Research Article Open Access

Impact of fixation method on catechin profile and antioxidant activity of Georgian green tea

Irakli Chkhikvishvili[1*], Temur Revishvili[2], N. Gogia[1], M. Esiashvili[1], Davit Chkhikvishvili[5], Sydney Whited[6], Danik Martirosyan[7], and Bakhva Dolidze[8]

[1]Institute of Medical Biotechnology, Tbilisi State Medical University, Tbilisi, Georgia; [2]Institute of Tea, Subtropical Crops, and Tea Industry of the Agrarian University of Georgia, Tbilisi, Georgia; [3]Boston University, Boston, MA, USA; [3]Functional Food Institute, San Diego, USA

***Corresponding Author:** Irakli Chkhikvishvili, Ph.D., Doctor of Biological Sciences, Professor, Institute of Medical Biotechnology, Tbilisi State Medical University, 33, Vaja Pshavela av., Tbilisi, 0186, Georgia

Submission Date: May 30th, 2025; **Acceptance Date:** July 21st, 2025; **Publication Date:** July 25th, 2025

Please cite this article as: Chkhikvishvili I., Revishvili T., Gogia N., Esiashvili M., Chkhikvishvili D., Whited S., Martirosyan D., Dolidze B. Impact of fixation method on catechin profile and antioxidant activity of Georgian green tea. *Bioactive Compounds in Health and Disease* 2025; 8(7): 257-268. DOI: https://doi.org/10.31989/bchd.v8i7.1659

ABSTRACT:

Background: Green tea (*Camellia sinensis*) is a widely consumed beverage across the globe. It is known for its health benefits, many of which can be attributed to high content of certain bioactive compounds, such as catechins. One example of a catechin is epigallocatechin gallate (EGCG), which has been found to hold antioxidant and anti-inflammatory properties. In Georgia, green tea is predominantly produced using the roasting method, which may not optimally preserve these beneficial compounds. To optimize the functional properties of Georgian green tea, understanding the impact of different fixation methods on its phytochemical profile and bioactivity is crucial.

Objectives: This study aimed to investigate and compare the total polyphenol (PF) content, individual catechin composition, and antioxidant activity of Georgian green tea using three distinct fixation methods: steaming, electromagnetic induction, and roasting. A secondary objective was to identify effective preservation methods by establishing a correlation between PF content and antioxidant capacity.

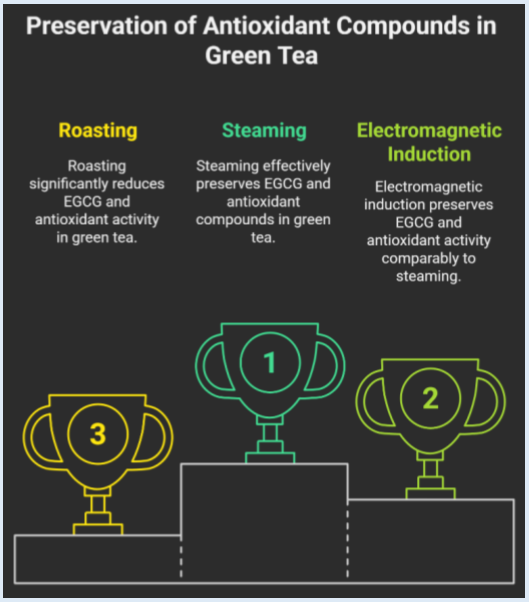
Methods: Green tea samples from a local Georgian variety were prepared using steaming, electromagnetic induction, and roasting fixation methods. Total PF content and antioxidant activity were determined using diphenylpicrylhydrazyl (DPPH) radical scavenging assay with standard methods. Individual catechins were quantified by high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity system with a Supelco-C18 column (25cm × 4.6mm, 5µm) at 35°C, using an acetonitrile and 1% acetic acid gradient mobile phase (10-90% to 20-80% over 20 minutes) and UV detection at 278nm.

Results: HPLC analysis revealed that EGCG content was comparable in green tea fixed by steaming and electromagnetic induction, but significantly higher than in the green tea fixed by roasting. The antioxidant activity of all tea extracts, as measured by the DPPH assay, showed a direct positive correlation with their total PF content. Among the methods, steaming and electromagnetic induction effectively preserved the antioxidant compounds in Georgian green tea, while roasting did not.

Novelty: This research uniquely provides a comparative analysis of three distinct fixation methods, electromagnetic induction, steaming, and roasting, on their effects on the comprehensive phytochemical profile and antioxidant activity of a specific Georgian green tea variety. It identifies electromagnetic induction and steaming as effective alternatives to roasting for preserving key bioactive catechins. Further, this study analyzed five different catechins, all sourced from Georgian green tea extracts, which has not been previously done. Overall, this study offers novel insights for optimizing processing to enhance the functional value of Georgian green tea products.

Conclusion: Green tea produced using electromagnetic induction and steaming fixation methods yields more EGCG and overall catechins than the roasting method. The strong correlation between total PFs and antioxidant activity underscores the importance of fixation in preserving these beneficial compounds. These findings provide valuable insights for the food industry, pharmaceuticals, and cosmetics, suggesting optimized processing methods to enhance the functional benefits of Georgian green tea.

Keywords: green tea, Georgian tea, polyphenols, catechins, antioxidant activity, fixation methods, electromagnetic induction, HPLC



Graphical Abstract: Impact of fixation method on catechin profile and antioxidant activity of Georgian green tea

©FFC 2025. This is an Open Access article distributed under the terms of the Creative Commons Attribution 4.0 License (http://creativecommons.org/licenses/by/4.0)

INTRODUCTION

Green tea is a beverage consumed around the globe, holding cultural significance in diverse geographic areas [15]. It is also associated with numerous, multi-faceted health benefits [1, 15]. These benefits are primarily attributed to its high content of catechins, a group of bioactive compounds which have been noted to treat and prevent chronic diseases and present antiviral properties [12-16]. Some examples of the catechins present in green tea are epicatechin (EC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) [2]. EGCG is the most abundant, so it plays a central role in providing the anti-inflammatory and antioxidant properties of green tea that maintain cellular health and prevent the onset of chronic disease [3-4].

Through these properties, catechins can alleviate cardiovascular, atherosclerotic, and hypertensive diseases. They are also able to reduce harmful cholesterol levels. Green tea consumption has also been linked to anticancer and anti-inflammatory effects through the modulation of NFκB and ERK of signaling pathways, inhibition of caspase-3 activity, preservation of mitochondrial membrane potential, and regulation of pro-apoptotic proteins [2, 5-6]. Furthermore, EGCG has been shown to prevent obesity and metabolic syndrome [7-11]. Furthermore, preclinical studies have shown they offer protective effects against environmental toxins [5].

These therapeutic effects highlight the importance of innovative fixation methods. The fixation process, which halts enzymatic oxidation during tea production, influences the tea's biochemical and antioxidant profiles. In Georgia, green tea is traditionally produced by roasting from local cultivars. However, this technique may not maintain optimal EGCG content, so the final product may exhibit reduced antioxidant capacity.

This study investigates the total polyphenol (PF) and individual catechin content, along with the antioxidant activity, of green tea prepared from Georgian raw materials using three different fixation methods (steaming, roasting, and electromagnetic induction). In order to determine effective preservation methods, a correlation between PF content and antioxidant capacity was established. This correlation provided insights into the functional benefits of the products of each of the fixation methods.

METHODS

Materials: The study used a local Georgian variety of green tea (*Camellia sinensis)***.** The study of total polyphenol content, individual catechin content, and antioxidant activity was conducted with varying methods of fixation: steaming, electromagnetic induction, or roasting. The analyses were conducted using standard techniques with the methods provided in literary sources [17-22]. Fresh, two or three-leafed tea shoots were collected from the experimental plantation, located in Anaseuli, Ozurgeti, Georgia, in July 2023. For shoots fixed with steaming, the following parameters were recorded: 0.5 – 0.6 MPa of pressure, 120 minutes, and 69,5% water content. For shoots fixed with induction, the temperature inside the equipment ranged from 150-155ºC, the fixation time was 180 minutes, and the water content was at 68%. Finally, for shoots fixed with roasting, the cylinder equipment temperature was between 220 - 230^{0}C, the fixation time was 240 minutes, and the water content was 67%. Fixed tea leaves were then processed by drying, so that the final water content was between 60-62%, rolling and shaping for 45-50 minutes, and a final drying to reach a 5% water content. The final drying was conducted at 85-90ºC for 120 - 130 minutes.

Catechin type and content were determined by high-pressure liquid chromatography (HPLC). Chromatographic analysis was performed on an Agilent 1260 Infinity (USA), using a Supelco-C18 chromatographic column (25cm x 4.6mm x 5µm), at a temperature of 35°C. Acetonitrile and 1% acetic acid dissolved in water were used to obtain the mobile phase. The gradient was carried out for 20 minutes, the ratio of acetonitrile and 1% acetic acid ranged from 10%:90% to 20%:80%. Purity of the product was confirmed using a single-signal ultraviolet detector at a wavelength of 278nm

Chemicals and reagents: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was sourced from Sigma-Aldrich (USA), and Folin-Ciocalteu reagent was obtained from Merck (Germany). All other reagents and chemicals used throughout the experiments were of analytical grade.

Sample preparation and extraction: Tea preparation usually involves a one-time distillation. Therefore, no data claims to completely reflect the precise number of compounds present in the pre-processed plant material.

For analysis, we took 0.5 g of each tea sample and added 35 ml of hot water. After waiting between 20 minutes and 2 hours, we extracted 60 µl from each sample. We then determined the antioxidant activity and phenolic compound content using the Folin-Ciocalteu method.

We took 0.5 g of each sample for analysis, added 50 ml of hot water, delayed extraction for 20 minutes, took 10 µl from each sample, and analyzed it on high-pressure chromatography.

Assessment of Total Phenolic Content: The tea extract samples' total phenolic content (TPC) was assessed using the Folin-Ciocalteu method, incorporating slight modifications to the traditional Folin-Denis procedure [19–21]. Gallic acid was used as the calibration standard. To perform the assay, 0.5 mL of the sample extract was combined with 0.5 mL of diluted Folin-Ciocalteu reagent (1:10 ratio with deionized water), followed by adding 1 mL of 20% sodium carbonate (w/v). The volume was adjusted with 8 mL of distilled water. The reaction mixture was allowed to stand at ambient temperature for 30 minutes, with periodic mixing to facilitate color development. Absorbance was recorded at 725nm using a UV-VIS double-beam spectrophotometer (UV Analyst-CT 8200). TPC was quantified using the linear regression equation of the gallic acid standard curve and is reported as milligrams of gallic acid equivalents (GAE) per gram of dry extract.

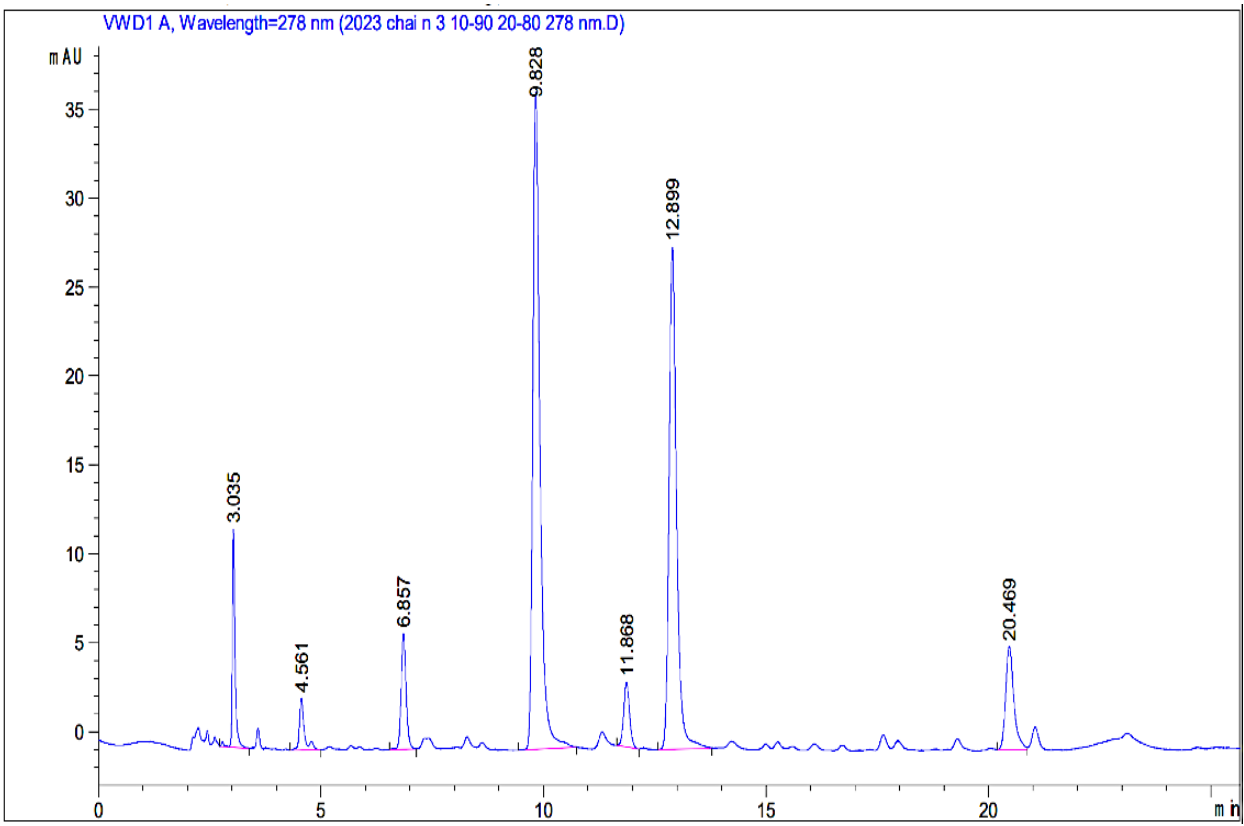
Evaluation of antioxidant capacity using the DPPH method: The antioxidant capacity of the tea samples, as well as a reference solution of ascorbic acid, was evaluated using a DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay as described previously in the literature [22]. The reaction consisted of 2 mL of a 1.0 mM DPPH solution prepared in methanol mixed with 1 mL of either the standard solution (75 µg/mL) or the sample extract. The mixture was gently agitated and incubated in the dark at room temperature for 20 minutes. Absorbance reduction was measured at 515 nm with a UV-Vis spectrophotometer. Gallic acid served as a positive control. A solution containing DPPH and ethanol (1 mL) without extract served as the blank. The percentage of free radical scavenging activity was calculated using the formula:

$$\% \text{ Scavenging} = 100 \times [(A\_control - A\_sample) / A\_control]$$

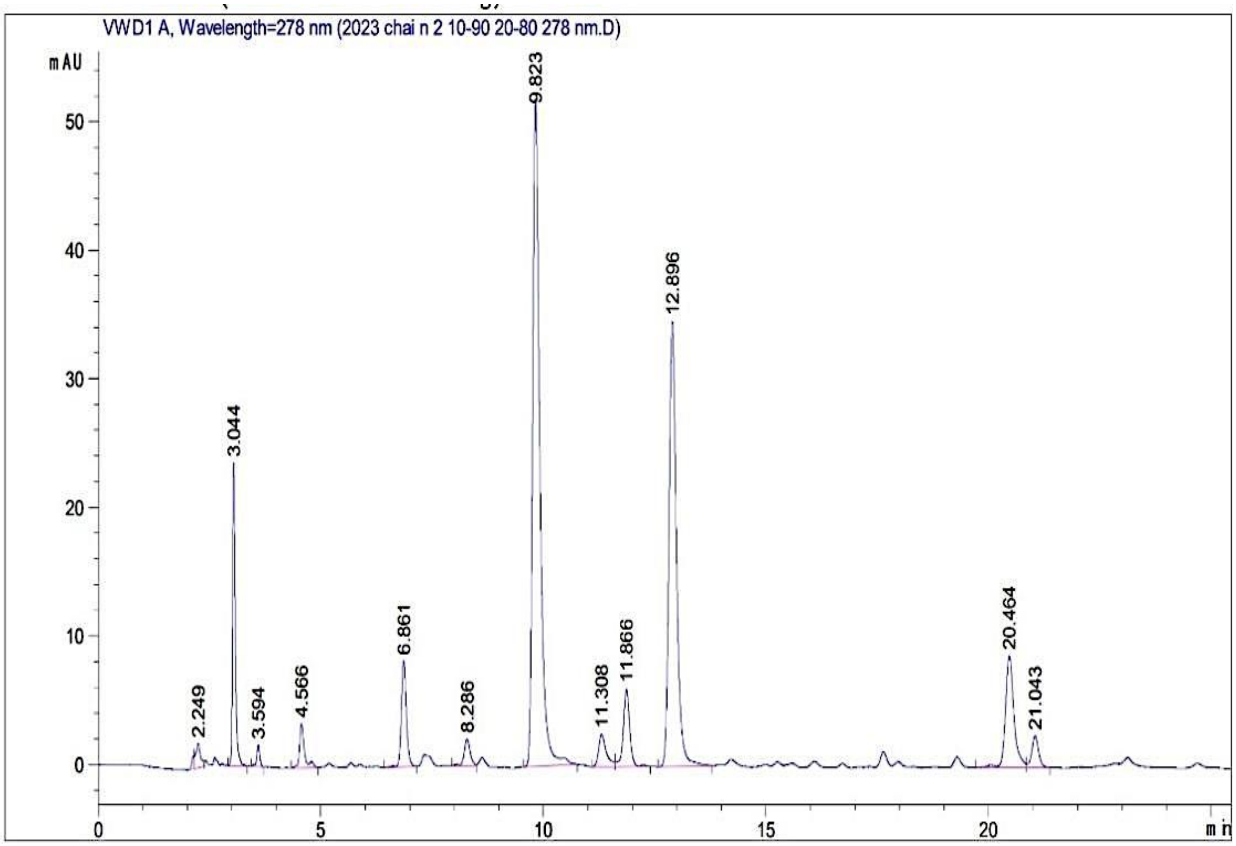
Statistical analysis: The results are expressed as mean ± SEM. The students' t-test was used to analyze the level of statistical significance between the groups. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

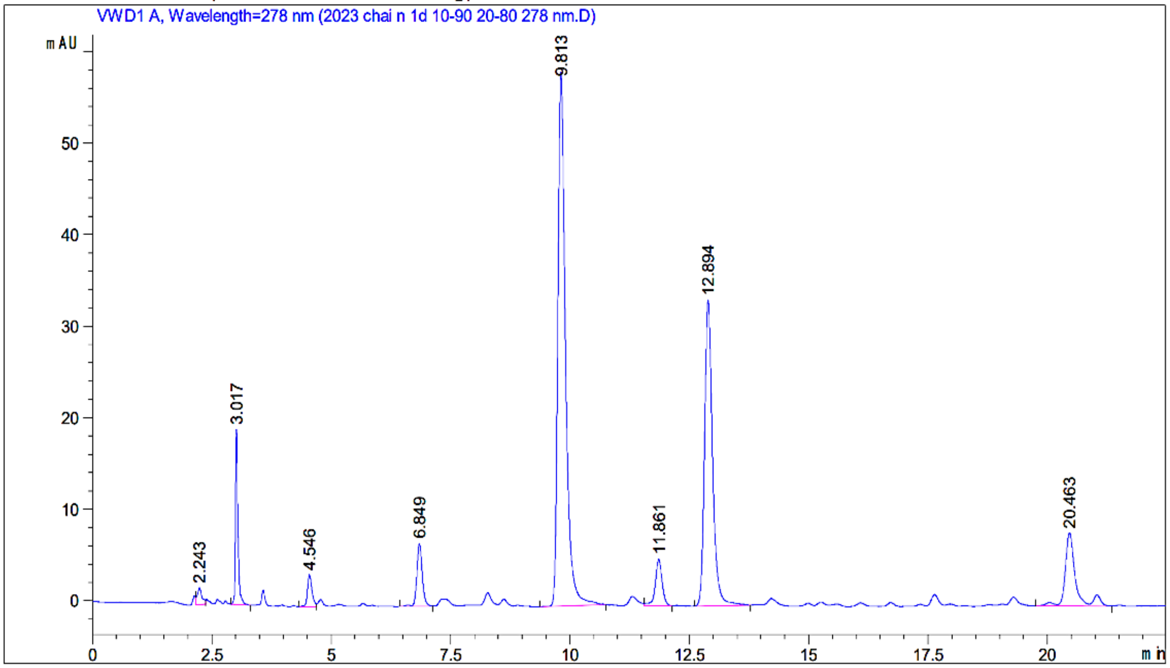
Figure 1 presents the results of the chromatographic separation of catechins from green tea extracts fixed by different methods.



A. Catechin separation in green tea fixed with roasting



B. Catechin separation in green tea fixed with induction



C. Catechin separation in green tea fixed with steaming

Figure 1. Chromatograms of experimental green tea. (A) fixed with roasting; (B) fixed with induction; (C) fixed with steaming.

The chromatograms are shown in Figure 1. They show the 5 major peaks (sample 2) of catechins by time retention (min): catechin – 4.56, epigallocatechin – 6.88, epicatechin – 11.86, EGCG - 12.89, ECG – 20.46, gallic acid – 3.04, and caffeine - 9.82. When interpreting results, it should be noted that our studies were conducted in the first extract of tea leaves, with water as the eluent, since consumers usually use a single extraction of tea. Accordingly, the catechin is relatively low compared to studies that have performed multiple extractions or used a mixture of alcohol and water as the eluent [2].

The chromatographic separation of catechins show that green tea fixed by steaming and induction had higher EGCG levels than the tea obtained by roasting. The quantitative determination of catechins confirms the high total catechins and EGCG content in tea extracts, fixed by steaming and induction. Tea fixed by induction retains simpler catechins and epigallocatechin than tea fixed by steaming (Fig. 2).

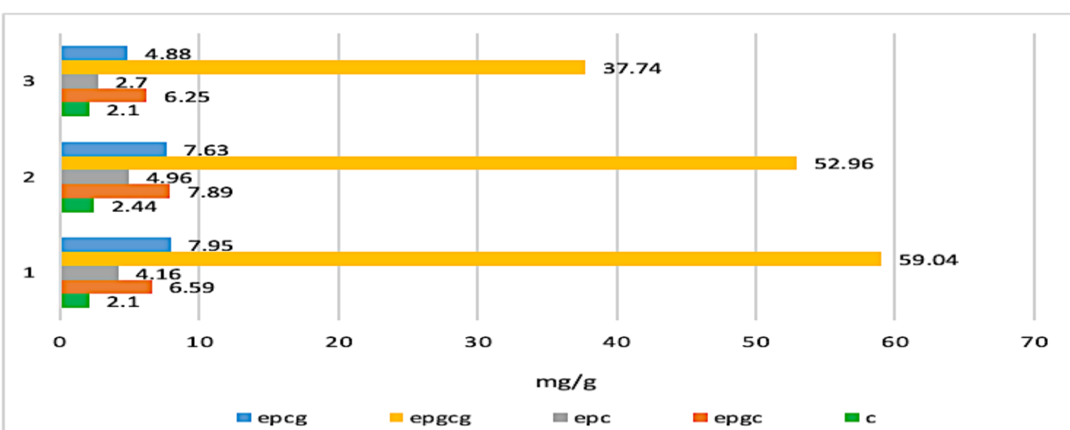


Figure 2. Comparative amounts of five catechins in experimental green tea extracts (mg/g). (1) fixed with steaming; (2) fixed with electromagnetic induction; (3) fixed with roasting. Abbreviations: epcg, ECG; epgcg, EGCG; epc, epicatechin; epgc, epigallocatechin; c, catechin.

It should be noted that with a slightly low amount of catechins in the fixation of tea leaves by electromagnetic induction, the amount of individual simple catechins: epicatechin, catechin, epigallocatechin is greater than when fixing with steam (Table 1).

Table 1. The amount of catechins in % in the first aqueous extract of tea leaves using different fixation methods.

Catechins	Catechin content in dry tea leaves (%)		
	Fixed with roasting	Fixed with electromagnetic induction	Fixed with steaming
Epigallocatechin gallate	3,7 ± 0.05	5,2 ± 0.06	5,90 ± 0.07
Epicatechin	0,27 ± 0.01	0,49 ± 0.02	0,41 ± 0.02
Catechin	0,21 ± 0.01	0,24 ± 0.01	0,21± 0.01
Epigallocatechin	0,62 ± 0.02	0,78 ± 0.03	0,65 ± 0.02
Epicatechin gallate	0,48 ± 0.01	0,76 ± 0.02	0,79 ± 0.03
Total catechins	5,28 ± 0.08	7,48 ± 0.1	7, 98 ± 0.11

The research results also show a direct correlation between the antioxidant activities of the studied tea extracts and the total amount of polyphenols. According to these indicators, the sample of green tea with fixed steam (1) stands out, slightly inferior to green tea fixed with electromagnetic induction (Fig. 3).

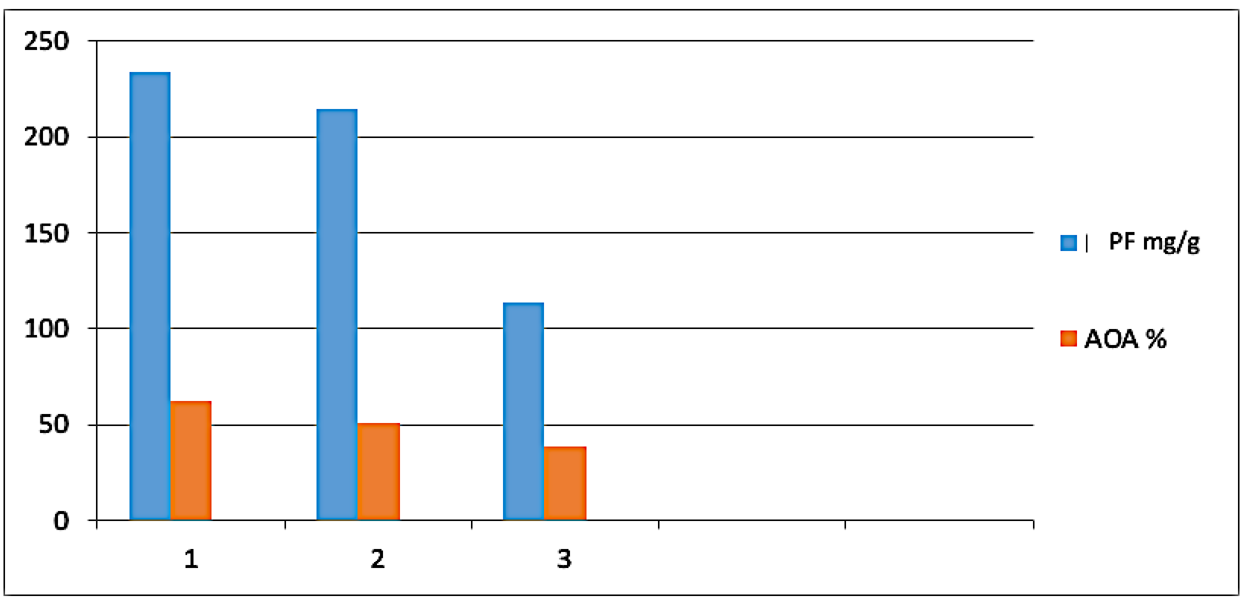


Figure 3. Total amount of polyphenols (PF), mg/g and antioxidant activity (AOA) %. (1) fixed with steaming; (2) fixed with induction; (3) fixed with roasting.

This study offers valuable research on fixation methods for preserving Georgian green tea, specifically comparing steaming, electromagnetic induction, and roasting methods. The polyphenol and catechin compositions were evaluated using HPLC and DPPH radical scavenging assays for each fixation technique [23-26]. The results indicated that steaming and electromagnetic induction better conserve the amount of bioactive compounds in green tea leaves over roasting, highlighting the role of fixation in preserving or destroying these compounds through enzymatic oxidation.

Moreover, the HPLC chromatograms displayed notably higher peaks in the steaming and electromagnetic induction methods than in roasting. This was affirmed by the findings of Zhu et al. (2021), who concluded that the roasting method reduces flavonoid and catechin content due to thermal degradation, despite enhancing the aroma profile [25]. Supporting this, Gulua et al. (2018) concluded that Georgian green tea has a high antioxidant activity when prepared with minimal thermal processing [26].

Furthermore, the DPPH radical scavenging assay directly correlated PF content and antioxidant activity. Preservation of PF allows green tea to reduce oxidative stress, modulate inflammation, reduce insulin resistance, and maintain lipid metabolism within the body [14, 26]. Moreover, high levels of EGCG content have shown promising effects in oral health, specifically periodontal diseases, due to its antibacterial and anti-inflammatory properties [27].

Steaming and electromagnetic induction provide green, energy-efficient methods for processing green tea. These methods offer sustainable and economically feasible applications that may be utilized in various industries. As noted in a previous study, Georgian green tea producers often face challenges with decreased production of tea products and abandoned plantations [28]. Therefore, introducing stronger fixation methods could benefit Georgia's economy and tea industry.

Functional Food Center's evaluation of Georgian green tea as a potential functional food product: The Functional Food Center (FFC) has pioneered a structured approach for evaluating functional foods, guided by standardized criteria and grounded in scientific evidence. This regulatory methodology involves a multi-phase process emphasizing candidate functional foods' scientific validity and health relevance [29]. Under the direction of Dr. Martirosyan, the FFC introduced a transformative definition of functional foods [30] and developed an integrated framework that includes key principles and strategic steps for their development and market readiness [31–32]. According to the FFC, functional foods are defined as: "Natural or processed foods that contain biologically active compounds, which, in defined, effective, non-toxic amounts, provide a clinically proven and documented health benefit utilizing specific biomarkers, to promote optimal health and reduce the risk of chronic/viral diseases and manage their symptoms" [30]. This science-based model provides a robust foundation for evaluating food products by assessing their bioactive components, clinical efficacy, safety profile, and mechanisms of action. Using this framework, the FFC has assessed numerous food items to determine whether they meet the criteria for functional foods. For those that do not yet qualify, the model outlines actionable steps for further development and validation [32–38]. This systematic, evidence-driven approach strengthens the scientific credibility of functional food classification and promotes the advancement of validated products in the global market.

According to the FFC's evaluation system, Georgian green tea demonstrates many health-promoting and functional properties. This study offers a novel analysis of Georgian green tea processed using three different fixation methods: steaming, electromagnetic induction, and roasting. Each method distinctly influences the polyphenol content, catechin composition, and antioxidant activity, assessed through HPLC analysis, which has not previously been applied to this tea variety. Notably, this research highlights electromagnetic induction as a promising new technique for optimizing catechin and EGCG levels, known for their positive health effects. Furthermore, the study includes a quantitative correlation analysis between antioxidant activity and polyphenol content, providing concrete evidence of Georgian green tea's functional qualities.

Despite the considerable health and economic potential of efficiently processed Georgian green tea, human clinical trials examining its bioavailability and long-term use remain limited [23]. Given its cultural and nutritional significance, Georgian green tea must be sustainably sourced to preserve heritage while maximizing its functional potential. Optimized fixation methods that retain high antioxidant levels may offer medical and cultural benefits, ultimately enhancing consumer health.

To understand the implications of prolonged consumption of Georgian green tea, oral bioavailability and possible drug interactions must be also evaluated. For example, there are potential interactions between *Camellia sinensis* and alprazolam, a commonly prescribed benzodiazepine for anxiety disorders [30]. The caffeine and catechins in green tea may alter the pharmacokinetics of alprazolam, affecting its metabolism, absorption, and excretion [30]. This interaction is believed to occur through the modulation of the CYP3A4 enzyme, a member of the cytochrome P450 family, which is involved in alprazolam metabolism. As a result, excessive green tea consumption may increase plasma levels of alprazolam, raising the risk of adverse effects like fatigue or impaired motor function [30]. While clinical trials are needed to confirm this interaction, anecdotal reports from patients and healthcare providers have raised valid concerns about concurrent green tea and alprazolam use.

Scientific innovation: This study introduces a significant scientific innovation by conducting a comprehensive comparative analysis of three distinct green tea fixation methods—steaming, roasting, and, notably, electromagnetic induction—on a specific Georgian variety of green tea. The unique contribution of this study lies in identifying electromagnetic induction as an effective alternative to traditional steaming for preserving key bioactive catechins, particularly EGCG, and maintaining high antioxidant activity. This comparative approach provides novel insights into the nuanced effects of different processing technologies on the phytochemical profile and functional properties of green tea, advancing the understanding of optimal preservation techniques for maximizing therapeutic value. These approaches may be utilized across industries.

Practical implications: The findings of this research hold substantial practical implications across several industries. For the food industry, identifying electromagnetic induction as a superior or comparable method to steaming offers a pathway for optimizing green tea processing to produce edible products with enhanced functional value and antioxidant benefits. Georgian green tea could also influence product development in the pharmaceutical and cosmetic industries, where antioxidant-rich ingredients are highly sought. Regardless of industry, these optimized processing methods can contribute to a more efficient and effective utilization of green tea raw materials, ultimately benefiting producers and consumers by delivering a product with maximized health-promoting compounds.

CONCLUSION

The study of catechins in experimental green tea extracts using the HPLC method showed that increased levels of EGCG remained in green tea produced using the electromagnetic induction and steaming methods, more so than the product made by the roasting process. Green tea obtained from fixation by electromagnetic induction has many total and simple catechins, with EGCG as the dominant catechin. The antioxidant activity of green tea extracts is directly correlated with the total amount of PFs.

List of Abbreviations: EGCG: epigallocatechin gallate; ECG: Epicatechin Gallate; EC: Epicatechin; HPLC: high-performance liquid chromatography; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; CYP3A4: Cytochrome P450 3A4 Enzyme; PF: Polyphenol; GAE: gallic acid equivalent; FFC: Functional Food Center

Acknowledgement and Funding: There was no external funding supporting this publication.

Conflict of Interest: The authors declare no conflicts of interest.

Author Contributions: IC and TR advised on the study, contributed to its conceptualization by focusing on the antioxidant activity of Georgian green tea, and provided guidance throughout the experimental process. TR was responsible for the selection, fixation, and processing of the tea samples, including applying the electromagnetic induction fixation method used for analysis. NG, ME, and DC measured the total polyphenol content and antioxidant activity in green tea samples processed by steaming, induction, and roasting. DC and IC conducted catechin analysis using HPLC and quantified five key catechins in the green tea extracts. TR and IC led the research execution, data collection, and figure preparation, and contributed to result interpretation and discussion. DM contributed to the writing and editing of the manuscript and summarized the research's scientific innovations and practical implications. SW assisted in drafting the manuscript, organized the literature review, and proofread the final version. BD advised on the study, oversaw the experimental phase, and contributed to interpreting the results.

REFERENCES

1. Beglaryan I, Gasparyan G, Khachatryan S, Yeritsyan S, Eloyan A, Jhangiryan T. The change in functional components in mulberry fruits (Morus alba L.) through the development of an optimal land transformation scheme for different land types. Bioact Compd Health Dis. 2025;8(3):113–29. DOI: https://doi.org/10.31989/bchd.8i3.1583
2. Capasso L, De Masi L, Sirignano C, Maresca V, Basile A, Nebbioso A, et al. Epigallocatechin Gallate (EGCG): Pharmacological Properties, Biological Activities and Therapeutic Potential. Molecules. 2025;30(3):654. DOI: https://doi.org/10.3390/molecules30030654
3. Chen L, Mo H, Zhao L, Zhao W, Yang L, Zhang M, et al. Therapeutic properties of green tea against environmental insults. J Nutr Biochem. 2017; 40:1–13. DOI: https://doi.org/10.1016/j.jnutbio.2016.05.005
4. Chkhikvishvili I, Revishvili T, Apkhazava D, et al. Catechins and antioxidant activity of tea products. Ann Agrar Sci. 2021;19(1):39–45.
5. Dutka P, Martirosyan D. The effectiveness of resveratrol in the management of childhood obesity. Funct Food Sci. 2023;3(10):193–216. DOI: https://doi.org/10.31989/ffs.v3i10.1244
6. Fu JF, Wang PP, Fu X, Tan CP, Chen C, Peng YP. Dietary Polyphenols in Obesity Management: Critical Insights on Health Benefits and Mechanisms of Action. J Food Sci. 2025;90: e70267. DOI: https://doi.org/10.1111/1750-3841.70267
7. G, Khalil M, Di Luca A, Abdallah H, D'Alessandro AG. Comprehensive Strategies for Metabolic Syndrome: How Nutrition, Dietary Polyphenols, Physical Activity, and Lifestyle Modifications Address Diabesity, Cardiovascular Diseases, and Neurodegenerative Conditions. Metabolites. 2024;14(6):327. DOI: https://doi.org/10.3390/metabo14060327
8. Gulua L, Nikolaishvili L, Jgenti M, Turmanidze T, Dzneladze G. Polyphenol content, anti-lipase and antioxidant activity of teas made in Georgia. Ann Agrar Sci. 2018;16(3):357–61. DOI: https://doi.org/10.1016/j.aasci.2018.06.006
9. He P, Cheng Q, Chen J, Wang Y, Du M, Lin X, et al. Green Tea Epigallocatechin-3-gallate Ameliorates Lipid Accumulation and Obesity-Associated Metabolic Syndrome via Regulating Autophagy and Lipolysis in Preadipocytes and Adipose Tissue. J Agric Food Chem. 2025;73(20):12272–91. DOI: https://doi.org/10.1021/acs.jafc.5c00973
10. International Organization for Standardization. ISO 14502-1: Determination of Substances Characteristic of Green and Black Tea—Part 1: Content of Total Polyphenols in Tea—Colorimetric Method Using Folin-Ciocalteu Reagent. Geneva: ISO; 2005.
11. International Organization for Standardization. ISO 14502-2: Determination of Substances Characteristic of Green and Black Tea—Part 2: Content of Catechins in Green Tea—Method Using High-Performance Liquid Chromatography. Geneva: ISO; 2005.
12. Kim SN, Kwon HJ, Akindehin S, Jeong HW, Lee YH. Effects of epigallocatechin-3-gallate on autophagic lipolysis in adipocytes. Nutrients. 2017;9(7):680. DOI: https://doi.org/10.3390/nu9070680
13. Kupina S, Fields C, Roman MC, Brunelle SL. Determination of total phenolic content using the Folin-C assay: single-laboratory validation, first action 2017.13. J AOAC Int. 2018;101(5):1466–72. DOI: https://doi.org/10.5740/jaoacint.18-0031

14. Lacerda UV, da Costa CVP, Cardoso RR, D'Almeida CTdS, do Carmo MAV, Lima AdS, et al. Antioxidant, Antiproliferative, Antibacterial, and Antimalarial Effects of Phenolic-Rich Green Tea Kombucha. Beverages. 2025;11(1):7. DOI: https://doi.org/10.3390/beverages11010007
15. Lee H, Kim H, Byun S. A review on the effect of green tea extract against obesity. Food Sci Biotechnol. 2025; 34:1661–78. DOI: https://doi.org/10.1007/s10068-024-01778-8
16. Marecek S, Martirosyan D. An assessment of clinical trials used in functional food science. Funct Foods Health Dis. 2023;13(2):22–35. DOI: https://doi.org/10.31989/ffhd.v13i2.1077
17. Martirosyan DM, Sanchez SS. Quantum Theory of Functional Food Science: Establishment of dosage of bioactive compounds in functional food products. Funct Food Sci. 2022;3(2):79–93. DOI: https://doi.org/10.31989/ffs.v2i3.915
18. Momose Y, Maeda-Yamamoto M, Nabetani H. Systematic review of green tea epigallocatechin gallate in reducing low-density lipoprotein cholesterol levels of humans. Int J Food Sci Nutr. 2016;67(6):606–13. DOI: https://doi.org/10.1080/09637486.2016.1196655
19. Ohishi T, Fukutomi R, Shoji Y, Goto S, Isemura M. The Beneficial Effects of Principal Polyphenols from Green Tea, Coffee, Wine, and Curry on Obesity. Molecules. 2021;26(2):453. DOI: https://doi.org/10.3390/molecules26020453
20. Paczkowska-Walendowska M, Grzegorzewski J, Kwiatek J, Leśna M, Cielecka-Piontek J. Green Tea: A Novel Perspective on the Traditional Plant's Potential in Managing Periodontal Diseases. Pharmaceuticals. 2025;18(3):409. DOI: https://doi.org/10.3390/ph18030409
21. Pilevar Z, Martirosyan D, Ranaei V, Taghizadeh M, Balasjin NM, Ferdousi R, et al. Biological activities, chemical and bioactive compounds of Echinophora platyloba DC: A systematic review. Bioact Compd Health Dis. 2024;7(2):95–109. DOI: https://doi.org/10.31989/bchd.v7i2.1283
22. Radeva-Ilieva M, Stoeva S, Hvarchanova N, Georgiev KD. Green Tea: Current Knowledge and Issues. Foods. 2025;14(5):745. DOI: https://doi.org/10.3390/foods14050745
23. Randisi F, Perletti G, Marras E, Gariboldi MB. Green Tea Components: In Vitro and In Vivo Evidence for Their Anticancer Potential in Colon Cancer. Cancers. 2025;17(4):623. DOI: https://doi.org/10.3390/cancers17040623
24. Revishvili T, Dolidze B, Andghuladze Z, Gobronidze E, Zhvania L. New energy-saving technology of green tea. Bull Georg Natl Acad Sci. 2023;17(4):111–7.
25. Rodwal I, Shinwari HR, Naizi MH, Sherzada H, Gopi M. Lactoferrin: Structure, biological functions as functional food and maintaining health. Funct Food Sci. 2024;4(12):495–507. DOI: https://doi.org/10.31989/ffs.v4i12.1407
26. Shanbo Z, Xinyao L, Yiwen X, Ee KH, Goh RMV, Huang Y, et al. A systematic approach to analyzing catechins and catechin derivatives in Ceylon black tea using liquid chromatography coupled with triple quadrupole mass spectrometry. Food Chem X. 2025; 28:102621.
27. Son J, Martirosyan D. Salient Features for GRAS Status Affirmation. Funct Food Sci. 2024;4(8):299–308. DOI: https://doi.org/10.31989/ffs.v4i8.1417
28. Srinivasa DGB, Kadiri SK. Interaction Risk: Green Tea Consumption in Patients Taking Alprazolam. Drug Metab Bioanal Lett. 2024;17(3):104–13. DOI: https://doi.org/10.2174/0118723128366248250206081121
29. Tsetskhladze M, Sulaberidze S. Green tea business in Georgia: Case of Guria region. BIO Web Conf. 2024; 114:01014. DOI: https://doi.org/10.1051/bioconf/202411401014
30. Ugoeze KC, Odeku OA. Antioxidants in Infectious Disease Management. In: Sindhu RK, Singh I, Babu MA, editors. Chapter 6. 2024. DOI: https://doi.org/10.1002/9781394270576.ch6
31. Wang T, Xu H, Wu S, Guo Y, Zhao G, Wang D. Mechanisms underlying the effects of the green tea polyphenol EGCG in sarcopenia prevention and management. J Agric Food Chem. 2023;71(25):9609–27. DOI: https://doi.org/10.1021/acs.jafc.3c02023
32. Wjbphs.com. Polyphenol content, antioxidant and anti-inflammatory activities of Georgian wine and green tea. 2025 [cited 2025 May 19]. Available from: https://wjbphs.com/content/polyphenol-content-antioxidant-and-anti-inflammatory-activities-georgian-wine-and-green-tea
33. Wu G, Wang M, Du Z, Li Z, Han T, Xie Z, Gu W. Tea polyphenol EGCG enhances the improvements of calorie restriction on hepatic steatosis and obesity while reducing its adverse outcomes in obese rats. Phytomedicine. 2025;141:156744. Available from: https://www.elsevier.com/locate/phymed
34. Yargatti R, Deshmane AR. Sensory impacts and consumer acceptability of using dates as an alternative functional sweetener in traditional Indian sweets. Funct Food Sci. 2025;5(1):30–45. DOI: https://doi.org/10.31989/ffs.v5i2.1522

35. Zhang SZ, Long X, Xing Y, Ee KH, Goh RMV, Huang Y, et al. A systematic approach to analyzing catechins and catechin derivatives in Ceylon black tea using liquid chromatography coupled with triple quadrupole mass spectrometry. Food Chem X. 2025; 28:102621.

36. Zhou JV, Martirosyan D. Functional foods for cholesterol management: A comparison between the United States and Japan. Funct Food Sci. 2024;4(6):228–50. DOI: https://doi.org/10.31989/ffs.v4i6.13

37. Zhu YM, Dong JJ, Jin J, et al. Roasting process shaping the chemical profile of roasted green tea and the association with aroma features. Food Chem. 2021; 353:129428. DOI: https://doi.org/10.1016/j.foodchem.2021.129428

38. Kwásny D, Borczak B, Zagrodzki P, Kapusta-Duch J, Prochownik E, Doskočil I. Antioxidant Activity, Total Polyphenol Content, and Cytotoxicity of Various Types of Starch with the Addition of Different Polyphenols. Molecules. 2025;30(11):2458. DOI: https://doi.org/10.3390/molecules30112458