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Soluble green tea production and determination of changes during the *in vitro* gastrointestinal system*

Çözünebilir yeřil çay üretimi ve *in vitro* gastrointestinal süreçteki deđişikliklerin belirlenmesi

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ABSTRACT

Objective: This study investigated the transformations and bio-accessibility of soluble green tea components throughout the digestive process by utilizing an *in vitro* gastrointestinal system simulation.

Material and Methods: Green tea was obtained from a local market considering the expiration date. In preliminary experiments, it was determined that the highest phenolic content was reached when 2% infusions were brewed at 80°C for 60 seconds. The prepared infusions were filtered, maltodextrin was added and freeze-dried at -59.5°C, 33 Pa for 48 hours. Color, total phenolic substance and antioxidant activity analyses were performed on green tea infusion (GT) and maltodextrin-coated green tea (SGT). In addition, changes in phenolic substance during the *in vitro* gastrointestinal process were investigated by HPLC.

Results: In the initial condition, only catechin and epicatechin contents were statistically significant ($p<0.01$) when comparing GT and SGT. Phenolic acid content, antioxidant activity, and phenolic compounds showed statistically significant differences in different regions of the gastrointestinal system. Additionally, changes in both green tea samples throughout the digestive process were statistically significant ($p<0.01$).

Conclusion: Significant alterations in phenolic content, antioxidant capacity, and polyphenolic composition were observed in GT and SGT samples; co-application of maltodextrin with other materials may enhance green tea phenolic stability and bioavailability.

ÖZ

Amaç: Bu çalıřma, çözünebilir yeřil çay bileřenlerinin sindirim sürecindeki dönüşümlerini ve biyoyararlanımını *in vitro* gastrointestinal sistem simülasyonu ile incelemiřtir.

Materyal ve Yöntem: Yeřil çay, son kullanma tarihi dikkate alınarak yerel bir marketten temin edilmiřtir. Ön deneylerde, 2% infüzyonların 80°C'de 60 saniye demlendiđinde en yüksek fenolik içeriđe ulařtıđı belirlenmiřtir. Hazırlanan infüzyonlar süzülerek maltodekstrin eklenmiř ve -59,5°C, 33 Pa'da 48 saat boyunca dondurarak kurutulmuřtur. Yeřil çay infüzyonu (YÇ) ve maltodekstrin kaplı yeřil çayın (MYÇ) renk, toplam fenolik madde ve antioksidan aktivite analizleri yapılmıřtır. Ayrıca, fenolik madde deđiřimi HPLC ile deđerlendirilmiřtir.

Arařtırma Bulguları: Bařlangıçta, YÇ ve MYÇ arasındaki kateřin ve epikateřin içerikleri istatistiksel olarak anlamlı bulunmuřtur ($p<0.01$). Gastrointestinal sistemin farklı bölgelerinde fenolik asit içeriđi, antioksidan aktivite ve fenolik bileřiklerde önemli farklılıklar gözlenmiřtir. Sindirim sürecinde her iki çay örneđinde de belirgin deđiřimler meydana gelmiřtir ($p<0.01$).

Sonuç: YÇ ve MYÇ örneklerinde fenolik içerik, antioksidan kapasite ve polifenolik bileřimde önemli deđiřiklikler gözlemlendi; maltodekstrinin diđer materyallerle birlikte uygulanması yeřil çay fenolik stabilitesini ve biyoyararlanımını iyileřtirebilir.

Keywords: DPPH, extraction, flavonoids, gastrointestinal system, green tea, *in vitro* polyphenols

Anahtar sözcükler: DPPH, ekstraksiyon, flavonoid, gastrointestinal sistem, yeřil çay, *in vitro* fenolik

INTRODUCTION

Tea, one of the most widely consumed beverages in the world after water, is commercially grown in more than 30 countries, especially in China, India, Kenya, Sri Lanka, and Türkiye (Akbulut et al., 2020). Tea is widely consumed worldwide for its unique color, taste, and health benefits derived from its bioactive components. The leaves of the tea plant, called *Camellia sinensis* L., are green in all seasons. Its high antioxidant activity is attributed to its phenolic compounds (Şatır, 2023). Among all major tea varieties, green tea has been associated with the most significant health benefits. It is one of the most widely consumed types of tea globally and has been steadily gaining popularity, currently accounting for approximately 20% of the 3 million tons of annual global tea production (Radeva-Ilieva et al., 2025). The most important of green tea polyphenols are classified into the 4 types: epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate (ECG), epigallocatechin (EGC) and epicatechin (EC) (Llczbínski & Bukowska, 2022). Green tea contains bioactive components such as flavonoids, tannins, methylxanthine alkaloids (caffeine, theophylline), and linoleic acid, as well as minerals such as calcium, magnesium, zinc, and selenium (Khatoon, 2023). EGCG supports the immune system by neutralizing free radicals and preventing cell damage. Green tea, which is obtained from the *Camellia sinensis* L. plant, contains more than 2000 active ingredients, including phenolic compounds, proteins, catechins, flavonoids, and volatile compounds. It is a functional beverage due to its health benefits (Singh et al., 2022a). Green tea, an unfermented tea, is produced by enzyme inactivation, which preserves the catechins in fresh leaves. Green tea's phenolic content and antioxidant capacity are higher than black tea (Akbulut et al., 2020). Catechins and polyphenols mainly attract attention with their potential health benefits, such as metabolic syndrome, protection against cancer, and prevention of obesity and diabetes (Singh et al., 2022a). In addition, green tea's anticarcinogenic and antioxidant properties may provide protective effects against cardiovascular diseases (Taş et al., 2005; Prasanth et al., 2019). Scientific studies have shown that green tea has many positive effects, such as weight control, improved physical performance, oral and bone health, and protection against ultraviolet radiation (Zhao et al., 2019). Green tea is consumed by brewing or straining bags in different cultures, and its health benefits increase with regular consumption (Taş et al., 2005). With all these features, green tea is a natural source that contributes to health beyond just a daily drink. Bioactive compounds such as phenolic substances can be significantly reduced during thermal processing and storage. In fact, the unsaturated bonds in the molecular structure of phenolic compounds can easily lead to degradation and loss of fragile components during storage, due to external factors such as heat, pH, exposure to oxygen and light (Medfai et al., 2023). Maltodextrin is one of the most widely utilized wall materials for preserving phenolic chemicals because of its low cost, ease of acquisition, film-forming capabilities, high water solubility, and low viscosity at significant concentrations (Navaro-Flores et al., 2020; Medfai et al., 2023). Maltodextrin has been found to be effective in formulating stable green tea extract microparticles through spray drying. These microparticles maintain their size, shape and antioxidant capacity over 60 days of storage at different pH values (Cruz-Molina et al., 2021). Silva et al. (2023) evaluated the physicochemical properties, antimicrobial activity, and toxicological safety of microcapsules obtained by spray-drying green tea extract with cashew gum and maltodextrin using the Zebrafish model. Chuysinuan et al. (2021) encapsulated green tea extract with cyclodextrin to form an inclusion complex, which was subsequently incorporated into a chitosan/polyvinyl alcohol (CS/PVA)-based hydrogel matrix. They assessed the physicochemical characteristics and antioxidant capacity of the resulting system.

The digestive system is a complex network responsible for digestion, absorption, and waste excretion (Kızıl, 2019). Digestion involves the physical and chemical breakdown of food, allowing the release of beneficial components to the body. The food matrix affects the release of nutrients and their transportation to target areas during digestion (Sensoy, 2021). The digestive system is controlled by a network of nerves and hormones that regulate digestion, secretion, absorption, and motility along a canal extending from the mouth to the anus (Baş, 2019). While digestion and absorption occur in the stomach and small intestine,

nutrients are separated, passed into the blood, and used for energy and growth in the body. Digestive enzymes and fluids support this process, and contractions provide motility and transport nutrients to target organs (Saka et al., 2016). Since *in vivo* studies are complex, expensive, and ethically limited, *in vitro* digestion models have been developed since the 1990s (Sensoy, 2021). *In vitro* digestion models are essential for designing and evaluating new food products. While static *in vitro* models simplify digestion and mimic biochemical conditions, these models have limitations. For example, differences in digestion parameters make it difficult to compare research results (Brodkorb et al., 2019). In addition, these models fail to simulate the dynamic aspects of the digestive process, especially mechanical forces and fluid dynamics (Li et al., 2020). Dynamic *in vitro* models provide more realistic digestion simulations. These models simulate the stomach and small intestine compartments and consider dynamic factors such as pH changes, enzyme secretion, and peristaltic forces (Minekus et al., 2014; Li et al., 2020). However, these models cannot mimic all physiological conditions of the digestive system (Sensoy, 2021). In general, dynamic digestion models aim to mimic all gastrointestinal tract compartments by providing a complete digestion simulation (Singh et al., 2022b). These models more accurately simulate the digestive process of nutrients while also considering the constant changes in environmental conditions (Fusco et al., 2022).

Upon examining the literature, it becomes apparent that studies conducted using *in vitro* gastrointestinal systems primarily focus on probiotic microorganisms and microbiological evaluations. For example, İnce-Palamutoğlu et al. (2023) evaluated the viability of lactic acid bacteria in the dynamic *in vitro* gastrointestinal system in home-type and industrial kefir samples. Similarly, Çomak-Göçer et al. (2024) examined the rheological, microbiological, and *in vitro* digestive properties of kefir produced with different kefir grains and commercial starter cultures. These studies provide essential findings for evaluating the microbiological and digestive properties of fermented products. On the other hand, studies on green tea generally focus on issues such as component stability, antioxidant capacity, and encapsulation technologies; structural changes during the digestive process and bioavailability levels are addressed to a limited extent. It is noteworthy that comprehensive studies on the chemical and biological transformations of green tea components during the digestive process, particularly using an *in vitro* gastrointestinal system model, are lacking. In this context, the study conducted is one of the pioneering studies that systematically evaluate the chemical and biological changes that natural and maltodextrin-coated green tea extracts undergo during the digestive process, providing original and scientifically valuable contributions to the literature on the understanding of the gastrointestinal behavior of functional ingredients.

The main objective is to evaluate the transformations and bioavailability of soluble green tea components during the stages of an *in vitro* gastrointestinal digestion simulation.

MATERIALS and METHODS

Materials

The study was conducted in the Food Chemistry Laboratory of the Department of Nutrition and Dietetics, Faculty of Health Sciences, Afyonkarahisar Health Sciences University. Green tea was obtained from the local market in Afyonkarahisar, Türkiye. The chemicals used for the analysis were analytical grade. This research has been approved by the Afyonkarahisar Health Sciences University Non-Interventional Scientific Research Ethics Committee with document number 2023/401 dated 1 September 2023.

Soluble Green Tea Production

Preliminary experiments showed that green tea (GT) prepared using 2% aqueous green tea infusions at 80°C of water for 60 seconds had the highest phenolic content. Green tea extracts obtained from the infusions were filtered through filter paper. Then, 2.5% maltodextrin was added and homogenized at 8000 rpm for 5 min. The samples were then freeze-dried for 48 hours at -59,5°C and 33 pa (INOFD-10S, Innova, China) (Figure 1).

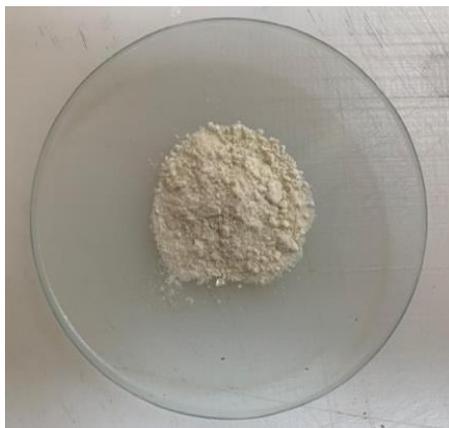


Figure 1. Maltodextrin-coated green tea.

Şekil 1. Maltodekstrin kaplı yeşil çay.

Methods

Establishment of *in vitro* gastrointestinal model

This *in vitro* digestive model simulates the mouth, stomach, and small intestine sections. A temperature-controlled water bath was used for the mouth section, and a double-jacketed reaction vessel kept at 37°C was used for the stomach and small intestine sections. Temperature and pH were continuously monitored. Digestion time was determined as 5 minutes in the mouth section, 2 hours in the stomach section, and 2 hours in the small intestine section. Secretion flow rates were controlled with adjustable peristaltic pumps. The pH balance of the stomach and small intestine sections was provided with 1 M Sodium Hydroxide (NaOH) and 1 M Hydrochloric Acid (HCl).

Mucin, α -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 5 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 (Minekus et al., 2014; Çomak Gökçer et al., 2021).

Total phenolic content

Tea samples were diluted 10-fold to determine the total phenolic content, and 0.1 ml of Folin-Ciocalteu reagent was diluted 1:2 with distilled water. Then, 0.3 ml of 2% Na₂CO₃ solution was added. The tubes were vortex-mixed and incubated in the dark and at room temperature for 2 hours. A blank solution was prepared by adding distilled water instead of 0.1 ml of tea sample. At the end of two hours, the absorbance of the resulting blue-green solutions was measured at 760 nm against the blank (Singleton & Rossi, 1965). The total phenolic content of the samples was expressed in mg gallic acid

equivalent (GAE)/g dry tea sample using a standard calibration curve ($r^2 > 0.99$) based on the gallic acid standard. The equation of the calibration curve is given below;

$$y = 0.0004x + 0.0009$$

$$r^2 = 0.9955$$

Color determination

In the study, infusion color was measured using an X-Rite Ci64x portable color spectrophotometer. Spectrophotometry is the best way to obtain data for color formulation and ensure quality by filtering light into very narrow color bands and analyzing their return signals. In addition, the spectrophotometer quantifies the red, green, and blue components of each measurement and determines the location of color in color space using L^* , a^* , b^* metrics (HunterLab, 2001; Schanda, 2018).

Antioxidant activity determination

Antioxidant activity was analyzed using the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical inhibition method (Brand-Williams et al., 1995). A DPPH solution was prepared by dissolving it in methanol at a concentration of 20 mg/L. 1.5 ml of DPPH radical was taken, 0.75 ml of tea samples were added to it, and the absorbance values at 517 nm at 0 and 30 minutes were read using a spectrophotometer (Klab Optizen Pop UV, Korea).

Antioxidant activity was calculated according to the formula.

$$\text{Antioxidant Activity (\%)} = (A_0 - A) / A_0 \times 100$$

Absorbance value at 0 minutes = (A_0); Absorbance value at 30 minutes = (A)

Determination of catechin composition in High-Performance Liquid Chromatography (HPLC)

Catechin composition of samples was determined according to the method applied by Wang et al. (2000). For this purpose, 1 mL samples were collected from the mouth (at 0 and 5 minutes), stomach (at 60 and 120 minutes), and small intestine (at 60 and 120 minutes) during the *in vitro* gastrointestinal system. The samples were centrifuged at 10000xg at 4°C for 1 hour. At the end of centrifugation, the extracts were filtered through a 0.45 µm membrane filter in the clear part, and the catechin composition was analyzed using HPLC (Thermoscientific). After mixing the brewed tea with the mobile phase 1:1 v/v and filtering the mixture through a 0.2 µm PVDF acrodisc syringe filter (Gelman, Ann Arbor, MI), tea flavanols were analyzed using HPLC. The filter discs were washed with 200 µl methanol, and the wash solution was also analyzed for flavanols with HPLC. The flavanol content separated from the filter disc was added to the data obtained from the tea analyses. Mobile phase A comprised acetonitrile, while mobile phase B comprised 960 ml of 0.1% acetic acid (pH 3.5) + 20 ml of acetonitrile + 20 ml of tetrahydrofuran. Flavanols were eluted and equilibrated with a gradient of 100% B at 0 min, 40% B at 45 min, and 100% B at 47 min. Final concentrations were calculated by comparing them with a known standard response. Detection was performed at 280 nm wavelength. An external standard method was used for the identification process, and catechin was used as a standard for this purpose. The amount of catechin in the samples was calculated with the help of the curve created with five different concentration standard solutions injected into the device under the same conditions as the samples (Henning et al., 2003).

Statistical analyses

The study was conducted with two replicates, and two parallel analyses were performed on each sample taken from each replicate. The results were evaluated with a one-way analysis of variance (ANOVA) using the statistical program IBM SPSS Statistics (Version 26). The Shapiro-Wilk test was used to determine whether the distribution of the analysis results was homogeneous. In cases where the distribution was homogeneous, the Tukey multiple comparison test was used to determine the difference between the means. In cases where it was not homogeneous, Dunnett's T_3 test was used (IBM Corp., 2019).

RESULTS and DISCUSSION

Color

The L^* , a^* , b^* values of GT and SGT samples used in the study were found to be 22.09 ± 0.11 , 1.85 ± 0.35 , 4.74 ± 0.26 and 23.2 ± 0.25 , 2.70 ± 0.14 , 3.07 ± 0.16 , respectively. The differences between them were statistically significant ($p < 0.05$). In accordance with Parvez et al. (2022), tea powders in the nanoencapsulation are lighter than tea extract, and the L^* value increases as the maltodextrin content increases by 5% to 15%. This suggests that maltodextrin contributes to the increased L^* values in encapsulated tea extracts (Zorzenon et al., 2020). Additionally, Parvez et al. (2022) showed that samples showing a tendency for red and yellow exhibited positive a^* and b^* values. The b value decreased with the concentration of 1:10. Similarly, in the present study, encapsulated green tea powders exhibited higher L values, while b values showed a decreasing tendency, consistent with the literature.

Total phenolic content

Green tea is essential for healthy nutrition due to its rich polyphenolic components and strong antioxidant properties. However, it has not been fully clarified how beneficial products such as green tea change throughout the digestive system. Gastrointestinal conditions such as stomach acids and bile can affect the phenolic compound values in green tea. Although human studies are ideal for determining changes, their applicability is limited due to technical, financial, and ethical limitations. On the other hand, animal studies offer an alternative solution, but they are generally not preferred due to the difficulty of methods such as surgical intervention. *In vitro*, gastrointestinal system models have become increasingly popular because they offer reproducibility and flexibility. These models are particularly important in determining how the phenolic substance content in green tea coated with green tea continues throughout the digestive system.

The total phenolic content values throughout the gastrointestinal system are given in Table 1. At the beginning of digestion, the total phenolic content in the mouth region of the GT sample was 112.19 ± 2.02 mg GAE/100mL while in the SGT sample, it was, it 106.74 ± 1.34 mg GAE/100mL at 0 minutes. It was determined that the difference between the total phenolic substance amount of these two tea samples at the beginning was not statistically significant. It was observed that the differences between the total phenolic substance amounts in the two tea samples were statistically significant only in the minor intestine at 0 and 120 minutes ($p < 0.05$). The change in the total phenolic substance amount of the GT and SGT throughout the gastrointestinal system was statistically significant ($p < 0.001$).

Tengse et al. (2017) conducted a study to optimize green tea extract and maltodextrin by spray drying at several percentages, yielding values of phenolic compounds between 33.48 and 58.19 mg EAG/g dry tea. Our phenolic content results are comparable to those of their study. Navarro-Flores et al. (2020) reported in their study that phenolic compounds were rapidly released for 5 minutes, and after 10 minutes, the release stabilized, and the microcapsules showed a complete phenol release of close to 97% for all treatments. They reported that the gum maltodextrin complex wall material provided a stronger phenolic compound interaction than the maltodextrin and protein complex, and there was a statistical difference between them. However, no significant difference was observed after 5 minutes. They reported that these results could be explained by the dispersion of the microcapsules in water, where the encapsulation agents are highly soluble.

Table 1. Total phenolic substance amount of tea samples throughout the *in vitro* gastrointestinal system**Çizelge 1.** *In vitro* gastrointestinal sistem boyunca çay örneklerinin toplam fenolik madde miktarı

GIS Tract	Total Phenolic Content (mg GAE/100mL)		
	GT	SGT	Sig.
Mouth 0. min	112.19±2.02 ^b	106.74±1.34 ^{de}	ns
Mouth 5. min	106.44±2.26 ^{bc}	102.12±0.17 ^e	ns
Stomach 0. min	109.58±3.68 ^b	105.43±3.18 ^{de}	ns
Stomach 60. min	130.66±3.35 ^a	112.96±5.11 ^c	ns
Stomach 120. min	126.40±4.36 ^a	131.32±0.25 ^a	ns
Small Intestine 0. min	99.99±3.18 ^{cd}	122.26±3.51 ^b	*
Small Intestine 60. min	98.09±2.52 ^d	102.83±1.51 ^{de}	ns
Small Intestine 120. min	113.61±0.33 ^b	109.11±0.67 ^{cd}	*
	***	***	

GIS: Gastrointestinal System

a-e: The difference between the means given with different letters in the same column is statistically significant

*p<0.05, ***p<0.001, ns: non-significant

Antioxidant activity

Antioxidant activity (%) values of the samples are given in Table 2. At 0 minutes, the antioxidant activity of the GT sample in the mouth region was 78.83±2.36, while it was 75.07±1.38 for the SGT sample. It was determined that the difference between the antioxidant activity values of these two tea samples at the beginning was not statistically significant. However, statistically significant differences were observed only in the stomach at 60 and 120 minutes (p<0.05). The overall change in antioxidant activity of the GT and SGT samples throughout the gastrointestinal system was found to be statistically significant (p<0.001).

Tengse et al. (2017), reported that the DPPH activity was higher at low temperature, while antioxidant activity decreased as the temperature increased due to a reduced interaction between antioxidants and free radicals. Results showed that total antioxidant activity, as measured by DPPH method, varied between 47.15% and 73.46%. In the experimental design, the highest DPPH scavenging activity was observed at 120°C with 25% green tea extract concentration at 1:2 core-wall ratio. Perez et al. (2022) *in vitro* antioxidant assays such as DPPH, reducing power, and metal chelating activity under a simulated gastrointestinal system demonstrated the bioactivity of the encapsulated with much higher values than the encapsulated form. Therefore, it can be predicted and concluded that the best way to improve the antioxidant potential of green tea extracts under simulated gastrointestinal conditions is to nano-encapsulate it using maltodextrin. The use of this nanotechnology may offer promising possibilities for the possible application of green tea extract as a new nutraceutical.

Table 2. Antioxidant activity of tea samples throughout the *in vitro* gastrointestinal system**Çizelge 2.** *In vitro* gastrointestinal sistem boyunca çay örneklerinin antioksidan aktivitesi

GIS Tract	Antioxidant Activity (%)		
	GT	SGT	Sig.
Mouth 0. min	78.83±2.36 ^a	75.07±1.38 ^b	ns
Mouth 5. min	84.40±0.79 ^a	80.15±5.81 ^a	ns
Stomach 0. min	79.46±7.97 ^a	82.31±1.77 ^a	ns
Stomach 60. min	51.18±0.10 ^b	25.28±1.86 ^d	**
Stomach 120. min	55.92±2.27 ^b	21.38±1.08 ^d	**
Small Intestine 0. min	53.13±5.61 ^b	58.57±2.07 ^c	ns
Small Intestine 60. min	23.96±0.98 ^c	25.07±0.99 ^d	ns
Small Intestine 120. min	22.14±2.76 ^c	25.35±4.14 ^d	ns
	***	***	

GIS: Gastrointestinal System

a-d: The difference between the means given with different letters in the same column is statistically significant

*p<0.05, **p<0.01, ***p<0.001, ns: non-significant

Galic Acid, Catechin, Epicatechin, Epigallocatechin Gallat and Naringin

Table 3 presents data on the concentrations of gallic acid and naringin phenolic compounds along the *in vitro* gastrointestinal tract of GT and SGT samples. While the gallic acid amount was determined as 0.96 ± 0.01 mg/100 mL in the mouth region of the GT sample at minute 0, while it was 0.94 ± 0.01 mg/100 mL in the SGT sample. No significant difference was found between the initial gallic acid amounts of the two tea samples ($p>0.05$). However, an important difference was observed in the measurements at minute 5 in the mouth region ($p<0.05$). Gallic acid concentration varied significantly between tea types across all gastrointestinal tract regions ($p<0.001$). Regarding naringin amounts, the initial concentration of the GT sample in the mouth region was 0.50 ± 0.01 mg/100 mL, while it was 0.51 ± 0.02 mg/100 mL in the SGT sample. There was no significant difference between these two samples at the initial measurement ($p>0.05$). However, the differences between the naringin amounts at all measurement points (0, 60, and 120 minutes) in the stomach region and the small intestine region were found to be statistically significant ($p<0.05$). Most of the changes in phenolic compound concentrations along the *in vitro* gastrointestinal tract of GT and SGT samples were statistically significant ($p<0.001$).

Table 3. Amount of gallic acid and naringin in tea samples throughout *in vitro* gastrointestinal system

Çizelge 3. *In vitro* gastrointestinal sistem boyunca çay örneklerindeki gallik asit ve naringin miktarı

GIS Tract	Gallic Acid Amount (mg/100mL)			Naringin Amount (mg/100mL)		
	GT	SGT	sig.	GT	SGT	Sig.
Mouth 0. min.	0.96 ± 0.01^a	0.94 ± 0.01^a	ns	0.50 ± 0.01^a	0.51 ± 0.01^a	ns
Mouth 5. min.	0.85 ± 0.01^b	0.75 ± 0.00^c	*	0.47 ± 0.01^b	0.46 ± 0.00^b	ns
Stomach 0. min	0.80 ± 0.02^c	0.80 ± 0.02^b	ns	0.45 ± 0.01^{bc}	0.36 ± 0.01^e	ns
Stomach 60. min	0.84 ± 0.02^b	0.82 ± 0.04^b	ns	0.36 ± 0.01^d	0.47 ± 0.02^b	ns
Stomach 120. min	0.84 ± 0.03^b	0.80 ± 0.01^b	ns	0.43 ± 0.01^c	0.39 ± 0.00^{cd}	**
Small Intestine 0. min	0.54 ± 0.01^d	0.53 ± 0.01^d	ns	0.15 ± 0.00^e	0.36 ± 0.01^e	*
Small Intestine 60. min	0.06 ± 0.00^e	0.70 ± 0.00^e	ns	0.45 ± 0.01^{bc}	0.38 ± 0.01^{de}	*
Small Intestine 120. min	0.02 ± 0.00^f	0.03 ± 0.00^f	ns	0.36 ± 0.00^d	0.42 ± 0.01^c	**
	***	***		***	***	

GIS: Gastrointestinal System

a-f: Differences between means given with different letters in the same row are statistically significant

* $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns: non-significant

Table 4 presents data on the concentrations of catechin, epicatechin, and epigallocatechin gallate (EGCG) phenolic compounds along the *in vitro* gastrointestinal tract of GT and SGT samples. Catechin levels, it was determined that the mouth region of the GT sample contained 1.02 ± 0.02 mg/100 mL catechin at minute 0. In contrast, this amount was 0.53 ± 0.00 mg/100 mL in the SGT. The difference between the initial catechin amounts of these two samples was statistically significant ($p<0.01$). However, the difference between the catechin concentrations in the stomach region at minute 60 was not significant ($p>0.05$). In all other regions, the amounts of catechin showed significant differences between tea types ($p<0.05$). The epicatechin amounts in the mouth region of the GT sample and SGT at minute 0 were determined as 0.12 ± 0.00 mg/100 mL and 0.23 ± 0.01 mg/100 mL, respectively. The difference between the initial epicatechin amounts was statistically significant ($p<0.01$). Except for the measurements at the 120th minute of the small intestine region, significant differences were found between the epicatechin amounts in all other regions ($p<0.05$). When evaluated in terms of EGCG concentration, the GT sample was determined as 10.62 ± 0.19 mg/100 mL at minute 0 in the mouth region, and the SGT sample as 10.05 ± 0.02 mg/100 mL. No significant difference was observed between these two samples at the beginning ($p>0.05$). However, the differences between the EGCG amounts measured at the 5th minute of the mouth region and the 120th minute of the small intestine region were statistically significant ($p<0.05$). Epigallocatechin gallate (EGCG) has been found to be partially degraded in stomach acid but exhibited higher bioactivity in the small intestine.

Table 4. Amount of catechin, epicatechin, and epigallocatechin gallate in tea samples throughout *in vitro* gastrointestinal system**Çizelge 4.** *In vitro* gastrointestinal sistem boyunca çay örneklerindeki kateşin, epikateşin ve epigallokateşin gallet miktarı

GIS Tract	Catechin Amount (mg/100mL)			Epicatechin Amount (mg/100mL)			Epigallocatechin Gallate Amount (mg/100 mL)		
	GT	SGT	Sig	GT	SGT	Sig	GT	SGT	Sig
Mouth 0. min.	1.02±0.02 _a	0.53±0.00 _c	**	0.12±0.00 _c	0.23±0.01 _c	**	10.62±0.19 ^a	10.01±0.13 _a	ns
Mouth 5. min.	0.64±0.01 _c	0.48±0.00 _d	**	0.25±0.01 _a	0.30±0.00 _a	**	10.89±0.23 ^a	10.05±0.02 _a	*
Stomach 0. min	0.68±0.02 _c	0.55±0.01 _c	*	0.22±0.01 _b	0.11±0.00 _e	**	10.25±0.35 ^a	9.78±0.29 ^a	ns
Stomach 60. min	0.54±0.01 _d	0.44±0.02 _e	*	0.11±0.01 _d	0.26±0.01 _b	**	9.87±0.25 ^{bc}	9.73±0.44 ^a	ns
Stomach 120. min	0.92±0.03 _b	0.85±0.00 _b	ns	0.21±0.01 _b	0.03±0.00 _g	**	9.87±0.35 ^{bc}	9.72±0.02 ^a	ns
Small Intestine 0. min	0.03±0.00 _g	1.06±0.04 _a	**	0.05±0.00 _e	0.17±0.00 _d	**	10.68±0.34 ^a	9.78±0.28 ^a	ns
Small Intestine 60. min	0.18±0.00 _e	0.36±0.00 ^f	***	0.03±0.00 ^f	0.09±0.00 ^f	*	9.52±0.25 ^c	8.95±0.13 ^b	ns
Small Intestine 120. min	0.12±0.00 ^f	0.48±0.00 _d	***	0.02±0.00 ^f	0.02±0.00 _g	ns	9.55±0.03 ^c	8.63±0.06 ^b	**
	***	***		***	***		**	**	

GIS: Gastrointestinal System

a-g: Differences between means given with different letters in the same row are statistically significant

*p<0.05, **p<0.01, ***p<0.001, ns: non-significant

As shown in Figure 2, the loss rates of antioxidant activity and analyzed polyphenol compounds were compared between the oral (0 min) and small intestine (120 min) digestion stages for GT and SGT samples. The findings revealed that the digestion stability of the compounds varied among the formulations. In terms of antioxidant activity, approximately 71.9% loss was observed in the GT sample, while 66.2% was observed in the SGT sample. These results indicate that both green tea samples were unable to retain their antioxidant activities to a great extent during *in vitro* digestion; however, the SGT sample showed slightly higher activity. Gallic acid exhibited a very high loss rate (approximately 98%) in both samples, indicating that it was degraded under digestive conditions. In contrast, the level of decrease in naringin levels was approximately 28.0% for the GT sample and 17.6% for the SGT sample. These low values indicate that naringin was significantly resistant during the digestion process. Catechin was lost by 82.4% in the GT sample, while it was only about 9.4% in the SGT sample. This remarkable difference suggests that SGT formulation may increase catechin stability during digestion. Epicatechin was lost by 83% in the GT sample and 91% in the SGT sample. This indicates that both tea samples are labile for Epicatechin, and Epicatechin is largely degraded under simulated gastrointestinal conditions. EGCG was found to be highly stable in digestive conditions in both samples, losing 10.1% in GT and 13.8% in SGT. As a result, the loss of antioxidant activity and polyphenol compounds during digestion varied depending on the compound and formulation. EGCG showed the highest stability, while Gallic acid showed a high loss. SGT showed lower loss rates except for Gallic acid and Epicatechin. These findings suggest that polyphenol stability during digestion is influenced not only by digestion conditions but also by formulation strategies and the type of carrier system employed.

Cruz-Molina et al. (2021) analyzed the content of phenolic compounds in the free extract at pH 6 was primarily epicatechin (68.12%), followed by epigallocatechin gallate (12.38%) and catechin (9.42%), based on the phenolic profiles of free and encapsulated green tea extracts during storage at various pHs. It was reported that no EGC was found at the 30-day storage end. A phenolic molecule may not be useful as an antioxidant or antibacterial agent if it is shown to be unstable during food processing conditions like heating, frying, or microwaving, or if it is present in foods exposed to high pH. For instance, the tea polyphenol epigallocatechin gallate, an ester of gallic acid, should be unstable as epigallocatechin is stable at high pH values while gallic acid is not (Friedman and Jürgens, 2000).

Green tea is an essential component of a healthy lifestyle due to its potent antioxidants and concentration of polyphenolic content. However, the transformation of these beneficial compounds throughout the digestive system has not yet been fully elucidated. Gastrointestinal conditions such as stomach acidity and bile may affect the structure and activity of phenolic substances in green tea. Although human studies are ideal for determining these changes, they are often impractical due to technical, financial and ethical limitations. Although animal models offer an alternative approach, they are generally not preferred due to the difficulty of methods such as surgical interventions. To overcome these challenges, *in vitro* gastrointestinal system models have become increasingly popular because they offer reproducibility and flexibility. The *in vitro* gastrointestinal system model is important in determining how the phenolic content of green tea coated with green tea changes throughout the digestive system. This study aims to understand the chemical transformation of green tea and maltodextrin coated green tea during the digestive process and to use this information to develop more effective consumption strategies for individuals seeking a healthy life. In parallel, recent studies have shown that EGCG, one of the major catechins in green tea, inhibits coronavirus replication by interfering with 3CL-protease activity (Jang et al., 2021). Therefore, understanding its stability and transformation during digestion is crucial for evaluating its biological effectiveness beyond antioxidant capacity.

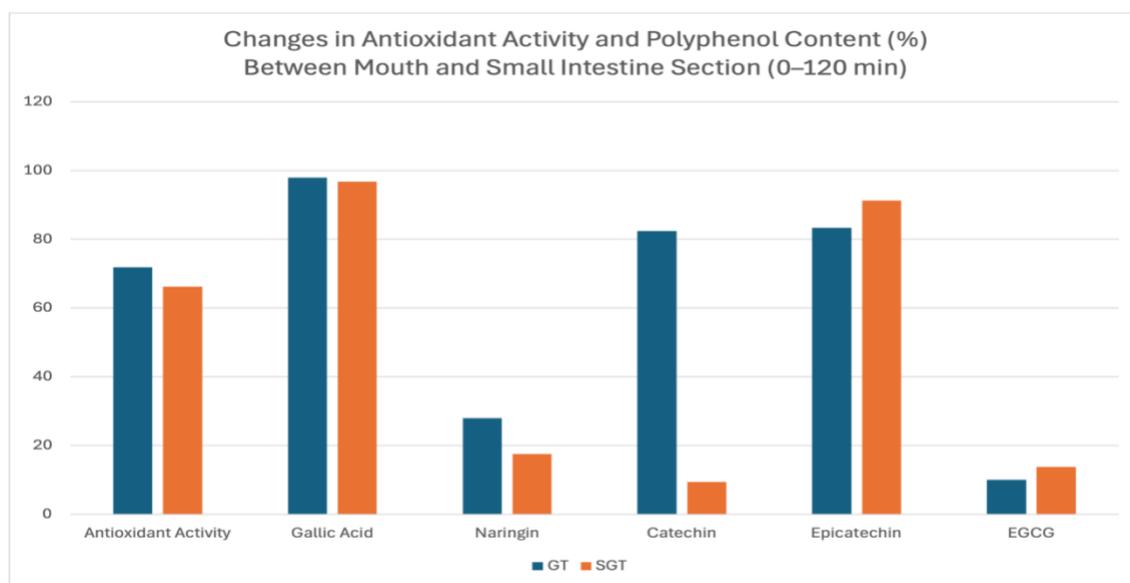


Figure 2. Changes in antioxidant activity and polyphenol content (%) between mouth and small intestine section (0-120 min)

Şekil 2. Ağız ve ince bağırsak bölümü arasında antioksidan aktivite ve polifenol içeriğindeki (%) değişiklikler (0-120 dk)

Results showed that green tea and maltodextrin coated green tea samples showed differences in total phenolic content, antioxidant activity and polyphenolic components throughout the *in vitro* gastrointestinal tract. Although the initial values were generally not statistically significant in both samples, significant changes were observed in different regions of the gastrointestinal tract. Especially in the small intestine stage, total phenolic content and antioxidant activity showed significant changes ($p < 0.05$). In addition, statistically significant changes were found in the amounts of components such as catechin, epicatechin, epigallocatechin gallate and naringin ($p < 0.001$). These findings suggest that maltodextrin coating may influence polyphenol stability and bioavailability and that their behavior in the gastrointestinal tract may differ. Liu et al. (2022) demonstrated that phenolic extracts isolated from tea seed oil exhibited significant antioxidant capacity in both *in vitro* and *in vivo* models. They emphasized that the antioxidant activity was closely related to the structural characteristics and concentration of phenolic compounds. This supports the notion that not only digestive conditions but also the physical form and protective matrices of phenolics (such as maltodextrin coating) can significantly influence their stability and

biological potential during gastrointestinal transit. Moreover, Ma et al. (2022) investigated Pu-erh tea, a fermented dark tea, and found that solid-state fermentation significantly altered the levels of phenolic compounds. While catechins, such as EGCG and EC, decreased markedly after fermentation, compounds like quercetin and kaempferol increased, thereby impacting the antioxidant capacity. These results support the idea that not only digestion conditions but also tea type and processing (fermentation vs. non-fermentation) can substantially affect the chemical stability and biological potential of tea polyphenols throughout the digestive process. This cross-validation highlights the importance of understanding tea matrix transformations under various physiological and processing conditions.

The chemical composition of green tea samples used as raw materials in the studies will vary depending on environmental factors such as the region where it is grown, climate, soil structure and harvest time, which will be a challenge in standardized product production. In addition, the changes that occur throughout the gastrointestinal system do not follow a linear decrease but instead fluctuate. Therefore, further research is needed with different coating materials and/or coating materials that can be used together in a mixture with maltodextrin.

CONCLUSIONS

This study reveals how the changes in the phenolic compound profiles of GT and SGT samples differ throughout the *in vitro* gastrointestinal system. The results show that the maltodextrin coating process may affect the stability and bioavailability of phenolic compounds. When examined for phenolic compounds such as total phenolic acid, gallic acid, catechin, epicatechin, epigallocatechin gallate, and naringin, it is observed that the coating process may be beneficial in preserving some compounds. Nevertheless, their initial concentrations and behavior in the gastrointestinal system may vary significantly with the coating material.

However, the effects of the coating process are particularly pronounced in the stomach and small intestine, highlighting the need to optimize the coating materials to increase the bioavailability of phenolic compounds. Furthermore, the variations in the behavior of different compounds emphasize the importance of understanding the dynamics of individual phenolic compounds in such biological systems.

In conclusion, the effects of maltodextrin coating on polyphenol stability and bioavailability of GT are apparent. This suggests that the behavior of GT in the gastrointestinal system is more complex than expected. The nonlinearity of the changes occurring during the digestive process emphasize the need for further in-depth research in this field. Therefore, future studies should explore a broader range of coating materials and maltodextrin combinations to gain a more comprehensive understanding of green tea component. Such studies will be critical for increasing the bioavailability and more effective use of essential nutrients such as green tea.

Data Availability

Data will be made available upon reasonable request.

Author Contributions

Conception and design of the study: MİP, RP; analysis and interpretation of data: MİP, RP, CK; statistical analysis: RP; visualization: MİP, RP; writing manuscript: MİP, RP, CK.

Conflict of Interest

There is no conflict of interest between the authors in this study.

Ethical Statement

Ethics approval was obtained from the Afyonkarahisar Health Sciences University Non-Interventional Scientific Research Ethics Committee (dated 1 September 2023 and no. 2023/401).

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