

Effect of Brewing Temperature on the Total Antioxidant Capacity of Green Tea

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Abstract The relationships between the total available antioxidant capacity of green tea and the brewing temperature and preparation time were studied, with the Trolox equivalent antioxidant capacity (TEAC) assay used to determine the antioxidant levels. The initial green tea mass-to-water volume ratio was set to 10 g/L, %-inhibition values were determined at various concentrations through serial dilution, and the slope of the relationship between %-inhibition and concentration was used to determine the TEAC value. The total antioxidant capacities of green tea determined under the experimental conditions adopted in this study are in the 214-819 $\mu\text{mol}_{\text{Trolox}}/\text{g}_{\text{green tea}}$ range, which is significantly higher than previously reported values for various common fruits and vegetables. The total available antioxidant capacity was found to increase with temperature at a rate of approximately 6 ($\mu\text{mol}_{\text{Trolox}}/\text{g}_{\text{green tea}}$)/ $^{\circ}\text{C}$ when brewed for 4 min. Green tea concentration, brewing time, and brewing temperature were experimentally determined to be the primary factors that determine the antioxidant capacity.

Keywords: green tea, antioxidant, TEAC, %-inhibition

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1. Introduction

Tea is globally the most consumed flavored beverage, having been used as a drink and for medicinal purposes for many centuries. Black tea is the most consumed tea in the USA, followed by green tea and oolong tea. All three teas are prepared from the leaf of the same *Camellia sinensis* plant, albeit differently [1]. Various teas have also recently become available in ready-to-drink designed packages, making them easily accessible to consumers. In addition, due to the high costs of healthcare services, an increasing number of people are becoming interested in nutritionally healthy foods and drinks [2]. As a result, the amount of tea consumed has steadily grown and is expected to increase annually by approximately 5.7%, resulting in a tea consumption of 0.4 kg per capita in the USA in 2021 [3].

Tea has been shown to have a variety of health benefits that are especially associated with high amounts of antioxidants (primarily polyphenols) that comprise 30% of the tea leaves [4]. Previous research confirmed that tea provides significantly higher antioxidant benefits than many other vegetables and fruits [5]. The large amounts of available antioxidants in tea respond to highly reactive oxidants *in vivo*, thereby reducing the risks of cancer and other common diseases, including cardiovascular disease, type-2 diabetes, and coronary disease [6]. A previous study showed that green tea has the highest antioxidant

capacity among common teas, including black and oolong teas [7].

While an increasing number of people consume teas on a daily basis, they mostly use simple ready-to-drink bottled or brewed teas using either commercial tea bags or tea leaves in accordance with the manufacturer's directions. While an earlier study showed that the temperature of the brewing water influences the available antioxidant capacity [8], no systematic study aimed at identifying the relationship between brewing temperature and the available antioxidant capacity of tea has been reported. Consequently, efforts have been performed to study the available antioxidant capacities of green tea prepared at various brewing temperatures and report our results herein.

2. Materials and Methods

The available antioxidant capacity of green tea was determined from tea solutions prepared at various brewing temperatures. Commercial green tea bags were purchased from a local market. The dried tea leaves were separated from the tea bags, and the required amount of tea for each experiment was placed in a 125-mL Erlenmeyer flask. For experiments at temperatures higher than room temperature, approximately two-thirds of a 1,000-mL beaker was filled with distilled water. The water was heated to the target temperature on a hot plate, after which 50 mL of the water was transferred to the Erlenmeyer flask containing the tea

leaves. The mixture was not mechanically stirred during the brewing period to simulate a typical brewing process. The reaction temperature was maintained for 4 min by placing the flask in the beaker holding distilled water. A tea-mass-to-water-volume ratio of 10 g/L was used in these experiments. A simulated cold-brew tea was prepared using water that had been refrigerated overnight. After 4 min of brewing, part of the solution was filtered through a Whatman number 5 filter paper, and the filtrate was used to determine the total antioxidant capacity. The brewing period was extended to 24 h for the simulated cold-brew tea.

The Trolox equivalent antioxidant capacity (TEAC) assay was used to determine the antioxidant capacities of the tea samples from the rate of disappearance of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radicals [ABTS^{*+}] [9,10]. The amount of ABTS radicals was determined from the absorption measured at 735 nm using an Aquamate 8000 UV-Vis spectrophotometer (Thermo Scientific). Fresh ABTS radical solutions were prepared daily and diluted until the absorbance was in the 0.7-1.2 reference range. TEAC values were determined

by comparing the slopes of the relationships between %-inhibition and tea concentration of the sample and the Trolox reference ($Slope_{Tea\ Sample} / Slope_{Trolox}$).

%-Inhibition is mathematically expressed by equation 1:

$$\%Inhibition = \frac{Abs_{Reference} - Abs_{Sample}}{Abs_{Reference}} \times 100 \quad (1)$$

where Abs_{Sample} and $Abs_{Reference}$ are the absorbances of the ABTS radical solutions with and without tea samples, respectively.

3. Results

The effect of the temperature of the brewing water on the antioxidant capacity of green tea was studied. The experimentally collected data and calculated %-inhibition values determination from the reference slope are listed in Table 1.

Table 1. Determining %-inhibition from the obtained absorbance

Trolox Conc. (μM)	Absorbance of the ABTS radical solution at 735 nm		Av. Abs	ΔAbs	% Inhibition
	trial 1	trial 2			
0.0	0.764	0.769	0.7665	NA	NA
31.3	0.740	0.744	0.7420	$0.7665 - 0.7420 = 0.0245$	$\left(\frac{0.0245}{0.7665}\right) \times 100 = 3.196$
62.5	0.713	0.717	0.7150	$0.7665 - 0.7150 = 0.0515$	$\left(\frac{0.0515}{0.7665}\right) \times 100 = 6.719$
125.0	0.653	0.657	0.6550	$0.7665 - 0.6550 = 0.1115$	$\left(\frac{0.1115}{0.7665}\right) \times 100 = 14.547$
250.0	0.563	0.573	0.5680	$0.7665 - 0.5680 = 0.1985$	$\left(\frac{0.1985}{0.7665}\right) \times 100 = 25.897$

ABTS radical absorbance decreased with increasing Trolox concentration, which indicates that ABTS radicals were reduced by the added Trolox, with the solution changing color to a lighter blue.

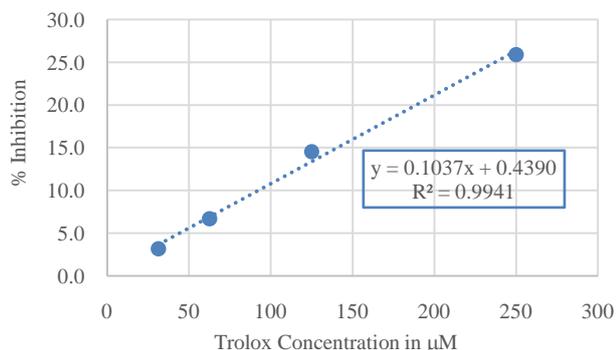


Figure 1. %-inhibition plotted as a function of the reference Trolox concentration

In this study, the slope was determined by MS Excel trendline analysis using the least-squares method. The reference ($\%inhibition/Trolox\ concentration$) slope was determined to be 0.1037 $\%inhibition/\mu\text{M}$. Figure 1 also reveals that %-inhibition is directly proportional to the Trolox concentration. Figure 2 shows %-inhibition as a function of green tea concentration, from which a slope of

22.14 $\%inhibition/(g_{green\ tea}/L)$ was determined at 4°C The TEAC value of the green tea at 4°C can be calculated from:

$$\frac{Slope_{TeaSample}}{Slope_{Trolox}} = \frac{22.14 \left(\frac{\%Inhibition}{g_{greentea} / L} \right)}{0.1037 \left(\frac{\%Inhibition}{\mu\text{M}_{Trolox}} \right)} = 213.5 \frac{\mu\text{mol}_{Trolox}}{g_{greentea}} \quad (2)$$

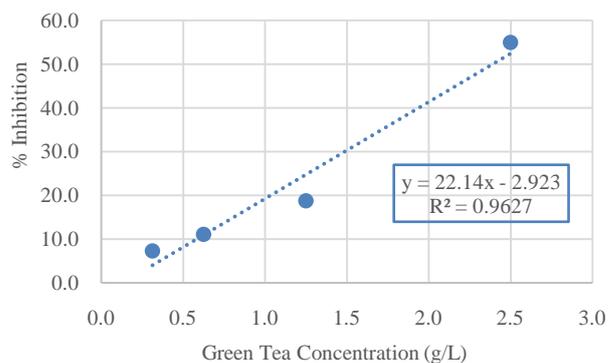


Figure 2. %-inhibition plotted as a function of the green tea concentration

TEAC values at other temperatures were determined using the same process. Figure 3 shows the effect of the brewing temperature on the available TEAC value of 4-min-brewed tea, which reveals that the extractable antioxidant capacity of green tea increases with increasing brewing temperature.

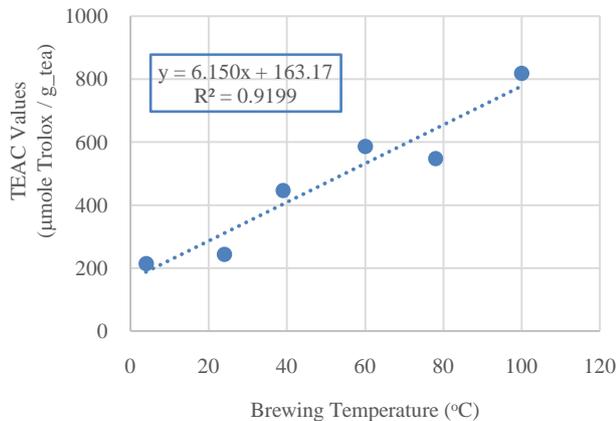


Figure 3. TEAC value as a function of brewing temperature

Additional tea was prepared at a tea-to-water ratio of 2.5 g/L at 4°C and stored in a refrigerator, with %-inhibition values determined for up to 24 h and compared with values obtained using the previously discussed procedure that involves brewing for 4 min at the same concentration.

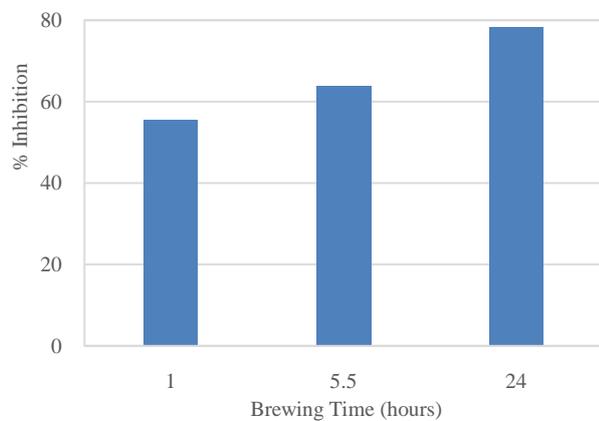


Figure 4. %-inhibition as a function of brewing time at 4°C

As shown in Figure 4, %-inhibition is affected by brewing time at 4°C, which confirms that the brewing period is an important factor that determines antioxidant capacity.

4. Discussion

The antioxidant capacities of green tea brewed at various temperatures was investigated. The TEAC assay used to determine the total dissolved antioxidant capacity was found to be well suited to this research purpose. The r^2 value of the reference relationship between Trolox concentration and %-inhibition slope was determined to be 0.9941, and all experiments showed r^2 values in the

0.9627–0.9928 range. The experimental results show that the disappearance rate of the ABTS radical is directly proportional to the green tea concentration.

This study confirmed that green tea is a good source of antioxidants. The TEAC values obtained in the 4–100°C brewing-temperature range were between 214 and 819 $\mu\text{mol}_{\text{Trolox}}/\text{g}_{\text{green tea}}$; these antioxidant capacities are significantly higher than those of common vegetables and fruits previously reported [11]. While green tea is a powerful source of antioxidants, the amount available depends significantly on the brewing temperature. The experimental results show that, when brewed for 4 min, the total available antioxidant capacity increases with brewing temperature at a rate of approximately 6 $\mu\text{mol}_{\text{Trolox}}/\text{g}_{\text{green tea}}/^\circ\text{C}$. Brewing time is an additional factor that affects antioxidant capacity. The %-inhibition (78.2%) of a 24-h-aged, 2.5-g-sample of tea brewed at 4 °C is approximately 40% higher than that (55.5%) of 1-h-aged green tea.

5. Conclusions

In this study, we showed that significant amounts of antioxidants are available from green tea over a wide range of brewing temperatures when brewed for 4 min, and that brewing time and temperature are two primary factors that determine the extractable antioxidant capacity.

Statement of Competing Interests

The authors have no competing interests.

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