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ORIGINAL RESEARCH PAPER

COMPARATIVE STUDY ON PHENOLIC COMPOUNDS OF GREEN TEA (*CAMELLIA SINENSIS L.)* PREPARED BY DIFFERENT BREWING METHODS

CANAN GÖKSU SÜRÜCÜ*[1], AYSU TOLUN[2], NEVZAT ARTIK[2]

[1]*Plant-Based Food Research Center, Field Crops Central Research Institute, Sehit Cem Ersever Street No:11, 06170, Yenimahalle,Ankara, Türkiye*

[2]*Department of Food Engineering, Ankara University, Bahçelievler, 319. Street., 06830 Gölbaşı, Ankara, Türkiye*

*Corresponding author: *canan.goksusurucu@tarimorman.gov.tr*

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Abstract

Tea consumers' choices are primarily influenced by taste, aroma, price, and brand while making tea selections; however, they are less likely to consider the phytochemical content and nutritional benefits of tea. Although studies on the phenolic content of green tea under various extraction conditions have been published, to the best of our knowledge, there are no studies on the impact of brewing duration and on the amount of phenolic compounds in the infusion. In this study, the effect of the amount of Turkish green tea (GT, 5-10 g) and brewing time (BT, 5-60 min) on phenolic compounds was evaluated using high-performance liquid chromatography (HPLC). According to the HPLC analysis of green tea infusions, 10 different phenolic compounds were determined: gallic acid (GA, 27.41-78.93 mg/L), gallocatechin (GC, 65.40-307.41 mg/L), catechin (C, 3.81-36.82 mg/L), epicatechin (EC, 177.29-366.71 mg/L), epigallocatechin gallate (EGCG, 645.85-1344.34 mg/L), epicatechin gallate (ECG, 16.60-44.81 mg/L), catechin gallate (CG, 0.245-0.564 mg/L), kaempferol (2.40-10.98 mg/L), quercetin-3-rhamnosylglucoside (Q3RG, 4.89-5.54 mg/L) and an alkaloid: caffeine (CAF, 390.63-785.79 mg/L). In addition, the total catechins and polyphenols were found to be in the range of 1325.00-2798.09 mg/L and 1357.31-2881.08 mg/L, respectively. It was observed that as the amount of green tea brewed increased, the phenolic compounds in the infusion increased in general, but this increase was not linear. There were variations regarding the caffeine and phenolic concentrations, which may be attributed to the brewing time. Therefore, it can be concluded that these compounds are exposed to degradation and/or epimerization as the brewing time increases.

Keywords: *Camellia sinensis* L., green tea, brewing time, phenolic compounds, caffeine, HPLC

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Introduction

Green tea (GT) is the most popular drink in Asia and it is gaining popularity worldwide due to its many health benefits and unique taste (Maurya *et al.*, 2020). Tea has antioxidant effects and contains small amounts of vitamins, minerals, lipids, proteins, carbohydrates and amino acids. Wide ranges of phytochemical components are also present, but polyphenols are primarily responsible for the flavor of the tea and beneficial health effects. According to numerous published pieces of research, the protective qualities of green tea against several diseases are due to its polyphenol content (Khan *et al.*, 2008; Maurya *et al.*, 2020; Wang *et al.*, 2021).

Flavor, aroma, brand and price have a major effect on the consumer when choosing tea; however, they are less likely to take into account the phytochemical composition and health advantages of tea. The active constituents present in green tea are powerful antioxidants called polyphenols. It is reported that green tea contains nearly 4000 bioactive compounds, one-third of which consists of polyphenols (Maurya *et al.*, 2020).

There is a disagreement over the best or ideal conditions for extracting as many phenolic components from tea infusions as possible (Rahim *et al.*, 2014). The availability of tea catechins is related to various factors such as cultivar, harvest time, brewing conditions, infusion time/temperature, particle size after grinding, number of extractions, storage time, and light exposure (Perva-Uzunalić *et al.*, 2006; Kyle *et al.*, 2007; Almajano *et al.*, 2008; Rusak *et al.*, 2008; Wakamatsu *et al.*, 2019). However, no studies have been reported on the impact of changing the amount of green tea used and the brewing time on the phenolic compounds in the infusion. Even though studies on the phenolic profile of green tea under different extraction conditions have been published, studies on the impact of brewing duration and amount on the phenolic compounds in the infusion have yet to be published to the best of our knowledge. Therefore, this study was primarily conducted to determine and compare the effect of infusion time and green tea amount on the phenolic compounds of green tea infusions.

Materials and methods

Raw material

Fresh GT leaves were collected from Türkiye's Eastern Black Sea Region in 2020 and processed into GT at the General Directorate of Tea Enterprises (CAYKUR) Green Tea Factory.

The catechin standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeine, gallic acid, orthophosphoric acid, HPLC- or analytical- grade acetonitrile and methanol were purchased from Fluka-Riedel-de Haën (BioChemica Fluka Chemie GmbH, Buchs, Switzerland). Quercetin-3-rhamnosylglucoside (Q3RG) was purchased from Wako (Pure Chem. Co., Osaka, Japan).

Extraction of phenolic compounds

To analyze the phenolic compounds, samples were prepared by brewing various quantities of fine particles (215±75 µm) of phenolics-rich GTs i.e.: 5 g (GT1), 7.5 g

(GT2), and 10 g (GT3) for a total of twelve times with 5-min increments (i.e., 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 min). The Turkish-style brewing technique utilized by Göksu Sürücü and Artık (2022) was applied. This process was carried out in a porcelain teapot with 250 mL of distilled and boiling water and then kept at boiling temperature. After this step, the tea infusions were quickly filtered through a Whatman no.1 filter paper under vacuum. The prepared samples were wrapped in an aluminum foil and stored at -18 °C until further analysis.

HPLC analysis of phenolic compounds

The analysis of phenolic compounds was carried out by HPLC system (Shimadzu, Kyoto, Japan) equipped with an autosampler, DAD detector, pumps (LC-20 AD), and system controller (SLC-10 A VP) by modifying the method proposed by Turkmen and Velioglu (2007). The stainless steel column was Phenomenex Luna C-18 (250x4.6 mm, 5 µm ID). Spectral data from the DAD system were recorded between 350 and 700 nm and peak areas were measured at 270 nm. The column temperature of 40 °C was maintained with a Shimadzu column oven (CTO-10ASVp, Shimadzu, Kyoto, Japan). The gradient HPLC elution was made of solvent A (acetonitrile) and solvent B (0.1% orthophosphoric acid in water (w/v)). The gradient elution profile was 7% A (isocratic) for 10 min. Then, mobile phase A was gradually increased to 17% at 57 min, to 22% at 80 min, and to 7% until 90 min. The flow rate was 1 mL/min. An aliquot of 20 µL was taken and injected into the HPLC column automatically. Green tea samples were filtered through a 0.45µm PTFE membrane filter (Chromafıl Xtra PTFE, 25 mm, 0.45 µm, Germany) before being injected into the HPLC unit. Quantification was performed from the peak area of each compound and its corresponding calibration curve. Stock standard solutions were prepared by dissolving the mixture in 80% methanol, at concentrations ranging between 0.25-250 mg/L and evaluated according to the linear calibration curves obtained. Correlation coefficient values were found to be in the range of 0.9956-1.000.

Statistical analysis

The experiments were conducted in duplicates and the data were subjected to analysis of variance (ANOVA). Tukey test was performed to analyze the correlation test and the comparison of means at a significant level of 1% ($p \leq 0.01$).

Results and discussion

Effect of brewing conditions on phenolic content and caffeine profile of GT infusions

In all samples, EGCG, ECG, C, EC, GC, and CG from the flavan-3-ol group and Q3RG and kaempferol from the flavonol glycoside group were detected. Caffeine, which is an alkaloid, and gallic acid, which is a phenolic acid, are other compounds detected in all samples in the study. Total catechin and total polyphenol values were also calculated. The distribution of the detected compounds, total catechins, and total polyphenols are shown in Table 1 according to the amount of green tea by taking into account the average values in the infusions. It was determined that the compounds detected in the study were statistically different ($p \leq 0.01$) depending on the amount of green tea and brewing time.

Table 1. The determined min and max levels of phenolic compounds and caffeine present in green tea infusions obtained during different brewing amounts and times.

Compounds	Minimum Concentration (mg/L)	Sample-Time (min)	Maximum Concentration (mg/L)	Sample-Time (min)
GA	27.41±0.22^{aA}	GT1-5	78.93±0.34^{bB}	GT3-55
GC	65.40±1.58^{aA}	GT1-5	307.35±12.94^{bB}	GT3-55
C	3.82±0.11^{aA}	GT1-40	36.82±0.53^{bB}	GT3-10
EC	177.30±6.19^{aA}	GT1-35	366.72±7.036^{bB}	GT3-55
EGCG	645.86±2.17^{aA}	GT1-5	1344.30±2.97^{bB}	GT3-35
ECG	16.60±0.52^{aA}	GT1-60	45.16±1.39^{bB}	GT3-15
CG	0.25±0.01^{aA}	GT1-60	0.57±0.02^{bB}	GT3-5
Kaempferol	2.41±0.09^{aA}	GT1-5	10.95±0.64^{bB}	GT3-30
Q3RG	4.89±0.99^{aA}	GT1-15	5.54±0.01^{bB}	GT3-35
CAF	390.63±3.00^{aA}	GT1-5	785.79±18.70^{bB}	GT3-55
Total catechins*	1325.00±11.83^{aA}	GT1-5	2798.10±28.11^{bB}	GT3-45
Total polyphenols**	1357.30±8.42^{aA}	GT1-5	2881.10±24.35f^{bB}	GT3-45

GA: gallic acid; GC: gallocatechin EC: (−)-epicatechin; EGCG: (−)-epigallocatechin gallate; ECG: (−)-epicatechin gallate CG: (−)-catechin gallate; Q3RG: quercetin-3-rhamnosylglucoside, CAF: caffein.* It was obtained by summing up the concentrations of catechins (GC, C, EC, EGCG, ECG, CG) in the infusions,** It was obtained by summing up the concentrations of catechins (GC, C, EC, EGCG, ECG, CG) with the concentrations of Q3RG, kaempferol, and gallic acid in the infusions. Different capital letters represent different mean values (±SD) ($p \leq 0.01$) for the green tea samples of a certain amount, while different lowercase letters represent different mean values (±SD) ($p \leq 0.01$) for the same samples at different times. GT1 and GT3 indicates 5 g and 10 g sample, respectively.

EGCG, EC and ECG are epistructured catechins and the changes in the infusion according to the brewing time and quantity are shown in Figure 1. EGCG was the major catechin infused in water among all catechins. The lowest and highest values were found to be 645.85 mg/L (GT1 for 5 min) and 1344.34 mg/L (GT3 for 35 min), respectively (Table 1). The amount of GT was very important to extract the maximum amount of EGCG into the infusion. As the amount of brewed green tea increased, the amount of EGCG in the infusion increased, but this increase did not occur at the same rate. The EGCG concentration was maximum during the brewing period of 35-50 minutes, and then it decreased. Changes in EGCG concentration in infusion were mainly due to the degradation of EGCG and/or epimerization to GCG.

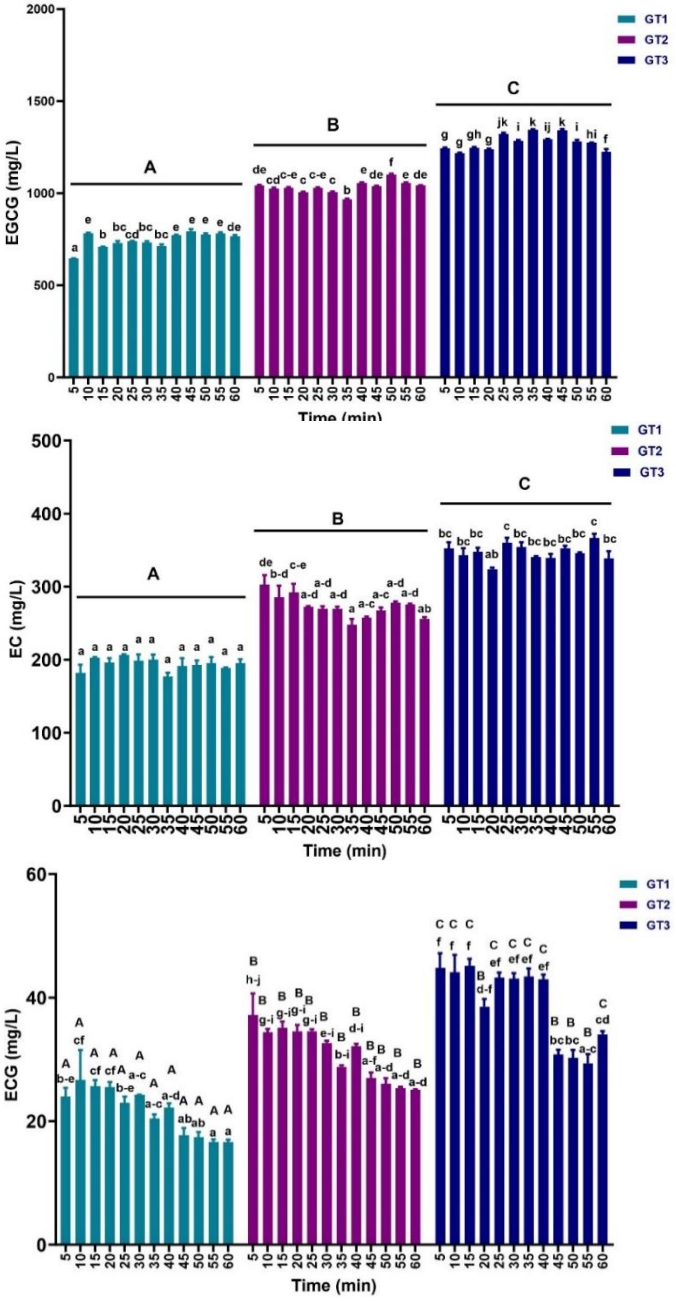
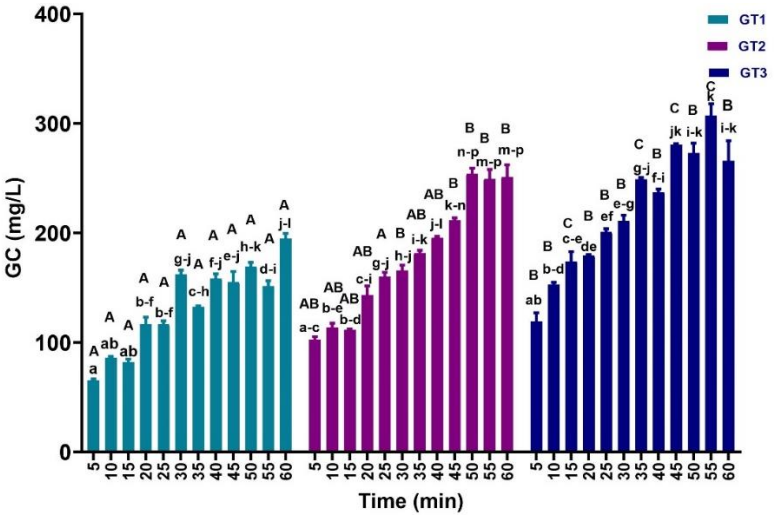


Figure 1. Effect of brewing time and green tea amount on the contents of epistructured catechins: EGCG: (−)-epigallocatechin gallate; ECG: (−)-epicatechin gallate EC: (−)-epicatechin. Different capital letters represent different mean values (±SD) ($p \leq 0.01$) for the green tea samples of a certain amount, while different lowercase letters represent different mean values (±SD) ($p \leq 0.01$) for the same samples at different times. GT1, GT2 and GT3 indicate 5 g, 7.5 g and 10 g sample, respectively. The "-" sign indicates the other lowercase letters in the letter range (e.g.: a-d for abcd).

EC, the second major catechin in green tea infusions, was determined at a minimum concentration of 177.29 mg/L (GT1 for 35 min) and a maximum concentration of 366.71 mg/L (GT3 for 55 min) (Table 1). As expected, the EC concentration increased as the amount of brewed green tea increased. The EC concentration reached its maximum level in 20 min for GT1, 5 min for GT2, and 55 min for GT3, and then decreased. Factors such as epimerization and/or degradation of EC to C could be effective in the transition of EC to infusion due to the large differences in the time needed to reach maximum concentration in green tea infusions and variations in concentration during the brewing period.

ECG was a stable catechin detected at a minimum concentration of 16.60 mg/L (GT1 for 60 min) and a maximum concentration of 45.15 mg/L (GT3 for 15 min) (Table 1). When the amount of green tea brewed increased, ECG concentration increased, and the amount of ECG was stable until the 40 minutes brewing. ECG concentration decreased as the brewing time increased. When the brewing time was 5-10 minutes, ECG was at the maximum concentration, and at 55-60 minutes, it was at the minimum concentration. This situation could be explained by the deterioration of ECG and/or epimerization to GC as time increases.

GC, C and CG are non-epistructured catechins and the changes in the infusion according to the brewing time and quantity are shown in Figure 2. GC was determined at a minimum concentration of 65.40 mg/L (GT1 for 5 min) and a maximum concentration of 307.41 mg/L (GT3 for 35 min) (Table 1). In general, as the brewing time and amount of green tea increased for GC, the concentration of the infusion increased and reached the maximum level at the brewing time of 50-60 minutes. Although some GC deteriorates during the brewing period, GC remains intact in the infusion and/or the amount of GC remains stable in the infusion as a result of the epimerization of the EGC to the GC. However, it is seen that the increase in the amount of green tea brewed and the amount of GC in the infusion are not at the same rate.



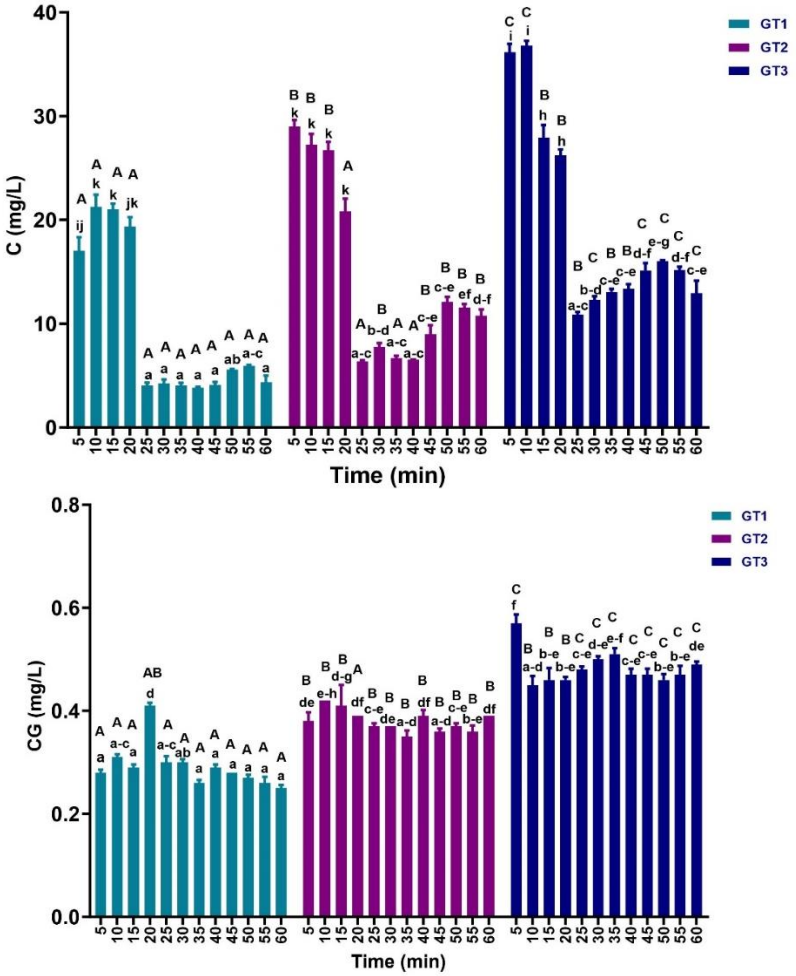


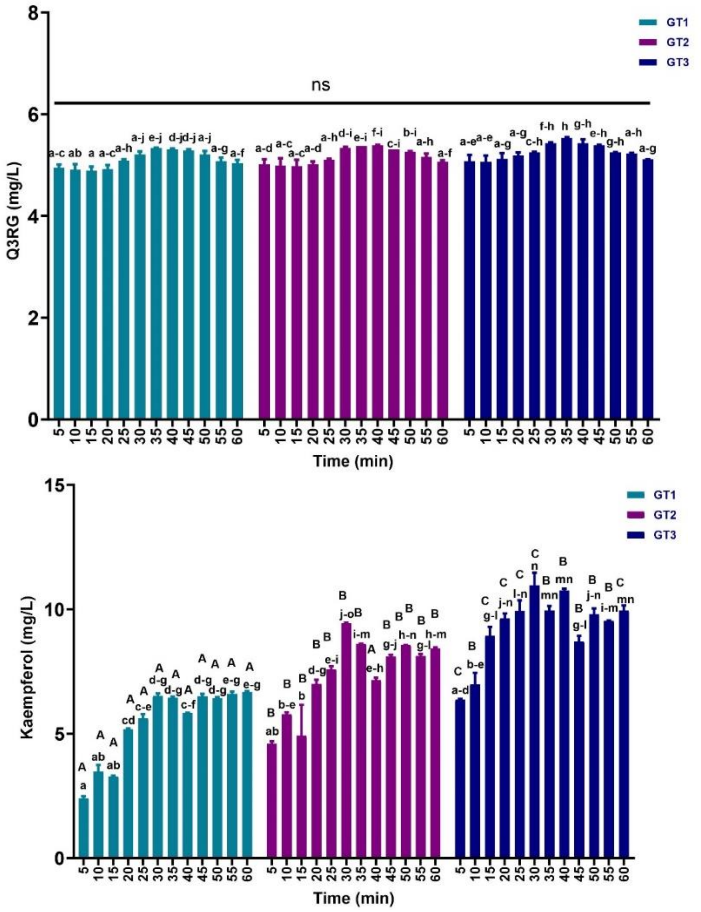
Figure 2. Effect of brewing time and GT amount on the contents of non-epistructured catechins. GC: (−)-gallocatechin; C: (−)-catechin; CG: (−)-catechin gallate. Different capital letters represent different mean values (±SD) ($p \leq 0.01$) for the green tea samples of a certain amount, while different lowercase letters represent different mean values (±SD) ($p \leq 0.01$) for the same samples at different times. GT1, GT2 and GT3 indicate 5 g, 7.5 g and 10 g, respectively. The "-" sign indicates the other lowercase letters in the letter range, (e.g.: a-d for abcd).

C, a minor catechin, was determined at a minimum concentration of 3.81 mg/L (GT1 for 40 min) and at a maximum concentration of 36.82 mg/L (GT3 - for 10 min) (Table 1). As the amount of brewed green tea increases, the C concentration in the infusion increases, but this increase did not occur at the same rate. The maximum concentration is reached within 5-10 minutes of brewing for GT1, GT2, and GT3. When the brewing time reaches 25 min, there is a great decrease in the amount of C, and some parts of C has deteriorated. The increases and decreases after the 25th min

may be due to the epimerization of EC and its conversion to C and/or the degradation of C.

CG was detected at a minimum concentration of 0.245 mg/L (GT1 for 60 min) and at a maximum concentration of 0.564 mg/L (GT3 for 5 min) (Table 1). As the amount of brewed green tea increases, CG concentration in the infusion increases, but these were not at the same rate. The CG concentration reaches its maximum level within 5-20 minutes of infusion. It reaches the minimum concentration in 60 minutes for GT1, 35 minutes for GT2, and 10 minutes for GT3. Considering that increases and decreases continue after these periods, especially for GT2 and GT3 as the brewing time increases, the amount in the infusion changes depending on the epimerization of ECG to CG and/or the deterioration of CG.

Q3RG, a flavonoid glycoside, was quite stable and was detected at a minimum concentration of 4.89 mg/L (GT1 for 15 minutes) and a maximum concentration of 5.54 mg/L (GT3 for 35 minutes). There was no statistical difference found between GT1, GT2 and GT3 in terms of quantity. Although the Q3RG concentrations in GT infusions were close to each other in terms of time, there was statistical differences (Figure 3).



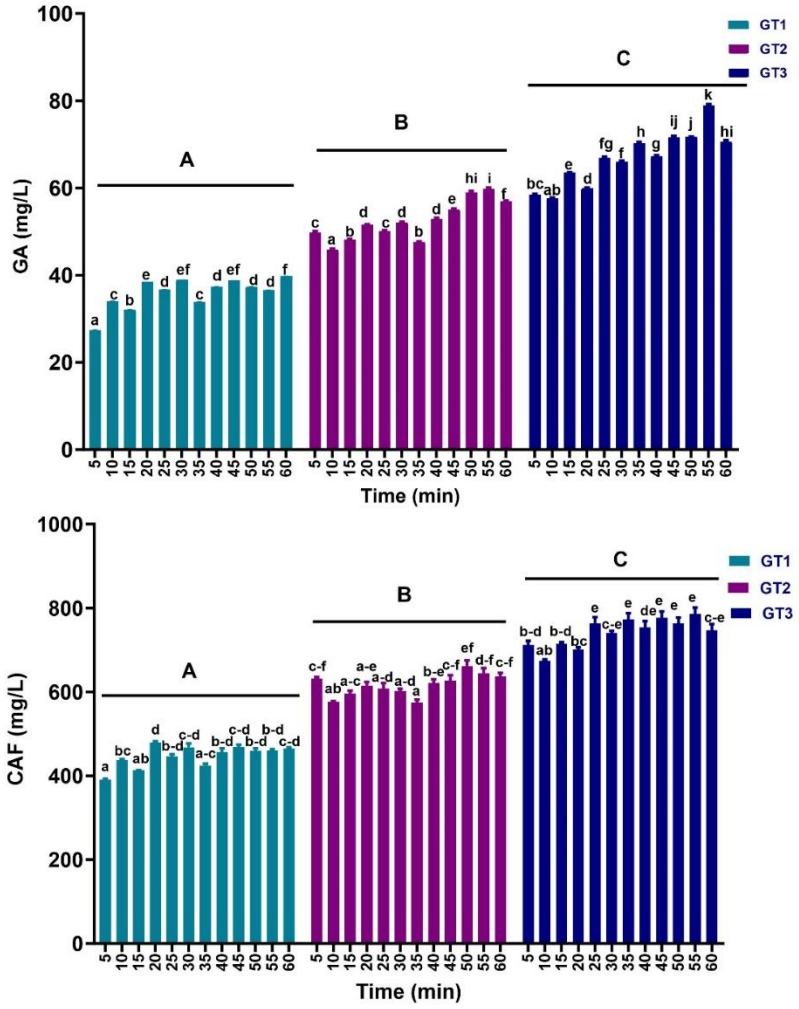


Figure 3. Effect of brewing time and green tea amount on the contents of Q3RG, kaempferol, GA and CAF. Q3RG: quercetin-3-rhamnosylglucoside GA: gallic acid; CAF: caffeine. Different capital letters represent different mean values (±SD) ($p \leq 0.01$) for the green tea samples of a certain amount, while different lowercase letters represent different mean values (±SD) ($p \leq 0.01$) for the same samples at different times. GT1, GT2 and GT3 indicate 5 g, 7.5 g and 10 g samples, respectively. The "-" sign indicates the other lowercase letters in the letter range (e.g.: a-d for abcd); ns: not significant.

The kaempferol compound was detected at a minimum concentration of 2.40 mg/L (GT1 for 5 min) and at a maximum concentration of 10.98 mg/L (GT3 for 30 min) (Table 1). As the amount of green tea brewed increased, the amount of kaempferol in the brew also increased. When the brewing time was up to 30 minutes, the kaempferol concentration in the infusion increased. After this period, kaempferol concentration varied (Figure 3). The increases and decreases after the 30th min may be due to the degradation of kaempferol.

GA, which is a phenolic acid, was found to be at a min concentration of 27.41 mg/L (GT1 for 5 min) and at a max concentration of 78.93 mg/L (GT3 for 55 min) (Table 1). The concentrations of GA in the infusion increased depending on the brewing amount. As the brewing time increased, the concentration of GA in the infusion showed variation (Figure 3).

The amount of CAF, an alkaloid compound, in the GT infusions was also studied, and the results are given in Figure 3. CAF was detected at a minimum concentration of 390.63 mg/L (GT1 for 5 min) and a maximum concentration 785.79 mg/L (GT3 for 55 min) (Table 1). The concentration of CAF in the infusion increased depending on the GT amount but it showed variations in the brewing time. The variations observed in CAF, GA, and kaempferol depending on the brewing time may be due to the degradation of these compounds in infusion.

The optimal brewing conditions for Turkish green tea were determined concerning extracting the highest amount of catechins was studied by Saklar *et al.* (2015) and green tea infusions were prepared at 75, 85, and 95 °C with brewing times of 1, 2, 3, 5, 10, 20, 30 and 45 min. The amounts of epistructured catechins (EGCG, 344.1-506.9 mg/L; EGC, 569.1-285.3 mg/L; ECG, 35.9-36.8 mg/L; EC, 39.6-63.1 mg/L), non-epistructured catechins (C, 23.1 mg/L; GC, 77.3 mg/L; GCG 36.3 mg/L, CG, 0 mg/L) caffeine (102.4-113.6 mg/L) in brewed tea samples were analyzed. Taking into account the quantity of the sample and the volume of infusion, EGCG, EC, ECG, and C concentrations are compatible with our findings. Unlike this study, a low concentration of CG was found in our study and the concentration of caffeine was found to be higher than in this study.

In the study conducted by Xu *et al.* (2018) by applying two different extraction methods to green tea, it was determined that it contained 10.35-38.50 mg/mL and 97.01-98.09 mg/mL polyphenol in extraction for 10 minutes and 40 minutes, respectively. In the same study, caffeine was 2.32-5.77 mg/mL, and EGCG 3.22-6.97 mg/mL through extraction for 0-40 minutes. The amounts of polyphenols, caffeine, and EGCG is higher than the values in our study.

Perva-Uzunalic *et al.* (2006) reported that the number of major catechins was determined as 191 g/kg ka, caffeine 36 g/kg ka; EGCG 129 g/kg ka; ECG 15.2 g/kg ka; EGC 46.0 g/kg ka; EC 0.9 g/kg ka. As flavonol, Q3RG was found to be 1.8 g/kg ka and kaempferol was found to be 2.6 g/kg ka. These values are higher than the values found in our study.

Effect of brewing conditions on the content of total catechin and total polyphenol profile of GT infusions

Total catechins and total polyphenols were also determined in this study, as shown in Figure 4. Total catechin amount was obtained by summing up the amounts of catechins (GC, C, EC, EGCG, ECG, and CG).

The changes observed in GA, CAF and Kaempferol depending on the brewing time may be due to the degradation of these compounds in infusion over time in the infusions. The total amount of catechins was determined at a minimum and minimum concentration of 1325.00 mg/L (GT1 for 5 min) and 2798.09 mg/L (GT3 for 45 min),

respectively (Table 1). The total amount of polyphenols was obtained by summing up the amounts of catechins (GC, C, EC, EGCG, ECG, and CG) in the infusions with the amounts of Q3RG, kaempferol, and GA. The total amount of polyphenols was determined at a minimum and maximum concentration of 1357.31mg/L (GT1 for 5 min) and 2881.08 mg/L (GT3 for 45 min), respectively (Table 1). In general, the total content of catechins and polyphenols fluctuate depending on the concentration of catechin in the infusion. However, the total catechin concentration was found to be constant after 35, 45, and 55 minutes of brewing ($p \leq 0.01$) (Figure 4).

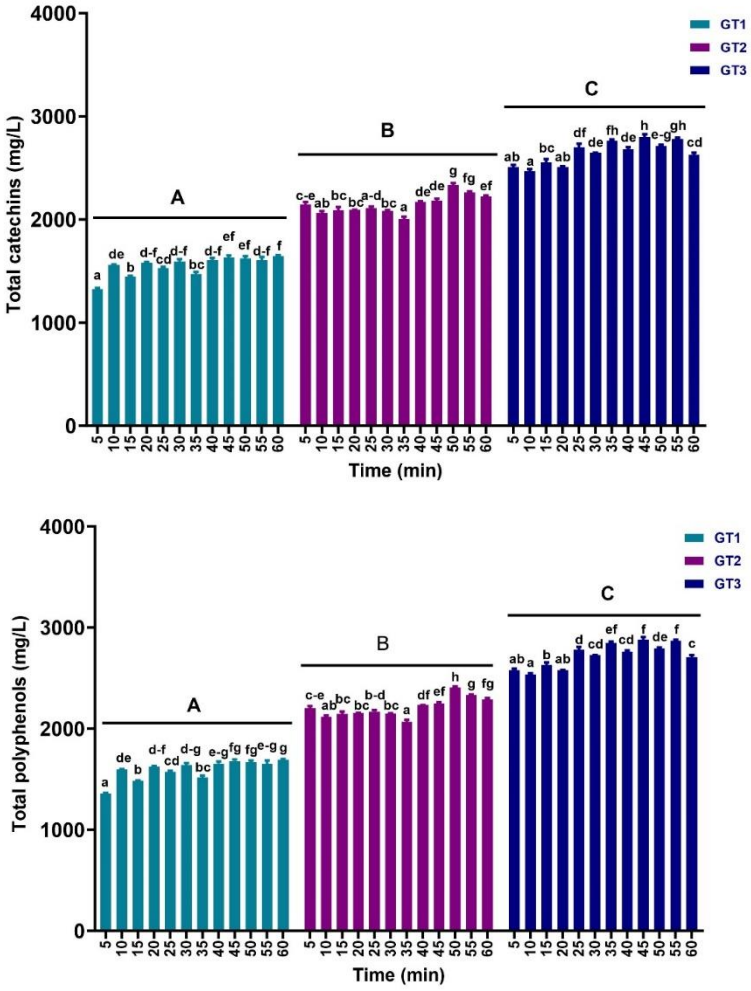


Figure 4. Effect of brewing time and green tea amount on the contents of total catechins and total polyphenols. Different capital letters represent different mean values (±SD) ($p \leq 0.01$) for the green tea samples of a certain amount, while different lowercase letters represent different mean values (±SD) ($p \leq 0.01$) for the same samples at different times. GT1, GT2 and GT3 indicate 5 g, 7.5 g and 10 g sample, respectively. The "-" sign indicates the other lowercase letters in the letter range (e.g.: a-d for abcd).

Saklar *et al.* (2015) reported total catechin concentrations were in the range of 745.3-977.1 mg/L and these results are lower than those found in our study. Our results are consistent with the values of total phenolic content (TPC) in the dried green tea leaves that were reported previously, as the TPC content varied from 11.40 to 19.58 g/100 g (Donlao and Ogawa, 2019). Xu *et al*. (2018) reported they found polyphenol concentrations in the range of 13.27-22.53 mg/mL. The amount of polyphenol is similar with our study. Chen et al (2021) reported the levels of total catechins gathered in the range of 110− 135 mg/g. C. Zhang vd. (2018) reported catechins content was found to range from 143.6 to 282.6 mg/g. Perva-Uzunalic *et al.* (2006) reported that the total polyphenol was found to be 196.2 g/kg. These values are higher than the values found in our study. Another study by Cao *et al*. (2019) reported that the polyphenol concentrations could be found in the range of 3.01-3.08 mg/mL, which is lower than the findings in our study. Extraction of catechins is related to many parameters such as infusion time/temperature, water/tea ratio, particle size after grinding, number of extractions, storage time, light exposure, etc. Given the different brewing times, tea/water ratios and GT used, the differences observed in these studies can be attributed to sample and preparation processes of GT infusions.

When all samples were evaluated in terms of quantity in general, as the amount of brewed green tea increased, the amount of compound infused increased as expected (Figure 4). However, the increase of compounds was not at the same rate as the amount of green tea brewed.

When all samples were evaluated in terms of time, as brewing time increased there was no linear increase in the concentration of phenolic compound and caffeine in the infusion (Figure 5). It was observed that the concentrations of these compounds showed variations during the brewing period. The maximum concentrations were reached when brewing for 55 minutes for EC, GC, gallic acid, and caffeine, 30-35 minutes for ECGC, Q3RG, and kaempferol, and 5-15 minutes for ECG, C, and CG. It can be concluded that the increase in concentrations in the infusion was observed for a specific time period of time, but these compounds degraded and/or epimerized after that time period.

In green tea infusion, there is oxidation and epimerization of catechins during heat treatment (Aoshima and Ayabe 2007, Kim *et al.* 2007). As the infusion time gets longer, tea catechins undergo chemical changes, and catechins with epimerized structures can turn into non-epimerized catechins (Saklar *et al.* 2015). Epimerization occurs at high temperatures. Wang *et al.* (2008) reported that while the concentration of catechin isomers increases at high temperatures, the concentration of catechins decreases. The decrease in the total amount of catechins has been shown as evidence of catechin degradation and it has been reported that tea catechins turn into their corresponding epimers in traditional brewing. EGCG, EGC, ECG, and EC are cis-structured and can be converted to their epimers, which are not epi-structured, namely GCG, GC, CG, and C, respectively. This epimerization between pairs of catechins can be reversed. The chemical structures of epi-structured catechins and non epi-structured catechins differ only between 2R, 3R (2,3-cis, epiform) and 2S,

3R (2,3-trans, non-epiform). Hsieh *et al.* (2018) reported that the concentration of EGCG in the solutions gradually rises from 20°C (6.14 g/mL) to 60°C (57.36 g/mL). The concentration decreases slightly at 80°C (36.13 g/mL) and then increases again at 100°C (44.85 g/mL). Temperature affected EGCG epimerization or degradation, and thus, its concentration in green tea fluctuated. Accordingly, the epi-structured or non-epi-structured catechins in the green tea infusions in our study transformed into each other depending on the temperature and time, and this transformation reaction was continuous during the brewing period. This explains the variations in the infusion depending on the brewing time and GT amount to reach the maximum and minimum concentration.

Conclusions

In terms of quantity, when the amount of green tea brewed increased, phenolic compounds and caffeine generally increased in infusions, as expected. However, this increase was not linear. There were differences in caffeine and phenolic concentrations that could not be attributed to brewing time. When the brewing time was the main analyzed factor, it was shown that as the brewing time increased, there was no linear increase in the concentration of phenolic compounds and caffeine in the infusion. They showed variations during the brewing time. The maximum concentrations were reached when brewing for 55 minutes for EC, GC, gallic acid, and caffeine, 30-35 minutes for ECGC, Q3RG, and kaempferol, and 5-15 minutes for ECG, C, and CG. This indicates that there was an increase in the concentration of phenolic compounds until a specific brewing time. Then these compounds deteriorated and/or epimerized as the brewing time increased.

Green tea is brewed according to the preferences of the consumer around the world. In our study, a high rate of phenolic compounds was liberated after 5-15 minutes of brewing. Consumers will be able to achieve maximum benefit in terms of phenolic compounds even when they consume green tea brewed for 5 minutes. Based on the total phenolic compounds data, the highest values were found in the infusion of green tea brewed for 35-45 minutes, but putting together the most recent scientific literature and the own experience of authors, green tea infused for such a long time was not sensorially acceptable. However, due to its high phenolic content, it is possible to use it externally for skin, hair, or other purposes other than consumption as food. In the study, the concentration of phenolic compounds in green tea infusions did not increase at the same rate as the amount of green tea brewed. It was observed that it decreased in some samples, but generally showed variations throughout the brewing period. In this regard, it could be concluded that consumers do not need to brew green tea more in order to get more benefits from it, and it may be more beneficial for them to increase their drinking frequency instead of increasing the amount.

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