black tea. Karori *et al.* (2007) reported 1900 µg AAE/g in white tea, while two similar studies found 110 µg AAE/g in chamomile (Sazegar *et al.*, 2011); and 639 µg AAE/g in a hibiscus tea (Ramadan-Hassanien, 2008).

The observed TPC, TFC, FRAP, and DPPH results for each tea in the present study may differ to some published values due to differences in tea cultivar, soil type, altitude of cultivation, post-harvest storage, processing conditions, and ontogenetic effects (Imran *et al.*, 2013; Pavlović *et al.*, 2013; Yang and Liu, 2013; Lee *et al.*, 2015; Soni *et al.*, 2015).

3.5 Antioxidant comparison with fresh orange and apple juice

3.5.1 TPC comparison with teas after 5 min infusion and freshly juiced orange and apple

As a dietary comparison, the total phenolic content of two commonly consumed juices, freshly squeezed orange and apple, was compared to that of each tea. The high proportion of phenolic antioxidant compounds in orange and apple juices and their health benefits have been widely reported (Preedy, 2014; Cuervo *et al.*, 2015; Hui and Evranuz, 2015). The World Health Organization recommends consuming at least five portions of fruit and vegetables per day to reduce the risk of diseases such as cancer, heart disease, and diabetes (WHO, 2015). Considering the significant consumption of tea worldwide, the total phenolic content of each tea was therefore compared to that of the juices as a measure of

its dietary antioxidant contribution. TPC was quantified using the same methodology detailed above.

Orange juice was found to have a TPC of 436.6 ± 10.9, and apple juice 603.33 ± 14.1 µg GAE/g. None of the five teas had a total phenolic content equal to that of apple juice (603.33 µg GAE/g). However, green and black teas (557.58 and 499.19 µg GAE/g respectively) both had a greater TPC than orange juice (436.6 µg GAE/g). Green tea had 92.42% the TPC of apple juice, and black tea had 82.74%. White, berry, and chamomile teas (190.24 ± 7.73, 98.86 ± 14.72, 75.31 ± 3.65 µg GAE/g) had a significantly lower TPC of 31.53%, 16.39%, and 12.48% respectively, in comparison to apple juice. In comparison to orange juice, white, chamomile, and berry teas had a TPC of 43.57%, 22.64%, and 17.25% respectively.

Figure 2 shows the comparative total phenolic content, and DPPH radical scavenging capacity of teas after 5 mins infusion, orange, and apple juice in µg AAE/g.

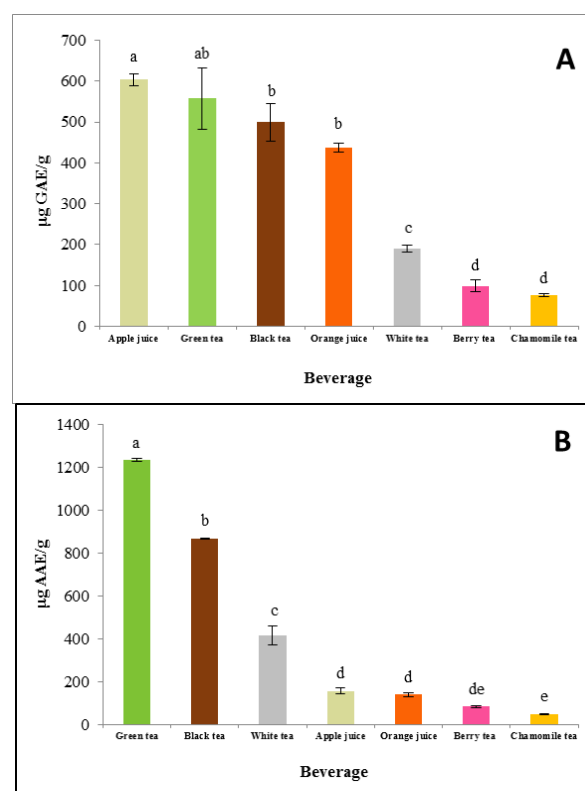Figure 2. (A) Comparative total phenolic content, and (B) DPPH radical scavenging capacity of teas after 5 min infusion, orange, and apple juice. Values are the mean of four replicates for each tea type ± standard deviation. Letters denote least significant difference between columns ($P \leq 0.05$)

3.5.2 DPPH radical scavenging capacity comparison with teas after 5 min infusion and freshly juiced orange and apple

As discussed, a comparison may be made between the dietary antioxidant contribution of phenolic antioxidants in orange and apple juices and that of tea. The DPPH radical scavenging capacity of freshly juiced orange and apple was quantified using the same methodology detailed in above. Orange juice was found to have a DPPH radical scavenging capacity of 138.42 ± 10.9, and apple juice 155.41 ± 14.1 µg AAE/g. Green, black, and white teas were found to have DPPH radical scavenging capacities significantly greater ($P \leq 0.05$) than that of apple juice. As percentage comparisons, green, black, and white tea capacities were 793.41%, 557.49%, and 266.14% greater than that of apple juice. Berry and chamomile teas had lower DPPH radical scavenging capacities than apple juice: 53.34% and 30.53% in comparison. A similar trend was seen in the comparison of the teas to the orange juice. Green, black, and white teas had DPPH radical scavenging capacities 890.79%, 625.91%, and 299.11% greater than the orange juice, while berry and chamomile teas were only 59.88% and 34.27% in comparison. From this data, green, black, and white teas have a significantly greater antioxidant capacity than both apple and orange juices per µg ascorbic acid equivalents per gram. This trend is common in published results for comparable DPPH assays (Ramadan-Hassanien, 2008). Paganga *et al.* (1999) found that two 150 mL servings of black tea had the same antioxidant activity as four whole apples, seven glasses of long life orange juice, twenty glasses of long life apple juice, or one glass of red wine (each 150 mL).

In respect of phenolic antioxidant content, the TPC and DPPH results show that *C. sinensis* teas are comparable to and in several cases greater than, the fruit juices studied. This is due to the presence of flavonoids in *C. sinensis* plants, which comprise 30-40% of the dry mass of tea leaves (Juneja *et al.,* 2013). The majority of tea flavonoids are flavan-3-ols (or flavanols). Examples include epigallocatechin in green tea, and theaflavin-3-gallate, in black tea. They are more potent antioxidants than those found in apples and oranges due to delocalised electrons in their alternating single and double (conjugated) bonds arranged in polyphenolic systems. If an electron from a flavanol molecule is lost to a free radical, the flavanol remains stable due to the sharing of electrons in its conjugated system (Kaur and Kapoor, 2001). For this reason, *C. sinensis* teas are more powerful dietary antioxidants than most fruits. HPLC analysis of caffeine.

HPLC peaks and retention times for the five tea varieties at each infusion time were compared to those of the caffeine standards. Caffeine content was extrapolated

from the standard curve equation generated. HPLC results for caffeine content per 200 mL serving of tea after 5 mins infusion are presented in Figure 3.
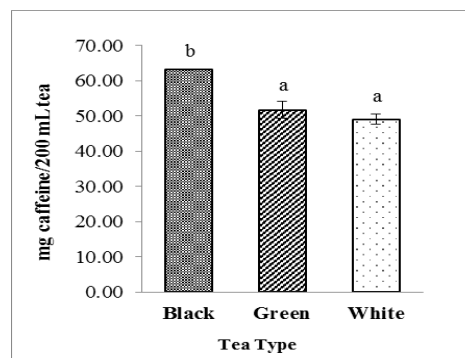

Figure 3. Comparative caffeine content of green, black, and white teas after 5 min infusion. Values are the mean of four replicates for each tea type ± standard deviation. Letters denote least significant difference between columns ($P \leq 0.05$)

After 5 mins infusion, black tea had the highest caffeine content of 63.3 ± 0.01; followed by green 51.7 ± 2.41; and white 49.1 ± 1.39 mg/200 mL. These values are in line with those published for black, green, and white teas (Astill *et al.*, 2001; Preedy, 2012). Chin *et al.* (2008) quantified caffeine contents of 14–61 mg per serving in white (lowest), green, and black teas (highest); and zero caffeine in herbal teas. Carloni *et al.* (2013) reported 15.6mg/200mL in white tea, 36.6 mg/200mL in green tea, and 32.6mg/200mL in black tea after seven minutes infusion. As expected, caffeine was not detected in chamomile or berry teas (Duke, 1997; Ali *et al.*, 2005; Guimarães *et al.*, 2013). In *C. sinensis* plants, caffeine is biosynthesised by the transfer of the methyl group from S-adenosyl methionine to methyl xanthine. The rate of this synthesis is increased during the withering/warm drying process of tea production (Panda, 2011). It was expected that black tea would have the greatest caffeine content since it is subjected to more withering than green and white teas. Although black, green, and white teas are a source of healthy dietary phenolics and antioxidants, excessive consumption can result in a caffeine intake above recommended levels. In order to avoid the side effects of excess caffeine consumption, such as heart palpitations, insomnia, and anxiety, a maximum of 300 mg caffeine per day is recommended for adults; and less than 200 mg/day during pregnancy (Preedy, 2012). 300 mg equates to approximately five servings of black tea or six servings of green or white tea. *In vitro* values determined in this study may differ from those *in vivo* since polyphenols undergo extensive modification during digestion via conjugation in the intestinal cells and liver by sulphation, methylation, and glucuronidation (Setchell *et al.*, 2003).
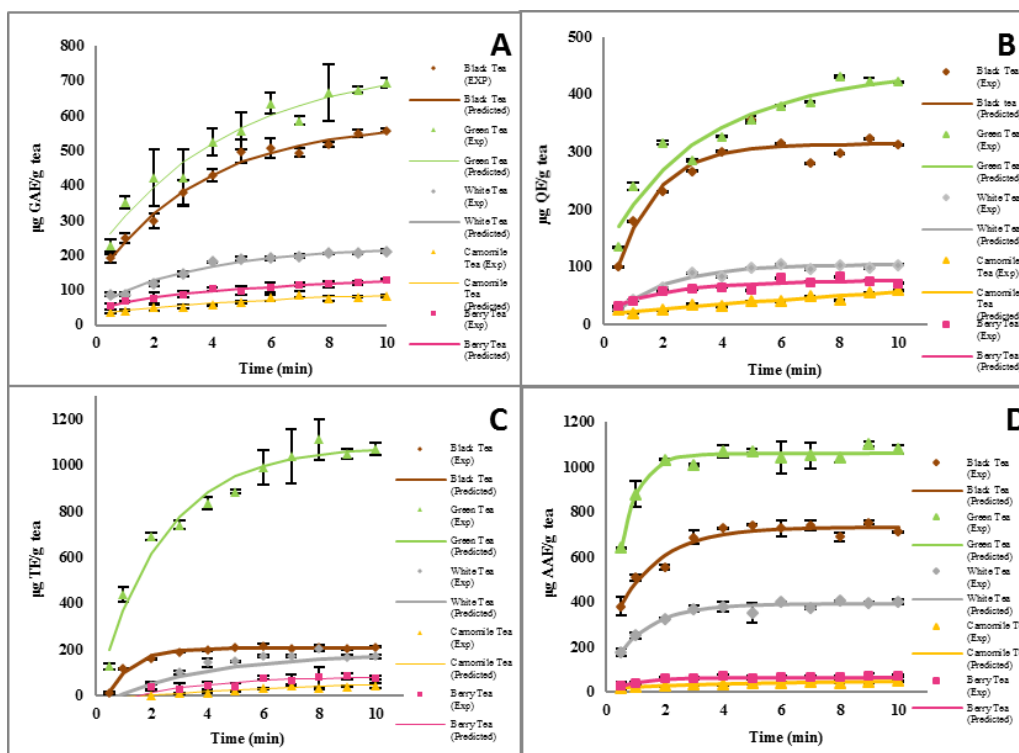
Figure 4. Experimental and predicted data (fractional conversion first order kinetics model) for the effect of infusion time on the TPC (A), TFC (B), FRAP (C), and DPPH radical scavenging capacity (D) of black, green, white, chamomile, and berry teas. Values are the mean of four replicates for each tea type ± standard deviation.

3.6 Kinetic study

A FCFO kinetics model was found to best fit all data, with the highest R^2 values, ranging from 77.49 to 98.12, and the lowest MSE value ranging from 0.26 to 5477.91. Kinetic parameter mean estimates, corresponding coefficient of determination, and mean square error of phenolic content and the antioxidant capacity increase in teas after 10 mins infusion are presented in Table 1.

A FCFO model was expected to fit each of the four assays since the raw data clearly demonstrated a steep initial increase, followed by a more constant content (Jaiswal *et al.*, 2012). Experimental and predicted data (FCFO kinetics model) for TPC, TFC, FRAP, and DPPH radical scavenging capacity of teas due to infusion over 10 mins are presented in Figure 4.

Kinetic studies for single tea cultivars have reported optimum infusion times in line with the present study. For example, Fernando and Soysa (2015) investigated the extraction kinetics of phenolic compounds, caffeine, catechins, and antioxidant activity as a function of time in black tea and found the optimum infusion time for the release of tea constituents was 2–8 mins. However, no kinetic data has been published for the range of teas used in the present study.

Kinetic modelling of phenolic and antioxidant behaviour in tea matrices as a function of infusion time

provides useful data for the prediction of organoleptic and nutritional losses and allows for the calculation of TPC, TFC, FRAP, and DPPH radical scavenging capacity in theoretical experiments at untested infusion times. For example, in the case of white tea, application of this model would determine whether a longer infusion time could result in increased phenolic content or antioxidant activity. Kinetic data may be used to optimise industrial and domestic brewing processes for maximum phytochemical and nutritional preservation.

4. Conclusion

Green, followed by black tea, had the highest TPC, TFC, FRAP, and DPPH scavenging capacity of all five teas. Although white tea is also produced from *C. sinensis*, it had significantly lower ($P \leq 0.05$) TPC, TFC, FRAP, and DPPH radical scavenging capacity. Chamomile and berry teas had significantly lower ($P \leq 0.05$) phenolic and antioxidant contents than green, black, and white teas. Black tea had the highest caffeine content, followed by green, then white tea. Changes in the phenolic content and antioxidant capacity of teas were modelled using zero, first, and FCFO kinetic models. FCFO model fitted the data, with the highest R^2 values and lowest MSE values for all teas. This data may be used to optimise industrial and domestic brewing processes for maximum phytochemical preservation. The optimum infusion time for maximum extraction of phenolic and antioxidant compounds for all teas was

Table 1. Kinetic parameter mean estimates (k), corresponding coefficient of determination (R^2), and mean square error (MSE) of phenolic content and antioxidant capacity increase in teas after 10 min infusion

			Tea Type				
			Black	Green	White	Chamomile	Berry
Total phenolic content	Zero Order	k	36.88	46.67	13.62	5.06	5.06
		R^2	86.68	77.74	85.80	85.00	87.01
		MSE	2445.14	7497.15	359.05	59.46	93.56
	First Order	k	0.08	0.08	0.07	0.07	0.07
		R^2	79.80	79.94	78.70	80.75	83.46
		MSE	3709.98	5335.20	538.66	75.43	119.06
	Fractional Conversion First Order	k	0.27	0.20	0.28	0.14	0.20
		R^2	97.02	91.21	97.61	88.05	93.80
		MSE	618.90	2663.89	68.24	54.17	47.57
Total flavonoid content	Zero Order	k	1.70	2.53	0.69	0.21	0.42
		R^2	55.83	76.43	70.56	86.77	64.75
		MSE	26.60	22.23	2.35	0.23	1.03
	First Order	k	0.06	0.07	0.07	0.09	0.06
		R^2	49.78	71.67	62.31	86.34	59.27
		MSE	30.25	27.20	2.97	0.24	1.21
	Fractional Conversion First Order	k	0.73	0.32	0.53	0.01	0.49
		R^2	91.49	86.38	97.17	86.16	82.77
		MSE	5.77	13.57	0.26	0.27	0.57
FRAP	Zero Order	k	13.79	83.82	18.80	5.94	11.79
		R^2	52.91	78.12	68.14	88.70	71.28
		MSE	1981.24	24639.17	1893.43	50.27	613.45
	First Order	k	0.07	0.09	0.15	0.19	0.19
		R^2	45.65	69.21	56.37	65.90	51.77
		MSE	2287.20	32333.30	2611.99	144.21	858.05
	Fractional Conversion First Order	k	1.16	0.43	0.29	0.10	0.35
		R^2	98.12	95.22	77.49	94.07	83.10
		MSE	90.36	5477.91	1510.09	33.05	337.59
DPPH radical scavenging capacity	Zero Order	k	28.62	28.19	18.03	2.84	3.50
		R^2	57.83	45.46	62.10	88.97	55.59
		MSE	7070.44	11079.80	2451.49	12.10	115.92
	First Order	k	0.04	0.03	0.05	0.07	0.05
		R^2	53.24	43.14	57.42	84.14	51.32
		MSE	7841.50	11564.23	2657.02	17.43	128.77
	Fractional Conversion First Order	k	0.68	1.76	0.81	0.19	0.84
		R^2	93.68	94.12	93.21	94.26	85.71
		MSE	1173.04	1292.82	496.04	6.80	36.73

determined to be 5 mins, as no significant increase in phytochemical content occurred after this time. Green and black tea are a significant source of dietary antioxidants having a greater TPC than fresh orange juice; while green, black and white teas had 7.93, 5.57, and 4.14 times greater DPPH radical scavenging capacity, respectively, than fresh apple juice; and 8.9, 6.2, and 2.9 times greater scavenging capacity than fresh orange juice.

Conflicts of interest

The authors certify that they have no affiliations with or involvement in any organisation or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or

beliefs) in the subject matter or materials discussed in this manuscript.

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