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# Effects of fixation methods on quality parameters of green tea

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## Summary

Thermal processes play an extremely important role in the formation of quality indicators of green tea. The main technological process is fixation, which can be carried out by various methods: roasting, steaming, hot air and electrophysical methods. Fixation is essential in green tea processing to inactivate the polyphenol oxidase enzyme. In Georgia, green tea is mainly made from local plant populations using the roasting method. Making green tea is an energy intensive process. The paper presents the results of research on the influence of fixation methods on quality, chemical and safety parameters of green tea. The results of the study of catechins in experimental green tea extracts by high-performance liquid chromatography (HPLC) showed that epigallocatechin gallate is equally present in green tea fixed by electromagnetic induction(Georgian patent 7427 B. 2022) and evaporation, and is superior to the product obtained by the roasting method. The antioxidant activity of tea extracts is in direct correlation with the total amount of polyphenols. According to the results of research, toxic elements: lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), copper (Cu), as well as: zinc (Zn), manganese (Mn), iron (Fe) absorption spectrometer (AAS 6 000), confirmed the high level of safety of Georgian tea in accordance with established standards.

**Key words**: green tea, fixation, energy saving technology, quality indicators

## Introduction

Green tea is produced from tender shoots and leaves of Camelia sinensis (L) O. Kuntze using special technological processes of fixing, pre-drying, rolling and drying. According to its chemical composition, pharmacological action and physiological activity, green tea is a more valuable and healing product than other types of products, due to the high content of polyphenols and catechins in it [1]. The optimal balance of the total amount of polyphenols, catechins and other quality-determining compounds in the tea leaves creates the best conditions for the maximum manifestation of the qualities of Georgian green tea [2]. Tea production is one of the most energy-intensive industries in the agricultural sector. In the cost of finished tea, a significant part, about 25% constitutes energy costs. Reducing energy costs and the cost of tea products is an important objective, which is indeed possible as a result of the use of alternative energy sources and innovative technologies.

In the formation of quality indicators of green tea, the role of thermal processes is extremely important. The purpose of the main technological process of fixation is to inactivate oxidative enzymes and fix the chemical compounds contained in the leaf in its original state, making the leaf elastic and eliminating the smell characteristic of raw tea. The fixation of raw materials is carried out in various ways: frying, steaming, humid hot air and electrophysical methods (high frequency current, infrared energy, electromagnetic heating). The essence of the technological processes of green tea, with its thermochemical basis, is the same in all tea-producing countries, the difference lies in the methods of processing, technological methods and equipment [3, 4].

Polyphenols, primarily catechins, shape the most important taste properties and biological activity of green tea. The comprehensive studies showed the high P-vitaminsactivity, antioxidant,anti-inflammatory, antimicrobial, antiviral, anticancer and anticoagulant activities of these substances. The ability of catechins shows to alleviate cardiovascular, atherosclerotic, and hypertensive diseases, to reduce harmful levels of cholesterol in the body. The health benefits of green tea are significantly determined by the positive effects and amount of epigallocatechin gallate. Tea consumption correlates with a low incidence of cardiovascular disease and cancer. Green tea epigallocatechin gallate has the pronounced ability to prevent obesity and metabolic syndrome [5 - 15].Tea consumption can play an important role in providing the human body with antioxidants. The antioxidant activity of tea is largely shaped by phenolic compounds and individual catechins, the amount and ratio of which depend on the plant variety, environmental conditions, the period of raw material production, the technological process and some other factors. Quality control of green tea involves determining the water extract, the total amount of polyphenols and catechins, and other compounds.In addition, the essential trace elements in humans can be supplemented through drinking tea because tea leaves contain potassium, manganese, selenium, boron, zinc, strontium, and copper [16].

The purpose of the work is to study the influence of fixation methods on the quality, chemical and safety indicators of green tea.

## Materials and Methods

The object of the study is an energy-saving device for fixing a tea leaf, created at the Institute of Tea, Subtropical Crops and the Tea Industry of the Agricultural University of Georgia (Georgian patent 7427 B. 2022) and a new green tea technology developed on its basis[17,18].During the experiments, for the comparative analysis of the obtained data, the existing methods and devices for heat treatment of tea with a traditional heat source, and an electric heater - a control option were used [19].The study of polyphenols and individual catechins was conducted in the following tea samples made by different methods of fixation: 1 - fixed with steaming, 2 - fixed with induction, 3 - fixed with roasting. The analyseswere conducted usingstandard methods and the methods provided in literary sources [20-25].Catechins were determined by high pressure liquid chromatography. Chromatographic analysis was performed on a high-pressure liquid chromatograph Agilent 1260 Infinity (USA), using a Supelco-C18 chromatographic column (25 cm × 4.6 mm, 5 μm), at a temperature of 35°C. To obtain the mobile phase, acetonitrile and 1% acetic acid dissolved in water were used, the gradient was carried out for 20 minutes, the ratio of acetonitrile and 1% acetic acid was from 10-90% to 20-80%, the detection of substances was carried out using a single-signal ultraviolet detector at a wavelength of 278 nm .

From raw materials produced in a different ecosystem, 70 m and 165 m above sea level (respectively, Likhauri and Anaseuli, Ozurgeti municipality), toxic elements were determined in 2 samples (Li and An) of green tea made with new technology: lead (Pb), arsenic (As ), cadmium (Cd), mercury (Hg) and copper (Cu)ю The analyses were performed using an Atomic Absorption Spectrometer (AAS 6 000) in accordance with the requirements for the quality and safety of food raw materials and food products.Zinc (Zn), manganese (Mn) and iron (Fe) have also been studied [16, 26].

***Chemicals.*** 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich USA. Folin-Ciocalteu reagent was obtained from Merck, Germany. All other chemicals used in the study were of analytical grade.

**Sample preparation and extraction**. Since setting up and consuming tea usually involves a one-time distillation, it should be noted from the beginning, Therefore, the data does not claim to be a complete reflection of the number of compounds present in the plant material.

For analysis, we took 0.5 g of each sample, added 35 ml of hot water, delayed extraction from 20 minutes to 2 hours, and took 60 μl from each sample and determined the antioxidant activity and fenolic compounds of Folin-Ciocalteu method.

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

***Determination of total phenolic content*** The total phenolic content of the tea fractions extracts were determined by using Folin-Ciocalteu reagent following a slightly modified method of Folin-Denis [8]. Gallic acid was used as a reference standard for plotting calibration curve. A volume of 0.5 mL of the tea extract was mixed with 0,5 mL of the Folin-Ciocalteu reagent (diluted 1:10 with de-ionized water) and were neutralized with 1 mL of sodium carbonate solution (20%, w/v) and was added 8mL DD water. The reaction mixture was incubated at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting blue color was measured at 725 nm using double beam UV-VIS spectrophotometer (UV Analyst-CT 8200). The total phenolic contents were determined from the linear equation of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract.

***Determination of antioxidant activity by DPPH- scavenging assay.*** The free radical scavenging activity of the tea extracts, also of the standard solution (ascorbic acid) were investigated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method as reported in the literature [8]. The assay mixture contained 2 mL of 1.0 mmol/L DPPH radical solution prepared in methanol and 1 mL of standard ( in concentration 75 µg/mL) or extract solutions. The solution was rapidly mixed and incubated in dark at room temperature for 20 min. The decrease in absorbance of each solution was measured at 515 nm using UV/Vis spectrophotomer. Gallic acid, a well known antioxidant was used as positive control while DPPH radical solution with 1 mL ethanol was taken as blank. The percentage of radical scavenging (%) was calculated by the following formula: % Free radical scavenging activity = 100 X [Absorbance of control - Absorbance of sample]/ Absorbance of control

***Statistical analysis*** The results are expressed as mean±SEM. Student's t-test was used to analyze level of statistical significance between groups. $P<0.05$ was considered statistically significant.

## Results and Discussion

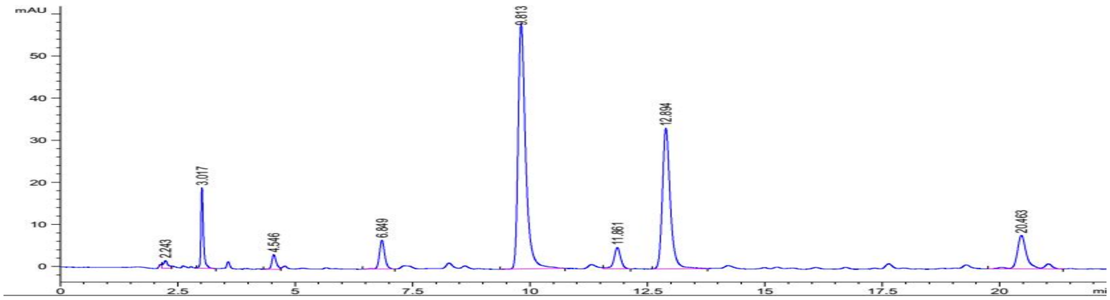
Table 1 shows the results of the study of the influence of fixation methods on the quality and chemical parameters of green tea.

The results of a comparative study of methods for fixing tea leaves showed that in terms of basic technological and technical indicators, the induction method is superior to the use of a device with an electric heater. At the same time, it should be especially noted the possibility of significant savings in energy costs for the fixation process[19].]. As a result of more intensive destruction of chlorophyll, under the conditions of a new technological process, the smell and taste of fresh greens is destroyed, the tea leaf acquires an olive-green color, which characterizes the quality of green tea. Changes are taking place in the composition of amino acids and polyphenols.

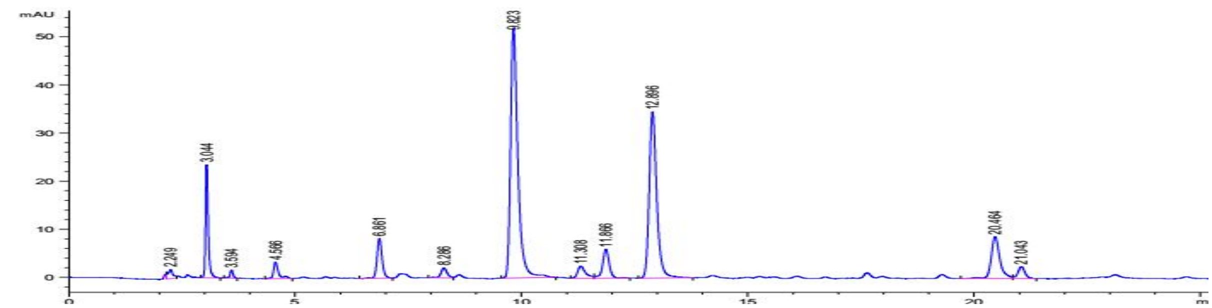
Table 1. Effects of fixation methods on the quality and chemical parameters of green tea

| Name of indicator | Tea leaf fixation method and equipment | |
|---|---|---|
| | Patent GEP 2022 7427 B (Experimental) | Industrial equipment (Control) |
| Water extract, % | 38,2 | 37,8 |
| Total polyphenols, % | 18,9 | 18,5 |
| Caffeine,% | 2,58 | 2,54 |
| Amino acids, mg/100 g | 1,41 | 1,32 |
| Chlorophylls,µg/mgµ | 2,1 | 2,3 |
| Organoleptic indicators:<br>- aroma and taste<br><br>- liquor | Subtle delicate aroma, pleasant taste with astringency<br>Transparent, light green, with a yellowish tinge | Delicate aroma, pleasant taste with astringency<br><br>Transparent, light green |

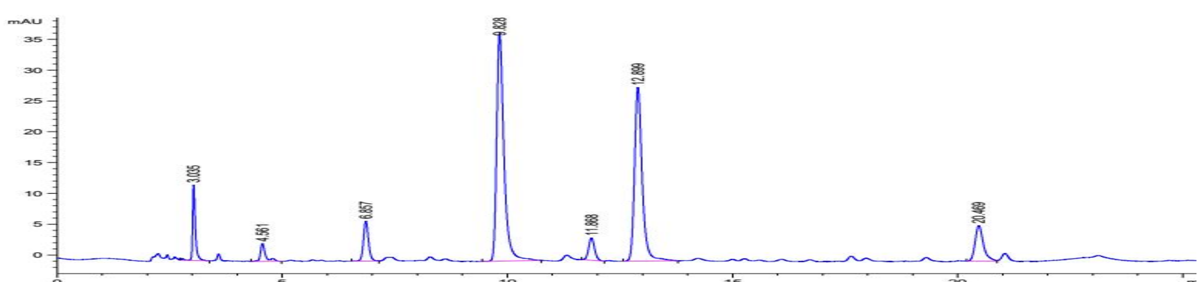
Figure 1. presents the results of the chromatographic separation of catechins from green tea extracts fixed by different methods.




1 - fixed with steaming




2 - fixed with induction




3 - fixed with roasting

Fig. 1. Chromatograms of experimental green tea

1 - fixed with steaming;2 - fixed with induction;3 - fixed with roasting.

The chromatograms shown in Figure 3. show the 5 major peaks (sample 2) of catechins by time retention (min): catechin – 4,56, epigallocatechin – 6,88, epicatechin – 1,.86, epigallocatechin gallate - 12.89, epicatechinal – 20,46 (gallic acid–3,04 and caffeine-9,82).

The results of the study of chromatographic separation of catechins show that the peak of epigallocatechin gallate is sharply defined and the most precisely quantifiable. In green tea fixed by the induction method and steaming, epigallocatechin gallate is practically equal and more present than that made by the roasting method.

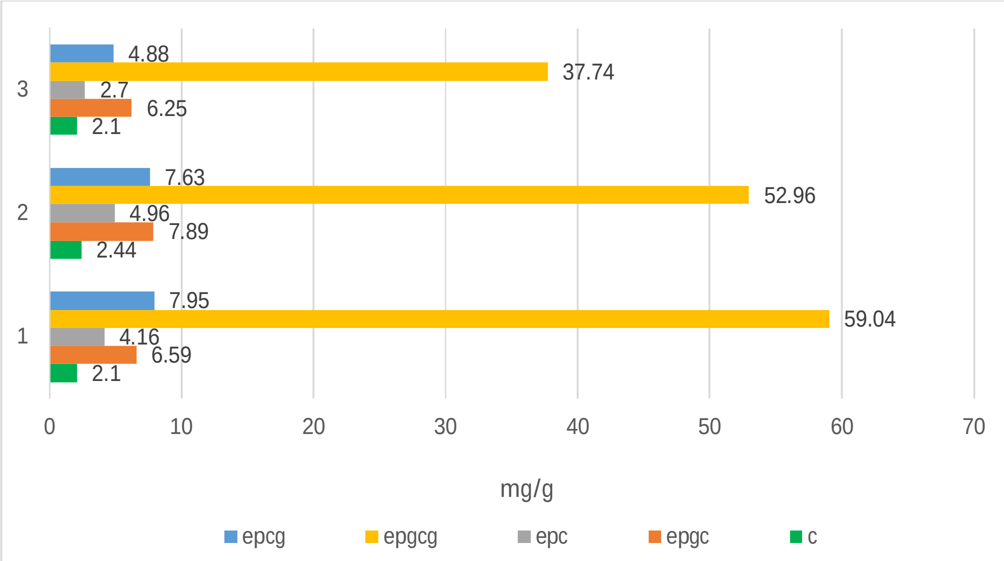



Fig. 2. Comparative amounts of five catechins in experimental green tea extracts (mg/g)
1 - fixed with steaming; 2 - fixed with electromagnetic induction; 3 - fixed with roasting.

Based on the results of quantitative determination of catechins, the high content of total catechins and epigallocatechin gallate in tea extracts fixed by induction and evaporation was confirmed. Induction-fixed tea retains more simple catechins than fixed with steaming (Fig. 2).

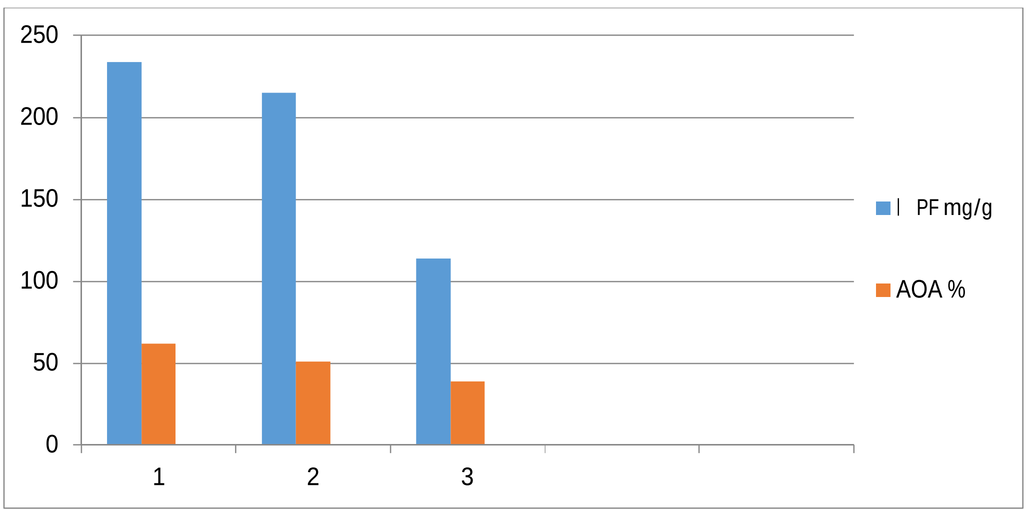



Fig. 3. Total amount of polyphenols (PF), mg/g and antioxidant activity (AOA). %

1 - fixed with steaming;2 - fixed with induction;3 - fixed with roasting.

The results of the conducted research 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 it is green tea fixed with electromagnetic induction (Fig. 3).

Table 2. Mass fraction of toxic elements in green tea test samples

| Name of indicator | Limit value | Results | |
|---|---|---|---|
| | | Green tea (Li) | Green tea (An) |
| lead (Pb), mg/kg | not more 10.0 | 0,001 | 0,001 |
| Arsenic (As), mg/kg | not more 1.0 | 0,059 | 0.066 |
| Cadmium (Cd), mg/kg | not more 1.0 | 0,035 | 0,029 |
| Mercury (Hg), mg/kg | not more 0,1 | 0,017 | 0,018 |
| Copper (Cu), mg/kg | not more 100,0 | 3,60 | 5,13 |

The results of the study of toxic elements lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg) and copper (Cu) showed that their concentrations in green tea made from raw materials of different natural conditions with new technology are practically identical and many times less than the permissible limit value. (Table 2).In addition, we additionally studied the following elements: zinc (Zn), manganese (Mn) and iron (Fe). The following results were obtained for the new technology green tea samples, respectively: (Li) and (An) : Zn: 0.397 - 0.374; Mn: 0.078-0.79 and Fe: 1.046-1.263.These data provide an opportunity to get more information about the safety of Georgian tea.

## Conclusion

1. The results of the study of catechins in experimental green tea extracts using the high-performance liquid chromatography method (HPLC) showed that epigallocatechin gallate is more quantity there n the green tea fixed by the induction method and by evaporation and is superior to the product made by the roasting method. Green tea obtained as a result of fixation by the induction method has a high amount of both total and simple catechins and the dominant catechin - epigallocatechingallate. The antioxidant activity of tea extracts is in direct correlation with the total amount of polyphenols.
2. The results of the study of toxic elements: lead (Pb), arsenic (As), cadmium (Cd), mercury (Hg), copper (Cu), as well as: zinc (Zn), manganese (Mn), iron (Fe) confirm the high-level safety of Georgian tea in accordance with established standards.

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# ფიქსაციის მეთოდების გავლენა მწვანე ჩაის ხარისხობრივ მაჩვენებლებზე

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[1]საქართველოს აგრარული უნივერსიტეტი, ჩაის, სუბტროპიკული კულტურების და ჩაის მრეწველობის ინსტიტუტი, ტექნიკის მეცნიერებათა დოქტორი, საქართველოს სოფლის მეურნეობის მეცნიერებათა აკადემიის აკადემიკოსი; [2]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, ბიოლოგიის მეცნიერებათა დოქტორი, პროფესორი; [3]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, აკადემიური დოქტორი; [4]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, მაგისტრი; [5]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, მაგისტრანტი; [6]საქართველოს აგრარული უნივერსიტეტი, ჩაის, სუბტროპიკული კულტურების და ჩაის მრეწველობის ინსტიტუტი, აკადემიური დოქტორი; [7] საქართველოს აგრარული უნივერსიტეტი, ჩაის, სუბტროპიკული კულტურების და ჩაის მრეწველობის ინსტიტუტი, აკადემიური დოქტორი

**აბსტრაქტი**

მწვანე ჩაის ხარისხობრივი მაჩვენებლების ჩამოყალიბებაში უაღრესად მნიშვნელოვან როლს ასრულებენ თბური პროცესები. მთავარი ტექნოლოგიური პროცესია ფიქსაცია, რომლის დანიშნულებაა ჩაის ფოთლის მჟანგავი ფერმენტების ინაქტივაცია და მასში შემავალი ქიმიური ნაერთების საწყის მდგომარეობაში ფიქსირება. მწვანე ჩაის დამზადება ენერგოტევადი პროცესია. ნაშრომში მოცემულია მწვანე ჩაის ხარისხობრივ, ქიმურ და უსაფრთხოების მაჩვენებლებზე ფიქსაციის მეთოდების გავლენის კვლევის შედეგები. ექსპერიმენტული მწვანე ჩაის ექსტრაქტების კატეხინების მაღალი ხარისხის თხევადი ქრომატოგრაფიის მეთოდით (HPLC) გამოყენებით შესწავლის შედეგებმა აჩვენა, რომ ელექტრომაგნიტური ინდუქციის მეთოდით (საქართველოს პატენტი 7427 B. 2022) და დაორთქვლით ფიქსირებულ მწვანე ჩაიში, ეპიგალოკატეხინ გალატი პრაქტიკულად თანაბრად არის წარმოდგენილი და აღემატება მოხალვის მეთოდით დამზადებულ პროდუქტს. ჩაის ექსტრაქტების ანტიოქსიდანტური აქტივობები პირდაპირ კორელაციაშია პოლიფენოლების საერთო რაოდენობასთან. ტოქსიკური ელემენტების: ტყვია (Pb), დარიშხანი (As), კადმიუმი (Cd), ვერცხლისწყალი (Hg), სპილენძი (Cu),და აგრეთვე: თუთია (Zn), მანგანუმი (Mn), რკინა (Fe) ატომურ-აბსორბციული სპექტრომეტრის (AAS 6 000) გამოყენებით. კვლევის შედეგები ადასტურებს ქართული ჩაის უსაფრთხოების მაღალ ხარისხს დადგენილი ნორმების მიხედვით.