

EFFECT OF BREWING TIME AND WATER HARDNESS ON FLUORIDE RELEASE FROM DIFFERENT IRANIAN TEAS

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ABSTRACT: The present study aimed to investigate the fluoride concentration in tea leaves of different types of commonly used Iranian tea and the influence of brewing time and water hardness on fluoride release during their infusion. The concentrations of fluoride, phosphate, sulfate, nitrate, and chloride were measured, after 3 to 120 min brewing time, in 100 tea samples, including white, green, oolong, and black teas. The daily fluoride intake was also assessed. The fluoride concentration was measured with the ion chromatography method. The results showed that the minimum and maximum concentrations of fluoride in the tea liquor, after 15 min infusion, were 1.46 and 2.9 mg/L in teas made with oolong tea leaf and green tea leaf, respectively. Fluoride release from the tea leaves into the infusion increased significantly with increased brewing time ($p < 0.05$). Fluoride release, after a 15-min brewing time, ranged from 43.7% for green tea bag to 73.5% for black tea leaf. Fluoride extraction was less in hard water.

Keywords: Brewing time; Daily intake of fluoride; Fluoride; Iranian tea; Water hardness.

INTRODUCTION

Tea is one of the most popular beverages in the world. It is estimated that more than 4 billion kg of tea are produced and consumed annually around the world.¹ The average consumption of tea in Iran is reportedly about 1.5 kg per person per year.² It is well known that the tea plant absorbs the fluoride ion (F) and heavy metals from the soil and can accumulate F in its leaves in the range of 100–200 mg F/kg.³⁻⁷ A significant amount of fluoride can easily be released into tea beverage during infusion⁹ and tea liquor can be a major source of fluoride intake by humans.⁴ The effects of ingested fluoride depend on the level of daily intake and fluoride becomes more toxic at higher intake levels.⁷ The upper limit of intake for preventing the development of clinically unacceptable dental fluorosis is not well known, but the value of 0.05–0.07 mg F/kg body weight/day is generally accepted as a reference level.¹⁰ It is, therefore, necessary to control the quality of the tea drunk.

The release of fluoride into tea infusion depends on several factors such as the degree of fermentation of the tea, the maturity of the tea leaves, the water type, the water hardness, the teapot material, and the duration of the infusion. Amongst these factors, the water hardness and the infusion duration are particularly important, as the amount of fluoride released into an infusion increases with increased brewing time. Infusion duration also affects the flavor and color of tea. Gao et al. reported that brewing time was the main factor affecting the extraction rate of fluoride.¹¹ Kalaycı et al. showed that water hardness has a reverse effect on

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the fluoride release rate.¹² Due to a lack of standardized experimental methods, it is difficult to compare the available data. With consideration of the high consumption of tea in Iran, the different brands of tea available in the domestic market, and the differences in the methods of preparing of tea for drinking, the present study was done to determine and compare, for commonly used different types of Iranian tea, the fluoride concentration in the tea leaves and the influence of brewing time and water hardness on fluoride release into the tea infusion. The concentrations, in the brewed tea, of four other ions, chloride, nitrate, sulfate and phosphate, were also determined by the ion chromatography method.

MATERIAL AND METHODS

A total of 100 tea samples of white, green, oolong and black tea including green tea leaf (GTL), oolong tea leaf (OTL), black tea leaf (BTL), green tea bag (GTB), and black tea bag (BTB) were purchased from manufacturer company (Refahe-Lahijan) and analyzed for their fluoride, nitrate, sulfate, and chloride contents. Three samples were taken from each type of the teas and each brewed for 3 to 120 min. Water samples, with levels of different water hardness, ranging from 0 to 350 mg CaCO₃/L, were prepared by adding adequate amounts of calcium and magnesium carbonate and bicarbonate to ultrapure water. For each type of tea, an infusion was prepared by adding 1.0 g of dried tea leaves into 100 mL boiling ultrapure water in a Teflon tea pot, and, for brewing, the mixture kept at 80°C for a pre-considered time in an incubator. At the end of the each infusion period, a 1 mL aliquot was taken from each sample and diluted to 100 mL with ultrapure water. The samples were then filtered through a 0.45 µm membrane and prepared for the measurement of anion concentrations. The ion concentrations were determined by ion chromatography using a Metrohm 882 compact IC plus, Metrohm AG, Switzerland. Each sample was analyzed twice. The determination of the F concentration in the dried tea leaves was performed according to the method used by Mahvi et al.¹³ All the obtained data were analyzed by SPSS.20 version. Furthermore, daily fluoride intake was assessed. All chemicals were of analytical reagent grade (Merck) and were used without further purification.

RESULTS AND DISCUSSION

The fluoride concentrations of five different types of green, oolong, and black teas in the dried tea samples and the samples from their tea liquor, after 3 to 120 min of brewing time, are shown in Table 1. The results showed that the average fluoride concentrations in the studied dried teas, ranged from 104 mg/kg for OTL to 205.5 mg/kg for GTB.

The tea leaves showed a lower fluoride content than the tea bags. As described in the materials and methods section, all teas were obtained from a single manufacturing company located in the north of the country, the main area for tea cultivation in Iran. Therefore, the broad variation between the fluoride concentrations in the different types of teas may be due to the differences in the irrigation water quality, the harvesting time, and the harvesting area. On the other hand, a high concentration of fluoride may be due to the use of inexpensive, low quality, very finely ground, and older tea leaves for making the tea bags. It is

known that the level of fluoride in the low grade, especially when finely ground, and older tea leaves is higher than in the young shoots.¹² This finding is in agreement with the results of Fung et al.⁸ and of Tokalioglu et al., who reported that over 2,000 mg F/kg can accumulate in old leaves compared to 250–360 mg F/kg in young leaves.¹⁵ After the first 15 min of brewing time, all the teas in the bag form showed lower concentrations of fluoride in the tea liquor than with the leaf infusions. However, with increasing brewing time, the amount of fluoride increased in the liquor from the tea bags' liquor compared to the infusions from tea leaves. This may be due to an effect of the paper bags delaying the release of ions into the liquor.

Table 1. Total fluoride content of dry teas (mg F/kg dry tea leaf) and tea liquors (mg F/L tea liquor) after different brewing times (min). Values for F in tea liquor are mean±SD

Tea type*	Total F content of dry teas (mg/kg dry tea leaf)	Total F content of tea liquor (mg F/L tea liquor) after different brewing times						
		Brewing times (min)						
		3	5	10	15	30	60	120
GTL	159.5	1.19 ±0.03	1.81 ±0.03	2.09 ±0.10	2.9 ±0.04	2.27 ±0.03	2.35 ±0.05	2.49 ±0.05
OTL	104	0.92 ±0.09	1.16 ±0.04	1.31 ±0.04	1.46 ±0.05	1.62 ±0.02	1.64 ±0.03	1.76 ±0.07
BTL	195.5	0.73 ±0.05	0.84 ±0.06	2.03 ±0.14	2.87 ±0.03	3.05 ±0.04	3.12 ±0.02	3.12 ±0.02
GTB	205.5	0.56 ±0.04	0.71 ±0.02	1.41 ±0.4	1.81 ±0.04	2.33 ±0.04	2.77 ±0.06	3.15 ±0.05
BTB	172.5	1.04 ±0.05	1.49 ±0.03	1.84 ±0.06	2.40 ±0.03	2.64 ±0.04	3.06 ±0.04	3.04 ±0.04

*GTL=green tea leaf; OTL=oolong tea leaf; BTL=black tea leaf; GTB=green tea bag. BTB=black tea bag.

Cao et al.¹⁶⁻¹⁷ showed that the paper bag did not have any effect on the tea filtrate ionic fluoride level. However, it is possible that, in the early stages of brewing, the paper bag has an effect in decreasing the F infusion into the tea liquor, and then, after reaching saturation of its fluoride-binding capacity, it becomes ineffective in reducing the effusion of F into the tea liquor. This area requires further investigation.

The extraction of F into tea liquor from dried tea was compared for the different types of teas and brewing times (Table 1). The concentration of F in the brewed teas increased with increased duration of brewing time. The F release depends on both the type of tea and the brewing time. There were significant differences in the amount of F released from the studied teas at all the time intervals ($p < 0.001$, $r = 0.63$). A *post hoc* Tukey test was also used to test for significant differences in F concentration between any two of brewing times. Based on this test, there were statistically significant differences between the F concentrations of the all studied teas at brewing times of 5, 10 and 15 min ($p < 0.05$). However, in spite of an

increase in the amount of F in the tea liquor, no significant differences were present after 15 min of brewing time ($p>0.05$). This trend, of increasing F levels, was also seen between the brewing times of 3 and 5 min. As a result, in some areas where a long brewing time is commonly used for preparing tea, an increased F intake can be a risk. This information can help with preparing guidelines for the brewing and safe consumption of the different types of tea.

For the studied teas, the average F concentrations in the tea liquors increased in the following order: OTL < GTB < GTL < BTB < BTL. The minimum and maximum average fluoride concentrations in the tea liquors were in OTL (1.41 ± 0.28 mg F/L) and BTL (2.25 ± 1.01 mg F/L), respectively. Based on one way ANOVA, there were statistically significant differences among the mean F concentrations in all five kinds of tea infusions ($p=0.002$, $F=4.6$). A *post hoc* Tukey test was also used for paired comparisons of the F concentrations in the five tea liquors. It was found from the test that there was a significant difference between the mean F concentration in the leaf and bagged tea liquors ($p<0.05$). Other studies have shown that the amount of fluoride in tea leaves differs.¹⁵⁻¹⁷ The differences may be related to the origin of the tea, the processing methods, and the tea brewing methods. However, no significant differences were found in the present study among the F levels in the dried tea samples ($p>0.05$).

The extraction of F from tea leaves into tea liquor was also compared for the different types of tea (Figure 1). After 15 min of brewing time, the proportion of the total F content of the dried tea released into the tea liquor ranged from 43.7% for GTB to 73.5% for BTL. Significant differences were found among the F levels in the dried teas and their infusions ($p=0.001$). Therefore, the degree of tea fermentation degree affects the amount of F in tea infusions and fermented teas can provide notable amounts of fluoride in the daily diet.

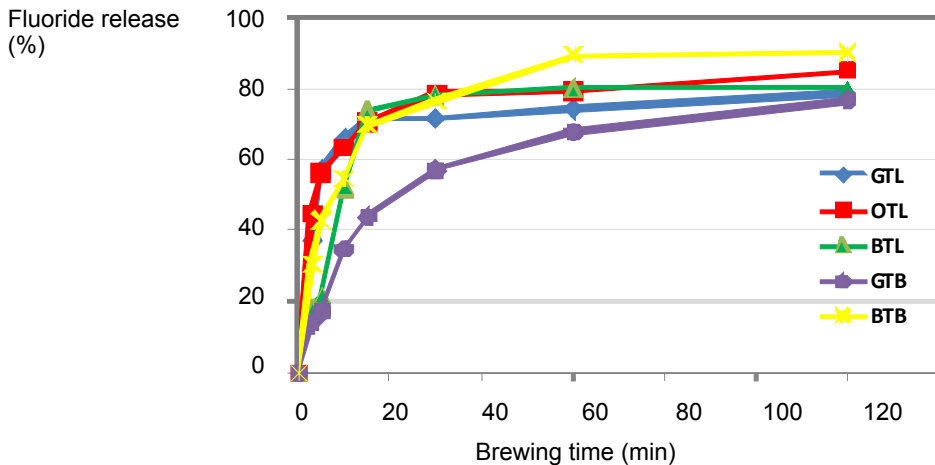


Figure 1. Total fluoride content of dried teas released into tea liquors after different brewing times. GTL=green tea leaf; OTL=oolong tea leaf; BTL=black tea leaf; GTB=green tea bag; BTB=black tea bag.

The concentrations of the phosphate, sulfate, nitrate, and chloride anions in the different tea infusions are presented in Figures 2–5. The results showed large

variations for all four anions: phosphate (range: 1.21–8.80 mg/L, mean: 3.32 ± 1.28 mg/L); sulfate (range: 0.23–3.38 mg/L, mean: 1.45 ± 0.76 mg/L); nitrate (range: 6.90–9.99 mg/L, mean: 8.12 ± 0.57 mg/L), and chloride (range: 7.20–9.09 mg/L, mean: 8.22 ± 0.32 mg/L). Tea can be an important source of these anions. The tea plant takes up these anions from the soil and accumulates them in its leaves. The high fluoride content in the tea leaves was associated with high levels of the phosphate, sulfate, nitrate, and chloride anions in the tea infusions (Figures 1–5). A substantial amount of these anions is released during the tea infusion and will be absorbed by consumers. Variations between these anions can be explained similarly to what was expressed for fluoride. None of the phosphate, sulfate, nitrate, and chloride anion levels exceeded the maximum levels allowable in foodstuffs. Based on the one way ANOVA test, there was a statistically significant difference between the mean concentration of all the studied anions in the studied tea infusions ($p < 0.001$).

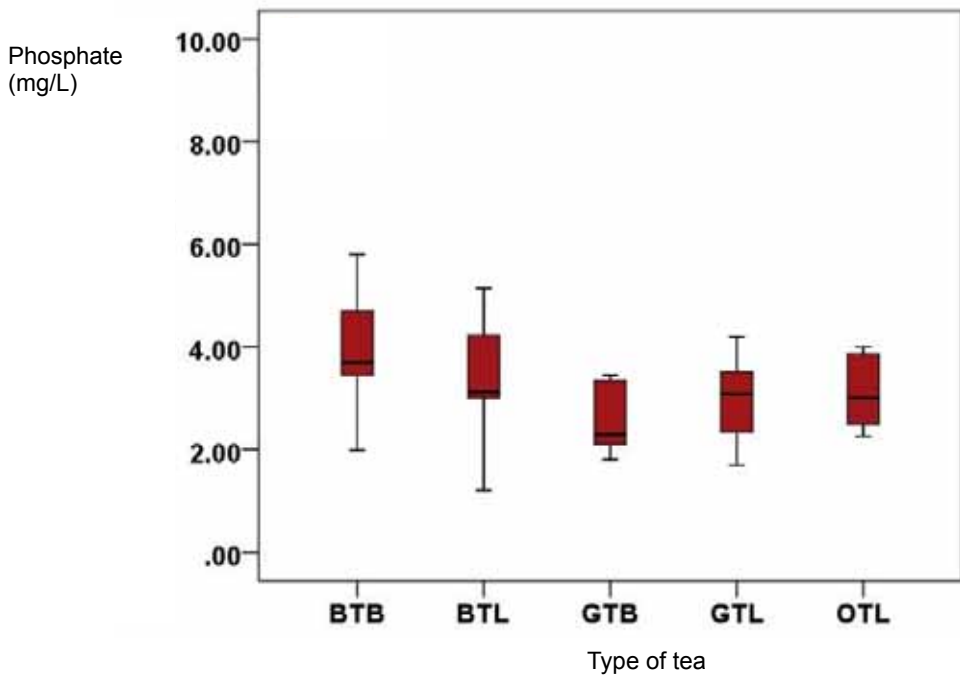


Figure 2. Phosphate content (minimum, mean, and maximum) in the studied tea infusions. BTB=black tea bag; BTL=black tea leaf; GTB=green tea bag; GTL=green tea leaf; OTL=oolong tea leaf.

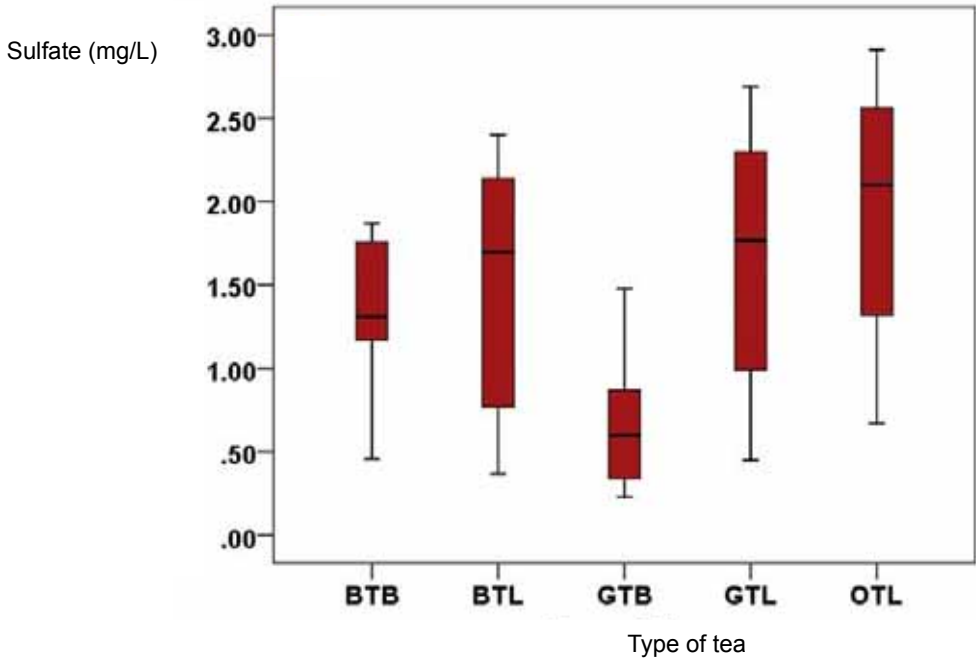


Figure 3. Sulfate content (minimum, mean, and maximum) in the studied tea infusions. BTB=black tea bag; BTL=black tea leaf; GTB=green tea bag; GTL=green tea leaf; OTL=oolong tea leaf.

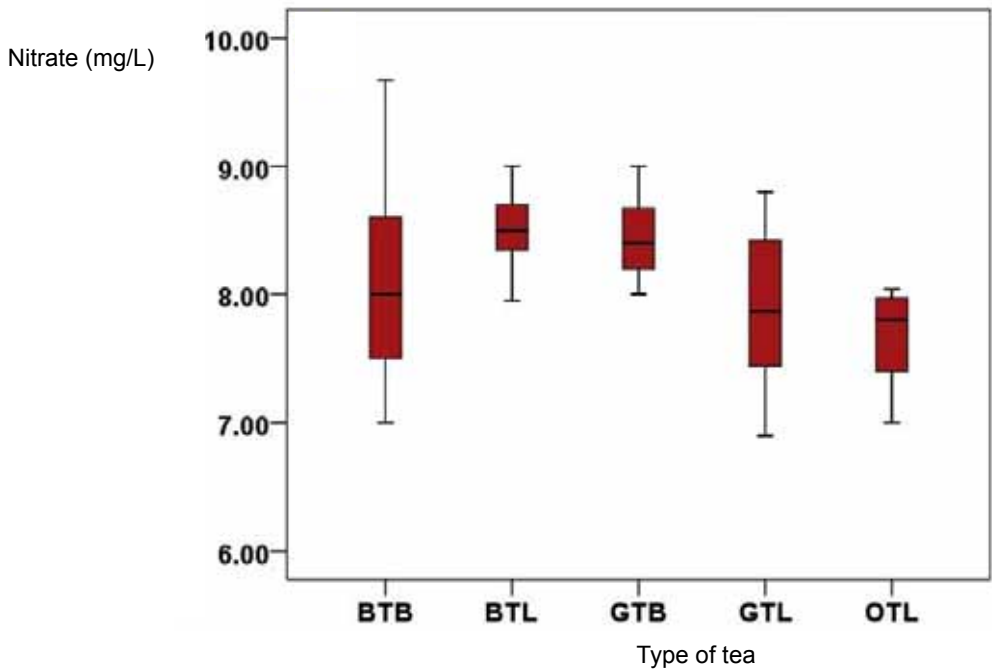


Figure 4. Nitrate content (minimum, mean, and maximum) in the studied tea infusions. BTB=black tea bag; BTL=black tea leaf; GTB=green tea bag; GTL=green tea leaf; OTL=oolong tea leaf.

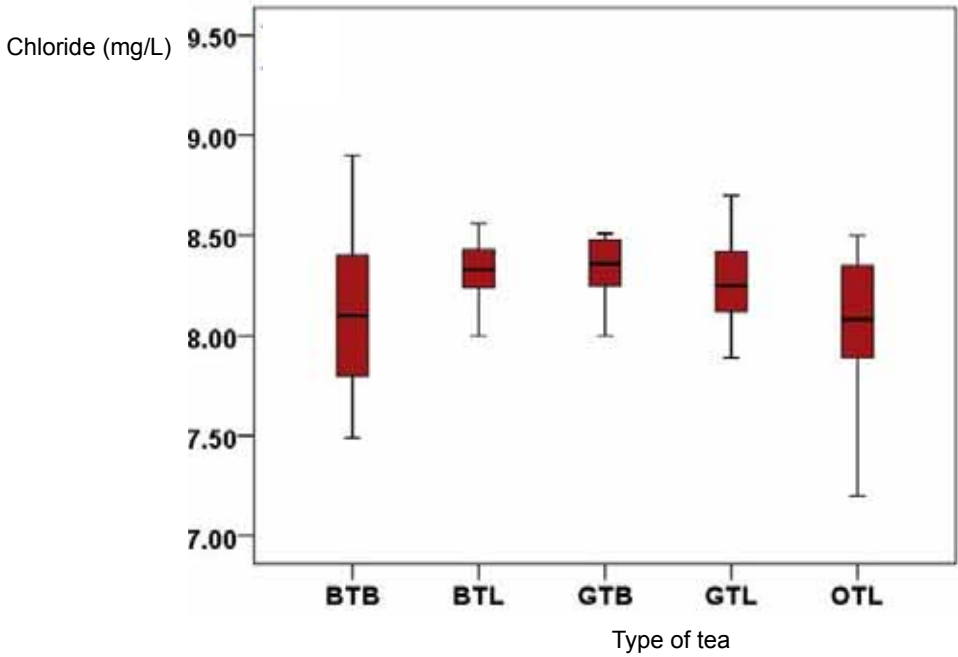


Figure 5. Chloride content (minimum, mean, and maximum) in the studied tea infusions. BTB=black tea bag; BTL=black tea leaf; GTB=green tea bag; GTL=green tea leaf; OTL=oolong tea leaf.

Figure 6 shows the effect of different degrees of water hardness on the extraction of fluoride from tea leaves.

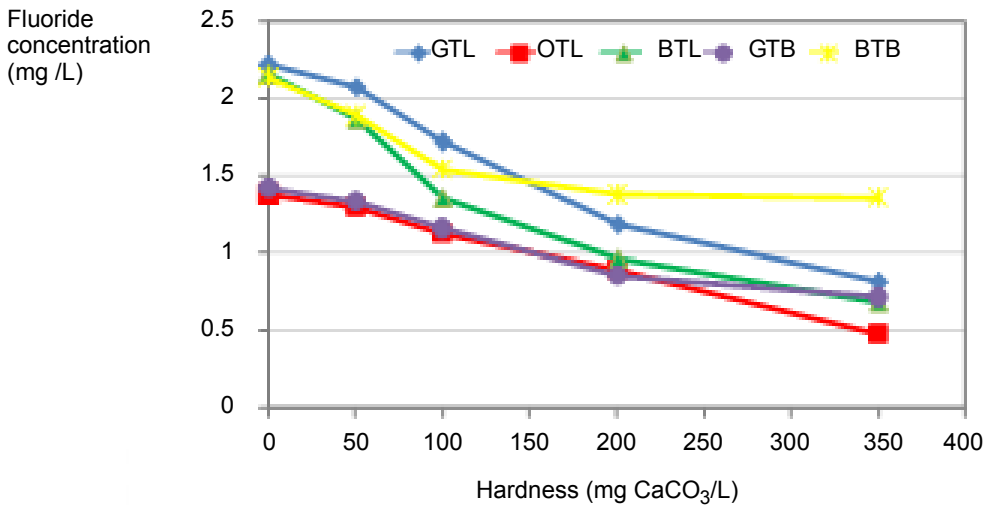


Figure 6. Effect of different degrees of water hardness on the extraction of fluoride from dried tea. GTL=green tea leaf; OTL=oolong tea leaf; BTL=black tea leaf; GTB=green tea bag; BTB=black tea bag.

The fluoride concentrations decreased as the water hardness increased. Accordingly, the fluoride content of tea liquors made with soft water will be higher than those made with hard water. The Tukey test showed there was no statistically significant difference between the F concentrations in the tea liquors with 0, 50 and 100 mg CaCO₃/L of water hardness (p>0.05) but a significant difference was present with water hardness above 100 mg CaCO₃ /L (p<0.05). The quality of water used for tea preparation affects not only the composition of the tea infusion, but also the color and the taste of the tea liquor. Hard water inhibits the extraction of some of the components of tea and is detrimental to the quality of the tea. Based on the experience of consumers, tea brewed in soft water appears brighter than if it is brewed in temporary hard water. Therefore, there is a tendency to use soft water for tea preparation in the community and this can be a risk for increasing fluoride intake. To avoid this problem and also for consumer satisfaction, the use of water with a natural mineral content that is neither too hard nor too soft is recommended. A few studies show that there is a correlation between fluoride and some inorganic constituents in groundwater.¹⁸

A high intake of fluoride can result in toxicity including skeletal and dental fluorosis in humans.¹² For this reason, the daily fluoride intake from the consumption of different amounts of the studied teas was determined (Table 2).

Table 2. Expected daily fluoride intake (mg/day) through the consumption of different quantities of tea

Tea type*	Expected daily fluoride intake (m g/day)			
	Quantity of tea consumed daily			
	1 cup	2 cups	3 cups	4 cups
GTL	0.46	0.92	1.37	1.83
OTL	0.29	0.59	0.88	1.18
BTL	0.57	1.15	1.72	2.29
GTB	0.36	0.72	1.08	1.44
BTB	0.48	0.96	1.44	1.92

*GTL=green tea leaf; OTL=oolong tea leaf; BTL=black tea leaf; GTB=green tea bag. BTB=black tea bag.

If a person consumes four 100 mL cups of tea per day (total of 400 mL) and each cup uses 2 g of tea leaves or one tea bag, the daily fluoride intake ranges from 1.18 mg F/day (for OTL) to 2.29 mg F/day (for BTL). According to the WHO, there is an increased risk of effects on the skeleton at total intakes above 6 mg F/day and when intakes are likely to approach or be greater than 6 mg/day it would be appropriate to consider setting a standard or local guideline concentration for fluoride in drinking water lower than 1.5 mg F/L.^{21, 22} In children, “safe and adequate daily dietary intakes of F” were estimated in 2006, by a Committee on Fluoride in Drinking Water of the National Research Council (NRC)²³ based on the recommended dietary allowances of the NRC,²⁴ ranging from 0.017–0.083 mg F/kg body weight/day at ages 0–0.5 yr (0.1–0.5 mg F/day) to 0.054–0.089 mg F/kg

body weight/day at ages 7–10 yr (1.5–2.5 mg F/day). The “safe and adequate” range for children at ages 4–6 yr was 1.0–2.5 mg F/day.²³ The “safe and adequate” values for older children and adults were: males 11–18 yr: 1.5–2.5 mg F/day; males 19–>51 yr: 1.5–4.0 mg F/day; females 11–18 yr: 1.5–2.5 mg F/day; and females 19–>51 yr: 1.5–4.0 mg F/day.²³ Thus, according to these guidelines, the consumption of 4 cups of tea, containing 1.18–2.29 mg F, prepared from all of the studied teas would be “safe” for children aged 4 yr and older and adults. However, the NRC Committee noted that the use of the term “safe and adequate daily dietary intake” should not be taken to imply that the present committee considered these intakes to be safe or adequate.²³

Due to importance of tap water for drinking and tea preparation purposes, the daily fluoride intakes from 4×100 mL cups of tea brewed with drinking water containing various fluoride levels were calculated (Table 3).

Table 3. Expected fluoride intake (mg) through consumption of 4 cups of tea brewed with drinking water with different fluoride levels

Tea type*	Expected fluoride intake (mg) through consumption of 4 cups of tea brewed with drinking water with different fluoride levels				
	Drinking water fluoride concentration (mg/L)				
	<0.1	0.1–0.5	0.5–1.5	1.5–4	4–7
GTL	<1.83	1.83–2.03	2.03–2.43	2.43–3.43	3.43–4.63
OTL	<1.22	1.22–1.38	1.38–1.78	1.78–2.78	2.78–3.10
BTL	<2.33	2.33–2.49	2.49–2.89	2.89–3.89	3.89–5.09
GTB	<1.48	1.48–1.64	1.64–2.04	2.04–3.04	3.04–4.24
BTB	<1.96	1.96–2.12	2.12–2.52	2.52–3.52	3.52–4.72

*GTL=green tea leaf; OTL=oolong tea leaf; BTL=black tea leaf; GTB=green tea bag. BTB=black tea bag.

In discussing how much tea may be safely drunk, Wong et al.,²⁵ noted the WHO optimal level for F intake in humans of 2–4 mg F/day, the United States Environmental Protection Agency, F intake recommendation, based on the maximum contaminant level, of 2.5 mg F/day for children and 4.0 mg F/day for adults, and, based on papers by Sha and Zheng,²⁶ and Cao et al.,²⁷ the total daily fluoride intake, when taken chronically, that may cause fluorosis in adults of over 13.0–14.5 mg F/day.²³ The National Research Council report on *Fluoride in drinking water: a scientific review of EPA’s standards* found that the predicted bone fluoride concentrations that can be achieved from lifetime exposure to fluoride at 4 mg/L (10,000 to 12,000 mg F/kg bone ash) fell within or exceeded the ranges of concentrations that have been associated with stage II and stage III skeletal fluorosis.²⁸ Carton found that, when iodine intake was adequate, human thyroid function was affected with fluoride exposures of 0.05–0.13 mg/kg body

weight/day, corresponding to 3.5 mg F/day for a 70 kg adult, and when iodine intake was inadequate, thyroid function was affected with fluoride exposures of 0.01–0.03 mg/kg body weight/day, corresponding to 0.7 mg F/day for a 70 kg adult.²⁹ He noted that seriously detrimental brain effects occurred in animals with a drinking water fluoride level of 0.3 mg/L, when associated with aluminium, and recommended that the Maximum Contaminant Level Goal (MCLG) should be zero.²⁹ Thus, the total fluoride content, from both the tea and the fluoride in the drinking water, in 4 cups of the studied teas (Table 3) may make a significant contribution to the daily dietary F intake of F, and, when combined with other dietary F from drinking water and food, may result in an intake in the range 3.5–4.0 mg F/day that has been the subject of concern related to thyroid and skeletal function. Lower intakes may be necessary to provide protection from adverse central nervous system effects. To be on the safe side, since the consumption of black tea is very common in this region and the amount of fluoride is high in drinking black tea, it is recommended to prepare tea with low fluoride bottled water or water treated for F removal.^{30,31}

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Environmental Health Research Center, Kurdistan University of Medical Sciences, for financial assistance, laboratory support, and material preparation.

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