J. Fac. Pharm. Ankara / Ankara Ecz. Fak. Derg., 47(3): 808-815, 2023
Doi: 10.33483/jfpau.1298235

ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE EFFECTS OF COLOR AND BRIGHTNESS OF BREWED BLACK TEA ON ITS FLUORIDE CONCENTRATION IN TÜRKİYE

TÜRKİYE'DE DEMLENMİŞ SİYAH ÇAYIN RENK VE PARLAKLIĞININ FLORÜR KONSANTRASYONUNA ETKİSİ

Üzeyir AKTUĞ[1]*, Yalçın DUYDU[2]

[1]Directorate of Plant Protection Central Research Institute, Ankara, Türkiye

[2]Ankara University Faculty of Pharmacy, Department of Toxicology, 06560, Ankara, Türkiye

ABSTRACT

Objective: *The main purpose of this study is to determine the effect of the tea brewing time on the total color, brightness, and fluoride ion concentration of tea infusions in Türkiye.*
Material and Method: *250 ml of boiling distilled water was added to 6 g of granulated tea sample and brewed against increasing time. The total color and brightness of the tea infusions were determined by a spectrophotometer at 460 nm, while a potentiometric method was used to determine the fluoride ion concentration.*
Result and Discussion: *We conclude that the concentration of fluoride ions and other bioactive compounds in the tea infusion is strongly dependent on the tea preparation and brewing preferences of consumers. According to our results, fluoride concentrations in tea infusions reach the plateau after 20 minutes in a Turkish way of tea brewing. Therefore, tea-brewing time is an important variable in calculating the daily fluoride intake for extreme tea-consuming populations like Türkiye.*
Keywords: *Black tea, brewed tea, extractable fluoride, percent brightness, total color*

ÖZ

Amaç: *Bu çalışmanın temel amacı, çay demleme süresinin Türkiye'deki çay infüzyonlarının toplam renk, parlaklık ve florür iyon konsantrasyonu üzerindeki etkisini belirlemektir.*
Gereç ve Yöntem: *6 g granül çay örneğine 250 ml kaynayan distile su ilave edilerek artan zamana karşı demlendi. Çay infüzyonlarının toplam rengi ve parlaklığı 460 nm'de bir spektrofotometre ile belirlenirken, florür iyonu konsantrasyonunu belirlemek için potansiyometrik bir yöntem kullanıldı.*
Sonuç ve Tartışma: *Çay infüzyonundaki florür iyonlarının ve diğer biyoaktif bileşiklerin konsantrasyonunun, tüketicilerin çay hazırlama ve demleme tercihlerine güçlü bir şekilde bağlı olduğu sonucuna vardık. Elde ettiğimiz sonuçlara göre, çay infüzyonlarındaki florür*

* **Corresponding Author / Sorumlu Yazar:** Üzeyir Aktuğ
e-mail / e-posta: uzeyiraktug@gmail.com, **Phone / Tel.:** +905322087917

Submitted / Gönderilme : 17.05.2023
Accepted / Kabul : 04.07.2023
Published / Yayınlanma : 20.09.2023

konsantrasyonları, Türk usulü çay demlemede 20 dakika sonra platoya ulaşmaktadır. Bu nedenle çay demleme süresi, Türkiye gibi aşırı çay tüketen toplumlar için günlük flor alımının hesaplanmasında önemli bir değişkendir.
Anahtar Kelimeler: *Demleme çay, ekstrakte edilebilir florür, siyah çay, toplam renk, yüzde parlaklık*

INTRODUCTION

The tea plant is an evergreen shrub or small tree of the *Camelia* genus, native to China, with dark green shiny leaves and white flowers. Processing of tea leaves allows the production of various types of tea: white, yellow, oolong, green, black, and pu-erh, depending on the extent of the oxidation/fermentation process [1]. Black tea is formed by oxidation process that is catalyzed by the enzyme polyphenol oxidase [2]. Tea is one of the most widely consumed beverages in the world and is second only to water [3]. Also, it is an antioxidant agent in daily consumption. However, considering the high consumption rate, the positive or negative health effects of tea can have serious consequences for public [4]. The positive effects of tea are quite remarkable. Pure catechins and phenolic acids contained in tea are a stronger antioxidant than vitamins C, E and β-carotene [5]. According to the studies published so far, it has been reported that tea has positive impacts on health such as protective effects on the cardiovascular system [6], anti-inflammatory [7], anticancer [8] and anti-diabetic [9]. Despite these beneficial effects of tea consumption, the fluoride content of tea might be a health concern for the highly tea-consumed populations due to the possibility of exceeding the safe daily fluoride intake level.

It has long been known that tea contains a certain amount of fluoride [10,11]. The amount of fluoride in tea may depend according to the geography and climatic conditions in which it is grown. The fluoride accumulated in the leaves of the tea plant is higher in the old leaves than in the young shoots [12]. Low-land tea contained more fluoride than high-land tea. Tea plucked in May has the highest fluoride content and the processing method does not affect the fluoride content of tea [13].

The World Health Organization recommends a maximum of 1.5 mg/l fluoride in drinking water. It has additionally been reported that if the amount of fluoride from other sources is more than 6 mg/day, this upper limit value of fluoride in drinking water might be set lower at the local scale [14]. The Food and Nutrition Board (FNB) has established Tolerable Upper Intake Levels (ULs) for fluoride from all sources for healthy individuals based on levels associated with dental and skeletal fluorosis. Accordingly, 10 mg is the daily UL of fluoride for both males and females aged over 9 [15].

There is no universally accepted method for the preparation and presentation of tea infusion. In some countries, tea is brewed by soaking a disposable tea bag (1.8-2.4 g) in 200-250 ml of hot water for 3-5 minutes. In Japan green tea, which is the most preferred, is presented with a ceremony. It is common in England to add a certain proportion of milk to black tea. Peppermint-flavored tea is preferred in North African countries, especially in Morocco. Türkiye is the country that consumes the most black tea in the world. Tea in Türkiye is served in traditional tea glasses called thin-waist tea glasses after brewing it in a traditional teapot and infuser. The most common way of brewing tea in Türkiye is displayed in Figure 1 [16,17].

Tea is a part of daily life and can be considered a cultural symbol and socialization tool in Türkiye. First of all, the color of the tea is essential for extreme consumers. The most preferred color is bright and dark red, which is named "tavşankanı" in Türkiye [18]. This color preference strongly influences the fluoride concentration of the tea, which contributes to the daily fluoride intake.

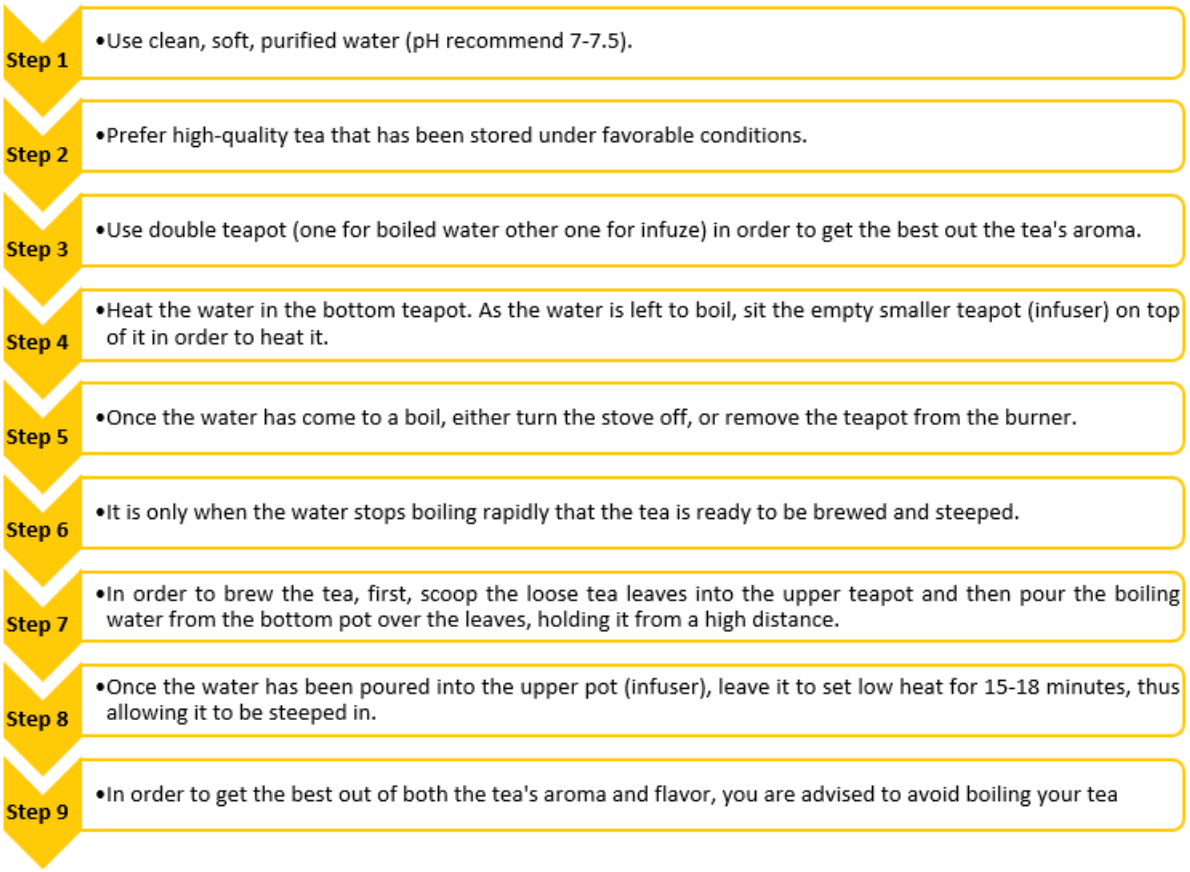

Figure 1. Outline of brewing black tea in Türkiye [15,16]

MATERIAL AND METHOD

Instruments and Chemicals

All chemicals used were analytical reagent grade. Fluoride ion selective electrode (Orion Fluoride Electrodes 9609BNWP) was immersed in the solutions and readings on the ion analyzer (Orion Star A324 pH/ISE Portable Multiparameter Meter) were recorded. Total ionic strength adjustment buffer (TISAB II, Orion 940909) regulates the ionic strength of samples and standard solutions and adjusts the pH, and also avoids interferences. The optical densities at 460 nm were measured with HITACHI U-1800 spectrophotometer.

Preparation of Tea Liquors

Tea liquors were prepared based on Roberts and Simit [18]. Accordingly, 250 ml of boiling water was added to 6 g of dry tea and brewed. After the specified brewing time, it was filtered through a cotton sieve and cooled to room temperature. It was carried out triple replicas every step. Tea liquor preparation steps are summarized in Figure 1.

The Determination of Total Color and Brightness in Tea Liquor

The determination of Total Color and Brightness in Tea Liquor was made according to Roberts and Simit [19]. Steps of method are summarized in Figure 2.

Roberts and Simit [19] have evaluated the sum of the optical densities of the A and B solutions as a measure of the color of the infusion. Thus the total color is calculated from the results of the measurements at 460 nm in the spectrophotometer with Equation 1.

$$\textbf{Total Color} = 6.25x(E_A + 2E_B) \qquad \text{(Equation 1)}$$

Roberts and Simit (1963) stated that the hue of the color, rather than the depth of color, is important in evaluating tea liquors and tea tasters refer to it as 'brightness'. The percent brightness values were calculated with Equation 2 from the results of the measurements at 460 nm in the spectrophotometer.

$$\textbf{Brightness\%} = 100xE_C/(E_A + 2E_B) \qquad \text{(Equation 2)}$$

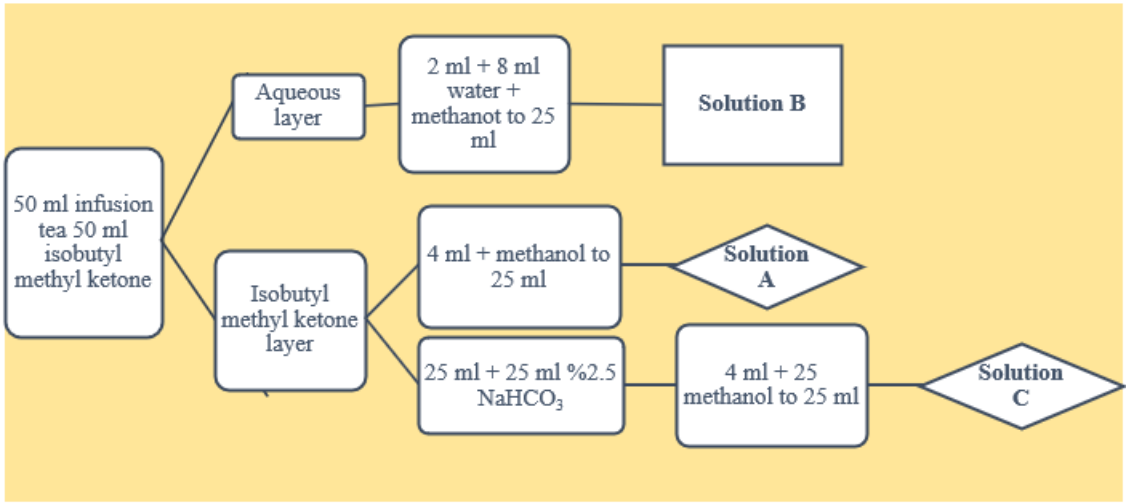

Figure 2. Sample preparation steps for Total Color and Brightness in Tea Liquor

Determination of Fluoride Ion in Tea Liquor:

Method 9214 [20] published by the United States Environmental Protection Agency (EPA) was used. The method is based on the potentiometric determination of total fluoride using a fluoride ion-selective electrode (ISE). The samples and fluoride standard solutions were diluted 1:1 with the TISAB II.

Calibration Solution:

For 1000 mg/l Fluoride (F^-) stock solution: 0.2210 g sodium fluoride (NaF, dried at 110°C for two hours and allowed to come to room temperature in a desiccator) is dissolved in 100 ml polyethylene measuring flask with distilled water.

For 100 mg/l Fluoride (F^-) standard solution: Dilute 10.0 ml of 1,000 mg/l fluoride calibration stock solution to 100 ml with water in a polyethylene volumetric flask.

Calibration curve was determined against the measured mV values, respectively, using the fluoride ion-selective electrode (ISE). Fluoride ion content of infusion tea samples was determined from the calibration curve (Table 1).

Table 1. Fluoride Calibration Standards

ml of 100 mg/l F^- Solution	Concentration when diluted to 50.0 ml (mg/l F^-)
0.050	0.100
0.150	0.300
0.500	1.00
1.500	3.0
5.000	10.0

RESULT AND DISCUSSION

Linearity of the Standard Curve

The linearity was obtained in the range of 0.1-10 mg/l fluoride concentration as Figure 3. The measured potential from fluoride ion selective electrode corresponding to the level of fluoride ion in solution is described by the Nernst equation.

$$\mathbf{E = S \log C + {}^{\circ}E} \qquad \text{(Equation 3)}$$

Where C is the mg/l fluoride yielding a millivolt potential of E, S is the slope, and ˚E is reference potential. A slope range 58.6 mV. The calibration curve was generated for each measurement with R^2=0.999.

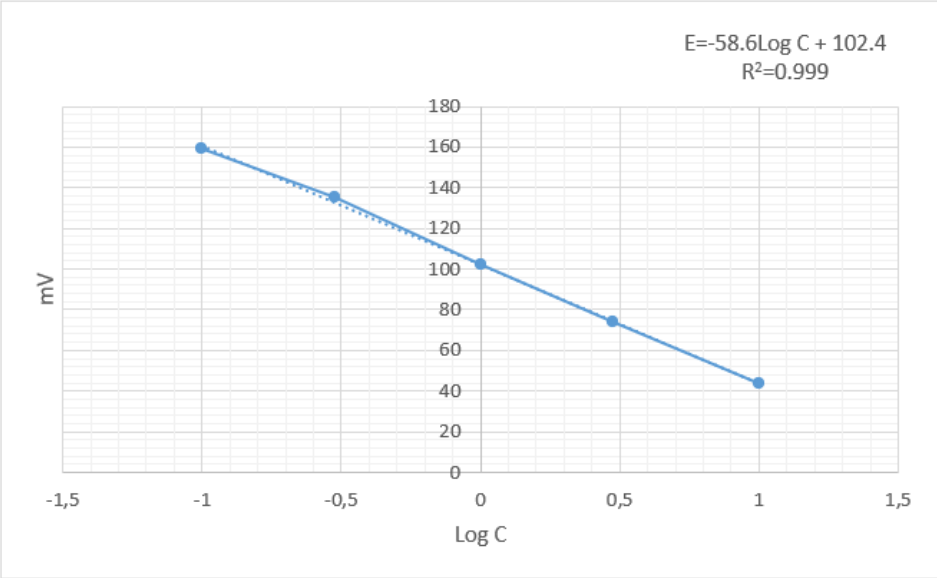

Figure 3. Standard calibration curve of fluoride

Time-dependent Change in Total Color and Brightness % in Tea Liquor

It was concluded that the Total Color parameter in the infused tea would not change significantly after the 10th minute of brewing time (Figure 4).

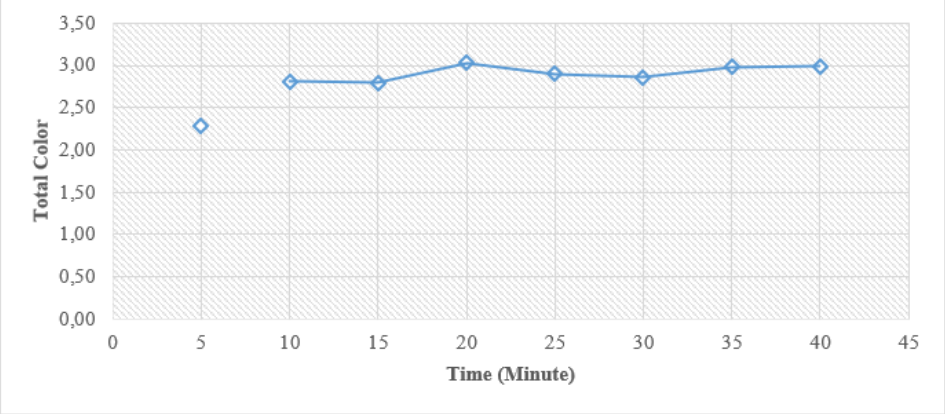

Figure 4. Effect of tea infusion on Total Color depending on brewing time

However, the Brightness % values of the tea infusions tended to decrease continuously. It is clear that this situation would affect consumption preference negatively. Therefore, it is recommended to consume the tea brewed in accordance with Turkish traditions within 30-35 minutes at most.

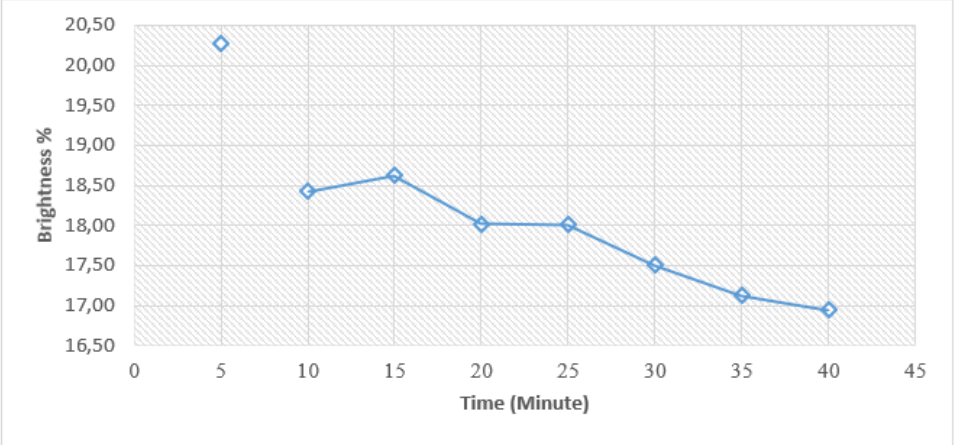

Figure 5. Effect of tea infusion on Brightness % depending on brewing time

There is a rapid increase in fluoride ion extraction immediately after the granule tea encounters boiling water (Figure 6). The rate of fluoride release increases relatively until the 5th minute, and the increases after the 15th minute are not significant. At the 15th minute, the fluoride ion concentration reaches its maximum and remains constant, while the brightness decrease is continuous.

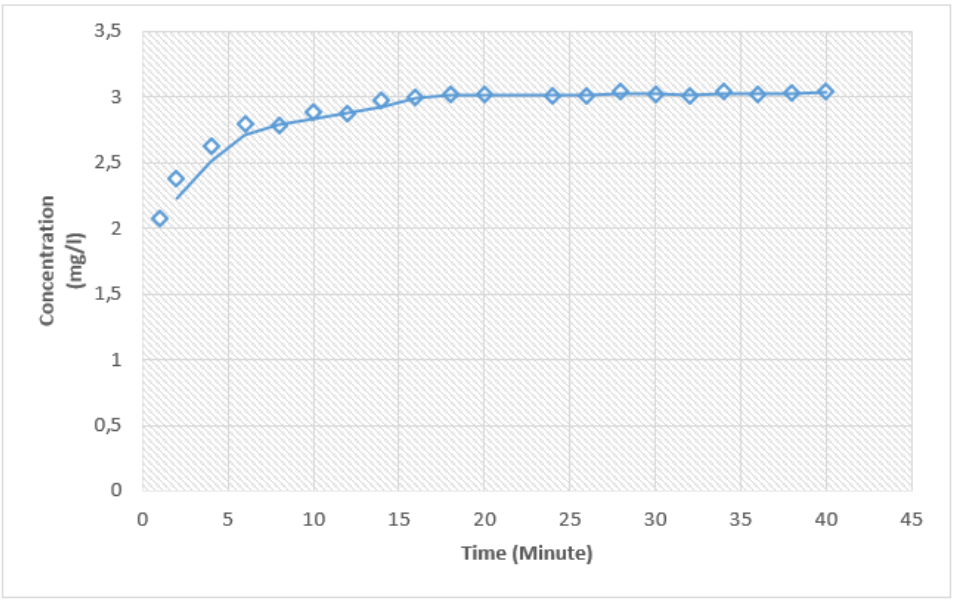

Figure 6. Effect on fluoride ion extraction depending on brewing time on tea infusion

The color of food is one of the most important features that directly affect the food preference and desires of consumers [21]. From the tea color point of view, it is not possible to evaluate consumer preferences using a standard analytical method. In Türkiye, it generally is asked the customer; how color do you take your tea, dark or light? The answer to this question is just a preference that varies from

person to person. However, the preferred color determines the fluoride concentration in tea and finally contributes to daily fluoride intake.

Tea naturally contains fluoride ions. According to the available reports, the fluorine content of granulated tea samples varies between 3.2 and 400 ppm in Türkiye [22]. This is quite a wide range and is the precursor to the fluoride ion that would migrate into the tea infusion. It is, of course, the color choice that would determine the exact chemical content of tea.

It is well known that fluoride has many beneficial effects on human health at a certain daily intake level. A high level of daily fluoride intake, however, may cause some health problems such as dental/enamel fluorosis and skeletal fluorosis. The safe daily fluoride intake level is especially significant for the populations consuming high levels of tea due to the high level of fluoride content of the black tea. The preparation and presentation methods of tea can also affect the concentration of fluoride in the tea infusion that would ultimately be consumed.

According to our results, it was concluded that the fluoride concentration reaches a plateau within 20 minutes of brewing (by Turkish traditions). Generally, the in-house brewing period of Turkish tea is much longer than 20 minutes. So, it means the tea infusion reaches its maximum fluoride concentration in the infuser. In our study, when brewing black tea (6 g/250 ml) by Turkish traditions, the fluoride concentration in the infuser reached a steady state after 20 minutes at 3 mg/l. After this stage, the color of the tea we prefer becomes important. Because the tea infusion in the teapot is diluted until the desired color is obtained in the tea glass. In general, the desired bright and dark red color (tavşankanı) is obtained in the tea glass by adding one portion of brewed tea from the teapot and one portion of boiling water. Thus, the fluoride concentration is diluted by half (this color preference is crucial from the daily fluoride intake point of view).

Using the above information, it is possible to calculate the daily fluoride intake that stems from tea consumption. Although the volumes of tea glasses in Türkiye vary between 100 and 175 ml, 125 ml is the most preferred glass for drinking tea. The final fluoride concentration will be 0.19 mg [3x(125/2)/1000] when we put one portion of brewed tea and one portion of boiling water in this tea glass. Heavy tea consumers drink 15-20 glasses of tea daily. The fluoride intake would be 3.8 mg/day (0.19x20) for these extreme consumers, which represents a worst-case exposure scenario. As mentioned before, 10 mg is the daily UL of fluoride for both males and females aged over 9 [15]. Accordingly, considering the population that consumes excessive tea in Türkiye, it is foreseen that the amount of fluoride that can be safely taken from sources other than tea is 6.2 mg.

This study should be considered as an attempt to assess the daily fluoride intake that stems from daily tea consumption. It is well known that different tea samples contain different amounts of fluoride. Therefore, in our next study, we have aimed to expand this study by using different tea samples to make a more precise exposure assessment for fluoride in Türkiye.

ACKNOWLEDGEMENTS

The authors are grateful to Ankara University (Project no: 21L0237007) for financial support.

AUTHOR CONTRIBUTIONS

Concept: Ü.A., Y.D.; Design: Ü.A., Y.D.; Control: Ü.A., Y.D.; Sources: Ü.A., Y.D.; Materials: Ü.A., Y.D.; Data Collection and/or Processing: Ü.A., Y.D.; Analysis and/or Interpretation: Ü.A., Y.D.; Literature Review: Ü.A., Y.D.; Manuscript Writing: Ü.A., Y.D.; Critical Review: Ü.A., Y.D.; Other: Ü.A., Y.D.

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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