

## Determination of the total phenolic content of commercially available green teas using an in vitro gastrointestinal system: a preliminary study

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### Abstract

This preliminary study evaluated changes in total phenolic content and catechin and epicatechin levels in commercially available green teas through an in vitro digestion. Samples (1.5% dry tea in 100 mL water at 80°C) were analyzed for total soluble solids, pH, and color. The initial total phenolic content ranged from 381.19–1779.50 µg gallic acid equivalent/mL, decreasing to 315.22–1266.37 µg/mL in the small intestine. Epicatechin decreased from 7.64–13.97 µg/mL to 4.53–9.19 µg/mL, while catechin dropped from 13.13–24.03 µg/mL to 5.43–14.03 µg/mL. Significant decreases occurred across digestion, indicating reduced bioaccessibility of green tea polyphenols that may influence antioxidant and health effects.

**Keywords:** catechin; HPLC; green tea; *in vitro* assays; phenolic compounds

### Introduction

Green tea, which is made from the upper leaves of *Camellia sinensis* (L.) Kuntze, is the second most widely consumed beverage in the world (after water). Throughout history, green tea has represented a source of pleasure and healing (Eruygur *et al.*, 2018). Consumed as an infusion, the beverage is popular and believed to be beneficial for general health, even at high doses (8–16 cups per day) (Lorenzo & Munekata, 2016). Its health benefits have attracted research interest, and >2000 active ingredients have been identified. Rich in bioactive substances such as phenolic compounds, catechins, and flavonoids, green tea is now at the forefront of health trends in Asia and the wider world (Singh *et al.*, 2022).

One of the most striking features of green tea is its high antioxidant capacity, which contributes to delaying aging by neutralizing free radicals, protecting against cancer, and preventing metabolic diseases. One particular compound—epigallocatechin-3-gallate (EGCG)—is considered to play a central role in the anticarcinogenic effects of green tea, and research in this area is expanding rapidly (Cavlak & Yağmur, 2016). Different types of tea are produced by various processes after the harvesting of *C. sinensis*, with green, black, and oolong tea being the most used worldwide (Zhang *et al.*, 2019). With its unfermented structure, green tea prevents catechin oxidation through its high antioxidant capacity; therefore, its consumption has become seen as a health investment and lifestyle choice in recent years (Akbulut *et al.*, 2020).

Green tea is also a functional food, as the polyphenols, flavonoids, and catechins offer a wide range of benefits, including supporting cardiovascular health, strengthening the immune system, and preventing chronic diseases such as diabetes. The method of consumption varies by culture, but the positive effects of green tea on human health are universally acknowledged (Taş *et al.*, 2005).

The recent increase in knowledge and desire for a healthy life, along with increasing interest in nutrition, has led to the widespread use of green tea, and its popularity has increased. Green tea is rich in polyphenolic compounds such as catechins, flavanols, and flavones, which have positive effects on health and exhibit antioxidant activity. The present study evaluated the change in phenolic content of green tea sold in current market tea bags throughout the gastrointestinal system *in vitro* using high-performance liquid chromatography (HPLC).

## Materials and Methods

### Materials

The green tea samples used in the study were obtained from a chain market in Afyonkarahisar city, Türkiye. The expiration dates of five different green teas that were sold in bags were considered. These products were coded as A, B, C, D, and E.

Samples were prepared in accordance with the preparation protocols indicated on the products, using 1.5% dry tea in 100 mL of water at 80 °C and infused for 3 minutes. Total soluble solids and color were analyzed after infusion, and the content of phenolic substances was determined at various points along the *in vitro* gastrointestinal tract.

### Methods

#### *Creation of an in vitro gastrointestinal system model*

The mouth, stomach, and small intestine were simulated using three consecutive, temperature-controlled, double-jacketed reaction vessels ( $37.0 \pm 0.1^\circ\text{C}$ ). The reaction vessel was integrated into a circulating water bath, and the pH and temperature of the bioreactors were monitored constantly during operation. The passage time through the *in vitro* gastrointestinal system was maintained at 2 min in the mouth region, 2 h in the stomach region, and 2 h in the small intestine region. Six peristaltic pumps with adjustable speeds were used to control the flow of simulated digestive system secretions and to ensure the passage from the mouth to the stomach and from the stomach to the small intestine. In addition, the pH of the stomach and small intestine

sections was kept constant using instant pH monitoring, with adjustment using 1M Sodium hydroxide (NaOH) and 0.2M Hydrochloric acid (HCl) when necessary.

Mucin, alfa-amylase, and a 40% NaOH solution were used to simulate salivary secretion. Simulated saliva (0.05 mL/g sample) was added to the oral environment at a flow rate of 5 mL/min, and all reagents were incubated at 37°C for 2 minutes. Gastric fluid simulation involved mucin and pepsin enzymes to promote acid denaturation of digested foods, with hydrochloric acid used to activate pepsin. Simulated gastric secretion (0.05 mL/g sample) was added to the stomach reactor at 0.25 mL/min. After digestion in the oral phase at pH 6.9, samples entered the stomach reactor at a 100 mL/min flow rate. Gradual acidification to pH 2.5 was achieved by adding 0.2 mL of 1 M HCl and 0.695 mL of water, with the HCl flow adjusted to 3.5 mL/min until reaching pH 2.5 and then reduced to 0.9 mL/min to simulate gastrin inhibition. Gastric digestion was maintained for 2 hours at 37°C. A double-jacketed reactor integrated with a circulating water bath ensured constant temperature. Pancreatin and bile salts were used to simulate small intestinal fluid. Simulated intestinal secretion (0.25 mL/g sample) was introduced at a 3 mL/min flow rate. Samples were transferred from the stomach (pH 2.5) to the small intestine reactor at 100 mL/min over 20 minutes. pH was gradually increased to 6.9 by adding 1 M NaOH at 0.65 mL/min. Intestinal digestion was maintained for 2 hours at 37°C. Temperature, digestion times, secretion compositions, and flow rates were set based on established gastrointestinal simulation protocols from the literature (Çomak Göçer *et al.*, 2016; Minekus *et al.*, 2014).

The enzymes used in the study (alfa-amylase, mucin, bile salt, pancreatin, and pepsin) were obtained from Sigma-Aldrich (St. *et al.*, USA). Chemicals (CaCl<sub>2</sub>, KCl, NaCl, NaHCO<sub>3</sub>, and NaOH) were supplied by AFG Bioscience (Northbrook, IL, USA), and HCl was supplied by Honeywell (Seelze, Germany).

#### *Determination of soluble solid content*

The soluble solid content at 20°C (°Brix) was measured using a hand-held refractometer (Fukui, Japan).

#### *Determination of pH*

The pH values were determined using a pH meter (Mettler Toledo, Columbus, OH, USA), which was calibrated before each use with buffer solutions of pH  $4.01 \pm 0.01$  and  $7.01 \pm 0.01$  (Norateks, Turkey).

#### *Determination of color*

Color determinations were performed using an X-Rite Ci64x portable color spectrophotometer (Optizen, Opt, Korea) to quantify the red, green, and blue components

of each sample. Information about the color was obtained using these data and the  $L^*$ ,  $a^*$ , and  $b^*$  metrics.

#### Total phenolic content analysis

Total phenolic content was determined using the Folin Ciocalteu test according to existing in-house protocols (Taga *et al.*, 1984). A total of 2.0 mL of 2%  $\text{Na}_2\text{CO}_3$  was added to each sample (100  $\mu\text{L}$ ) and incubated at room temperature for 2 min. After incubation, 100  $\mu\text{L}$  of 50% Folin Ciocalteu reagent was added, and the solution was mixed well. After incubating at room temperature (21°C) in the dark for 30 min, absorbance at 720 nm was measured using a spectrophotometer (Klab Optizen Pop UV, Korea) (Shannon *et al.*, 2018). The total phenolic content of the samples was expressed in  $\mu\text{g}$  gallic acid equivalent (GAE)/g dry tea or  $\mu\text{g}$  GAE/mL of sample with the help of a standard calibration curve ( $r^2 > 0.99$ ) drawn using a gallic acid standard.

#### High-performance liquid chromatography

Samples (1 mL) were homogenized in 9 mL pure methanol, then centrifuged at  $3007 \times g$  for 15 min, and the residues were extracted twice. After centrifugation, the supernatants were mixed and used as a crude extract for determination of antioxidant activity and phenolic content. Phenolic compounds were analyzed according to a published method with slight modifications (Albishi *et al.*, 2013). A Thermo Scientific HPLC Dionex Ultimate 3000 system with an automatic injector (WPS-3000SL), a TCC-3000SD column oven, and an MWD-3000 Multiple Wavelength photodiode array detector was used for analysis. A C18 column was used ( $4.6 \times 150\text{-mm}$  ID, 3- $\mu\text{m}$  particle size). The mobile phase was 0.1% formic acid in purified water (A), and the mobile phase was 100% methanol (B). The following gradient program was used: (B) = 10% (0–8 min), 25% (8.1–16 min), 45% (16.1–25 min), 45% (25.1–28 min), 80% (28.1–45 min), and 100% (45.1–60 min). The crude extract (10  $\mu\text{L}$ ) was injected into the column at a flow rate of 1 mL/min and a column temperature of 25°C. Absorbance at 280 nm was monitored to detect peaks (Göksu Sürücü *et al.*, 2024).

#### Statistical analysis

All samples were analyzed in duplicate, and two parallel analyses were performed. Data were evaluated using one-way analysis of variance (ANOVA). The Shapiro-Wilk test was used to determine whether the distribution of results was homogeneous. The difference between the means was evaluated using the Tukey multiple comparison test for homogeneous distribution of data, and Dunnett's T3 test was used when data were not homogeneously distributed. The statistical significance level was taken as  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$  for all calculations and interpretations, and hypotheses were established in two

directions. Statistical analyses were carried out using SPSS version 26.0 (SPSS 2020-IBM Inc., Chicago, IL, USA).

## Results

Color analysis revealed samples B and E to be similar in terms of brightness ( $24.66 \pm 0.28$  and  $24.62 \pm 0.13$ , respectively), but these two samples were significantly different from all other samples ( $p < 0.001$ ). Sample B was similar to samples A and D in red and green levels, but significantly different from all other samples in these variables ( $p < 0.001$ ). All samples were significantly different from each other in yellow and blue levels ( $p < 0.001$ ). Sample C exhibited the lowest red-green value ( $0.66 \pm 0.25$ ), while sample E had the lowest yellow-blue value ( $6.05 \pm 0.06$ ) (Figure 1).

The total soluble solid contents of the green tea samples did not differ significantly (Table 1). However, while samples B and C had similar pH values, the other samples were significantly different in terms of pH. Samples D and E, A and B, and B and C were found to be similar in phenolic content ( $p < 0.001$ ). Samples D and E had the highest phenolic contents, while sample C had the lowest.

Table 2 gives the total phenolic contents of green teas in each region of the *in vitro* gastrointestinal system model after brewing. Samples D and E exhibited the highest phenolic content in both the mouth and intestine regions, while sample C had the lowest phenolic content in both these regions. The phenolic content of samples B and D decreased significantly in the transition from the stomach to the small intestine ( $p < 0.01$ ), while no statistically significant changes were observed between the stomach and small intestine for samples A, C, or E. Phenolic compounds are released in the acidic environment of the stomach because they are bound in the food matrix. When pepsin is added under normal digestive conditions and food remains for 1 h in the stomach, an increase in phenolic compound content can indicate that enzymatic reactions are breaking the chemical bonds between phenolic compounds and proteins, carbohydrates, and other components, leading to the release of these compounds. Based on the values obtained from the five groups, Pearson's correlation analysis demonstrated a very strong positive association between the Mouth and Small Intestine phases ( $r = 0.996$ ,  $p = 0.0003$ ). This finding indicates that the measured values in the Mouth phase were highly consistent with those observed in the Small Intestine phase. Notably, the results reflect the total phenolic amount ( $\mu\text{g}$  gallic acid equivalent/mL) of green tea samples along the *in vitro* gastrointestinal system.



Figure 1. Color chart of green tea samples.

Table 1. Total soluble solids, pH, and total phenolic content of green tea samples.

Sample	Total soluble solids content (°Brix)	pH	Total phenolic substance amount*
A	2.15 ± 0.07	5.83 ± 0.00 <sup>a</sup>	50.30 ± 1.63 <sup>b</sup>
B	2.10 ± 0.00	5.73 ± 0.00 <sup>c</sup>	37.61 ± 0.18 <sup>bc</sup>
C	2.15 ± 0.07	5.72 ± 0.00 <sup>c</sup>	27.15 ± 0.98 <sup>c</sup>
D	2.20 ± 0.00	5.77 ± 0.01 <sup>b</sup>	125.32 ± 11.02 <sup>a</sup>
E	2.15 ± 0.07	5.57 ± 0.01 <sup>d</sup>	113.08 ± 3.52 <sup>a</sup>
Sig.	ns	***	***

\*Total phenolic content (µg gallic acid equivalent/g dry tea).  
A–E: Difference between means, different letters in the same column indicate statistical significance.  
Ns: nonsignificant.

**Table 2.** Total phenolic amount ( $\mu\text{g}$  gallic acid equivalent/mL) of green tea samples along the *in vitro* gastrointestinal system.

Sample	Mouth	Stomach	Small intestine	Sig
A	720.33 $\pm$ 42.06 <sup>aB</sup>	601.77 $\pm$ 86.20 <sup>bC</sup>	609.01 $\pm$ 24.75 <sup>bB</sup>	*
B	541.29 $\pm$ 29.84 <sup>aC</sup>	498.18 $\pm$ 28.10 <sup>aC</sup>	382.15 $\pm$ 67.20 <sup>bC</sup>	***
C	381.19 $\pm$ 31.23 <sup>aD</sup>	321.77 $\pm$ 7.99 <sup>bD</sup>	315.22 $\pm$ 11.79 <sup>bC</sup>	***
D	1779.50 $\pm$ 165.81 <sup>aA</sup>	1642.89 $\pm$ 113.91 <sup>aA</sup>	1266.37 $\pm$ 168.67 <sup>bA</sup>	***
E	1662.58 $\pm$ 49.39 <sup>aA</sup>	1288.54 $\pm$ 220.68 <sup>bB</sup>	1151.78 $\pm$ 141.77 <sup>bA</sup>	**
Sig	***	***	***	

a–c: Difference between means. different letters in the same row indicate statistical significance.  
A–E: Difference between means, different letters in the same column indicate statistical significance.

**Table 3.** Catechin amount ( $\mu\text{g/mL}$ ) of green tea samples along the *in vitro* gastrointestinal system.

Sample	Mouth	Stomach	Small intestine	Sig
A	22.08 $\pm$ 2.14 <sup>aA</sup>	19.98 $\pm$ 1.74 <sup>aB</sup>	14.03 $\pm$ 1.74 <sup>bA</sup>	*
B	13.13 $\pm$ 1.34 <sup>aB</sup>	11.13 $\pm$ 1.09 <sup>aD</sup>	5.43 $\pm$ 1.10 <sup>bB</sup>	**
C	23.94 $\pm$ 1.80 <sup>aA</sup>	21.02 $\pm$ 1.47 <sup>aB</sup>	11.89 $\pm$ 1.47 <sup>bAB</sup>	***
D	24.03 $\pm$ 2.12 <sup>aA</sup>	21.74 $\pm$ 1.73 <sup>aA</sup>	11.92 $\pm$ 1.73 <sup>bAB</sup>	**
E	14.00 $\pm$ 1.98 <sup>aB</sup>	14.28 $\pm$ 1.61 <sup>aC</sup>	7.18 $\pm$ 1.61 <sup>bAB</sup>	*
Sig	***	***	*	

a–c: Difference between means. different letters in the same row indicate statistical significance.  
A–E: Difference between means, different letters in the same column indicate statistical significance.

The amounts of catechin in the samples at each stage in the model gastrointestinal tract are provided in Table 3. Sample B had the least catechin in all regions; the highest catechin amounts were recorded from sample D in the mouth and stomach regions, and from sample A in the small intestine region. The differences between mouth and stomach region values were not statistically significant for any samples, while the differences between these and small intestine values were statistically significant. This is because green tea catechins are bound to the cellular structure or other compounds (protein, polysaccharides) in the leaves. Bound catechins may be released during exposure to acidic pH and enzymes such as pepsin in the stomach, causing an increase in the amount of catechin in the stomach environment. The decrease in catechins in the small intestine may be due to structural transformations occurring in the neutral/slightly basic pH conditions of this region (pH 6–7.5). Based on the values obtained from the five groups, Pearson's correlation analysis revealed a strong positive correlation between the Mouth and Small Intestine phases ( $r = 0.919$ ,  $p = 0.027$ ). This finding indicates that catechin amounts ( $\mu\text{g/mL}$ ) measured in the Mouth phase were strongly associated with those observed in the Small Intestine phase of the *in vitro* gastrointestinal system.

In this study, analyses of five different commercial products (A, B, C, D, and E) were conducted to investigate the changes in catechin and epicatechin compounds throughout the *in vitro* gastrointestinal tract. Initial catechin amounts were determined in the mouth phase (0 min) of each sample. The samples were then passed through the *in vitro* gastrointestinal system, and the dissolved catechin amounts at 120 min of the small intestinal phase were analyzed by HPLC. Based on the obtained data, bioaccessibility potential was calculated using the formula "Bioaccessibility (%) = (Dissolved catechin amount in the small intestinal phase/Initial catechin amount)  $\times$  100."

Figure 2 shows significant differences in catechin bioaccessibility among the products. The highest bioaccessibility was determined in sample E at 32.8%. Despite having the lowest catechin content in the mouth phase, sample E is notable for its high bioaccessibility. This suggests that the product matrix or the form of the catechin compound may have a decisive effect on solubility and stability. Similarly, sample C stood out as another product with high absorption potential, with a bioaccessibility of 32.7%. In contrast, sample B had the lowest bioaccessibility of 17.4%, which could be interpreted as either

catechins being unstable under digestive conditions or the product content negatively affecting solubility.

A high catechin content alone does not necessarily imply high bioaccessibility. The presence of other ingredients and pH levels in products can affect the solubility and stability of catechins during digestion. Therefore, when assessing the health effects of catechin-containing products, not only the total content but also the bioaccessibility potential of these compounds should be taken into consideration. In this context, focusing formulation studies on bioaccessibility may be a crucial strategy for enhancing the efficacy of functional foods.

The amount of epicatechin in each sample at each stage is shown in Table 4. Sample B exhibited the lowest epicatechin value in all regions. Sample D was found to contain the highest amount of epicatechin in the mouth and

small intestine regions, while sample C contained the highest amount of epicatechin in the stomach region. Epicatechin values decreased significantly throughout the *in vitro* gastrointestinal system, except in the case of sample C ( $p < 0.001$ ).

Upon evaluating the analysis results expressed in Figure 3, it was determined that all samples exhibited bioaccessibility rates ranging from 51.17% to 62.65%. The highest bioaccessibility rate, 62.65%, was observed in sample C, indicating that this product retained its epicatechin content throughout the digestive process. On the other hand, sample B had the lowest bioaccessibility value of 51.17%, although even this rate indicates a generally high level.

The obtained data indicate that epicatechin is more stable and more soluble in the digestive tract compared

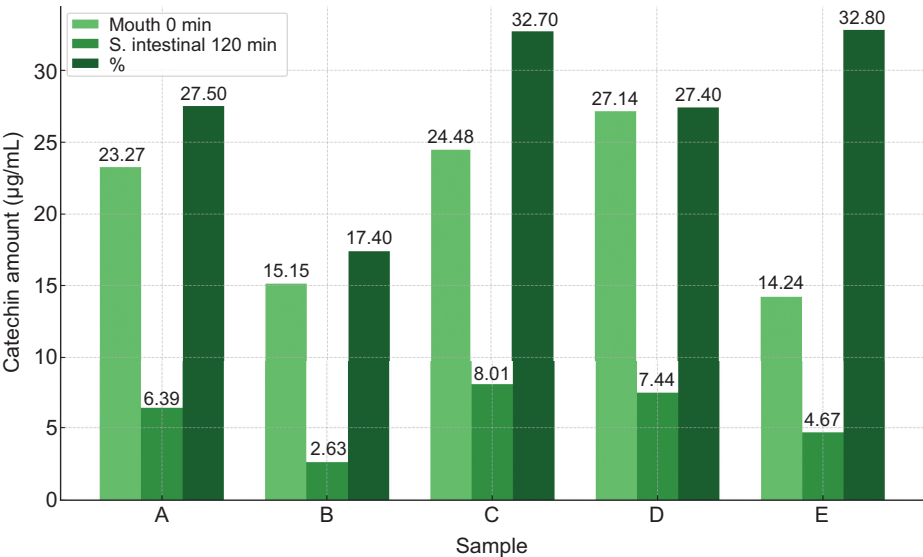


Figure 2. Catechin amount by sample and digestion stage.

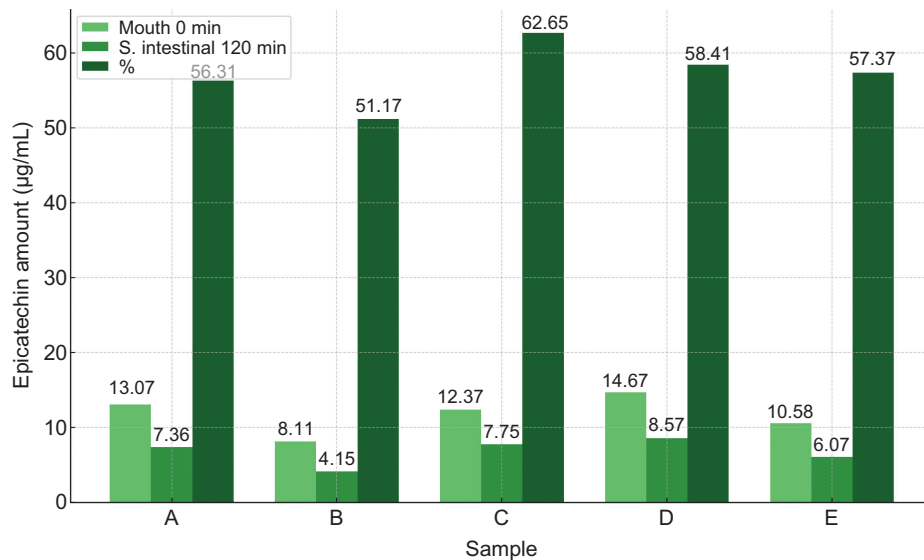
Table 4. Epicatechin amount (µg/mL) of green tea samples along the *in vitro* gastrointestinal system.

Sample	Mouth	Stomach	Small intestine	Sig
A	12.49 ± 0.67 <sup>aB</sup>	10.81 ± 0.42 <sup>bA</sup>	8.15 ± 1.37 <sup>cAB</sup>	***
B	7.64 ± 0.54 <sup>aD</sup>	6.54 ± 0.26 <sup>bC</sup>	4.53 ± 0.92 <sup>cC</sup>	***
C	12.36 ± 0.02 <sup>aB</sup>	11.27 ± 0.52 <sup>aA</sup>	8.30 ± 1.23 <sup>bAB</sup>	***
D	13.97 ± 0.81 <sup>aA</sup>	11.22 ± 1.09 <sup>bA</sup>	9.19 ± 1.40 <sup>cA</sup>	***
E	10.10 ± 0.55 <sup>aC</sup>	8.56 ± 0.33 <sup>bB</sup>	6.80 ± 1.12 <sup>cB</sup>	***
Sig	***	***	***	

a–c: Difference between means. different letters in the same row indicate statistical significance.

A–E: Difference between means, different letters in the same column indicate statistical significance.





**Figure 3.** Epicatechin amount by sample and digestion stage.

to catechin. All products retained more than half of their initial epicatechin content at the end of the small intestinal phase, demonstrating a very favorable bioaccessibility profile. These findings indicate that the functional effects of epicatechin-containing products are related not only to their total content but also to their ability to maintain a form suitable for absorption in the digestive tract. The comparatively greater stability of epicatechin observed in this study may be explained by structural differences between catechin and epicatechin, such as hydroxylation and galloylation patterns, as well as their distinct binding affinities to proteins and polysaccharides.

## Discussion

Green tea can contribute to healthy nutrition due to its high content of polyphenolic components and strong antioxidant properties. However, the changes that these beneficial compounds undergo throughout the digestive system are not yet fully understood. Gastrointestinal conditions such as stomach acidity and bile can affect the structure and function of phenolic substances. Human studies are optimal for determining these changes; however, they are not always feasible due to technical, financial, and ethical limitations. Animal models offer an alternative approach, though they involve complex methods such as surgical intervention. *In vitro* gastrointestinal system models have become increasingly popular as an alternative that circumvents these issues and offers reproducibility and flexibility. Understanding how the phenolic content of green tea from tea bags that are currently on the market changes during the digestive process

is crucial to optimize the health benefits. The present study, therefore, aimed to elucidate the chemical transformations that compounds in green tea undergo during digestion, which may contribute to the development of more effective consumption strategies for individuals seeking a healthy life.

Color is a key attribute influencing food acceptability, as consumers often judge quality at first sight. For this reason, manufacturers must carefully consider both the inherent color characteristics of their products and possible changes during processing. In this study, color analysis of green tea bags revealed notable differences among brands, likely reflecting variations in raw material and processing. Samples B and E showed similar brightness, indicating comparable light reflectance, while sample C exhibited a greener tone and sample E a tendency toward blue tones. Such differences highlight the influence of production techniques, leaf type, and post-processing conditions on the visual quality of green tea.

Agca *et al.* (2020) reported the amount of gallic acid to be highest in water-extracted green tea (1.341 mg/100 mL), while Al-Ghafari *et al.* (2016) reported green tea and green tea fortified with lemon to contain 678.7 and 957.3 µg GAE/10 mg tea, respectively. Brewing time has been found to affect total catechin and polyphenol contents, with HPLC analysis of green tea infusions revealing minimum and maximum values of 1325.00–2798.09 mg/L and 1357.31–2881.08 mg/L (Göksu Sürücü *et al.*, 2024). The results of the study indicate the total phenolic content of green tea leaves to be lower than previously reported, which may be attributed to differences

in extraction conditions and other parameters such as the source of material, amount of solid material used for infusion, temperature, and infusion time.

Donlao and Ogawa (2019) reported the total phenolic content of green tea to be between 11.40 and 19.58 g/100 g, while Saklar *et al.* (2015) determined the total catechin and epicatechin contents to be  $16.66 \pm 0.004$  and  $0.16 \pm 0.004$  g/100 g dry tea, respectively, in Turkish green tea infusions. Henning *et al.* (2003) analyzed eight different green teas and determined catechin content to be  $0.0 \pm 0.0$  mg/100 mL in four products, and between  $3.4 \pm 0.5$  and  $5.8 \pm 0.9$  mg/100 mL in the other four. The same study revealed the epicatechin content to be  $0.0 \pm 0.0$  mg/100 mL in one product and ranging from  $2.9 \pm 0.0$  to  $13.3 \pm 0.1$  mg/100 mL in the other seven. We found that catechin and epicatechin decrease in the transition from stomach to small intestine region for all samples. This supports the findings of Shu *et al.* (2019), who reported the amounts of catechin and epicatechin in green tea powder to be  $9.64 \pm 0.19$  and  $10.43 \pm 0.18$ , respectively, in the stomach region and  $1.81 \pm 0.1$  and  $0.75 \pm 0.05$ , respectively, in the small intestine region. In line with these findings, Annunziata *et al.* (2018) demonstrated that after simulated gastrointestinal digestion of different teas (green, white, black), the total phenolic content significantly increased at the colon stage compared to the duodenal phase, highlighting the role of microbial metabolism in phenolic bioaccessibility. Taken together, these results suggest that while catechin and epicatechin levels decrease in the upper gastrointestinal tract, colonic microbial fermentation may contribute to the release or transformation of bound phenolics, partially compensating for these losses.

We found the total phenolic content to differ significantly between the five samples that we analyzed. Qin *et al.* (2022) reported the total phenolic content of green tea to be  $68.66 \pm 0.48$  mg GAE/g dry matter and observed a similar significant decrease in phenolic content throughout simulated digestion (reducing to  $47.75 \pm 0.68$  mg GAE/g dry matter 1 h after addition of pepsin and  $41.41 \pm 0.50$  mg GAE/g dry matter in the small intestine stage). A similar study using an in vitro system reported that in vitro digestion significantly affects the total polyphenol content of green tea, with total polyphenol content decreasing from  $1425 \pm 0.12$  mg GAE/g dry extract in the salivary phase to  $13.93 \pm 0.25$  mg GAE/g dry extract in the gastric phase and  $12.77 \pm 0.06$  mg GAE/g dry extract in the intestinal phase (Laib *et al.*, 2021). Similarly, Shu *et al.* (2019) reported the total phenolic content of green tea powder to decrease from 190 mg GAE/g dry extract before digestion to 30 mg GAE/g dry extract after simulated digestion. Governa *et al.* (2021) further confirmed that during in vitro simulated digestion of green, black, and Oolong teas, the stability

and bioaccessibility of phenolic compounds, particularly EGCG, were markedly altered, with EGCG undergoing greater degradation than other catechins. This suggests that gastrointestinal digestion substantially modifies phenolic compound bioaccessibility, independent of the initial extract composition.

*In vitro* gastrointestinal digestion has also been reported to cause total polyphenol concentration in green tea to decrease by 6.12% (Wu *et al.*, 2015). Consistent with this, Tenore *et al.* (2015) assessed not only simulated digestion but also intestinal permeation and plasma protein interactions of tea polyphenols, showing that white tea preserved the highest polyphenol content and exhibited the greatest intestinal bioaccessibility compared with green and black tea. Similarly, Chen *et al.* (2013) investigated nine commercial tea juices and demonstrated that simulated gastrointestinal digestion generally reduced total polyphenol content, underscoring the degradative impact of digestion on tea constituents. Our *in vitro* study supports this, showing gastrointestinal digestion to reduce the phenolic content of green tea, indicating that these bioactive compounds are sensitive to the environmental conditions in the digestive tract (high pH, dissolved oxygen, and presence of oxygen-reactive species).

Although this study provides valuable preliminary information on the phenolic composition and bioaccessibility of green tea using the in vitro gastrointestinal system, it has some limitations. The analysis was restricted to five commercially available samples obtained from a single market, which may limit generalizability. Moreover, the brewing conditions applied (1.5% at 80 °C for 3 min) followed standardized preparation instructions but may not fully reflect the variability in consumer practices. In addition, analyses were performed in duplicate, representing technical rather than biological replicates, which may affect statistical robustness. These factors should be considered when interpreting the results, and future research including a wider variety of products and additional biological replicates will be important to strengthen the generalizability and reliability of the findings.

Taken together, these findings contribute to the literature by providing one of the first comparative evaluations of commercially available bagged green teas using an in vitro gastrointestinal system. Unlike many previous studies focusing mainly on extracts or total phenolic content, this study highlights the differential stability of catechin and epicatechin throughout digestion and incorporates bioaccessibility calculations to better reflect the physiological relevance of these compounds. Such an approach strengthens the practical implications of the findings for both consumers and the food industry.



## Conclusions

This preliminary study is novel in that it evaluates commercially available bagged green teas, emphasizing catechin and epicatechin stability across digestive stages and applying bioavailability calculations to assess their potential health impact. This study further highlights that the phenolic composition and bioaccessibility of green tea can vary substantially between products, which may influence their antioxidant potential and health benefits. Beyond reporting differences among samples, these findings emphasize that high initial phenolic content does not necessarily translate into high bioaccessibility, underlining the importance of considering digestive stability when evaluating the functional properties of green tea. These results can inform tea formulation strategies aimed at improving polyphenol stability and consumer guidance on how product choice and preparation conditions may affect health outcomes. While the present study used an in vitro gastrointestinal system, future research involving a wider variety of green tea products and human intervention trials will be essential to confirm these findings and provide evidence-based recommendations for consumers and the food industry.

## Data Availability Statement

The data obtained in this study are available from the corresponding author upon request.

## Author Contributions

Conceptualization, M.İ.P., R.P., C.K. and S.S.; methodology, M.İ.P. and R.P.; software, R.P., and C.K.; validation, M.İ.P., R.P., and C.K.; formal analysis, M.İ.P., R.P., and C.K.; investigation, M.İ.P., and R.P.; resources, M.İ.P., and S.S.; data curation, M.İ.P., and R.P.; writing—original draft preparation, M.İ.P., and R.P.; writing—review and editing, C.K., and S.S.; visualization, M.İ.P.; supervision, M.İ.P.; project administration, M.İ.P. All authors have read and agreed to the published version of the manuscript.

## Conflicts of Interest

The authors declare no conflicts of interest.

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