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Effect of infusion time on phenolic compounds and caffeine content in black tea

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ABSTRACT

Tea is a quite popular and largely consumed beverage in several continents. It is a rich source of phenolic compounds, however, few studies have addressed the availability of these compounds associating with the infusion time of the drink. The objective of this study was to determine total phenolic compounds in several types of black tea either in granule or bag form and to verify the effect of infusion time on the availability of these compounds. The infusion times were varied until 30 min and measurements were done initially at 2.5 min interval up to 10 min whereafter measurements were done at 10 min interval. Eight different brands of tea were analyzed and among these 5 were in tea bag form and 3 in granule form. The total phenolic content was determined by using Folin–Cicoalteau reagent and measurement done in a spectrophotometer. Identification and quantification of some phenolic compounds and caffeine were done in a system of High Performance Liquid Chromatography coupled with Diode Array Detector. Higher concentrations of propyl gallate (43 mg.g^{-1}), caffeine (67 mg.g^{-1}), gallic acid (0.9 mg.g^{-1}), catechin (48 mg.g^{-1}) and rutin (12 mg.g^{-1}) were found in Brazilian samples while chlorogenic acid (4 mg.g^{-1}) and *p*-coumaric acid (7 mg.g^{-1}) were found in Indian teas. Chlorogenic acid was not detected in Brazilian teas at all infusion times including the maximum of 30 min tried in this study. It was concluded that these teas are good sources of dietary phenolic compounds and the content of these compounds in the beverage is higher with the longer infusion time.

1. Introduction

Antioxidants can be defined as substances that retard or inhibit oxidation of oxidizable substrates. These include substances such as α-tocopherol (Vitamin E), β-carotene, ascorbate (vitamin C) and phenolic compounds (flavonoids). Flavonoids are a class of antioxidant phenolic compounds present in plants. The use of natural antioxidants such as phenolic compounds present in most plants inhibits the formation of free radicals which have been associated with a lower incidence of diseases related to oxidative stress such as cardiovascular, cancerous and neurodegenerative diseases (Javanmardi, Khalighi, Kashi, Bais, & Vivanco, 2002; Kim, Jeong, & Lee, 2003; Liao, Kao, & Hiipakka, 2001; Lu & Yeap, 2002; Mendel & Youdim, 2004; Wiseman, Waterhouse, & Korver, 2001).

Tea is one of the most widely consumed beverages in the world and the oldest, being referred in the literature as one of the best sources of phenolic compounds (Lima, Mélo, & Lima, 2004) and has attracted much attention in recent years due to its antioxidant capacity and abundance in the diet of thousands of people throughout the world. In general tea is taken after infusion with water which contributes to the extraction of phenolic compounds, which are considered beneficial to human health (Bancirova, 2010; Bunkova, Marova, & Nemec, 2005; Higdon & Frei, 2003; Mendel & Youdim, 2004). The major constituents of tea polyphenols are catechins with a structure of flavan-3-ol and their polymerized products. In *oolong* black tea, most of catechins are oxidized and polymerized by enzymes derived from tea leaves during the fermentation process (Coggon, Moss, Graham, & Sanderson, 1973).

Although some studies report that the presence of phenolic compounds in tea in different conditions of infusion (Wang & Helliwell, 2001; Yang, Hwang, & Lin, 2007) has been undertaken earlier, no study details the changes in phenolic compounds related with the extension of infusion time for a longer period. Thus the objective of the present work was to study the effect of infusion time of several brands of tea (origin of production being Brazil, India and Britain) on the presence of phenolic compounds so as to enhance knowledge on its best utilization from nutritional stand point.

2. Materials and methods

2.1. Tea

Several brands of black tea (*Camellia sinensis*) available in the market were used, however, their names are not being disclosed. Two tea bag samples from Brazil, two from India and one from Britain (each of

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Table 1
Effect of infusion time on the total phenolic compounds content in different teas.

Tea* Samples	Total phenolic compounds content (mg gallic acid g^{-1}) at different periods of infusion								
	0 min	2.5 min	5 min	7.5 min	10 min	15 min	20 min	25 min	30 min
A1	$13.7^{g}\pm0.01$	$117.1^{d}\pm0.01$	$120.4^{c}\pm0.01$	$97.8^{f}\pm0.01$	$102.2^{e}\pm0.01$	$189.7^{a}\pm0.02$	$106.9^{e}\pm0.01$	$168.4^{b}\pm0.01$	$75.1^{f}\pm0.01$
A2	$6.4^{g}\pm0.01$	$86.8^{f}\pm0.01$	$133.9^{d}\pm0.01$	$129.6^{e}\pm0.01$	$149.7^{c}\pm0.01$	$153.2^{b}\pm0.01$	$177.5^{a}\pm0.01$	$158.8^{b}\pm0.01$	$144.3^{c}\pm0.01$
A3	$18.1^{f}\pm0.01$	$22.5^{f}\pm0.01$	$132.9^{c}\pm0.01$	$58.3^{e}\pm0.01$	$50.3^{e}\pm0.01$	$299.4^{a}\pm0.01$	$88.5^{d}\pm0.01$	$130.2^{c}\pm0.01$	$219.7^{b}\pm0.01$
A4	$55.4^{g}\pm0.01$	$62.7^{f}\pm0.05$	$240.4^{b}\pm0.01$	$67.1^{f}\pm0.01$	$138.7^{e}\pm0.04$	$258.5^{a}\pm0.01$	$174.7^{d}\pm0.02$	$172.2^{d}\pm0,01$	$189.0^{c}\pm0.01$
A5	$151.8^{f}\pm0.07$	$210^{e}\pm0.01$	$270.2^{c}\pm0.08$	$244.7^{d}\pm0.02$	$241.0^{d}\pm0.01$	$262.0^{b}\pm0.04$	$272.8^{b}\pm0.06$	$268.7^{b}\pm0.05$	$291.2^{a}\pm0.08$
A6	$73.7^{f}\pm0.01$	$95.6^{e}\pm0.01$	$205.6^{d}\pm0.02$	$253.1^{b}\pm0.04$	$274.0^{b}\pm0.07$	$284.5^{a}\pm0.01$	$239.4^{c}\pm0.03$	$182.3^{d}\pm0.01$	$311.0^{a}\pm0.01$
B1	$138.4^{d}\pm0.01$	$110.2^{f}\pm0.01$	$274.3^{b}\pm0.01$	$97.8^{f}\pm0.06$	$225.7^{b}\pm0.04$	$163.2^{c}\pm0.02$	$129.0^{e}\pm0.01$	$159.7^{c}\pm0.01$	$308.5^{a}\pm0.01$
B2	$123.0^{d}\pm0.05$	$169.1^{c}\pm0.01$	$170.2^{c}\pm0.01$	$180.0^{a}\pm0.03$	$178.5^{b}\pm0.01$	$181.9^{a}\pm0.05$	$179.7^{a,b}\pm0.01$	$179.4^{b}\pm0.03$	$182.3^{a}\pm0.02$

Means in each row followed by different subscript letters were significantly different ($p \leq 0.05$).
* A1, A2 = Tea bags from Brazil; A3, A4 = Tea bags from India; A5 = Tea bag from Britain; A6 = Tea in granule form from Britain; B1, B2 = Tea in granule form from India.

these containing 2 g) along with black tea in granular form — one from Britain and two samples from India were analyzed in this study.

2.2. Standard reagents

The standard chemicals used were chlorogenic acid, propyl gallate, gallic acid, rutin trihydrate, caffeine, catechin and *p*-coumaric acid. All these reagents were obtained from Sigma (St Louis, MO, USA). All solutions were prepared with deionized ultra pure water obtained from Elga Purelab Classic system.

2.3. Preparation of samples

Each bag of tea containing about 2 g was added to 200 mL of hot deionized water (100 °C). Aliquots of these were withdrawn at different times of infusion varying from 0 to 30 min. Initially the samples for analysis were withdrawn at each 2.5 min interval up to 10 min whereafter samples were collected at each 5 min intervals until a final infusion time of 30 min. Later the infusions were filtered through 0.22 μm syringe filter and analyzed in HPLC system.

2.4. Determination of total phenolic compounds content

Total phenolic compounds content was determined following the adopted Folin–Ciocalteu colorimetric method (Singleton, Orthofer, & Lamuela-Raventos, 1998). In 1 mL of tea extract after infusion, 14.5 mL of 2% sodium carbonate solution in NaOH 0.1 M was added. The samples were left in water bath maintained at 37 °C for 10 min whereafter 1 mL of Folin–Ciocalteu's reagent diluted in water (1:2) was added and the absorbance was measured at 765 nm after 1 h incubation at room temperature. Total phenolic compounds content was expressed as gallic acid equivalents.

2.5. HPLC analysis

The analyses were performed using a Shimadzu LC-20AT HPLC system consisting of a pre-column (LC Column, CLC G — C18, Shim-Pack) and the analytical column (250 mm × 4.6 mm × 5 μm, Shimadzu, Japan), a pump (LC-20AT), an autoinjector (SIL-20A, Shimadzu, Japan) and a diode-array detector (SPD-M20A, Shimadzu, Japan).

The flow-rate of mobile phase (water–acetonitrile–methanol–ethyl acetate–acetic acid, 89:6:1:3:1, v/v) was maintained at 0.7 mL/min (Saito, Welzel, Suyenaga, & Bueno, 2006). The wavelength adjusted for detection was 254 nm (propyl gallate, caffeine and gallic acid) and 280 nm (clorogenic acid, *p*-coumaric acid, catechin and rutin) using the detector DAD/UV–VIS and other conditions being pressure of 66 Kgf/cm^2 analysis performed at 21 °C. The samples were filtered through a syringe filter of 0.22 μm (Phenomenex) and 20 μL of this extract was injected for HPLC-DAD analysis.

Six concentrations varying from 60 to 20,000 ng of the standard phenolic compounds (propyl gallate, caffeine, gallic acid, chrogenic acid, catechin and rutin) of 20 μL were injected into HPLC system and analysis performed in identical conditions. Correlation coefficient for each compound was obtained by plotting the concentration data of the compound against peak area. The curves for all the standard compounds were prepared by using data obtained in triplicate analyses while the mean values of triplicate analyses were used for determining the compound's concentration in tea samples. These results revealed linearity in the calibration curves and very high values (more than 0.99) of correlation coefficients.

2.6. Statistical analysis

The statistical analysis was performed using SAS software (SAS Institute, Cary, NC) Version 9.1.3. Significant differences between the mean values of different characteristics were determined by applying Tukey's test for multiple comparisons at the probability of 5% ($p \leq 0.05$).

3. Results and discussion

Standard solutions prepared by varying concentrations from 60 to 20,000 ng of the phenolic compounds (propyl gallate, caffeine, gallic acid, chrogenic acid, catechin and rutin) were analyzed and the results were processed to obtain the calibration curves.

3.1. Effect of infusion time on total phenolic compounds and caffeine contents in blacks teas

Tests were conducted to determine the effect of heating time (0, 2.5, 5, 7.5, 10, 15, 20, 25 and 30 min) on the concentration of total phenolic compounds content and Table 1 presents the data expressed as mg gallic acid ((GAE).g^{-1}) obtained on different tea samples. Statistical analysis of these data revealed that the total phenolic compounds content in all different black tea infusions, prepared at 100 °C differs significantly ($p<0.05$) until 10 min whereafter majority of the samples do not differ in its content significantly ($p<0.05$). One important conclusion that can be derived is that in general, in all tea samples there was an increase in total phenolic compounds until 5 min of infusion, followed by a decrease in its value on 7.5 min of infusion. However, after this period, there was again an increase in total phenolic compounds until 15 min. The Indian sample (B2) tends to stabilize its content soon after 7.5 min of infusion while Brazilian teas (A1 & A2) tend to decrease after 25 min. This phenomenon of increase and decrease in various

Fig. 1. HPLC chromatograms (X axis — Time in min; Y axis — Absorbance) of various black tea samples after 15 min of infusion showing peaks of propyl gallate (1), gallic acid (2), caffeine (3), chlorogenic acid (4), *p*-coumaric acid (5), catechin (6) and rutin (7).