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Effect of Different Brewing Methods on Some Physicochemical Properties of Green Tea (*Camellia sinensis*)

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ABSTRACT

Different brewing methods can lead to changes in the physicochemical properties of green tea. In this study, the physicochemical properties of green tea brewed with different amounts (5, 7.5 and 10 grams) and times (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 minutes) were investigated. Green tea produced by Çaykur was used in the study. Mineral analysis of green tea samples was performed with ICP-OES, and color analysis was performed with Minolta Spectrophotometer. The antioxidant activity was assessed using the DPPH method and the total phenolic content was determined using the Folin–Ciocalteu method. In terms of mineral content, it was determined highest levels of mineral content (mg/kg) for Al, Ca and Mg (10 g-25 minutes); Fe, Mn and Na (10 g-25 minutes); and B (10 g-50 minutes) as 16.005, 4.099, 24.075, 0.120, 13.855, 1.320 and 0.164, respectively. It was determined lowest levels of mineral content (mg/kg) for Al, Ca, Mg, and Mn (5 g-5 minutes); B and Fe (5 g-10 minutes), and Na (5 g-15 minutes) as 2.756, 1.193, 3.324, 1.23, 0.025, 0.002 and 0.48, respectively. In terms of color, it was determined that the lowest and highest *L** values are 39.97 (10 g-45 minutes) -54.23 (5 g-35 minutes), lowest and highest *a** values are -2.24 (5 g-5 minutes) (-4.70) (7.5 g-35 minutes) and lowest and highest *b** values are 4.91 (5 g-5 minutes)- 23.98 (10 g-60 minutes). The antioxidant activity value (inhibition %) in green tea was found to be the range of 28.51 % (5 g, 45 minutes)- 47.95 (10 g- 40 minutes), and the total phenolic content (mg GAE/mL) was found to be the range of 15.99 (5 g, 5 minutes)- 35.61 (10 g, 15 minutes). The findings determined that green tea brewed with different amounts and times showed statistical differences in terms of color, mineral content, antioxidant activity, and total phenolic content.

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Introduction

Tea is a popular beverage that can be served hot or ice-cold worldwide. Black, green, oolong, and white teas, which are among the most commonly consumed teas, are made from the leaves and buds of the *Camellia sinensis* (L.) (family *Theaceae*) plant and are classified according to differences in processing, harvesting, and the degree of oxidation of polyphenols in fresh tea leaves (Sharangi 2009; Unachukwu et al. 2010).

Tea infusion has a distinct flavor, aroma, and essential dietary components for human health (El-Shahawi et al., 2012). Green tea catechins have gotten much attention lately, both in the scientific and consumer worlds, for their many health advantages, spanning from cancer to weight reduction and functionality like anti-oxigenicity, antimutagenicity, and anticarcinogenecity (Yoshida et al., 1999; Bansal et al., 2012).

Tea is also an important source for human health in various macro and microelements. Regular tea consumption can meet the daily need for many mineral elements (Fernández et al., 2002; Ercişli et al., 2008).

Researchers have looked into the effects of brewing conditions such as infusion time (Kyle et al., 2007), water temperature (Perva-Uzunalic et al., 2006), and tea varieties (Henning et al., 2009) on the antioxidant capacity of infusions because of the rising popularity in drinking green tea for health reasons. Although the relationship between in vitro and in vivo antioxidant capacity is still debated (ARS 2010), assessing the antioxidant capacity of green tea infusions is likely to represent the overall phenolic content in each infusion, and hence reflect potential health benefits.

Availability of tea catechins is related to various factors as cultivar conditions and drying process (Almajano et al. 2008) and even brewing conditions (Rusak et al., 2008; Kyle et al., 2007; Perva-Uzunalic et al., 2006). As a result, there is debate concerning the optimum or better conditions for extracting as many antioxidant compounds as possible from tea infusions (Rahim et al., 2014). The extraction of catechins (the principal chemicals responsible for tea antioxidant capacity) is affected by several factors, including infusion time/temperature, particle size after grinding, number of extractions, storage time, and light exposure, among others. According to Wakamatsu et al. (2019), catechin content is affected by harvest time, some catechins are better extracted with increasing temperature, and catechin content may also alter the taste of tea infusion.

In this study, the effects of different amounts and times on the specified physicochemical properties were scrutinized by examining the mineral content, color, antioxidant activity, and total phenolic contents of green tea brewed in 3 different amounts (5-7.5 and 10 g) and 12 different times (5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 minutes) and it was aimed to guide both consumers and researchers in terms of providing optimum benefit.

Material and Method

Material

In the study, green tea from the 2016 harvest year, which was obtained from Çaykur, was used as material.

Extraction of phenolic compounds (brewing/infusing) and preparation of samples

Samples were prepared by brewing green teas to analyze mineral content, color, total phenolic content, and antioxidant activity for a total of 12 times with 5-minute increments between 5 to 60 minutes and amounts of 5-7.5 and 10 g. While preparing the sample for total phenolic content and antioxidant activity analysis, ground green tea with a particle diameter in the range of 150-280 µm was used. The brewing method applied by Gürses and Artık (1982) was revised, and in a porcelain teapot, 250 ml of distilled boiling water was used for brewing, and the temperature was kept at boiling level. After brewing, the tea infusions were quickly filtered through cotton and the Whatman No:1 under the vacuum. The prepared samples were wrapped in aluminum foil and stored at -18°C until analyzed.

Method

Mineral Content Analysis

Mineral content analysis was performed with an ICP-OES (Varian 720- ES, Walnut Creek, CA, USA). The ICP-OES measurement conditions were set to 1.15 kW of power, 15.0 L/min plasma flow, 2.25 L/min auxiliary flow, and 0.8 L/min nebulizer flow.

Color Analysis

The color of samples was determined using the Minolta Spectrophotometer CR-400 (Minolta Camera Co., Japan) according to the CIE Lab-scale (CIE, 1974) as $L^*a^*b^*$ values. The instrument was calibrated with a standard white calibration plate before color measurements. L^* value (100=light, 0=dark), a^* value (+a=redness, − a = greenery) and b^* value (+ b = yellowness, −b=blueness) values were determined in all infusions (Hunter 1942, Liu et al. 2018).

Antioxidant Activity Analysis

The DPPH free radical reduction assay measured the total antioxidant activity of green tea samples. Green tea infusions were diluted 100-fold, and 50µl of the sample was mixed with 1950 µl of DPPH solution in methanol. Instead of extract, distilled water was utilized as a control. The reaction mixture was vortex-mixed and let to stand for 60 minutes at 25°C in the dark, following which the absorbance was measured using a spectrophotometer at 517 nm with methanol as a blank. Antioxidant activity was expressed as percentage inhibition (inhibition %) of the DPPH radical and was determined by the following equation (Turkmen et al., 2006):

$$\text{AA. (\%)}= \frac{\text{Abscontrol-Abssample}}{\text{Abscontrol}} \times 10$$

Total Phenolic Content Analysis

The number of phenolic compounds was determined by the Folin-Ciocalteu method proposed by Singleton and Rossi (1965) and modified in accordance with Turkmen et al. (2006). Samples diluted 100-fold and 0.2 mL sample and 1 mL (10 % v/v) Folin-Ciocalteu reagent was placed in a test tube and mixed. 0.8 mL (10 % w/w) sodium carbonate solution was added and left to stand at 25 °C in the dark for 60 minutes; absorbances of the reaction of the mixture were measured at 765 nm using a spectrophotometer (Hitachi U-1800, Hitachi High Technologies America, Inc.). The calibration curve for gallic acid (range from 1.25 to 75 mg/L) was prepared, and the findings were calculated using the calibration curve's regression equation ($y = 0.0112x+ 0.0132$, $R^2 = 0.9994$) and were represented as mg gallic acid equivalents per gram of sample gallic acid (mg/mL).

Statistical Analysis

The experiments were carried out in duplicates for mineral content analysis and triplicate for the rest. Analysis of variance (ANOVA) and correlation test was performed using JMP statistical analysis program (Version 13.0.0, SAS Institute Inc., Cary, NC, USA). The LSD, a multiple comparison test, was used to compare the means of the variables that showed significant variation ($P \le 0.01$).

Findings and Discussion

Mineral Content

The ICP-OES was used to determine the concentrations of a total of 15 mineral contents in the samples: macro elements: K, Ca, Mg, Na, and microelements: Fe, Mn, Cu, Zn, B, Ni, Pb, Co, Cr, Al, and Cd, respectively. Cd, Co, Cr, Cu, Ni, Pb, and Zn measurements were found below the detection limits while K was above it. Regarding Al, B, Ca, Fe, Mg, Mn, and Na values (mg/kg) within the device's detection limits are shown in Tables 1, 2, and 3.

In the study, the amount of Aluminum (Al), Boron (B), Calcium (Ca), Iron (Fe), Magnesium (Mg), Manganese (Mn) and Sodium (Na) were found (mg/kg) as 2.756-16.005, 0.024-0.164, 1.193-4.099, 0.02- 0.120, 3.324-24.880, 1.23-13.855 and 0.497-1.320, respectively (Table 1, 2, 3). The difference between the mineral content distributions of the green tea samples was statistically significant ($P \le 0.01$). As the amount of brewed green tea increased, the amount of mineral content transferred to the infusion increased. However, there is an increase up to 10-15. minutes in general, the increase and decrease after this period show variation. This situation manifests that there is an exchange of contents between the tea pulp and the tea infusion.

Table 1. Mineral content changes of green tea brewed at different amounts and times (Al, Ca, and Mg)

Mineral Content / Green Tea Amount			Time (d)							
			5	10	15	20	25	30	35	40
Al (mg.kg^{-1})	Quantity (g)	5	2.756^{k*}	2.950^{jk}	6.355^{fk}	3.767^{k}	5.742^{gk}	6.650^{ek}	5.820^{gk}	$9.342^{bı}$
		7.5	5.039^{hk}	6.831^{ek}	8.561^{cj}	5.114^{hk}	$8.719^{cı}$	11.540^{af}	5.617^{gk}	9.87^{bh}
		10	7.369^{ek}	6.991^{ek}	5.041^{hk}	6.895^{ek}	16.005^{a}	12.293^{ae}	7.555^{ek}	14.81^{ac}
		Min.	2.68	2.09	4.06	3.42	4.51	4.87	3.02	7.66
		Max.	7.4	7.47	10.12	8.09	16.06	15.04	8.62	15.05
		Mean	5.05	5.59	6.65	5.26	10.16	11.33	6.33	11.33
		SD	2.09	2.09	2.21	1.74	5.44	3.04	2.02	3.03
		SE	0.85	0.85	0.9	0.71	2.22	1.24	0.82	1.24
Ca (mg.kg^{-1})	Quantity (g)	5	1.193^{g*}	1.632^{eg}	1.745^{eg}	1.502^{eg}	1.501^{eg}	1.611^{eg}	1.743^{eg}	2.272^{cg}
		7.5	1.716^{eg}	2.444^{bg}	1.834^{dg}	1.724^{eg}	2.196^{dg}	2.163^{dg}	1.480^{fg}	2.064^{dg}
		10	1.762^{dg}	1.908^{dg}	1.589^{eg}	1.725^{eg}	4.099^{a}	2.722^{af}	1.679^{eg}	3.203^{ad}
		Min.	1.18	1.24	1.44	1.32	1.39	1.5	1.46	1.51
		Max.	1.89	2.53	1.87	1.94	5.6	3.08	1.87	3.81
		Mean	1.56	1.99	1.72	1.65	2.6	2.17	1.63	2.51
		SD	0.3	0.46	0.17	0.21	1.58	0.57	0.14	0.75
		SE	0.12	19	0.68	0.09	0.65	0.23	0.06	0.31
Mg (mg.kg^{-1})	Quantity (g)	5	3.324^{h*}	4.257^{gh}	10.446^{bh}	5.521^{fh}	11.140^{bh}	11.865^{bh}	10.477^{bh}	18.070^{ad}
		7.5	6.939^{eh}	11.430^{bh}	13.095^{bh}	7.160^{dh}	13.932^{ah}	19.340^{ac}	8.455^{ch}	17.170^{ae}
		10	10.370^{bh}	10.140^{bh}	6.311^{eh}	9.795^{bh}	24.075^{a}	19.540^{ab}	11.425^{bh}	24.615^{a}
		Min.	3.12	4.2	4.7	4.8	6.07	7.95	4.32	12.51
		Max.	10.39	12.35	15.44	11.81	24.32	24.13	16.63	24.91
		Mean	6.88	8.61	9.95	7.49	16.38	16.91	10.11	19.95
		SD	3.2	3.53	4.14	2.67	7.91	5.64	4.15	4.68
		SE	1.31	1.44	1.69	1.09	3.23	2.3	1.7	1.91

Mineral Content / Green Tea Amount		45	50	55	60	Min.	Max.	Mean	SD	SE
Al (mg.kg^{-1})	Quantity (g)	7.640^{ek}	5.830^{fk}	7.821^{ek}	8.392^{ag}	2.68	9.35	6.09	2.35	0.48
		$8.961^{cı}$	8.240^{dk}	9.842^{bh}	10.478^{ah}	4.07	12.92	8.23	2.98	0.61
		14.805^{ab}	14.175^{ac}	10.058^{bh}	10.960^{ag}	4.06	16.06	10.4	3.82	0.8
		5.68	4.93	7.38	814					
		15.01	14.31	10.66	11.8					
		9.66	9.41	9.24	9.94					
		3.88	4.35	1.3	1.49					
		1.73	1.77	0.53	0.6					
Ca (mg.kg^{-1})	Quantity (g)	2.149^{dg}	1.725^{eg}	2.060^{dg}	3.695^{ac}	1.18	3.92	1.9	0.65	0.13
		1.843^{dg}	2.056^{dg}	2.269^{cg}	2.728^{af}	1.46	3.39	2.04	0.51	0.11
		3.855^{ab}	2.946^{ae}	1.939^{dg}	2.297^{cg}	1.44	5.6	2.47	1.01	0.21
		147	1.45	1.83	2.07					
		3.87	3.43	2.51	3.91					
		2.62	2.24	2.09	2.91					
		1.01	0.72	0.23	0.78					
		0.41	0.3	0.09	0.32					
Mg (mg.kg^{-1})	Quantity (g)	15.965^{af}	11.299^{bh}	16.815^{ae}	18.040^{ad}	3.12	18.45	11.43	5.61	1.15
		15.654^{af}	14.753^{ag}	18.94^{ac}	19.610^{ab}	5.18	21.96	13.87	6.06	1.24
		24.880^{a}	24.205^{a}	17.99^{ad}	19.835^{ab}	4.7	25.27	16.93	6.96	1.42
		9.35	8.22	16.05	17.49					
		25.27	24.5	20.91	21.73					
		18.83	16.75	17.92	19.16					
		6.16	7.35	1.81	1.63					
		2.51	3.01	0.74	0.66					

*The difference between values marked with different letters in the same column and row is statistically significant ($P \leq 0.01$).

In their study, Poyrazoğlu and Gürses (2004) found that the amount of mineral content infused in black teas is directly proportional to the amount of tea, that the increase in the amount of mineral content continues until the brewing time is 30-40 minutes, and that the increases and decreases after this period are between the tea pulp and tea infusion due to content exchange.

The dietary reference index values of these minerals belonging to Ca, Na, Fe, Mg, and Mn in adult men and women were reported as 1.0-2.5 g, 1.5-2.3 g, 18-45 mg, 320-420 mg, and 11 mg, respectively (IOM, 2006). In the same study, the intake limit of Boron for adults was stated as 20 mg/day, and the maximum intake dose of Al was determined as 1 mg/kg by the European Food Safety Authority (EFSA, 2004).

Table 2. Mineral content changes of green tea brewed at different amounts and times (B, Fe, and Mn)

Mineral Content / Green Tea Amount		Time (d)								
			5	10	15	20	25	30	35	40
B (mg.kg^{-1})	Quantity (g)	5	0.047^{fj*}	0.025^{j}	0.050^{ej}	0.024^{j}	0.053^{ej}	0.060^{dj}	0.056^{dj}	0.093^{ch}
		7.5	0.051^{ej}	0.059^{dj}	0.062^{dj}	0.033^{hj}	0.073^{dj}	0.104^{dj}	0.045^{gj}	0.091^{ci}
		10	0.063^{dj}	0.053^{ej}	0.030^{ij}	0.046^{gj}	0.148^{ac}	0.109^{af}	0.059^{dj}	0.158^{ab}
		Min.	0.04	0.024	0.02	0.021	0.03	0.04	0.02	0.06
		Max.	0.63	0.062	0.075	0.05	0.149	0.144	0.085	0.167
		Mean	0.053	0.046	0.034	0.091	0.091	0.053	0.114	0.105
		SD	0.008	0.017	0.02	0.013	0.053	0.036	0.02	0.037
		SE	0.003	0.007	0.008	0.005	0.022	0.014	0.008	0.015
Fe (mg.kg^{-1})	Quantity (g)	5	0.015^{ef*}	0.002^{f}	0.022^{df}	0.005^{f}	0.022^{df}	0.024^{cf}	0.021^{df}	0.048^{cf}
		7.5	0.021^{df}	0.033^{cf}	0.031^{cf}	0.008^{ef}	0.043^{cf}	0.063^{cf}	0.015^{ef}	0.042^{cf}
		10	0.028^{cf}	0029^{cf}	0.019^{ef}	0.023^{ef}	0.119^{ef}	0.081^{ac}	0.028^{cf}	0.113^{a}
		Min.	0.011	0.001	0.011	0.005	0.006	0.012	0.001	0.023
		Max.	0.029	0.039	0.039	0.0235	0.141	0.121	0.041	0.127
		Mean	0.021	0.022	0.024	0.012	0.061	0.056	0.021	0.068
		SD	0.006	0.016	0.011	0.009	0.053	0.04	0.014	0.04
		SE	0.003	0.006	0.004	0.004	0.022	0.016	0.006	0.016
Mn (mg.kg^{-1})	Quantity (g)	5	1.23^{k*}	1.554^{jk}	3.882^{ek}	2.180^{ik}	4.129^{dk}	4.484^{dk}	4.155^{dk}	7.389^{di}
		7.5	2.501^{hk}	3.979^{ek}	4.927^{dk}	2.759^{gk}	6.1265^{dk}	8.304^{dk}	3.387^{fk}	7.408^{di}
		10	3.747^{ek}	3.713^{ek}	2.544^{hk}	3.805^{ek}	13.16^{ac}	9.502^{ad}	4.538^{dk}	13.665^{ab}
		Min.	1.16	1.54	2.01	1.93	2.51	2.98	1.78	4.99
		Max.	3.78	4.35	5.91	4.56	13.27	12.98	6.53	13.75
		Mean	2.49	3.08	3.78	2.91	7.81	7.42	4.03	9.49
		SD	1.14	1.23	1.48	0.98	4.83	3.46	1.6	3.58
		SE	0.47	0.5	0.6	0.4	1.97	1.41	0.65	1.46

Mineral Content / Green Tea Amount		45	50	55	60	Min.	Max.	Mean	SD	SE
B (mg.kg^{-1})	Quantity (g)	0.077^{dj}	0.057^{dj}	0.086^{dj}	0.101^{hp}	0.21	0.106	0.06	0.027	0.005
		0.084^{dj}	0.079^{dj}	0.102^{ag}	0.117^{ad}	0.023	0.141	0.075	0.034	0.007
		0.156^{ab}	0.164^{a}	0.095^{bg}	0.110^{ae}	0.02	0.167	0.099	0.049	0.01
		0.05	0.04	0.08	0.09					
		0.159	0.165	0.113	0.141					
		0.099	0.094	0.109	0.11					
		0.045	0.056	0.011	0.017					
		0.018	0.022	0.004	0.006					
Fe (mg.kg^{-1})	Quantity (g)	0.036^{cf}	0.031^{cf}	0.031^{cf}	0.045^{cf}	0.001	0.063	0.025	0.017	0.003
		0.037^{cf}	0.038^{cf}	0.055^{bf}	0.076^{ad}	0.005	0.097	0.038	0.027	0.006
		0.120^{a}	0.105^{bf}	0.052^{bf}	0.053^{bf}	0.016	0.014	0.064	0.042	0.009
		0.012	0.012	0.026	0.042					
		0.131	0.106	0.067	0.097					
		0.064	0.058	0.046	0.058					
		0.047	0.04	0.014	0.02					
		0.019	0.017	0.006	0.008					
Mn (mg.kg^{-1})	Quantity (g)	6.314^{dk}	4.289^{dk}	6.788^{dj}	7.545^{di}	1.16	7.7	4.5	2.32	0.47
		7.188^{di}	6.520^{dk}	8.195^{cg}	9.119^{ae}	2.12	10.79	5.87	3.05	0.62
		13.855^{a}	13.705^{ab}	7.739^{ch}	9.155^{ae}	2.01	14.28	8.26	4.55	0.93
		3.72	3.31	6.42	7.39					
		14.28	13.87	9.32	10.79					
		9.12	8.17	7.57	8.61					
		4.3	4.87	1.08	1.35					
		1.76	1.99	0.44	0.55					

* The difference between values marked with different letters in the same column and row is statistically significant ($P \leq 0.01$)

Considering the dietary reference intakes of Ca, Na, Fe, Mg, and Mn in the study, it was seen that it has corresponded 0.119-0.41%, 0.033-0.088%, 0.111-0.667%, 1.039-7.775% of the daily requirement for 100 ml consumption, respectively. Considering B's recommended daily intake limit, it meets 0.12-0.82%. For Al, it was seen that it meets 4.59-26.68% of a 60 kg adult. Since the relationship of Al with Alzheimer's disease, this result seems remarkable. According to EFSA (2004), there is a daily intake of 28.6-214 µg/kg Al in Europe. Due to the acidophilic nature of the tea plant, the amount of Al in the leaves of the tea plant grown in acidic soils makes a serious contribution to the daily intake. In a study conducted by Sweileh et al. (2014), tea infusions had a mean concentration of 312 ± 18 µg/g Al but brewed tea infusions had lower concentrations (2.1 ± 0.1 mg/L).

Table 3. Mineral content changes of green tea brewed at different amounts and times (Na)

Mineral Content / Green Tea Amount			Time (d)							
			5	10	15	20	25	30	35	40
Na (mg.kg^{-1})	Quantity (g)	5	0.841^{ad*}	0.723^{ad}	0.896^{ad}	0.494^{d}	0.949^{ad}	0.699^{ad}	0.712^{ad}	1.039^{ad}
		7.5	0.771^{ad}	1.236^{ad}	0.750^{ad}	0.526^{cd}	0.763^{ad}	1.025^{ad}	0.520^{cd}	0.892^{ad}
		10	0.720^{ad}	0.623^{bd}	0.487^{d}	0.562^{cd}	1.162^{ac}	1.078^{ad}	0.580^{bd}	1.239^{ab}
		Min.	0.71	0.51	0.43	0.36	0.43	0.53	0.33	0.57
		Max.	0.92	1.36	1.06	0.69	1.18	1.36	1.09	1.25
		Mean	0.78	0.86	0.71	0.53	0.96	0.93	0.6	1.06
		SD	0.08	0.32	0.23	0.11	0.29	0.33	0.26	0.26
		SE	0.03	0.13	0.09	0.05	0.12	0.14	0.11	0.1
Mineral Content / Green Tea Amount		45	50	55	60	Min.	Max.	Mean	SD	SE
Na (mg.kg^{-1})	Quantity (g)	1.138^{ad}	0.840^{ad}	1.075^{ad}	1.307^{a}	0.33	1.6	0.89	0.28	0.057
		0.812^{ad}	0.889^{ad}	1.007^{ad}	1.227^{ab}	0.36	1.36	0.87	0.31	0.064
		1.320^{a}	1.358^{ad}	0.881^{ad}	0.968^{ad}	0.43	1.53	0.91	0.33	0.068
		0.68	0.51	0.86	0.92					
		1.5	1.53	1.15	1.6					
		1.09	1.03	0.99	1.17					
		0.28	0.37	0.11	0.25					
		0.12	0.15	0.04	0.1					

* The difference between values marked with different letters in the same column and row is statistically significant ($P \leq 0.01$)

Another study reported that Al concentrations in brewed tea ranged from 3-4 mg/L (Saiyed and Yokel 2005). Hardisson et al. (2017) reported that high tea consumption could meet 50 % of daily Al intake.

Derun (2014) found the amount of Ca in green tea as 9.271 mg/kg, Mg as 12.61 (mg/kg), and Mn as 116.5 mg/kg in her research conducted by brewing 2 g of green tea with 50 ml of water. The Mn concentration was found as 105.86 % and 217.95 % excess of the daily requirement. The ratio of other minerals is below the daily requirement. Fe and Na minerals were not found. In the same study, Cu, Zn, Co, and Cr minerals were determined as 2.386, 3.202, 0.048, and 0.148 mg/kg, respectively. In the study, the amount of Mg is consistent with Derun (2014), and the concentrations of other minerals are divergent. Brzezicha-Cirocka et al. (2015) found the Mg and Ca ratios as 1.7 mg Mg/200 mL and 0.3 mg Ca/200 mL, respectively. The Mg ratio found is consistent with this study, and the amount of Ca is divergent. Reto et al. (2007) reported the amounts of Ca, Fe, K, Mn and Na in green tea in Portugal as 1.9-3.5, 0.022-0.128, 92-151, 0.52-1.9, and 35-69 mg/kg, respectively. Ca and Fe ratios are similar to the study; other values are divergent. Shen and Chen (2008) reported the amount of Co, Cr, Cu, Fe, Mg, and Zn in green teas in Taiwan as 59.3, 12.4, 22.9, 10.9, 34.6, and 60.7 mg/kg, respectively. These values are divergent from the study. Malik et al., (2008) reported 8 element concentrations of green teas of Ca (1.45-6.10 mg/kg), Cu (0.079-0.201 mg/kg), Fe (0.005-0.078 mg/kg), K (277-503 mg/kg) Mg (15.3-24.5 mg/kg), Mn (2.05-10.9 mg/kg) and Zn (0.261-0.366 mg/kg) in Czech. The amounts of Ca, Fe, Mn, and Mg are consistent with this study. In a study conducted by Gürses and Artık (1982) on black Turkish teas, Fe was between 3.5-12.5 mg/kg, Cu 3.82-21.36, for Ca 13-110 mg/kg. They found the K concentration to be 1837-3538 mg/kg and Na concentration to be 89-223 mg/kg. These values are also higher than the findings in the study.

Since these studies were conducted with different types of teas with different characteristics and grown in different regions, It is thought that the differences between the studies originate from these reasons.

Color Analyses (L*a*b* values)

Data on L^* (whiteness or brightness/darkness) a (+, redness/-, greenness) and b (+, yellowness/-, blueness) values according to the CIE system are presented in Table 4. The difference between $L^*a^*b^*$ values of green tea samples was found to be statistically significant ($P \leq 0.01$).

L^* value is the highest brewing 5 g green tea for 35 minutes (54.23). The lowest value was found brewing 10 g green tea for 45 minutes (38.97). In terms of L^* value, the measurement results do not show a broad range with 46.18-54.23 for brewing 5 g of green tea, 43.42-51.83 for brewing 7.5 g of green tea, and 38.97-48.62 for brewing 10 g of green tea (Table 4). The L^* value in the range of 38.97-54.23 suggests that, regardless of the time, the change in the whiteness/brightness amount varies according to the highest and lowest concentrations in the infusion due to content exchange.

As it is known, negative (-) a^* value point out the green color, and it is seen that the highest value (-4.70) is reached with brewing 7.5 g of green tea for 35 minutes and the lowest value (-2.24) is reached with brewing 5 g of tea for 5 minutes. The a^* values of the samples for 5 g of green tea (-2.24-(-4.27) for 7.5 g of green tea (-2.84-(-4.70) and for 10 g of green tea infusion with (-3.11-(-4.27)) the measurement results do not show a wide range (Table 4). In terms of a^* value, the results with (-2.24)- (-4.27) suggest that regardless of the time, the greenness value changes according to the highest and lowest concentration in the infusion due to content exchange.

In terms of the b^* value, it is seen that the highest b value is reached by brewing 10 g of green tea for 60 minutes (23.98), and the lowest b^* value is obtained by brewing 5 g of tea for 5 minutes (4.91). The b^* values of the samples do not show a wide range with 4.91-17.23 for brewing 5 g green tea, 7.54-20.69 for brewing 7.5 g green tea, and 12.91-23.98 for brewing 10 g green tea (Table 4). b^* values in the range of 4.91-23.98 point out that the yellowness value increased as the amount and time increased, and the changes were in a relatively wide range.

Table 4. *L*a*b** values of green tea brewed at different amounts and times

*L*a*b**/Amount of green tea (g)			Time (d)							
			5	10	15	20	25	30	35	40
L	Green Tea (g)	5	46.18^{ck*}	51.50^{ac}	52.63^{ab}	52.46^{ab}	51.50^{ac}	45.43^{dk}	54.23^{a}	48.85^{af}
		7.5	48.02b^{g}	51.34^{ac}	51.83^{ac}	47.71^{bi}	51.17^{ad}	48.34^{bg}	49.47^{ae}	48.10^{bg}
		10	41.59^{kl}	48.62^{ag}	46.24^{ck}	47.51^{bj}	46.62^{gl}	4302^{il}	42.10^{el}	43.81^{e}l
		min.	37.41	44.59	44.3	42.93	43.14	39.59	40.4	37.58
		max.	50.2	54.88	55.71	55.41	54.59	51.88	58.13	51.82
		mean	45.26	50.49	50.23	49.23	49.76	45.6	48.6	46.92
		SD	3.47	3.38	3.93	3.58	3.5	4.13	5.52	3.85
		SE	1.01	0.97	1.13	1.03	1.01	1.192	1.59	1.11
*a**	Green Tea (g)	5	-2.24^{a*}	-2.69^{ab}	-4.14^{gm}	-3.66^{dh}	-4.16^{gm}	-3.63^{dg}	-4.58^{lm}	-3.43^{df}
		7.5	-2.84^{bc}	-2.81^{ab}	-3.70^{ei}	-4.03^{gl}	-3.79^{fj}	-4.04^{gl}	-4.70^{m}	-4.02^{g}
		10	-3.11^{bd}	-3.68^{dh}	-3.96^{fk}	-4.16^{gm}	-4.21^{gm}	-4.18^{gl}	-4.11^{fj}	-3.86^{fj}
		min.	-3.29	-3.94	-4.42	-4.43	-4.56	-4.6	-4.92	-4.85
		max.	-2.03	-2.44	-3.6	-3.43	-3.59	-3.17	-3.88	-2.49
		mean	-2.73	-3.06	-3.93	-3.95	-4.05	-3.95	-4.46	-3.76
		SD	0.42	0.5	0.3	0.3	0.29	0.39	0.31	0.64
		SE	0.12	0.14	0.08	0.09	0.08	0.11	0.09	0.18
*b**	Green Tea (g)	5	4.91^{r*}	5.43^{qr}	15.37^{ei}	12.78^{jm}	14.65^{fj}	13.33^{im}	16.59^{dg}	17.23^{de}
		7.5	8.38^{op}	7.54^{pq}	11.94l^{n}	16.15^{dh}	12.05^{kn}	14.39^{gk}	20.69^{b}	19.72^{bc}
		10	13.01^{jm}	12.91^{jm}	16.85^{df}	16.69^{dg}	15.86^{b}	21.48^{ab}	21.87^{fj}	14.81^{fj}
		Min.	4.8	5.3	11.71	10.89	11.65	12.01	15.8	13.41
		Max.	13.55	13.68	17.82	17.44	17.01	22.4	22.47	20.86
		Mean	8.76	8.62	14.7	15.2	14.18	16.4	19.71	17.25
		SD	3.49	3.31	2.28	1.99	1.8	3.87	2.43	2.22
		SE	1.01	0.96	0.66	0.57	0.52	1.11	0.7	0.64

*L*a*b**/Amount of green tea (g)		45	50	55	60	Min	Max	Mean	SD	SE
L	Green Tea (g)	52.54^{ab}	47.98^{bh}	48.60^{ag}	46.95^{bk}	39.59	58.13	49.9	4.23	0.61
		43.70^{fl}	43.97^{el}	48.08^{bg}	43.42^{fl}	47.93	54.83	47.93	3.65	0.53
		38.97l	45.21^{ek}	41.87^{hl}	42.25^{hl}	33.89	51.79	43.98	3.79	0.55
		33.89	40.93	39.84	40.85					
		55.56	49.52	53.88	51.04					
		45.07	45.07	46.18	44.21					
		6.6	6.6	4.83	3.68					
		1.91	1.9	1.39	1.06					
*a**	Green Tea (g)	-4.07^{gl}	-3.40^{cf}	-3.88^{fj}	-4.27^{jm}	-4.92	-2.03	-3.68	0.69	0.1
		-4.21^{hm}	-3.85^{fj}	-3.71^{ej}	-4.09^{gl}	-4.85	-2.44	-3.81	0.6	0.08
		-3.19^{be}	-4.52^{km}	-4.10^{km}	-4.27^{im}	-4.73	-2.51	-3.95	0.49	0.07
		-4.33	-4.73	-4.47	-4.63					
		-2.51	-3.22	-3.5	-3.77					
		-3.82	-3.92	-3.9	-4.21					
		0.59	0.51	0.25	0.28					
		0.17	0.15	0.07	0.08					
*b**	Green Tea (g)	14.43^{gj}	10.38^{no}	13.11^{im}	16.62^{dg}	4.8	17.86	12.9	4.02	0.58
		20.14^{bc}	14.16^{hl}	11.79^{mn}	16.45^{dh}	6.83	21.13	14.45	4.25	61
		19.98^{bc}	21.16^{b}	17.98^{a}	23.98^{a}	11.97	26.6	18.05	3.9	0.56
		13.38	9.88	11.2	15.22					
		26.6	21.92	19.32	24.81					
		18.18	15.23	14.29	19.01					
		4.27	4.7	2.85	3.76					
		1.23	1.35	0.82	1.085					

* The difference between values marked with different letters in the same column and row is statistically significant ($P \leq 0.01$).

Changes in the *L*a*b** values for all three quantities (5, 7.5, and 10 g) of green tea during the brewing period indicate, regardless of the time, a change in the highest and lowest concentrations in the infusion as a result of substance exchange.

In a study conducted by Liu et al. (2018) at different times, particle sizes, ratios, and temperatures in green teas, the *L** value was found to be 65.9-73.6, a * value was found to be 5.47- (-0.99), and the *b** value was found to be 1.32-18.6. Although the *a** and *b** values found in the study are compatible with this study, it is seen that the *b** value is higher in some samples, and the *L** value is low.

Kadiroğlu and Dıblan (2017) determined the *L*a*b** values in the green tea they brewed at different concentrations and times in the range of 69.98-94.67, -7.39-(-3.39), and 11.34-78.25, respectively. *a** and *b** values were compatible in our study, and the *L** value was found below.

Table 5. Antioxidant activity (Inhibition %) values of green tea brewed at different amounts and times

Antioxidant Activity Inhibition (%)/AGT			Time (d)							
			5	10	15	20	25	30	35	40
Antioxidant Activity	Green tea amount (g)	5	32.05^{no}*	31.04^{oq}	30.07^{or}	30.31^{or}	31.49^{np}	29.13^{qr}	29.36^{pr}	29.19^{pr}
		7.5	43.36^{de}	38.43^{ık}	37.20^{jl}	40.83^{fg}	36.80^{kl}	39.16^{hj}	35.35^{lm}	39.38^{hj}
		10	46.61^{ab}	42.41^{ef}	41.49^{eg}	43.70^{ce}	45.94^{ac}	44.09^{ce}	45.10^{bd}	47.95^{a}
		Min.	31.6	30.59	30.59	29.92	31.43	28.91	29.08	27.9
		Max.	46.72	42.52	42.02	44.02	46.05	44.2	45.21	48.06
		Mean	40.67	37.29	36.56	38.28	38.08	37.46	36.6	38.84
		S.D.	6.62	5	4.83	6.11	6.33	6.6	6.88	8.17
		SE	2.21	1.67	1.61	2.04	2.11	2.2	2.29	2.72
Antioxidant Activity Inhibition (%)/AGT (g)			45	50	55	60	Min.	Max.	Mean	SD	SE
Antioxidant Activity	Green tea amount (g)		28.51^{r}	29.63^{pr}	29.64^{pr}	33.56^{mn}	27.9	33.61	30.38	1.46	0.24
			43.81^{ce}	42.75^{ef}	42.58^{ef}	39.99^{gı}	35.29	43.87	39.97	2.69	0.45
			47.45^{a}	40.73^{fi}	43.59^{de}	42.13^{eg}	33.61	48.06	44.29	2.72	0.45
			28.4	29.24	29.58	33.45					
			47.56	44.87	43.87	42.35					
			39.92	37.7	38.6	38.56					
			8.7	6.86	6.74	3.87					
			2.9	2.25	2.25	1.29					

* The difference between values marked with different letters in the same column and row is statistically significant (P≤0.01). AGT: Amount of Green Tea

Table 6. Total phenolic content of green tea brewed at different amounts and times (mg GAE/mL)

Total phenolic content/Green tea amount (g)			Time (d)							
			5	10	15	20	25	30	35	40
Total Phenolic Content	Green tea amount (g)	5	15.99^{s}	20.01^{o}	18.05^{pr}	21.98^{n}	17.87^{qr}	17.24^{r}	15.93^{s}	18.82^{pq}
		7.5	28.08^{jl}	27.18^{lm}	27.78^{jl}	27.15^{lm}	26.62^{m}	27.21^{lm}	27.69^{km}	28.85^{ij}
		10	33.70^{be}	33.29^{df}	35.61^{a}	32.51^{fg}	31.56^{g}	33.49^{cf}	35.10^{a}	33.88^{bd}
		Min.	15.79	19.71	17.57	21.86	17.21	16.68	15.7	18.46
		Max.	33.82	33.91	36.41	32.84	31.85	33.81	35.25	34.63
		Mean	25.92	26.83	27.15	27.2	25.35	25.98	26.24	27.18
		SD	7.841	5.78	7.63	4.57	6.01	7.11	8.37	6.67
		SE	2.61	1.93	2.54	1.52	2.01	2.37	2.79	2.22
Total phenolic content/Green tea amount (g)			Time (d)							
			45	50	55	60	Min.	Max.	Mean	SD	SE
Total Phenolic Content	Green tea amount (g)	5	19.00^{op}	22.51^{n}	22.72^{n}	18.11^{pr}	15.7	23.02	19.02	2.31	0.39
		7.5	28.55^{ık}	30.25^{h}	29.32^{hı}	27.84^{jl}	26.32	30.61	28.05	1.01	0.18
		10	33.89^{ab}	34.77^{ab}	32.60^{eg}	34.51^{ac}	31.23	36.41	33.74	1.22	0.2
		Min.	18.55	22.21	22.3	17.21					
		Max.	34.63	35.07	33.11	34.98					
		Mean	27.18	29.18	27.45	26.82					
		SD	6.56	5.38	4.31	7.16					
		SE	2.19	1.79	1.44	2.39					

* The difference between values marked with different letters in the same column and row is statistically significant (P≤0.01)

Determination of Antioxidant Activity by DPPH Method

Antioxidant activities of green tea brewed with different amounts and times were determined as Inhibition % by the DPPH method. The difference between the inhibition % values of green tea samples was found to be statistically significant (P≤0.01).

As shown in Table 5, the highest antioxidant activity value was obtained by brewing 10 g of green tea in 40 minutes (47.95), the lowest value was obtained by brewing 5 g of green tea for 45 minutes (28.51). The inhibition % values for 5, 7.5 and 10 g ranged between 28.51-33.56, 36.80-43.81 and 40.73-47.95, respectively (Table 5). Antioxidant activity increased as the amount of green tea increased. For three amounts (5, 7.5 and 10 g) of green tea, the changes in antioxidant activity during the 5–60-minute brewing interval suggest that, regardless of time, there is a difference between the highest and lowest concentrations in the infusion as a result of content exchange. Lin et al. (2008) found that by infusing green tea leaves with cold (4°C, 24 hours) and hot water (90°C, 20 min), inhibition % values have been in the range of 31.7- 36.3 % and 29.1-34.0%, respectively.

In a study conducted by Velioğlu (2007) on fresh green tea leaves and green extracted with different solvents, the antioxidant activity value of the extracts was found to be 16.1-22.2 g aa/100 g. Balcı and Özdemir (2016) found the antioxidant activity value as 0.48-1.16 mg/mg^{-1} DPPH. Göksu (2011) reported the antioxidant activity of green tea infusion were found to be in the range of 87.2-95.68%. Considering the different tea types, extraction solvents, and antioxidant activity methods, the findings are consistent with the studies.

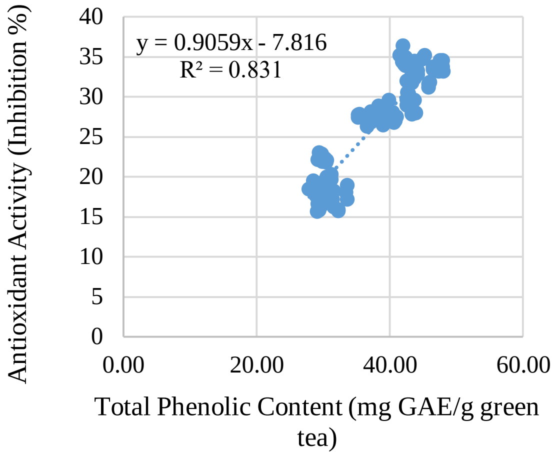

Figure 1. Correlation between antioxidant activity and total phenolic content (P≤0.01).

Determination of Total Phenolic Content by Folin-Ciotalteu Method

The total phenolic content values (mg GAE/mL) are shown in Table 6. The difference between the total phenolic content of the green tea samples was found to be statistically significant (P≤0.01).

The highest total phenolic content was obtained by brewing 10 g of green tea for 15 minutes (35.61 mg GAE/mL), and the lowest was obtained by brewing 5 g of tea for 5 minutes (15.99 mg GAE/mL). The total phenolic content of 5, 7.5 and 10 g determined range from 15.99-22.51, 26.62-30.25 and 31.56-35.61 mg GAE/mL, respectively (Table 6). For three amounts (5, 7.5 and 10 g) of green tea, the changes in antioxidant activity during the 5–60-minute brewing interval suggest that, regardless of time, there is a difference between the highest and lowest concentrations in the infusion as a result of content exchange.

In the study conducted by Liu et al. (2018), the total phenolic contents in green teas brewed in different ways were determined to range from 0.50-1.70 mg GAE/mL. Velioğlu (2007) reported the total phenolic content as 155.9-183.3 mg GAE/g dry tea due to extraction with water. Balcı and Özdemir (2016) found the total amount of phenolic contents as 68.1-131.3 mg/g dry weight in their study. Kadiroğlu and Dıblan (2017) found the total phenolic contents in green tea as 3.09-39.15 mg GAE/mL. Considering different tea types, extraction solvents, and calculation methods, the findings are consistent with our study.

Relationship Between Total Phenolic Content and Antioxidant Activity

A positive correlation was obtained between the antioxidant properties of green tea infusions (R^2=0.831) and their total polyphenol content (Figure1). Turkmen et al. (2006) found this correlation as 0.96-0.98 in a study conducted with different solvents in black teas. In a study conducted by Velioğlu (2007) on fresh green tea leaves (FGT) and green tea (GT) extracted with different solvents, there was a good correlation between the antioxidant activity and polyphenol content of the extracts (R^2 for FGT =0.9376, R^2 for GT=0.9783) was determined.

Conclusion

The changes in the mineral content, color, antioxidant activity, and total phenolic content of green tea were brewed with three different amounts (5, 7.5, and 10 g) and 12 different times (5-minute intervals from the 5th to the 60th minute) has been revealed with this study. It was observed that the values of color, mineral content, antioxidant activity, and total phenolic content increased as the amount of brewed green tea increased. It is thought that the differences in the amount of mineral content with the examples in the literature are due to geographical differences and changes in climatic conditions. Although it has no significant contribution to the intake of primary mineral contents except Mn, it has been observed that it covers approximately 26.68 % of the daily Al intake limit (1 mg/kg) of a 60 kg adult in terms of aluminum intake. A positive correlation between antioxidant activity and phenolic content (R^2=0.831) is found. For three amounts (5, 7.5, and 10 g) of green tea, the changes in antioxidant activity during the 5-60 minutes brewing interval suggest that, regardless of time. There is a difference between the highest and lowest concentrations in the infusion due to content exchange. Based on total phenolic content and antioxidant activity data, consumers may be advised to drink 10 g of green tea brewed for 35-40 minutes.

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