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Original article

The Influence of Preparation Methods on Phenolic Compounds Content in Green Tea Samples from the Libyan Market

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Abstract
Tea leaves are rich in polyphenols, including catechins and flavonoids, which exhibit potent antioxidant properties. These compounds play a critical role in combating oxidative stress, a key factor in the development of chronic diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. The extraction of these bioactive compounds from tea offers a sustainable source of antioxidants with significant health benefits. This study investigates the phenolic content of green tea samples available in the Libyan market, with a focus on the impact of traditional Libyan preparation methods. Using the Folin–Ciocalteu method, the phenolic profiles of seven green tea brands were quantified. The study also examines the effect of traditional Libyan preparation techniques, particularly the removal of the first tea boiling, on the concentration and efficacy of these compounds. Results reveal significant variability in phenolic content across different tea samples and preparation methods, emphasizing the importance of retaining the first boiled tea to preserve bioactive composition. This research provides valuable insights into optimizing tea preparation to maximize health benefits, offering practical guidance for consumers, and promoting the integration of traditional preparation methods into modern health practices .
Keywords: Green Tea, Phenolic Compounds, Folin-Ciocalteu, Gallic Acid, Preparation Methods.

Introduction

Tea (*Camellia sinensis*) is one of the most widely consumed beverages globally, famous for its rich content of polyphenolic compounds, such as catechins, and flavonoids [1]. These compounds are known for their antioxidant properties, which help alleviate oxidative stress and reduce the risk of chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders [2-4]. The phenolic content of tea is influenced by several factors, including the type of tea (green or black), growing conditions, and preparation methods. Inhibition of tumor genesis by extracts of green tea and polyphenols present in tea has been proved in different animal models, including those for cancers of the lung, skin, oral cavity, esophagus, stomach, small intestine, colon, liver, pancreas, prostate, bladder, and mammary glands [5].

In Libya, tea is a staple beverage, often prepared using traditional methods that involve boiling tea leaves and discarding the first infusion. This practice, while culturally significant, may impact the concentration of bioactive compounds in the final beverage. Despite the widespread consumption of tea in Libya, there is limited research on the phenolic content of commercially available tea brands and the influence of local preparation techniques on these compounds .

The phenolic content in tea differs significantly across different brands and types. Studies have shown that green tea generally contains higher levels of polyphenols compared to black and oolong teas.[6] For instance, green tea has been reported to contain up to 3585 mg/L of gallic acid equivalents (GAE) of total phenols, with epigallocatechin gallate (EGCG) being the most abundant catechin [7],[8]. In contrast, black tea, which undergoes fermentation, has lower phenolic content but contains theaflavins and the arubigins, which also contribute to its antioxidant activity [9,10].

Green Tea is known for its high catechin content and exhibits the highest antioxidant activity among tea kinds. For example, a study on Argentine green tea found total polyphenol concentrations ranging from 21.02% to 14.32% GAE [4]. Local preparation methods knowingly impact the phenolic content and bioavailability of tea. Traditional techniques, such as boiling and immersing, can alter the concentration of bioactive compounds. For example, in Libya, tea is often prepared by boiling tea leaves and discarding the first infusion, a practice that may reduce the phenolic content in the final beverage [10]. High temperatures and prolonged steeping times can degrade heat-sensitive polyphenols, reducing their antioxidant activity. However, optimal extraction conditions (e.g., 80°C for 5–30 minutes) can maximize phenolic content. This study aims to quantify the phenolic content of green tea samples from the Libyan market and evaluate the impact of traditional Libyan preparation methods on the retention of these bioactive compounds. By providing insights into the variability of phenolic content and the effects of preparation techniques, this research seeks to optimize tea consumption for enhanced health benefits .

Materials and Methods

Sample Collection

Seven commercially available green tea brands were purchased from local markets in Libya. Samples were selected to represent a range of popular brands consumed in the region (Table 1).

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Table 1. Green tea brands obtained in this study

Brands	Samples
Brand A	Green tea
Brand B	Aljeed
Brand C	Alkabsheen
Brand D	Almasa.
Brand E	Tenarion.
Brand F	Alsahem
Brand G	Algafela

Preparation Methods

Traditional Libyan tea preparation involves boiling tea leaves in water and discarding the first infusion. To evaluate the impact of this method, two preparation techniques were compared: 1) Traditional Method (Decoction method): Tea leaves were boiled in water for 10 minutes, and the first infusion was discarded and used for the determination of phenolic compounds. A second infusion was prepared using the same leaves and used for the quantification of phenolic compounds. 2) Standard Method (Infusion method): Tea leaves were steeped in hot water (80°C) for 10 minutes without discarding the first infusion.

Quantification of Phenolic Compounds

The total phenolic content (TPC) of the tea samples was determined using the Folin–Ciocalteu method. Gallic acid was used as a standard, and results were expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of dry tea leaves.

Results

All tea samples were subjected to the Folin-Ciocalteu assay for the determination of phenolic compounds among different tea preparation methods. Significant variability in TPC was observed among the seven tea brands in green tea samples.

Galic acid calibration curve

The calibration curve showed a high linearity with an R^2 value of 0.9967 (Figure 1), showing good correlation between gallic acid concentration and absorbance.

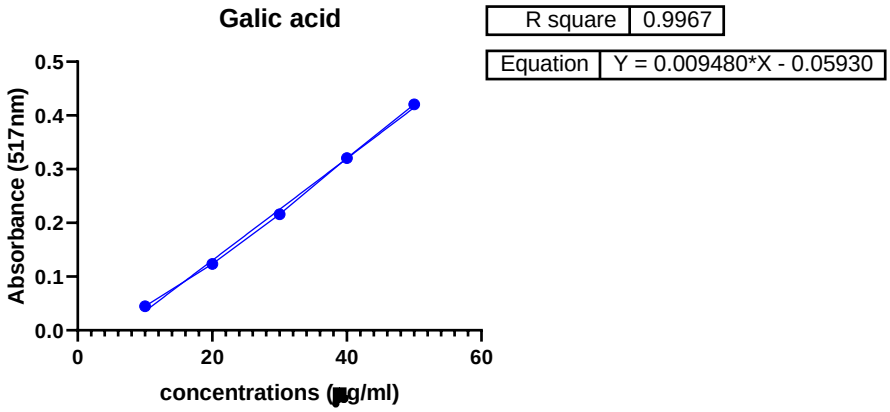

Figure 1. Different concentrations of Gallic acid

The traditional Libyan preparation method, which involves discarding the first infusion, resulted in a significant reduction in TPC compared to the standard method. On average, the TPC of the second infusion was 25-30% lower than that of the first infusion. This suggests that discarding the first boiling removes a substantial portion of the bioactive compounds

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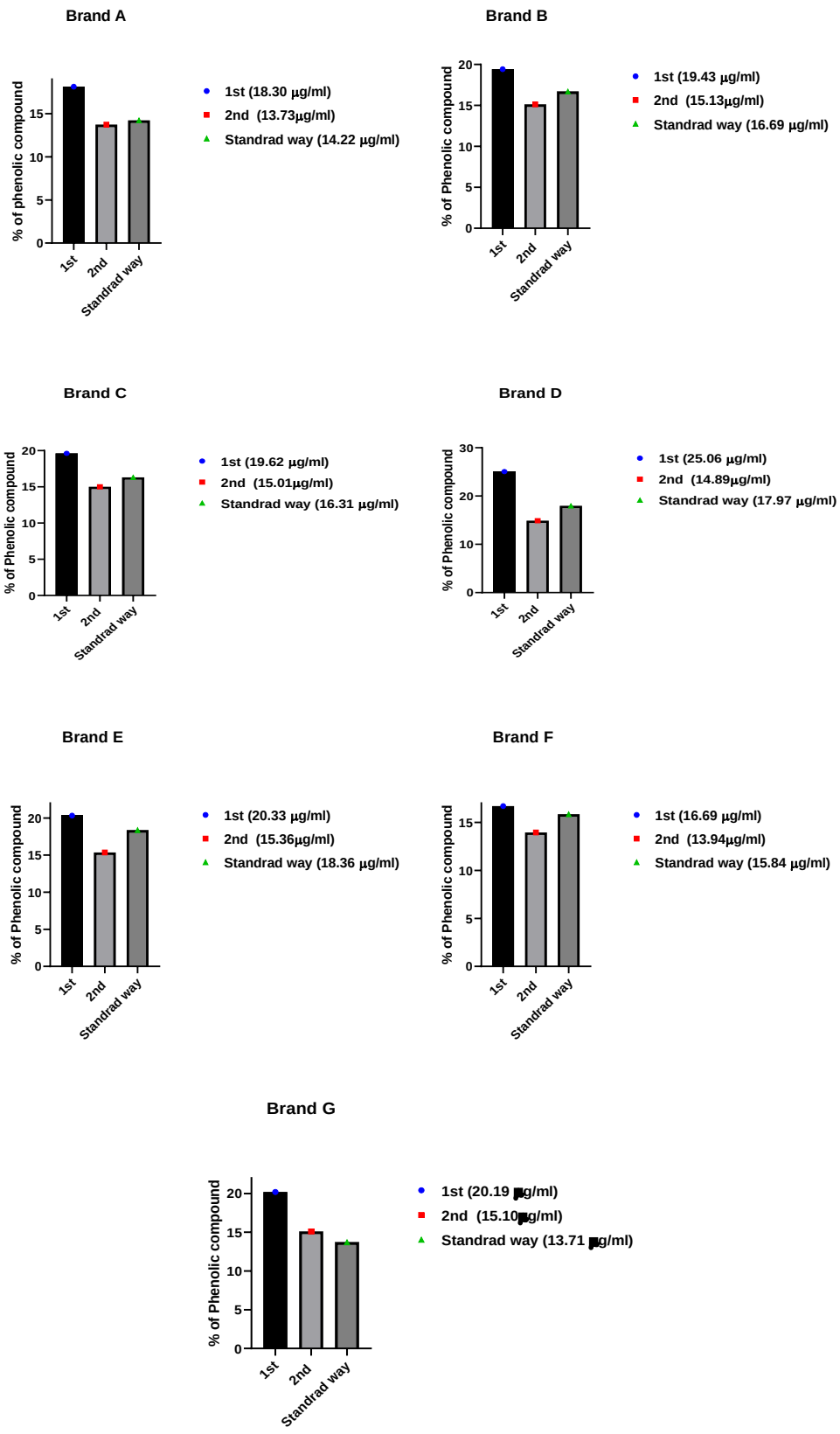

Figure 2: ***Phenolic content of different tea samples with different preparation methods, 1st and 2nd columns represents first infusion and second infusion for tea samples that prepared by decoction method as mentioned above in materials and methods section, whereas, standard way column represents tea samples that prepared by infusion method which mentioned in materials and methods section***

Discussion

The findings of this study highlight the significant variability in phenolic content among commercially available tea brands in the Libyan market. Green tea samples consistently exhibited higher phenolic content because they undergo minimal oxidation, preserving their polyphenol compounds [5]. To calculate phenolic

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content, a standard curve is created using a reference compound, and Gallic acid (GA) is the most commonly used standard. Gallic acid has several hydroxyl groups that readily decrease the FC reagent, ensuring a strong and reproducible signal [9]. In this study, the calibration curve showed a high linearity with an R^2 value of 0.9967 (Figure 1), showing good correlation between Gallic acid concentration and absorbance. The data of this study showed a considerably high phenolic content in tea samples subjected to first boiling, proposing that this initial extraction step plays a vital role in the release of phenolic compounds (Figure 2). This data is consistent with previous studies showing that temperature and extraction time greatly affect the solubility and stability of polyphenols in tea [10]. The brand D(25.06µg/ml) shows high phenolic content among all tea samples. This could be attributed to that the boiling water enhances the rapid diffusion of phenolic compounds from tea into water. The time of boiling is a very crucial factor that affects the quantity of phenolic compounds. The first boiling typically lasts 3–5 minutes, which may be the best duration for extreme phenolic release before degradation begins. On the other hand, long heating (e.g., second or third boiling) could lead to thermal degradation of sensitive polyphenols (e.g., epigallocatechin gallate, EGCG)
The results in Figure 2 point to that traditionally prepared tea samples display lower phenolic content than tea samples subjected to first boiling. This difference can be attributed to dissimilarities in extraction efficacy, thermal degradation, and chemical stability of polyphenols.
The preparation method, which involves discarding the first infusion, was found to significantly reduce the phenolic content of the final beverage. This practice, while culturally ingrained, may diminish the potential health benefits of tea by removing a substantial portion of its bioactive compounds. These results underscore the importance of retaining the first infusion to maximize the antioxidant properties of tea .

Conclusion

This study provides valuable insights into the phenolic content of green tea samples available in the Libyan market and the impact of traditional preparation methods on these compounds. The findings suggest that retaining the first infusion during tea preparation can enhance the retention of bioactive compounds, thereby maximizing the health benefits of tea. These results have practical implications for consumers seeking to optimize their tea consumption for health purposes and highlight the need for further research on the cultural and health aspects of traditional tea preparation methods.

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