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Effect of brewing method on quality parameters and antioxidant capacity of black tea

Zeynep B. Guzel-Seydim[1,2], Atif Can Seydim[1,2], and Annel K. Greene[2*]

[1]Department of Food Engineering, Suleyman Demirel University, Isparta, Turkey; [2]Animal and Veterinary Sciences Clemson University, Clemson, SC USA

[*]**Corresponding Author:** Annel K. Greene**,** Department of Animal and Veterinary Sciences 129 Poole Agricultural Center, Clemson University, Clemson, SC USA

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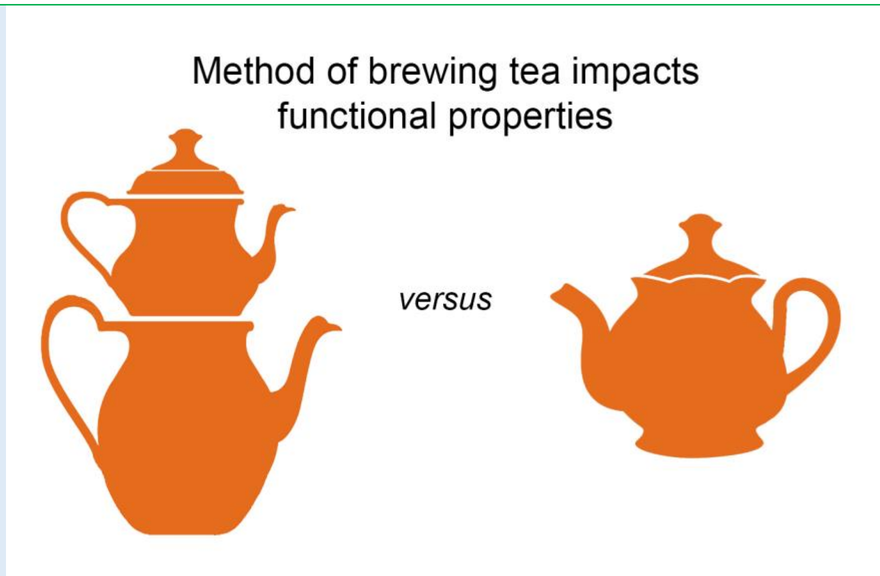
ABSTRACT

Introduction: The traditional method for brewing tea in Turkey involves use of a two-vessel apparatus known as a "caydanlik." The caydanlik allows for tea to be brewed for a longer time than typical brewing methods and involves introducing boiling water to tea leaves and steeping them in a teapot as the temperature declines over 15 minutes. In comparison, the typical tea brewing method used in other parts of the world involves steeping tea leaves in a single vessel teapot for 5 minutes. This study evaluated the effects of these two methods of brewing using two brands of tea.

Results: Results of this experiment indicate that use of the caydanlik and the accompanying longer brewing time significantly changed key quality parameters and increased the antioxidant capacity of the brewed black tea beverage. Use of the caydanlik method resulted in significantly higher ($P < 0.05$) theaflavins, thearubigins, theabrownins, and total polyphenols in the resultant tea than in tea brewed in a traditional teapot. Additionally, analysis of color values of the brewed tea indicated the L and b-values were significantly ($P < 0.05$) lower in the caydanlik brewed tea while a and hue values were significantly higher ($P < 0.05$). However, chroma values were not significantly different ($P < 0.05$) between the two methods.

Conclusions: In addition to influencing color, flavor and aroma, the brewing method also influenced the functional properties of prepared black tea. Use of the traditional two-vessel caydanlik for tea preparation increased concentrations of beneficial functional components in the tea.



Keywords: Black tea, Caydanlik, Brewing, *Camelia sinensis*, ORAC, Total Phenolic Content

INTRODUCTION

Black tea leaves are used to produce tea, an aromatic and widely known beverage consumed throughout the world. Tea consumption has increased 10% in the last decade according to the Tea Association of USA [1]. In particular, black tea is the most popular variety of tea consumed worldwide. Both black and green teas are produced from the leaves of *Camelia sinensis*. However, during black tea production, enzymatic browning occurs after leaves of *Camelia sinensis* are crushed and dried. During this oxidation step, some of the natural catechins in the leaves form theaflavins, thearubigins and theabrownins [2]. Theaflavin and thearubigin increase a brewed tea's color, mouth-feel and body, and contribute to the briskness of the tea. These compounds are of such importance to the characteristic flavor and aroma of tea that methods of optimizing fermentation levels have been studied [3]. Feng et al. (2019) investigated the effect of manufacturing processes on the aroma composition of tea leaves and reported that black tea has a concentration of 710 µg/g of volatile compounds whereas green tea has 20 µg/g [4]. Therefore, the taste and color parameters of the tea increase during processing of the leaves to produce black tea.

The commonly known black tea beverage is made by brewing (steeping) tea leaves in hot water. In a study by Cleverdon et al. [5], it was determined that steeping tea bags for 5 minutes released the most polyphenol content and produced a tea beverage with high antioxidant levels. Makanjuola et al. (2020) reported that extraction temperature, time, and concentration (tea to solvent ratio) significantly impacted the levels of flavonoids and polyphenols extracted from the tea. An aqueous extraction temperature of 92.01ºC was optimal for extraction of flavonoids and 95.75ºC was optimal for polyphenols [6]. Hiep et al. (2020) used subcritical water extraction of epigallocatechin gallate from green tea leaves and determined that maximum yield of extraction and optimal parameters for extraction occur at 120ºC for 6 min [7].

Tea polyphenols have functional properties including cancer prevention, antioxidant activity, anti-inflammatory activity, and lipid metabolism regulation. Tea polyphenols have been used in disease treatment as antioxidants and research has identified that polyphenols are involved with molecular signaling pathways. Additionally, tea polyphenols are used for the prevention and treatment of a number of diseases including high blood pressure, neurodegenerative diseases, diabetes, cancer, and many more [8].

Catechin can modulate cell signaling pathways including regulating apoptosis and angiogenesis, leading to cell death in various types of cancer. Epigallocatechin-3-gallate (EGCG) has been shown to inhibit the initiation, promotion, and progression stages of cancer. EGCG has a synergistic effect with chemoprotective agents, reduces toxicity and promotes anti-cancer effects. However, the bioavailability of epigallocatechin is a concern. Studies on delivering EGCG via nanotechnology-based technologies are being explored [9]. In a recent study, EGCG blocked the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) entry into cells. EGCG has broad anti-viral activity against several unrelated viruses: it competes with heparan sulfate or sialic acid in viruses for binding to target cells. Herpes simplex virus-1, Type A influenza virus, adenovirus, reovirus, vaccinia virus and other viruses are inhibited by EGCG's interaction with viral surface proteins, preventing attachment. The SARS-CoV-2 spike protein to receptor interaction is inhibited as well, indicating the potential of EGCG for developing drugs that will be effective against COVID-19. Furthermore, EGCG has anti-inflammatory effects that may help protect against inflammation and cytokine storms in COVID-19. However, the absorption rate via drinking green tea is likely too low to produce high enough plasma levels and improved delivery means are needed [10].

In the present study, the antioxidant capacity of black tea was investigated using two distinctive brewing techniques. Traditional Turkish tea brewing is conducted by a different method than the tea brewing method typically used worldwide. Turkish tea brewing utilizes an apparatus known collectively as a caydanlik (Figure 1). During tea brewing, tea leaves are placed in the upper teapot or "demlik" of the caydanlik and good quality, potable water is placed in the lower vessel. After heating the water in the lower vessel to boiling point, it is transferred to the upper vessel to steep the tea leaves. The lower vessel water is replenished and brought to a boil on a stove or other heating device. The stove temperature is then reduced to allow the lower vessel water to remain at a simmer, which maintains the heat in the upper vessel, much as a double boiler pot is used in cooking. The caydanlik allows fairly constant temperatures during tea brewing over an extended period of at least 15 minutes and produces a tea concentrate that is diluted as desired when served. The beverage produced by the Turkish tea brewing method is strong and flavorful.

Figure 1. Turkish caydanlik tea brewing apparatus.

It was hypothesized that tea brewed by the Turkish method may contain higher levels of beneficial antioxidant and phenolic compounds than tea generated by the traditional tea brewing method in which tea leaves are introduced to boiling water and allowed to steep for a shorter period of time.

The aim of this study is to determine the effect of different brewing techniques on quality parameters and antioxidant capacity of black tea. To our knowledge, there is no research published on this subject. This paper demonstrates the importance of brewing technique regardless of tea variety.

METHODS

Chemicals and Reagents: Ethyl acetate (99.8% HPLC grade, Aldrich, St. Louis, MO, USA) butyl alcohol (Omnisolv, MilliporeSigma, St. Louis, MO, USA), absolute ethanol (Mallinckrodt, St. Louis, MO, USA), sodium bicarbonate (ACS grade; Mallinckrodt, St. Louis, USA), potassium sodium tartrate tetrahydrate (ACS grade, BDH, VWR, Radnor, PA, USA), ferric chloride 6-hydrate (ACS grade; Mallinckrodt), and oxalic acid (Sigma, St. Louis, MO, USA) were purchased. Folin-Ciocalteu reagent was purchased from Merck (Darmstadt, Germany). The reagents 2,2'-azinobis (3-ethlybenzthiazolin-6-sulfonic acid) diammonium salt (ABTS), 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®), 2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH), fluorescein disodium salt, and gallic acid were purchased from Acros Organics (Morris Plains, New Jersey, USA).

Black Tea Samples and Sampling Design: Two brands of tea were purchased from U.S. markets on three different days, ensuring that different lots were obtained. The teas were an American brand of loose orange pekoe black tea (Lipton™, Florham Park, NJ, USA) and a Turkish brand of loose black tea (Rize Turist™ black tea, Caykur Tea Company, Rize, Turkey).

For each of the two brands, three tea packages were each brewed three times by two different brewing methods. The two methods were: 1) the most commonly used teapot brewing method in which boiling water was introduced to the tea leaves and steeped for 5 minutes and 2) the Turkish method using a caydanlik and brewing for 15 minutes. Therefore, the experimental design involved 2 different tea brands x 3 different tea packages from each brand x 2 brewing methods (treatments) x 3 repetitions of each.

Preparation of Black Tea Beverage with Different Brewing Techniques

Brewing Method 1: Black tea leaves (12.5 g) were added into freshly boiled water (1000 mL) in a regular American/English style teapot. Tea was brewed for 5 minutes without supplementary heat according to the typical brewing technique. The temperature of the water in the teapot was recorded using Ecklund-Harrison 38 mm CNS needle thermocouples (Ecklund-Harrison Technologies, Fort Meyers, FL) that were installed in the teapot to record the liquid's temperature. A CalPlex datalogger and CalSoft™ software (TechniCAL, Metairie, LA) were used to record temperature data. Samples of brewed black tea were collected from the teapot. This brewing technique was labeled as Method 1. Lipton™ tea brewed by Method 1 was labeled Lip1 and Turist™ tea brewed by Method 1 was labeled Tur1.

Brewing Method 2: Black tea leaves (25 g) were placed in the upper vessel of the caydanlik. Water (1500 mL) was placed into the bottom vessel of the caydanlik. The upper tea vessel was placed on top of the lower vessel and placed onto the stove. After the water in the lower vessel began rapidly boiling, 1000 mL of the boiling water was transferred to the upper teapot vessel. Another 1000 mL of fresh water was added to the lower vessel and the entire stacked teapot (caydanlik) assembly was returned to the stove. Once the water in the lower vessel was boiling again (as evidenced by steam exiting the vent hole in the lower vessel), the temperature of the stove was reduced to allow the water in the lower vessel to simmer. The tea was brewed a total of 15 minutes beginning from the time of initial water introduction to the tea leaves. The water temperature in the upper and lower vessels was recorded using Ecklund-Harrison 38 mm CNS needle thermocouples (Ecklund-Harrison Technologies, Fort Meyers, FL) that were installed in the vessels to record the liquid's temperature. A CalPlex datalogger and CalSoft™ software (TechniCAL, Metairie, LA) were used to record temperature data. After brewing, samples of brewed black tea were collected from the upper vessel of the caydanlik and diluted with simmering water from the lower vessel at a ratio of 1 to 1, meaning the brewed tea was diluted 50%. Hence, the tea leaf to water volume ratios used in the two brewing methods were identical. The brewing technique in the caydanlik was labeled as Method 2. Lipton™ tea brewed by Method 2 was labeled Lip2 and Turist™ tea brewed by Method 2 was labeled Tur2. The same source of water was used for both Method 1 and Method 2 brewing.

Sample Collection: Samples of brewed tea from both brewing methods were collected in screw cap Pyrex™ glass tubes and frozen (-20°C) until used for further analysis.

pH and Moisture Content: The pH of brewed tea samples was measured using a pH meter (Thermo Scientific Orion 5-Star, Beverly, MA USA). Tea moisture was measured using the ISO 1573 vacuum oven method [11].

Theaflavins (TF), Thearubigins (TR), Theabrownins (TB)**:** Measurement of theaflavins (TF), thearubigins (TR) and theabrownins (TB) were conducted according to the spectrophotometric method of Yao et al. [12]. For the blank, 95% (v/v) ethanol was measured at 380 nm in a Spectronic 20D+ (Thermo Spectronic, Rochester, NY, USA). The tea was extracted using four different extraction techniques as described by Yao et al. (2006), and the extracts were labeled as Ea, Eb, Ec, and Ed. Moisture content and the Ea, Eb, Ec, and Ed values of the tea samples were used to calculate the TF, TR and TB percentages as per Yao et al. [12].

Total polyphenols: The total polyphenol content was measured according to the method of Yao et al. [12] using the prescribed tartarate and buffer solutions and measured in a Spectronic 20D+ (Thermo Spectronic, Rochester, NY, USA) spectrophotometer at 540 nm. Results were calculated according to the formula provided by Yao et al. [12].

Total Phenolic Content: The total phenolic content of the tea samples was determined spectrophotometrically according to the Folin-Ciocalteu method using 2N Folin reagent [13]. After the addition of Folin-Ciocalteu reagent to the sample, the mixture was allowed to react for 6 min. The reaction was stopped with 1.25 mL of 7% sodium carbonate in distilled, deionized water. The color was allowed to develop for 90 min, and the absorbance was determined at 760 nm using a Spectronic 20D+ spectrophotometer (Thermo Spectronic, Rochester, NY, USA). The measurement was compared to a standard curve of gallic acid concentrations and expressed as milligrams of gallic acid equivalents (GAE)/L.

Oxygen Radical Absorbance Capacity (ORAC) Assay: The total antioxidant activity of tea samples was measured by the Oxygen Radical Absorbance Capacity (ORAC) method. The ORAC assay was carried out according to the hydrophilic ORAC-Fluorescein method [14-16]. This method kinetically measures the total antioxidant capacity of the samples using 2, 2'-Azobis (2-amidinopropane) dihydrochloride's damage to a fluorescent compound, which changes the fluorescent intensity. Briefly, 20 µL of a water blank, Trolox standard, and appropriate dilutions of tea samples in 75 mM phosphate buffer were added to triplicate wells in a black, clear-bottom, 96-well microplate. A volume of 200 µL of 0.96 µM fluorescein in phosphate buffer was added to each well and incubated at 37°C for 30 min. Then, 25 µL 2, 2'-Azobis (2-amidinopropane) dihydrochloride was added to each well. To measure the decay of fluorescence, a Synergy™ HT Multi-Detection Microplate Reader with injectors (BioTek Instruments, Winooski, VT, USA) was used with a 485 nm, 20 nm bandpass excitation filter and a 528 nm, 20 nm bandpass emission filter. The Trolox® was used as a standard and ORAC values were kinetically calculated according to Cao and Prior (1999) using KC4™ Data Reduction Software (BioTek Instruments, Winooski, VT, USA) [14].

Color Analysis: Reflected color measurement of black tea samples was performed using a Minolta Colorimeter (CR-400, Konica Minolta Sensing, Inc., NJ, USA) in combination with a sample holder (CR-A505) and a specimen holder (CM-A96). A glass cell made of optical glass (CM-A99) with an optical path of 20 mm was used and Hunter L, a, b-values of tea samples were recorded. Color data were then, mathematically converted to hue angle [arc tan (a*/b*)] and Chroma values [(a*2 +b*2)$^{1/2}$].

Statistical Analysis: The study was replicated three times using three separate batches for each of the two tea brands and the two brewing methods. The experimental results were analyzed by analysis of variance (ANOVA) using the Linear Model Procedure (PROC GLM) procedure of SAS software for treatment effects. Pooled means of Lip1 and Tur1 and of Lip 2 and Tur2 were compared with the Least Square Means (LSMEANS) statement with PDIFF option when the statistical significance level was 0.05% ($P < 0.05$) [17]. This was done to compare the effect of Method 1 versus Method 2 of brewing.

RESULTS AND DISCUSSION

Time-Temperature Profiles during Brewing with Different Methods: The mean temperatures that occurred during the two brewing methods over time and across both brands and all replicates are shown in Figures 2 and 3. In this study, the first brewing method involved adding boiling water to tea leaves and brewing tea for 5 minutes without supplementary heat. As shown in Figure 2, the brewing temperature decreased from slightly over 90°C to 85°C during the 5 min brewing time.

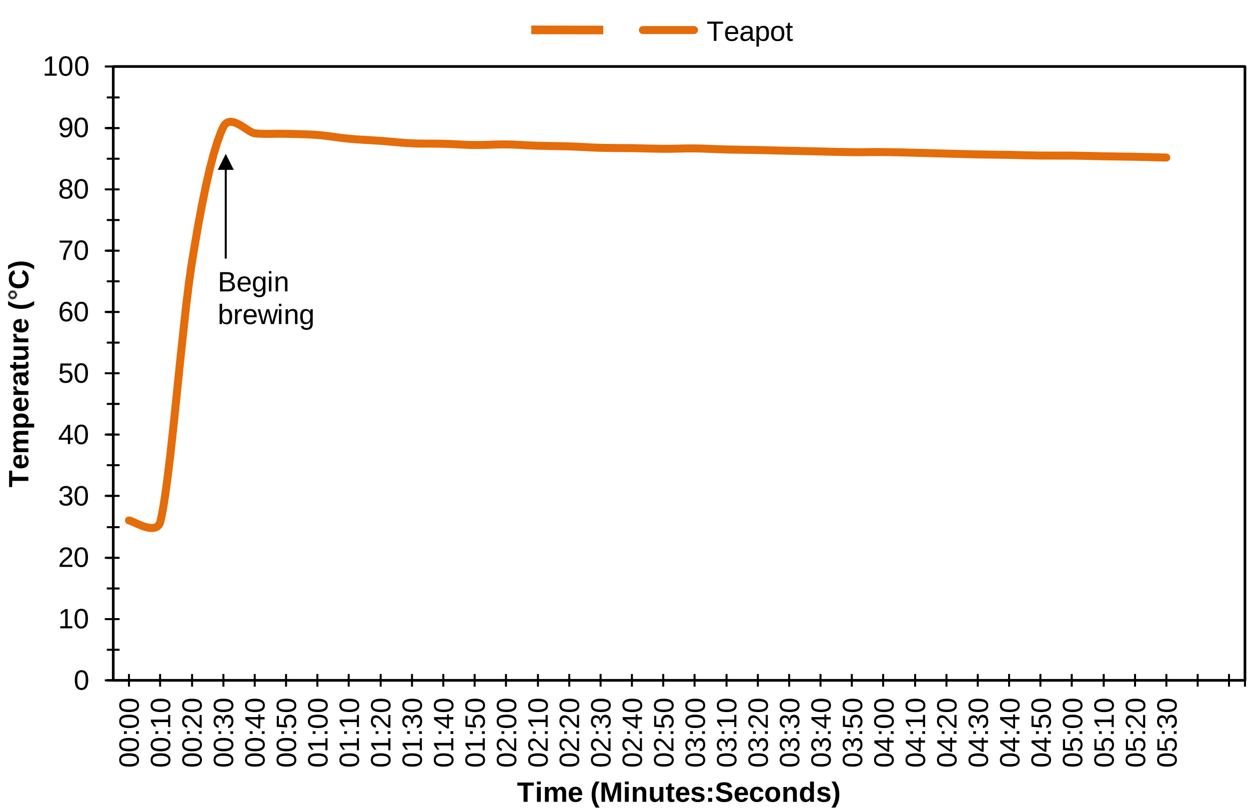


Figure 2. Mean time-temperature profile for brewing tea in a standard teapot in which boiling water was introduced to the tea leaves and steeped for 5 minutes.

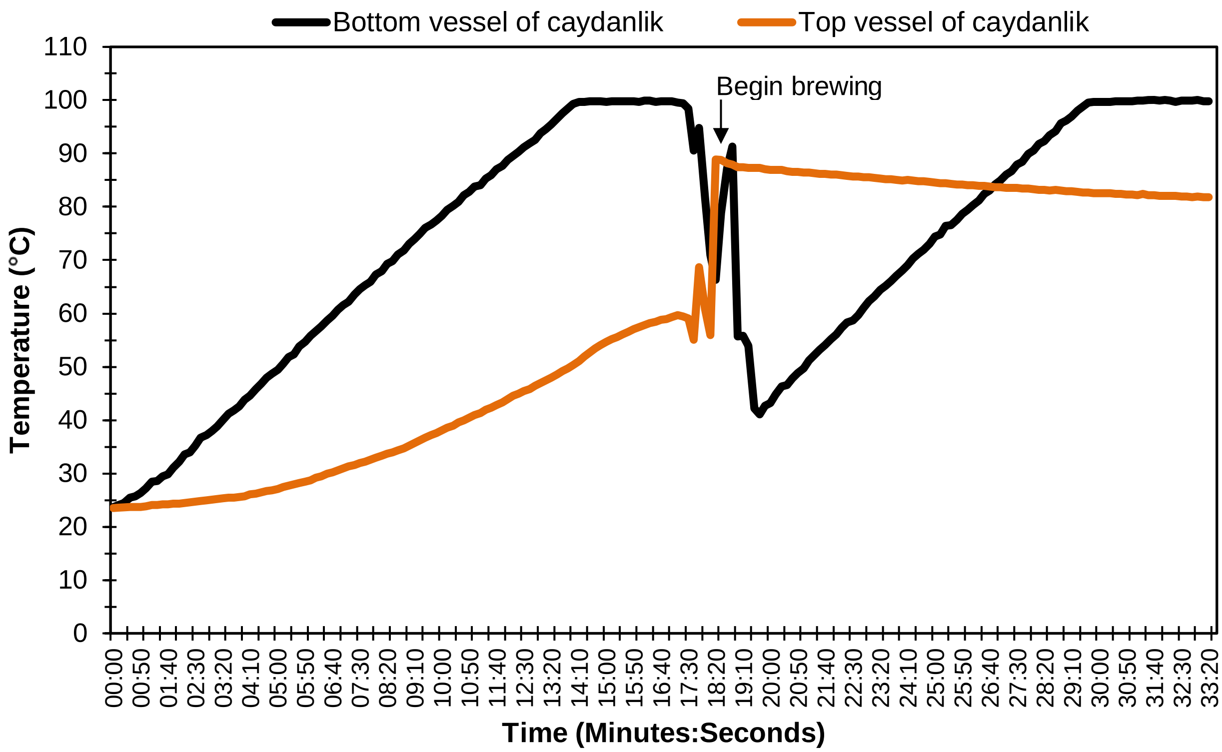


Figure 3. Mean time-temperature profile for brewing tea in a caydanlik in which tea leaves were measured into the upper vessel and water was brought to a boil in the lower vessel. Upon boiling, the heated water from the lower vessel was introduced to the tea leaves in the upper vessel. Additional water was added to the lower vessel and the unit was returned to the heating unit. The tea in the upper vessel was steeped for 15 minutes.

The second brewing method involved the use of the caydanlik method which is commonly used in Turkey. Tea leaves were placed in the top vessel of the caydanlik. Water in the bottom vessel was brought to a boil. During the first 17 min, as the water temperature in the bottom vessel increased, the temperature inside the upper vessel also increased (Figure 3). After the water in the lower vessel came to a rolling boil, the boiling water was transferred to the top vessel. Additional water was added to replenish the bottom vessel and the caydanlik was placed back on the heating unit. During the subsequent 15-minute brewing, the temperature of the brewing tea in the upper vessel gradually decreased from approximately 89°C to approximately 82°C (Figure 3).

pH and Moisture Content: The mean pH ± standard deviation of the brewed black tea made with the Lipton™ brand was 5.06 ± 0.22 for Lip1 and 5.06 ± 0.0.07 for Lip2. For brewed black tea made with the Turist™ brand of tea, the mean pH ± standard deviation was 5.20 ± 0.07 for Tur1 and 5.13 ± 0.0.05 for Tur2. There was no significant difference (P>0.05) in pH due to tea variety or brewing technique.

Moisture content was measured for use in the theaflavin, thearubigin and theabrownin calculations. Mean moisture content of all samples of Lipton™ tea samples and Turist™ tea samples were 6.99% and 6.08%, respectively.

Theaflavins (TF), Thearubigins (TR), Theabrownins (TB) and Total Phenolics**:** The percent theaflavins (TF), percent thearubigins (TR), percent theabrownins

(TB) and percent total polyphenols ± standard deviation values of Lip1 were 0.45 ± 0.17, 3.80 ± 1.28, 5.08±1.65, and 10.86 ± 1.88, respectively, while that of Tur1 were 0.18±0.07, 3.78 ± 3.92, 3.89 ± 0.57, and 5.52 ± 0.81, respectively. The percent TF, percent TR, percent TB, and percent total polyphenols values ± standard deviation of Lip2 were 0.69 ± 0.48, 7.61 ± 5.16, 7.35± 3.56, and 20.29 ± 2.01, respectively while that of Tur2 were 0.31±0.05, 5.58 ± 4.47, 6.76 ± 1.76, and 9.03 ± 3.13, respectively. There were significant differences between the results according to the brewing method (P<0.05). The mean results of all replicates of both tea brands by brewing method for TF, TR, TB, and total polyphenol values are shown in Figure 4. TF, TR, TB and total polyphenol content were significantly higher (P<0.05) in black tea samples made by Method 2 than by Method 1.

Enzymatic browning, which results in the formation of TF, TR, and TB, is a crucial parameter during black tea leaf processing [2]. TR are major components of black tea and have anti-inflammatory and anti-cancer activities. Wang et al. (2018) reported a new analysis method to prepare and define TR for health studies [18]. TR are formed during the fermentation of black tea.

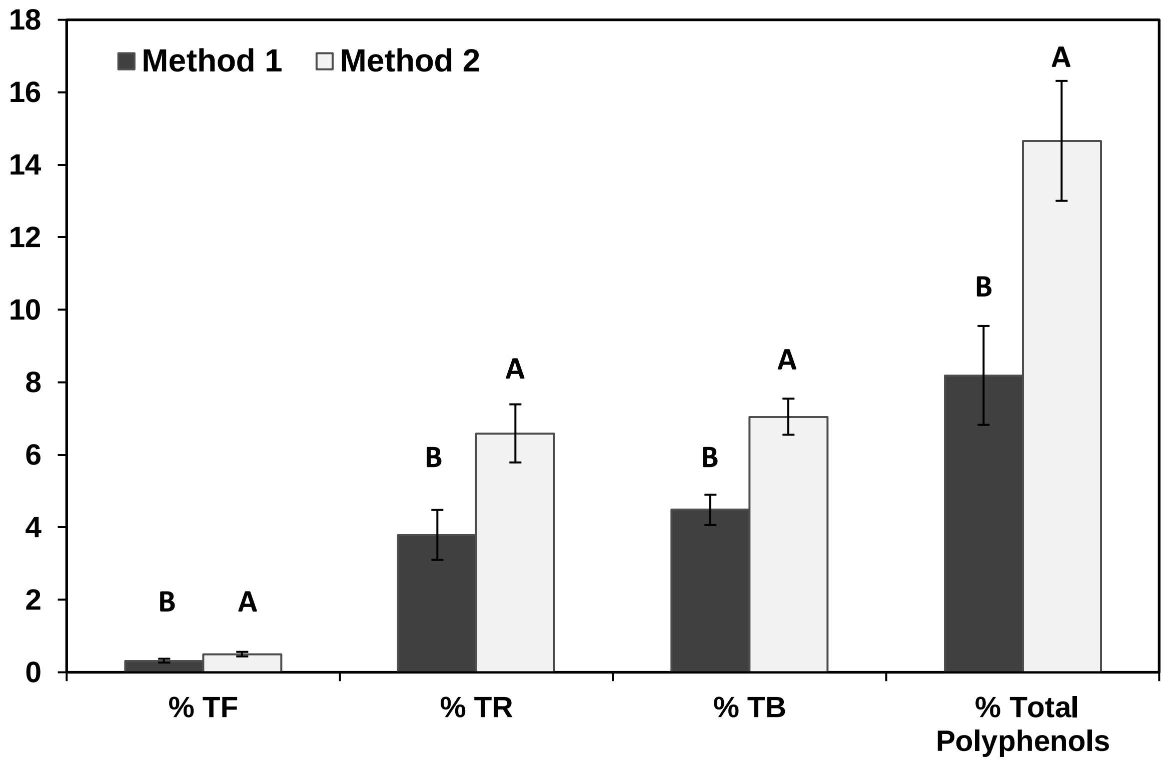


Figure 4. Mean % aroma (theaflavins [TF], thearubigins [TR], and theabrownins [TB]) and total polyphenol values ± standard error of black tea regardless of the brand as brewed by Method 1 and Method 2.

[A,B] Within each measured aroma value, columns with different letters are significantly different (P<0.05)

Total Phenolic Content and Oxygen Radical Absorbance Capacity (ORAC): Total phenolic content ± standard deviation was 534.62 ± 125.24 mg GAE/L, 284.66 ± 78.65 mg GAE/L, 687.00 ± 176.11 mg GAE/L, and 453.92 ± 135.61 mg GAE/L, respectively, in Lip1, Tur1, Lip2, and Tur2 samples. The ORAC value ± standard deviation of Lip1 and Tur1 tea samples were 66.05 ± 13.56 µM TE/mL and 34.38 ± 7.20 µM TE/mL, respectively, whereas for Lip2 and Tur2, the values were 91.11 ± 19.79 µM TE/mL and 63.57 ± 22.72 µM TE/mL, respectively. There were significant differences between the antioxidant activities of tea brands (P<0.05). The mean results of all replicates of both tea brands by brewing method indicated the total phenolic and antioxidant capacity (ORAC) values were significantly higher (P<0.05) in the longer-brewed, black tea samples of Method 2 (Figure 5).

Williamson (2017) reported the role of polyphenols in chronic disease prevention, including reducing the risk of Type 2 diabetes [19]. Cleverdon et al. (2018) suggested that most of tea polyphenols are released in 5 minutes [5]. However, the present study results indicate that antioxidant activity significantly increased using the longer Method 2 brewing technique in the caydanlik as compared to the shorter Method 1 brewing technique in the teapot. Therefore, regardless of tea brand, the Method 2 brewing technique using the caydanlik, with a longer brew time, significantly increased the antioxidant activity level of the prepared tea (P<0.05).

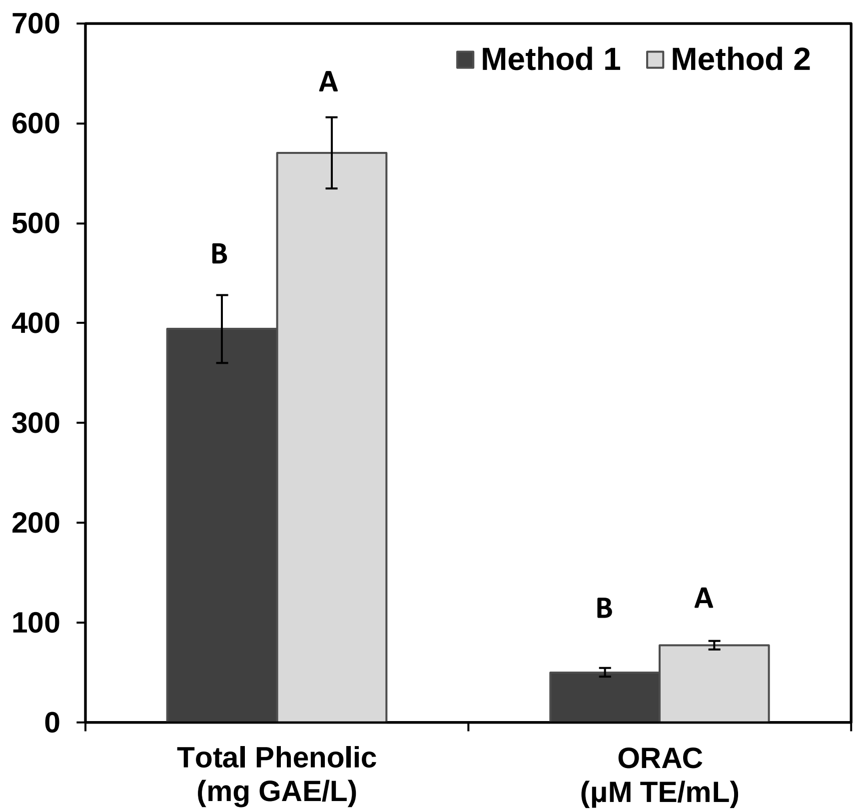


Figure 5. Mean total phenolic content (GAE/L) and antioxidant activity (ORAC) (µM TE/mL) values ± standard error of black tea regardless of the brand as brewed by Method 1 and Method 2.

[A,B,] Within each measured value, columns with different letters are significantly different (P<0.05)

Color Analysis: Hunter L-values ± standard deviation for Lipt1 and Tur1 samples were 38.25 ± 1.63 and 40.27 ± 0.95, respectively, and for Lip2 and Tur2 samples were 34.33 ± 3.44 and 34.85 ± 2.21, respectively. L-values ± standard deviation significantly decreased with the longer brewing time for the same brand of tea; brewing time affected the Hunter L-values (P<0.05). A decline in L value resulted in darker tea color for the Method 2 tea brewed in the caydanlik. The mean results ± standard error for Hunter L-values of all replicates of both tea brands by brewing methods are shown in Figure 6. Results indicated that Hunter L-values were significantly higher (P<0.05) in Method 1 brewing.

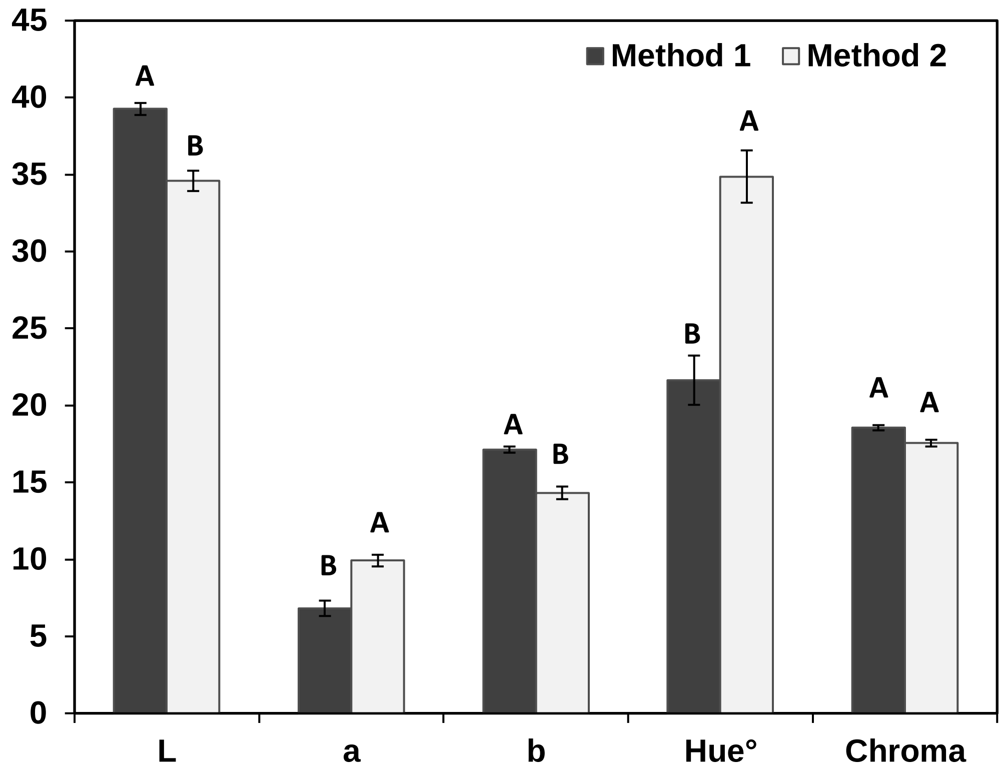


Figure 6. Mean Hunter color and calculated Hueº and Chroma values ± standard error of black tea regardless of the brand as brewed by Method 1 and Method 2.

A,B Within each color value, columns with different letters are significantly different (P<0.05)

Hunter a-values (redness) ± standard deviation of samples of Lip1 and Tur1 were 8.38±1.30 and 5.26±1.51, respectively. For the Lip2 and Tur2 samples, Hunter a-values were 10.20±1.96 and 9.65±1.24, respectively. Mean Hunter a-values ± standard deviation were statistically different across both brands and both methods with Lip2 tea samples having significantly higher a-values (P<0.05) than the other samples. The mean results of all replicates of both tea brands compared by brewing methods indicated that Hunter a-values were significantly higher (P<0.05) in Method 2 brewing (Figure 6).

The Hunter b-values (yellowness) ± standard deviation for Lip1 and Tur1 were 17.01 ± 0.93 and 17.24 ± 0.77, respectively; and for Lip2 and Tur2, the values were 14.89 ± 1.92 and 13.74 ± 1.40, respectively. The mean results of all replicates of both tea brands by brewing methods indicated that Hunter b-values were significantly decreased with longer brewing time (P<0.05) (Figure 6). It was noted that the increasing a-values and decreasing b-values for both tea types resulted in a dark-orange colored liquid.

Hue angle and chroma values for samples were calculated. Hue values of Lip1, Tur1, Lip2, and Tur2 were 26.23 ± 4.27, 17.04± 5.29, 34.55 ± 8.50, and 35.20 ± 6.07, respectively. The hue values of Lip 1 and Tur1 were significantly different (P<0.05). The highest hue values were observed for Lip2 and Tur2 and results indicated brewing Method 2 significantly increased (P<0.05) hue value due to the loss of "yellowness." The mean results of all replicates of both tea brands by the brewing method indicated a significant difference (P<0.05) in the hue angle for tea prepared in the caydanlik (Figure 6).

Chroma, also known as saturation, is defined as the intensity or purity of color [20]. Chroma values calculated for this study did not significantly differ (P>0.05) between tea brewing methods.

Chroma values of Lip1, Tur1, Lip2, and Tur2 samples were calculated as 19.01 ± 0.73, 18.10 ± 0.33, 18.23 ± 0.66, and 16.88 ± 0.65, respectively. There was no significant difference (P>0.05) between Lip1, Tur1, and Lip2 chroma values, but there was a significant difference (P<0.05) with the Tur1 values. The mean results of all replicates of both tea brands by brewing method indicated there was no significant difference (P>0.05) in chroma values by brewing method (Figure 6).

An increased hue angle corresponds to an increase in color intensity. In this study, the mean results of all replicates of both tea brands by brewing method indicated a significant difference (P<0.05) in hue angle for tea brewed in the caydanlik for 15 min as compared to tea prepared by the traditional teapot method for 5 min.

CONCLUSION

The present study determined that using the caydanlik brewing method produces a black tea product with higher theaflavins, thearubigins, theabrownins and total polyphenol values than tea brewed via the brewing technique typically used worldwide.

Results of this experiment indicate that the use of the caydanlik and the accompanying longer brewing time typically used in the Turkish tea brewing method, can significantly change the quality parameters and increase the antioxidant capacity of the brewed black tea beverage. Therefore, a higher-quality functional food beverage can be produced using the caydanlik for brewing black tea.

List of Abbreviations: ANOVA: analysis of variance, LSMEANS: least-square means, PROC GLM: linear model procedure, Lip: Lipton™, ORAC: oxygen radical absorbance capacity, TF: theaflavins, TR: thearubigins, TB: theabrownins, Tur: Turist™

Competing Interests: The authors declare no competing interests.

Author Contributions: ZGS, ACS, and AKG prepared the tea and collected samples and temperature data. ZGS and ACS carried out the analyses with assistance from AKG. ACS and ZGS conducted the statistical analyses. ZGS, ACS, and AKG drafted the manuscript. All authors read and approved the final manuscript.

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