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Availability of Reactive Oxygen Species Scavengers in the Conventional Tea and Coffee

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Abstract

The antioxidant capacities of black coffee and two different teas such as green tea and black tea were determined using the trolox equivalent antioxidant capacity (TEAC) method. The green tea showed approximately 40% higher antioxidant capacity compared with the other two beverages, and the obtained TEAC values for green tea, black tea, and coffee were 8.79, 6.20, and 6.30 μmol trolox/mL of the sample, respectively. When the teas were prepared with cold water (20℃ ± 1℃), the total available antioxidant capacity reduced significantly, with TEAC values of green tea and black tea of 1.34 and 1.42 μmol trolox/mL of the sample, respectively. Per individual serve, hot teas and coffee could provide approximately four- to sixfold more antioxidants compared with teas prepared with cold water. With the kinetics experimental data, the 2,2′-aziono-bis(3-ethylbenzthiazoline-6-suphonic acid) radical scavenging rate by green tea with cold water can be expressed as a pseudo-first-order reaction. The obtained pseudo-first-order reaction rate constant was 0.147 min^{-1}, indicating that the reduction rate of undesirable reactive oxygen species by green tea is significantly faster than that of other reported water-soluble vitamins.

Keywords: TEAC, antioxidant capacity, reactive oxygen species, tea, coffee.

Introduction

Reactive oxygen species are free radicals and are produced continuously through human metabolism. These reactive oxygen species can damage essential components of the human body such as DNA, lipid, and protein, demoting health[1]. Antioxidants are molecules that can protect cells from the reactive oxygen species. Therefore, antioxidants are considered as valuable resources to reduce the risks of various chronic diseases such as cancer, diabetes, anti-rheumatic, and cardiovascular diseases[2-6].

Tea has been well known as the leading antioxidant beverage. Because of its powerful antioxidant capacity, it has been one of the most popular drinks in the world. Black tea and green tea are the most widely consumed in the United States, with approximately 80.0% and 19.5% of total tea consumption, respectively[7,8]. These two tea leaves come from the same plant of *Camellia sinensis*, with different preparation: black tea is prepared via fermentation, whereas green tea is not[9].

Recent study showed that coffee is the leading antioxidant resource for Americans not because of its high antioxidant capacity but because of its high consumption[10]. On the basis of statistical records, approximately 54% of 18 years or older of the U.S. population drinks coffee every day at a rate of three cups a day[11].

Although there have been many studies about the antioxidant capacity of various teas and coffees, limited information is available for the direct comparison of antioxidant capacity between these two leading beverages. In this research, the available antioxidant capacities of two main teas such as black tea and green tea and traditional coffee were determined. In addition, the temperature effects of the preparation temperature on the total available antioxidant capacity were also studied. The scavenging rate constant for the 2,2′-aziono-*bis*(3-ethylbenzthiazoline-6-suphonic acid) ($ABTS^{\bullet+}$) radical cations was determined to predict the reduction rate of harmful reactive oxygen species.

Material and Methods

Teas and coffee were obtained from the local market. Samples of tea and coffee were prepared following manufacturers' instructions. The coffee was prepared by brewing using a conventional coffeemaker. The ratio of coffee mass (g) to water volume (L) was 60 g/L. Both black and green tea solutions were prepared by steeping 2 g of tea bag in 250 mL of water for 5 min. The water temperatures were either 95°C ± 1°C or 20°C ± 1°C. All the prepared solutions were filtered using Fisher P4 qualitative filter paper to separate particles from the solutions. The prepared solutions were used to determine antioxidant capacity. Samples of fresh teas and coffee were prepared for each experiment.

Trolox equivalent antioxidant capacity (TEAC) assay[12] was used to determine the total available antioxidant amounts from the three typically prepared beverages. The $ABTS^{\bullet+}$ radicals were prepared by mixing the same volume of 14 mM ABTS

solution with 4.9 mM potassium persulfate solution. Fresh solution was prepared at least 6 hours before the experiments and stored in an amber bottle. The developed solution was diluted with water treated using reverse osmosis until the absorbance at 735 nm was approximately 0.8 using a Varian Cary 50 UV/Vis spectrophotometer. To obtain TEAC values, each prepared solution was diluted, and the ability of scavenging blue ABTS•$^+$ was determined by monitoring the absorbance after 6 minutes of reaction between 0.1 mL of sample and 2.9 mL of diluted ABTS•$^+$ solution. With the determined absorbance, the percentage of inhibition was determined with the following equation:

$$\% \text{ inhibition} = \frac{\Delta ABS_{ABTS^{\bullet+}}}{A_{referencxe}} \times 100\%$$

where $A_{\text{reference}}$ is the absorbance of reference, and $\Delta ABS_{ABTS\bullet+}$ is the difference of absorbance between the sample and the solvent. The percentage of inhibition of trolox solutions was also obtained as a standard. The slopes of the percentage of inhibition as a function of concentration for each sample and trolox solution were determined. The TEAC values represent the ratio between the slope of sample solution and the slope of trolox solution $\left(\frac{\text{Slope}_{\text{sample}}}{\text{Slope}_{\text{trolox}}}\right)$. Because the samples and the trolox solution have units of milliliters per liter and micromole per liter, respectively, the TEAC values calculated in this studies have a unit of micromole of trolox per milliliter of the sample. Additional experiments were performed to study the extraction rate of antioxidant capacity from green tea as a function of steeping time with cold water (20°C ± 1°C). The concentration used for kinetic study was 1 g/L, and samples were taken every 2 minutes, followed by filtration before mixing with ABTS•$^+$ radicals. After the 6-minute reaction period, the absorbance was determined. The potential interferences by the color of prepared samples were corrected by subtracting the absorbance of the solution prepared with 0.1 mL of sample and 2.9 mL of water if necessary. All the experiments run in either duplicate or triplicate.

Results and Discussion

The total amounts of the antioxidant capacity of the three different beverages were determined and compared using TEAC values. The TEAC value of this research indicates the equivalent amount of trolox per milliliter of prepared sample solution. Figure 1 shows the percentage of inhibition of trolox as a function of concentration. The slope of linear plot is 0.1193 (% inhibition/μM), with a standard deviation of 0.0055 (n = 8), which was used as the standard slope in comparing the slope of each prepared beverage sample. The determined TEAC values of prepared hot teas and coffee solutions are shown in figure 2. The results indicate that hot green tea (TEAC, 8.79) has an approximately 40% higher antioxidant capacity compared with hot black tea (TEAC, 6.20) and coffee (TEAC, 6.30). This observation was consistent with the previous study showing that the green tea has higher antioxidant capacity compared with black tea[13]. In our research, however, the results did not show dramatic different antioxidant capacity between green tea and black tea, whereas the previous study showed a sixfold higher antioxidant capacity with green tea than that with black tea. It should be noted that coffee without additives showed antioxidant capacity as high as black tea prepared with hot water. Coffee also showed significantly higher antioxidant capacity than teas prepared with cold water. The TEAC values of black tea and green tea prepared with cold water were 1.41 and 1.34 μmol/mL, respectively. The antioxidant capacity for two different teas prepared with cold water did not show any significant difference.

Figure 3 shows the percentage of inhibition by green tea as a function of steeping time in cold water. It clearly shows that approximately 55% of total cold water extractable antioxidant was obtained within 5 minutes, and after 10 minutes of steeping period, the extracted amount of antioxidant started to become constant. Considering the results shown in figures 2 and 3, the maximum extractable antioxidant capacity from green tea with cold water could be significantly less than the extractable antioxidant capacity from teas with hot water within 5 minutes. The total amounts of extracted free radical scavengers per one serving of bleak tea, green tea, and coffee are shown in table 1.

Additional efforts were performed to determine the exact amount of antioxidant extracted from green tea with cold water. It has been reported[11] that the extinction coefficient of ABTS•$^+$ radicals at 734 nm is 1.5×10^4 mol^{-1} L cm^{-1}. Using the Beer–Lambert law, ABTS•$^+$ radical concentrations were calculated as a function of time, and pseudo first order rate constant was determined as shown in figure 4. It shows that the ABTS•$^+$ radical disappearance rate can be well fitted with the pseudo first order integrated method, and the obtained pseudo first order rate constant was 0.147 min^{-1}. The rate constant for green tea indicates that even green tea prepared with cold water plays actively as free radical scavengers, and its reaction rate is much faster than those with other water-soluble vitamins reported in previous studies[1].

With the findings of this research and the previous report[13,14], tea is considered as the primary antioxidant provider worldwide. In North America, however, coffee could be the leading resource not only because of its high consumption but also because of its high antioxidant capacity. It also should be noted that this study has focused on the pure teas and black coffee since the effects of consumption of dairy products such as milk and spray drying with teas and coffee have not been fully understood regarding its potential inhibition on the antioxidant activities in human metabolism with different experimental observations[15-19].

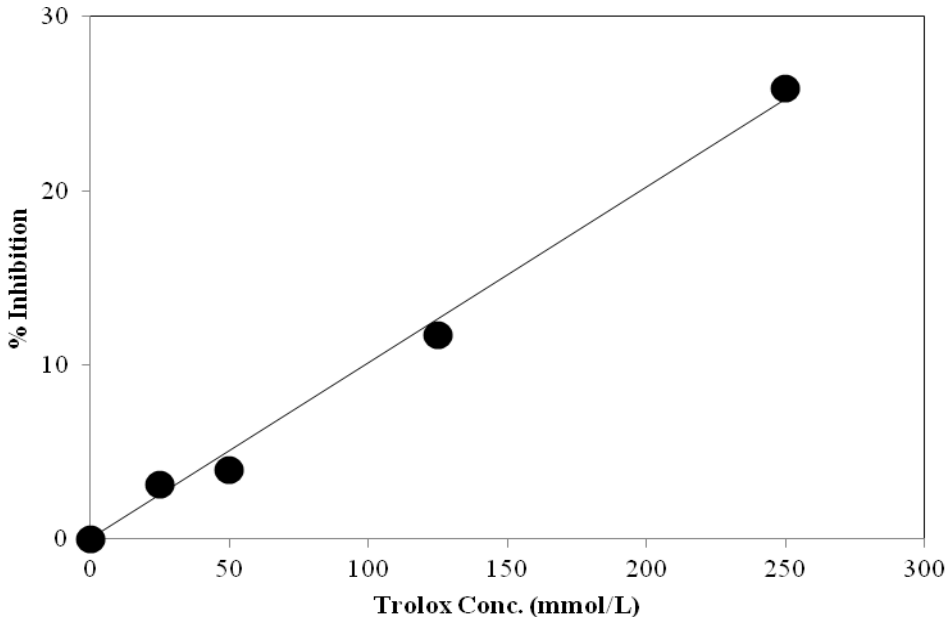

Figure-1
Percent (%) Inhibition as a function of standard trolox concentrations in µM at 734 nm

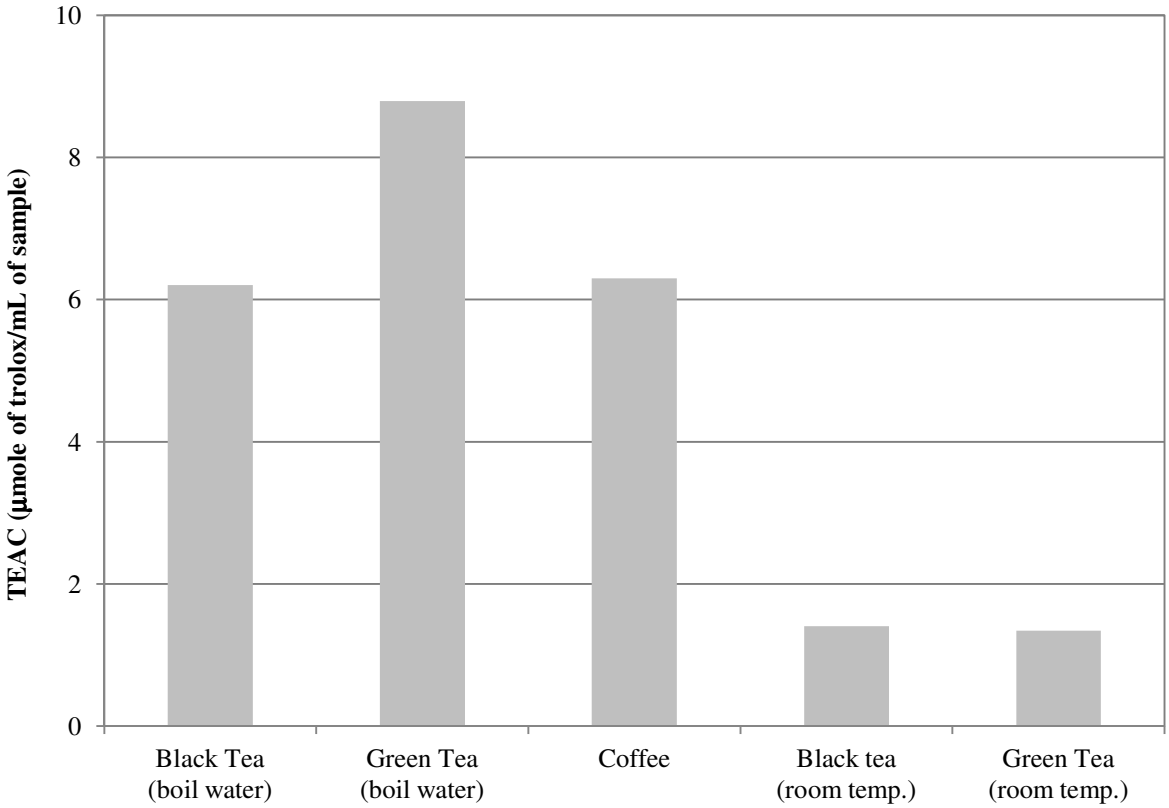

Figure-2
TEAC values of coffee and teas prepared at two different temperatures (20°C and 95°C)

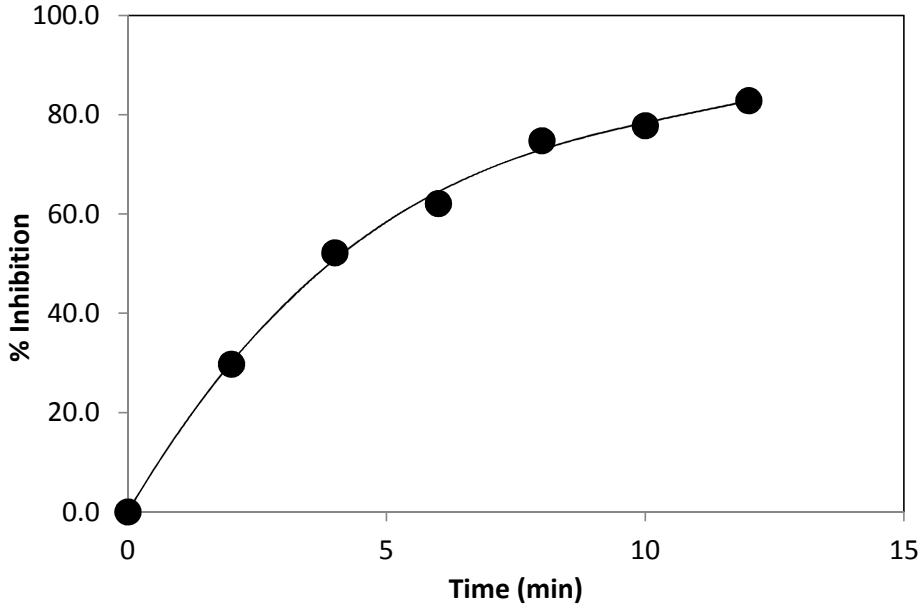

Figure-3
Percent (%) inhibition as a function of green tea steeping time at 20 ^{o}C (mass to volume ratio: 8g/L)

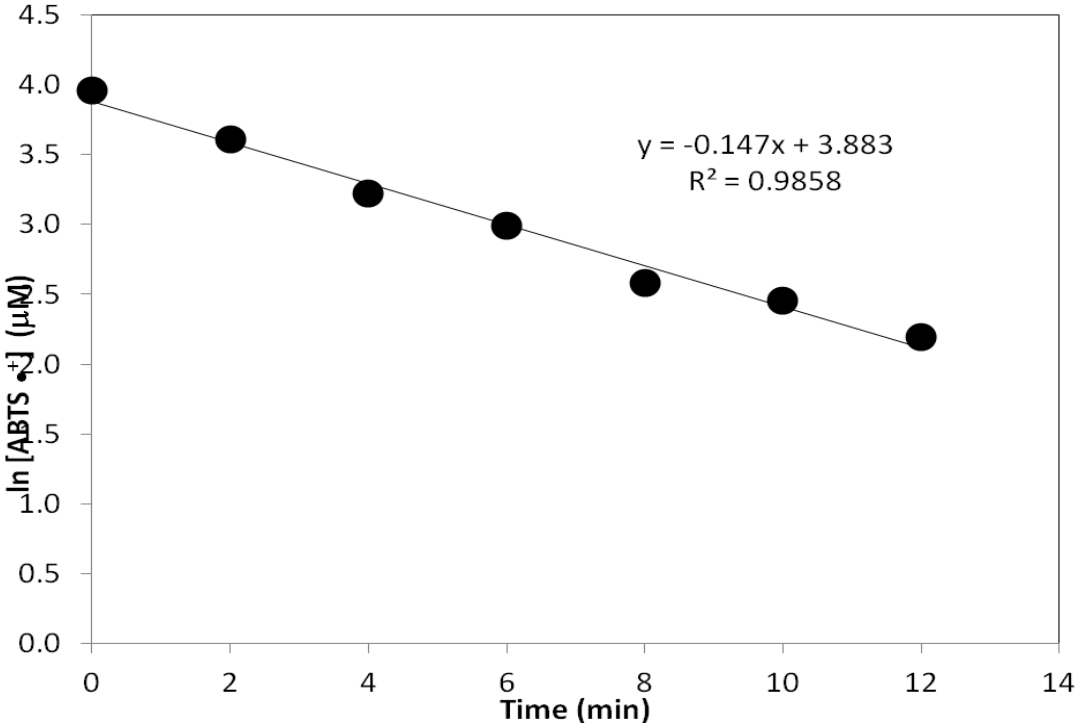

Figure-4
Pseudo first order plot for ABTS$^{\bullet+}$ radical cation disappearance

Table-1
Comparison between the extracted amounts of potential reactive oxygen species scavenger per serving size of conventionally prepared tea and coffee

Beverage	mg of extracted antioxidant (mg ± SD / serving size)	Number of samples (n)
Green tea (95°C ± 1°C)	172.36 ± 46.52 mg	6
Black tea (95°C ± 1°C)	127.11 ± 16.38 mg	7
Coffee	101.81 ± 10.78 mg	8
Green tea (20°C ± 1°C)	25.22 ± 3.02 mg	4
Black tea (20°C ± 1°C)	28.22 ± 5.18 mg	4

Conclusion

Coffee, black tea, and green tea are the most popular beverages in the world aside from water. The antioxidant capacities of these three beverages prepared following the manufacturers' recommendations showed a high availability of antioxidants. The highest antioxidant capacity was observed in the hot green tea, and similar antioxidant capacities were observed in hot black tea and coffee. The study also found that the antioxidant capacities of black tea and green tea decreased significantly when the teas were prepared with cold water. The TEAC values for both teas were not significantly different. Black tea, green tea, and coffee prepared with hot water could provide approximately four- to sixfold more antioxidants compared with teas prepared with cold water.

Although the antioxidant mechanisms have not been clearly understood in vivo process, pseudo first order kinetic experiments using green tea with cold water confirmed that in vitro, the harmful reactive oxidant species can be removed faster by green tea than other common water-soluble vitamins.

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