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# Influence of shooting period and extraction conditions on bioactive compounds in Turkish green tea

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**Abstract**

The aim of this study was to investigate the influence of different extraction temperatures (75, 85 and 95 °C) and times (3, 5, 10, 15 and 20 min) on bioactive compounds in Turkish green tea produced and classified as Turkuaz, Kardelen and Antikyeşil by ÇAYKUR (the Turkish Tea Board) over three shooting periods. Green tea extracts were subjected to analyses of extraction yield, antioxidant capacity, total phenolic and flavonoid contents, and the phenolic and flavonoid composition. Total phenolic and flavonoid contents and the antioxidant capacity of samples increased when extraction temperatures and times were increased. Extraction yield, total phenolics, total flavonoids and the antioxidant capacity of the green teas were found in the range of 21.52-37.40 g 100 $g^{-1}$, 68.13-131.31 mg GAE $g^{-1}$ dw, 17.97-32.04 mg CE $g^{-1}$ dw and 0.48-1.16 mg dw $mg^{-1}$ DPPH, respectively. The average amounts of individual catechins, i.e. catechin, epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate, gallocatechin gallate, gallocatechin and catechin gallate were found in the range of 8.91-17.09, 4.29-9.55, 28.03-59.42, 8.02-14.61, 38.05-69.66, 2.53-18.53, 2.30-12.97 and 0.04-1.78 mg $g^{-1}$ dw, respectively.

**Keywords:** antioxidant capacity; catechin; extraction conditions; shooting periods; Turkish green tea.

**Practical Application:** This paper presents different extraction conditions in Turkish green tea of bioactive compounds such as phenolic and flavonoids. The results indicated that optimum green tea brewing for the consumers.

## 1 Introduction

Young shoots of tea bushes are processed into different products, such as black (fermented), green (unfermented) and oolong (semi - fermented) tea. Among these, green tea contains more catechins, which have received considerable interest for their potential benefits in human health for their antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic and food preservative properties (Wang & Helliwell, 2001; Cabrera et al., 2003, 2006; Higdon & Frei, 2003; Perva-Uzunalić et al., 2006; Pharn-Huy et al., 2008; Yuan et al., 2011; Vuong et al., 2012). Green tea processing involves steaming, rolling, drying, sorting and packing. A critical step in this process is enzyme inactivation by steaming (Şahin-Nadeem et al., 2007).

The tea crop becomes ready for harvesting at nearly the same time in all plantations of the country. Tea is plucked three times between May and October, depending on weather conditions. In other words, there are three shooting periods in a season (Ozdemir et al., 1993).

Green tea contains about 30 g 100 $g^{-1}$ polyphenols by dry weight, which includes flavonols, flavonoids and phenolic acids. Catechins (about 10% of the dry weight basis) are the most prevalent flavonoids in green tea (Komes et al., 2010). They are divided into four primary compounds, i.e. epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), epigallocatechin gallate (EGCG), with four secondary compounds i.e. catechin (C), catechin gallate (CG), gallocatechin (GC), and gallocatechin gallate (GCG). EGCG is the predominant catechin present in green tea leaves (Perva-Uzunalić et al., 2006).

There are many studies on green tea polyphenols especially associated with extraction conditions such as solvent type, extraction time, extraction temperature, agitation and loading percentage (Hertog et al.,1993; Khokhar & Magnusdottir, 2002; Perva-Uzunalić et al., 2006; Xi et al., 2009; Komes et al., 2010). Also according to Wang & Helliwell (2001), the extraction efficiency of polyphenols depends on the extraction time, temperature, pH and type of solvents.

In general consumer practice for the preparation of green tea, loose leaves of tea are brewed in just boiled water. A second way of preparing green tea is to brew a teabag in boiled water for 3-5 min. In the second procedure, the consumers may not get the full health benefits of green tea beverage because the infusion of the catechins is not optimal under household brewing conditions (Yuan et al., 2011; Vuong et al., 2012).

According to the literature survey, there has been no detailed comparison study related to the extraction conditions and their effects on the catechins. The present study was undertaken to identify the amount of bioactive components of extracted Turkish green tea. Different extraction temperatures (75, 85 and 95 °C) and times (3, 5, 10, 15, and 20 min) were selected to investigate their influences on the total phenolic content (TPC), total flavonoid

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content (TFC), antioxidant capacity (AC) and catechin content of green tea, which were classified as Turkuaz, Kardelen and Antikyeşil from three shooting periods.

## 2 Materials and methods

### *2.1 Materials*

Green tea (*Camellia sinensis L.*) samples of the Kardelen, Antikyeşil and Turkuaz types were obtained from the Turkish tea board (ÇAYKUR) during three shooting periods of the 2010 tea season. Turkish green teas were separated into different categories according to size.

Fine particles which were sieved through a Middleton pakka were designated "Kardelen (Sample A)". The remaining tea in the Middleton pakka was separated through a winnower according to their specific gravity. The tea with a higher specific gravity was designated "Turkuaz (Sample C)" and those with a lower specific gravity were designated "Antikyeşil (Sample B)". The bulk density values of the A, B and C green tea samples were determined as 354 kg $m^{-3}$, 146 kg $m^{-3}$ and 304 kg $m^{-3}$, respectively (Şahin-Nadeem et al., 2013). The catechin standards and other chemicals were purchased from Sigma-Aldrich.

### *2.2 Preparation of green tea extracts*

Extraction was accomplished according to the procedure of Gürses & Artık (1987). 2.83 g of the green tea samples were immediately added to 250 mL of water conditioned at the extraction temperature in a round-bottom flask. The mixture was extracted by steady shaking with an orbital shaking water bath (GFL, Germany) at 150 rpm to submerge every particle in the water and to perform forced convective diffusion. The mixtures were uniformly maintained at three different temperatures (75, 85 and 95 °C), which were checked using a thermocouple throughout each extraction process. The extraction was performed in a time-dependent manner (3, 5, 10, 15 and 20 min). The extracts were filtered (Whatman No. 42), cooled and stored at - 18 °C until analysis.

### *2.3 Determination of extract yield*

15 mL of the filtered extracts were dried in an oven (Memmert, Germany) at 65°C until a constant weight. Extraction yield was calculated using Equation 1:

$$EY(g\ /\ 100g) = (W_1 x\ 15)\ /W_2 \quad (1)$$

where $EY$ is the extraction yield, $W_1$ is the weight of the dried extract and $W_2$ is the dry matter of tea (Gürses & Artık, 1987).

### *2.4 Determination of total phenolic content*

The total phenolic content analyses were performed according to Torun et al. (2014). For this purpose, 0.5 mL of the extract was treated with 2.5 mL of 0.1 N Folin-Ciocalteu reagent and 2 mL of $Na_2CO_3$ (75 g/L). This mixture was incubated at 50 °C for 5 min and cooled immediately. The absorbance of the final solution was recorded with a spectrophotometer (Shimadzu UV - Vis 160A, Japan) at 760 nm. The results were expressed as gallic acid equivalents (GAE) (g gallic acid/L).

### *2.5 Determination of total flavonoid content*

2.5 mL of distilled water and 150 µL of a 5% $NaNO_2$ solution were added into 0.5 mL of the extract sample, then allowed to stand for 5 min after vortexing. After that, 300 µL of a 10% $AlCl_3$ solution was added to the solution and allowed to stand for 5 min again. A further 1 mL of 1 M NaOH was added and the final volume was made up to 5 mL with distilled water. Sample absorbance was measured at 510 nm by using a spectrophotometer (Shimadzu UV-Vis 160A, Japan). The results were expressed as (+) - catechin equivalents (CE) (g (+) - catechin $L^{-1}$) (Chang et al., 2006).

### *2.6 Determination of antioxidant capacity using DPPH*

DPPH (2,2 Diphenyl – 1 - picryhydrazyl radical) radical scavenging was assessed according to the literature procedure (Gadow et al., 1997; Maisuthisakul et al., 2007). 100 µL of the diluted extract (prepared at four different concentrations to provide 10 to 90% inhibition) were added into 4 mL of freshly prepared DPPH solutions (6 x $10^{-5}$ M in MeOH). The mixtures were then shaken and placed in the dark at room temperature for 30 min. Absorbance values of the final solutions ($A_s$) were recorded at 516 nm using a spectrophotometer (Shimadzu UV-Vis 160A) with respect to the control (distilled water instead of extract in the DPPH solution) solution ($A_c$). The inhibition percentage of the DPPH radical was calculated by using Equation 2:

$$Inhibition\ (\%) = \left[(A_c - A_s)/A_c\right] x100 \quad (2)$$

The extract concentration providing 50% inhibition ($IC_{50}$) was calculated by plotting the concentration versus inhibition percentage.

### *2.7 Determination of catechin contents by HPLC*

The tea catechin content of the extracts was determined according to the method of Zuo et al. (2002). All green tea extracts were filtered through a membrane filter (0.45 µm Macherey Nagel, Germany), and 20 µL of each sample were injected for HPLC analysis.

The chromatographic separation was performed using a solvent delivery system (20AD, Shimadzu, Japan) coupled with an auto - sampler (SIL-20A Prominence, Shimadzu, Japan), column (LiChroCART® 250-4 250 x 4 mm 5 µm Nucleosil® 100-5 C 18). Individual peaks were detected by an SPD-M20A Diode Array Detector (Shimadzu, Japan) which was controlled by LC solution software. The mobile phase was HPLC grade methanol (solvent A) and consisted of 0.2% trifluoroacetic acid (solvent B) at a flow rate of 1 mL $min^{-1}$. Elution was performed with a gradient started at 5% A for 1 min, followed by a linear increase of solvent A to 63% in 28 min, after which the mobile phase composition was brought back to the initial conditions in 5 min for the next run. Chromatograms were recorded at 280 nm. Catechin compounds were identified by comparing the retention times and spectral data with authentic standards.

Calibration curves were obtained at detection for all catechins using a series of standard solutions over a concentration range from 1 to 100 mg $L^{-1}$. All calibration curves were linear over the concentration ranges tested with correlation coefficients ≥ 0.998.

### *2.8 Statistical analysis*

The experiment was set up as a factorial design for the extraction temperature and time (3 × 5), using duplicate samples. The data were then subjected to analysis of variance, and appropriate means separation was conducted using Duncan's multiple - range test using the SAS System for Windows V7 software (SAS Institute Inc., Cary, NC, USA).

## 3 Results and discussion

### *3.1 Extraction yield*

All of the experimental factors (tea class, extraction temperature and time) had a significant ($P < 0.01$) effect on the extraction yield of the samples, with a range of 21.52-37.40 g 100 $g^{-1}$ (Table 1). The Sample A showed the highest extraction yield at 95 °C for the 20 min extraction procedure (Table 1), which may be related to the particle size of the samples. Indeed, the Sample A had smaller particles than the others, hence the extraction equilibrium time decreased. However, the extraction yield of the samples increased with increasing the temperature and time. Extraction yield plays an important role in the quality of green tea by impacting the colour, taste and flavour. In this study, the equilibration time of the extraction yield was found to be around 5 min. From this point of view, especially in samples of the third shooting period, green tea should be extracted for over 5 min before consumption.

Yao et al. (2004) reported the water extraction yield of commercial green teas as 32.84%. According to Perva-Uzunalić et al. (2006) the extraction yield depends on the extraction temperature of the green tea sample, and is usually in the range of 29.2- 43%. In addition, Ozdemir et al. (1993) reported that the extraction yield of Turkish black teas of the first shooting period is higher than samples of the second and third shooting periods. Our findings are in agreement with the above studies. Small differences may be observed due to differences in tea origin, the number of coarse leaves, the extraction procedure, particle size and growing conditions of the tea.

### *3.2 Total phenolic and flavonoid content*

The total phenolic and flavonoid contents of the samples, which contained nearly 25% TPC, were found in the range of 68.13-131.31 mg GAE $g^{-1}$ dw and 17.97-32.04 mg CE $g^{-1}$ dw as shown in Table 2 and 3, respectively. The highest TPC and TFC values were observed in Sample A extracted at 95 °C for 20 min. Previous studies reported the total phenolic content of green tea as 11.42-27.10 g GAE 100 $g^{-1}$, which corresponds to 114.2-271.0 mg GAE $g^{-1}$ (Astill et al., 2001; Turkmen et al., 2006; Karori et al., 2007; Anesini et al., 2008). These results are slightly lower as compared to previously reported results for green tea, but this may be related to the extraction conditions, tea clone, geographic origin, growing conditions, processing conditions, differences in plucking season and plucking standardisation.

In Turkey, the first shooting period starts in May, and tea is plucked three times until October, depending on the weather conditions. During the nearly seven months of winter, tea undergoes a seasonal dormancy period. Consequently, Turkish tea bushes of the first shooting period contain a higher amount of total phenolic and flavonid content in the apical bud and leaves than the following shooting periods. The total phenolic and flavonoid contents of these parts are higher than the other leaves and stalks of the tea plant. As a matter of fact, the total phenolic and flavonoid contents of samples obtained from the first shooting period were higher than those from the second and the third shooting period. Moreover, in the second and the third shooting periods, both leaves and buds are harvested. Therefore,

**Table 1**. Duncan's multiple - range test results of extraction yield values (g/100 g).

| | | | | | | |
|---|---|---|---|---|---|---|
| **First shooting period** | **Class (n = 30)** | **Sample A** | **Sample B** | **Sample C** | | |
| | | $37.4^{a} \pm 0.4$ | $31.7^{b} \pm 0.6$ | $29.3^{c} \pm 0.7$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $31.2^{c} \pm 0.8$ | $32.5^{b} \pm 0.7$ | $34.7^{a} \pm 0.9$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | $28.7^{e} \pm 1.2$ | $31.7^{d} \pm 1.0$ | $33.3^{c} \pm 0.9$ | $34.5^{b} \pm 0.8$ | $35.72^{a} \pm 0.82$ |
| **Second shooting period** | **Class (n = 30)** | **Sample A** | **Sample B** | **Sample C** | | |
| | | $34.8^{a} \pm 0.8$ | $28.3^{b} \pm 0.8$ | $25.7^{c} \pm 1.2$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $26.9^{c} \pm 1.2$ | $29.8^{b} \pm 1.1$ | $32.1^{a} \pm 1.0$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | $22.5^{d} \pm 1.3$ | $28.1^{c} \pm 1.4$ | $31.5^{b} \pm 1.1$ | $31.5^{b} \pm 1.0$ | $34.39^{a} \pm 1.09$ |
| **Third shooting period** | **Class (n = 30)** | **Sample C** | **Sample B** | **Sample A** | | |
| | | $24.4^{b} \pm 1.1$ | $25.4^{b} \pm 0.7$ | $30.4^{a} \pm 0.7$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $26.1^{a} \pm 1.0$ | $27.1^{a} \pm 1.0$ | $27.1^{a} \pm 1.0$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | $21.5^{c} \pm 1.0$ | $23.5^{c} \pm 1.3$ | $27.9^{b} \pm 0.9$ | $29.5^{a,b} \pm 0.8$ | $31.38^{a} \pm 0.53$ |

Results are means ± standard error. The values within a column with different superscript letters are significantly ($P < 0.01$) different.

**Table 2**. Duncan's multiple - range test results of total phenolic content (mg GAE/g dw).

| | | | | | | |
|---|---|---|---|---|---|---|
| First shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 130.8$^{a}$ ± 4.0 | 113.0$^{b}$ ± 3.2 | 101.7$^{c}$ ± 4.5 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 103.15$^{c}$ ± 3.1 | 113.4$^{b}$ ± 4.3 | 128.9$^{a}$ ± 4.7 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 90.6$^{d}$ ± 5.5 | 109.2$^{c}$ ± 5.2 | 118.6$^{b}$ ± 4.6 | 126.1$^{a}$ ± 4.2 | 131.3$^{a}$ ± 4.3 |
| Second shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 134.8$^{a}$ ± 4.3 | 100.2$^{b}$ ± 4.3 | 97.3$^{b}$ ± 5.6 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 101.2$^{c}$ ± 5.8 | 110.1$^{b}$ ± 5.7 | 121.0$^{a}$ ± 5.1 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 72.2$^{d}$ ± 5.8 | 103.2$^{c}$ ± 6.3 | 119.8$^{b}$ ± 5.9 | 123.7$^{b}$ ± 4.1 | 134.9$^{a}$ ± 3.9 |
| Third shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 109.9$^{a}$ ± 4.5 | 89.3$^{b}$ ± 3.2 | 92.8$^{b}$ ± 4.7 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 90.2$^{b}$ ± 4.0 | 98.1$^{a,b}$ ± 4.5 | 103.7$^{a}$ ± 4.7 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 68.1$^{d}$ ± 3.5 | 90.1$^{c}$ ± 5.1 | 100.4$^{b,c}$ ± 3.6 | 106.3$^{b}$ ± 4.2 | 121.8$^{a}$ ± 3.6 |

Results are means ± standard error. The values within a column with different superscript letters are significantly (P < 0.01) different.

**Table 3**. Duncan's multiple - range test results of total flavonoid content (mg CE/g dw).

| | | | | | | |
|---|---|---|---|---|---|---|
| First shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 31.8$^{a}$ ± 0.5 | 28.3$^{b}$ ± 0.7 | 25.0$^{c}$ ± 0.9 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 26.0$^{b}$ ± 0.7 | 29.6$^{a}$ ± 0.8 | 29.4$^{a}$ ± 1.0 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 23.7$^{e}$ ± 1.2 | 27.1$^{d}$ ± 1.1 | 28.8$^{c}$ ± 0.8 | 30.2$^{b}$ ± 0.7 | 32.0$^{a}$ ± 0.7 |
| Second shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 31.8$^{a}$ ± 0.8 | 25.6$^{b}$ ± 1.9 | 22.7$^{c}$ ± 1.1 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 23.7$^{b}$ ± 1.2 | 27.8$^{a}$ ± 1.2 | 28.6$^{a}$ ± 1.0 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 19.9$^{d}$ ± 1.5 | 25.4$^{c}$ ± 1.4 | 28.2$^{b}$ ± 1.2 | 29.2$^{b}$ ± 1.0 | 31.6$^{a}$ ± 1.0 |
| Third shooting period | Class (n = 30) | Sample A | Sample B | Sample C | | |
| | | 27.1$^{a}$ ± 0.8 | 21.1$^{b}$ ± 0.8 | 21.9$^{b}$ ± 0.9 | | |
| | Temperature (n = 30) | 75˚C | 85˚C | 95˚C | | |
| | | 21.1$^{c}$ ± 0.9 | 23.3$^{b}$ ± 0.8 | 25.7$^{a}$ ± 0.9 | | |
| | Time (n = 18) | 3 min | 5 min | 10 min | 15 min | 20 min |
| | | 18.0$^{e}$ ± 1.1 | 21.3$^{d}$ ± 1.1 | 23.6$^{c}$ ± 0.8 | 25.8$^{b}$ ± 0.9 | 28.2$^{a}$ ± 0.7 |

Results are means ± standard error. The values within a column with different superscript letters are significantly (P < 0.01) different.

a decrease was observed in the total phenolic and flavonoid content at each shooting periods in this study. There are also examples available in the literature about black tea, green tea and buds, which are in agreement with our results (Ozdemir et al., 1993; Singh et al., 1999). However Şahin-Nadeem et al. (2007) reported that green tea obtained from the first and second shooting periods had higher phenolic contents than obtained from subsequent shooting periods.

The effect of temperature was also studied, which showed a positive effect on the extraction efficiencies of the total phenolic and flavonoid contents. In a previous study, an increase in extraction temperature increased the phenolic and flavonoid contents of green and black tea (Astill et al., 2001; Khokhar & Magnusdottir, 2002; Ziaedini et al., 2010). Also, the results showed that nearly 50% of the total phenolic and flavonoids of the samples are extracted in the first three minutes.

### *3.3 Antioxidant capacity*

Antioxidant capacity is related to the phenolic compounds of green tea. It reflects the amount of active components ($IC_{50}$) scavenging 50% of the DPPH radical. The $IC_{50}$ values of the green tea samples were found in the range of 0.48-1.16 mg dw mg$^{-1}$ DPPH, as shown in Table 4. The lowest $IC_{50}$ value, thereby the highest antioxidant capacity, was determined in the Sample A extracted at 95 °C for 20 min, from the first shooting period. The antioxidant capacity of the samples was significantly (P < 0.01) affected by the extraction temperature and time. Similar results were found

in some previous studies (Samaniego-Sanchez et al., 2011; Komes et al., 2010).

### *3.4 Catechin content*

Catechins are responsible for the health effects, bitterness and astringency of green tea. The catechin content in Sample A of the first shooting period (showing the highest total phenolic and flavonoid contents in this study) were identified by HPLC and the evaluated results are given in Table 5. The catechins are composed of catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), epigallocatechin gallate (EGCG), gallocatechin gallate (GCG), gallocatechin (GC) and catechin gallate (CG).

The amounts of individual catechins, i.e. C, EC, EGC, ECG, EGCG, GCG, GC and CG, were found in the range of 8.91- 17.09, 4.29-9.55, 28.03-59.42 mg $g^{-1}$ dw, 8.02-14.61 mg $g^{-1}$ dw, 38.05-69.66 mg $g^{-1}$ dw, 2.53-18.53 mg $g^{-1}$ dw, 2.30-12.97 mg $g^{-1}$ dw and 0.04-1.78 mg $g^{-1}$ dw, respectively (Table 5). EGCG was the major constituent of the Sample A green tea, as shown in Table 5. It is well-known that EGCG composes nearly 60% of the total catechins in tea (Khokhar & Magnusdottir, 2002; Zaveri, 2006). The EGCG content was not affected significantly ($P < 0.01$) by an extraction time beyond three minutes.

The results show that the C, EC, EGC, ECG, EGCG and GC contents decreased when the extraction temperature was increased from 75 °C to 85 °C. Ziaedini et al. (2010) reported that EGC and EC decrease when the temperature is increased from 80 °C to 90 °C, which they associated with degradation, oxidation or epimerization of these compounds. Similar results were also reported by Perva-Uzunalić et al. (2006).

**Table 4**. Duncan's multiple - range test results of $IC_{50}$ values (mg dw/mg DPPH).

| | | | | | | |
|---|---|---|---|---|---|---|
| **First shooting period** | **Class (n = 30)** | **Sample A** | **Sample B** | **Sample C** | | |
| | | $0.8^{a} \pm 0.0$ | $0.6^{b} \pm 0.0$ | $0.5^{c} \pm 0.0$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $0.7^{a} \pm 0.0$ | $0.7^{a} \pm 0.0$ | $0.5^{b} \pm 0.0$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | $0.8^{a} \pm 0.0$ | $0.7^{b} \pm 0.0$ | $0.6^{c} \pm 0.0$ | $0.6^{c} \pm 0.0$ | $0.5^{d} \pm 0.0$ |
| **Second shooting period** | **Class (n = 30)** | **Sample A** | **Sample B** | **Sample C** | | |
| | | $0.9^{a} \pm 0.1$ | $0.8^{b} \pm 0.0$ | $0.6^{c} \pm 0.0$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $0.8^{a} \pm 0.0$ | $0.8^{b} \pm 0.0$ | $0.7^{b} \pm 0.0$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | 1.1a ± 0.1 | $0.8^{b} \pm 0.0$ | $0.7^{c} \pm 0.0$ | $0.7^{c} \pm 0.0$ | $0.5^{d} \pm 0.0$ |
| **Third shooting period** | **Class (n = 30)** | **Sample A** | **Sample B** | **Sample C** | | |
| | | $1.0^{a} \pm 0.0$ | $0.8^{b} \pm 0.0$ | $0.7^{c} \pm 0.0$ | | |
| | **Temperature (n = 30)** | **75˚C** | **85˚C** | **95˚C** | | |
| | | $0.9^{a} \pm 0.0$ | $0.8^{b} \pm 0.0$ | $0.8^{b} \pm 0.0$ | | |
| | **Time (n = 18)** | **3 min** | **5 min** | **10 min** | **15 min** | **20 min** |
| | | $1.1^{a} \pm 0.1$ | $0.9^{b} \pm 0.0$ | $0.7^{c} \pm 0.0$ | $0.7^{c} \pm 0.0$ | $0.6^{d} \pm 0.0$ |

Results are means ± standard error. The values within a column with different superscript letters are significantly ($P< 0.01$) different.

**Table 5**. Individual catechins content of Sample A of the first shooting period as a function of extraction temperature and time (mg/g dw).

| | Temperature | Time (min) | GC | EGC | C | EGCG | EC | GCG | ECG |
|---|---|---|---|---|---|---|---|---|---|
| **Sample A** | **75˚C** | 3 | $3.8^{a} \pm 0.0$ | $41.9^{a} \pm 0.0$ | $12.5^{a} \pm 0.0$ | $56.5^{a} \pm 0.1$ | $8.9^{a} \pm 0.0$ | $2.5^{a} \pm 0.0$ | $10.9^{a} \pm 0.0$ |
| | | 5 | $4.2^{b} \pm 0.0$ | $42.1^{a} \pm 0.9$ | $12.9^{a,b} \pm 0.0$ | $59.4^{b} \pm 0.1$ | $9.0^{a,b} \pm 0.0$ | $3.0^{b} \pm 0.0$ | $11.7^{a,b} \pm 0.0$ |
| | | 10 | $4.7^{c} \pm 0.0$ | $42.6^{a} \pm 0.0$ | $13.9^{b} \pm 0.0$ | $59.8^{b} \pm 0.0$ | $9.3^{b,c} \pm 0.0$ | $3.6^{c} \pm 0.0$ | $11.9^{a,b} \pm 0.1$ |
| | | 15 | $5.3^{c} \pm 0.0$ | $43.2^{b} \pm 0.1$ | $14.6^{c} \pm 0.0$ | $60.1^{b} \pm 0.3$ | $9.5^{c} \pm 0.0$ | $4.2^{d} \pm 0.0$ | $12.6^{a,b} \pm 0.0$ |
| | | 20 | $5.9^{d} \pm 0.0$ | $44.1^{a,b} \pm 0.2$ | $14.8^{c} \pm 0.0$ | $61.7^{b} \pm 0.1$ | $9.5^{c} \pm 0.0$ | $4.6^{e} \pm 0.0$ | $13.0^{b} \pm 0.0$ |
| | **85˚C** | 3 | $2.3^{a} \pm 0.0$ | $28.0^{a} \pm 0.3$ | $8.9^{a} \pm 0.0$ | $38.0^{a} \pm 0.3$ | $4.3^{a} \pm 0.1$ | $2.4^{a} \pm 0.0$ | $8.0^{a} \pm 0.1$ |
| | | 5 | $2.6^{a} \pm 0.0$ | $28.7^{a} \pm 0.0$ | $9.8^{a,b} \pm 0.0$ | $41.2^{a,b} \pm 0.2$ | $4.8^{a,b} \pm 0.1$ | $2.8^{a} \pm 0.0$ | $8.6^{a} \pm 0.0$ |
| | | 10 | $3.6^{b} \pm 0.0$ | $28.9^{a} \pm 0.1$ | $10.0^{a,b} \pm 0.0$ | $41.8^{a,b} \pm 0.2$ | $5.9^{a,b,c} \pm 0.0$ | $4.1^{b} \pm 0.0$ | $9.3^{a} \pm 0.0$ |
| | | 15 | $5.1^{b} \pm 0.0$ | $29.0^{a} \pm 0.1$ | $10.7^{b} \pm 0.0$ | $41.9^{a,b} \pm 0.0$ | $6.7^{b,c} \pm 0.0$ | $5.2^{c} \pm 0.0$ | $9.3^{a} \pm 0.0$ |
| | | 20 | $5.4^{c} \pm 0.0$ | $30.3^{a} \pm 0.1$ | $10.9^{b} \pm 0.0$ | $43.3^{b} \pm 0.2$ | $6.8^{c} \pm 0.0$ | $5.4^{c} \pm 0.0$ | $9.4^{a} \pm 0.0$ |
| | **95˚C** | 3 | $3.8^{a} \pm 0.0$ | $49.6^{a} \pm 0.9$ | $12.7^{a} \pm 0.3$ | $51.4^{a} \pm 1.2$ | $6.8^{a} \pm 0.1$ | $4.0^{a} \pm 0.1$ | $9.5^{a} \pm 0.2$ |
| | | 5 | $4.7^{b} \pm 0.0$ | $49.9^{a} \pm 0.0$ | $13.4^{a} \pm 0.2$ | $66.2^{b} \pm 0.1$ | $7.4^{a,b} \pm 0.1$ | $6.7^{b} \pm 0.0$ | $13.4^{b} \pm 0.0$ |
| | | 10 | $7.8^{c} \pm 0.0$ | $50.0^{a} \pm 0.1$ | $16.1^{a} \pm 0.0$ | $67.2^{b} \pm 0.0$ | $7.6^{b} \pm 0.0$ | $11.7^{c} \pm 0.0$ | $14.1^{b} \pm 0.0$ |
| | | 15 | $12.2^{d} \pm 0.0$ | $53.9^{a} \pm 0.1$ | $16.4^{a} \pm 0.0$ | $69.0^{b} \pm 0.0$ | $8.0^{b} \pm 0.0$ | $16.5^{d} \pm 0.1$ | $14.1^{b} \pm 0.0$ |
| | | 20 | $13.0^{d} \pm 0.0$ | $59.4^{a} \pm 0.1$ | $17.1^{a} \pm 0.1$ | $69.7^{b} \pm 0.1$ | $8.1^{b} \pm 0.0$ | $18.5^{e} \pm 0.0$ | $14.6^{b} \pm 0.0$ |

Results are means ± standard error. The values within a column with different superscript letters are significantly ($P < 0.01$) different.

## 4 Conclusion

The bioactive compounds of green tea were significantly affected by both extraction temperature and time. It can be concluded that increasing the extraction temperature and time increases the amount of total phenolic and flavonoid contents as well as the antioxidant capacity of tea extracts. The highest total phenolic and flavonoid contents and antioxidant capacity values were obtained from the first shooting period in Sample A which were extracted at 95 ºC for 20 min. The highest amounts of total phenolic and flavonoid contents and antioxidant capacity were observed in the samples obtained from the first shooting period rather than the second and the third shooting periods. The most interesting finding of this study was the decline in catechins when the temperature was increased from 75 °C to 85 °C. These results also show that 5 minutes are enough to brew Turkish green tea in order to extract almost 80% of the total phenolics.

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