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Daily Fluoride Intake from Iranian Green Tea: Evaluation of Various Flavorings on Fluoride Release

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ABSTRACT: With increased awareness of the health benefits of the compounds in green tea, especially polyphenols, its consumption is rising. The main purpose of this study is to determine the effect of different additives on the released fluoride into tea liquor and also daily fluoride intake. The concentrations of fluoride, nitrate, sulfate, and chloride were measured in 15 different flavored green teas (Refah-Lahijan). The fluoride and other anion concentrations were measured by ion chromatography method. The data were analyzed with Statistical Package for the Social Sciences version 16.0. The results showed that the minimum and maximum concentrations of fluoride in the green tea infusions were 0.162 mg/L (cinnamon-flavored green tea) and 3.29 mg/L (bagged peach-flavored green tea), respectively. The mean concentration of fluoride in the green tea leaves was 52 mg/kg, and approximately 89% of the fluoride was released from the green tea leaves into the infusions after brewing. The fluoride concentrations varied significantly among the examined green teas ($P < 0.05$). However, the additives had no significant effect on the fluoride release into the infusions ($P > 0.05$). Finally, drinking of the studied green teas cannot make a significant contribution to the daily dietary intake of F for consumers.

KEYWORDS: fluoride, daily intake, green tea, tea infusion, flavorings

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Introduction

Green tea has been used as a medicine for thousands of years in China. Recently, its health benefits have caused its increased consumption worldwide.[1] Green tea is proved to have antioxidant, antimutagenic, antidiabetic, anti-inflammatory, antibacterial and antiviral, and anticarcinogenic activities. Such properties lead to prevention and/or even therapy of different cancers, protection of skin against ultraviolet light, and oral health.[2] The unique health benefits of green tea are due to its processing type, which retains the maximum amount of antioxidants and polyphenols in its dried leaves. However, these compounds are responsible for the bitter taste of green tea that may be unfavorable for some consumers. To mask the bitter taste of green tea, various additives have been used. For example, jasmine flowers are used in China.[1,3] In Iran, people usually use jasmine, rose, mint, cardamom, dried or fresh lemon, and saffron for this purpose More favorable taste causes more consumption. It is noteworthy that there is a large amount of fluoride in the tea plant (around 100–200 mg/kg),[4] 98% of which has been accumulated in its leaves and can be easily released into the tea infusion.[1] Thus, tea liquor can be a major source of fluoride. Although fluoride is an important micronutrient in small concentrations and reduces dental caries among children and adults, it becomes toxicant at high concentrations and may cause dental and skeletal fluorosis.[5–8] Therefore, increased consumption of green tea can increase the risk of fluorosis and osteoarthritis.[1,3] Hayacibara et al have introduced 0.05–0.07 mg F per kg body weight per day as a generally accepted reference.[9] However, its maximum clinically acceptable content has not yet been well known.

Besides fluoride, there are other inorganic ions in tea composition, such as nitrate, sulfate, chloride, and phosphate. These anions have a vital role in human health. For example, chloride causes muscle irritability. Phosphates are an important element in energy cycle and tissue growth and recovery[8] Nitrates widen arteritis, and it seems that they reduce cardiovascular diseases.[10] However, high concentration of these anions may have dangerous effects on the human body. Therefore, the Joint FAO/WHO Expert Committee on Food Additives has set an acceptable daily intake (ADI) of 0–3.7 mg/kg body weight for nitrate[11] and 10 g/kg body weight for phosphate.[12] However, it has left the ADI of sulfate as *not specified*.[13] The US Institute of Medicine has established an adequate intake of 1.8–2.3 g/day for chloride.[14]

In spite of the significant health impacts of tea and its complex compositions,[15] it is important to know that adding different compounds may change the acidity of the aqueous environment and cause an increase or a decrease in the released fluoride from tea leaves. The main objective of this study was to determine the level of released fluoride from plain and various essences of processed green tea leaves into their brewed liquor. The concentrations of other ions such as chloride, nitrate, sulfate, and phosphate were also determined in brewed tea by ion chromatography method.

Material and Methods

Tea samples. In this descriptive–analytical study, leaf and bagged forms of plain green tea and 15 different types of flavored green tea were purchased from a manufacturer (Refahe-Lahijan) in northern Iran and analyzed for their fluoride, nitrate, sulfate, chloride, and phosphate concentrations. Eight samples were taken from each type of the tea and brewed according to the manufacturer's instructions.

Experiments. For each type of tea, the infusion was prepared as follows:. For dried tea leaves, 1.0 g of each type of tea leaves was added into 100 mL of boiling ultrapure water in a Teflon tea pot, and the mixture was kept at 80 °C in the incubator for brewing according to the manufacturer's recommended time.

For bagged teas, first, bags were cut and the net weight of the teas inside of each bag was weighted. They were heavier than 1 g; therefore, to overcome this problem and to have real situations of using bagged teas, depending on the net weight of teas inside the bags, relatively larger amounts of water were used for infusion of original tea bags. The subsequent procedures were the same as for the tea leaves.

At the end of the infusion period, the tea liquor was stirred and a 1-mL aliquot was taken from each sample and diluted to 100 mL with ultrapure water. Then, the samples were filtered through a 0.45 µm membrane filter and prepared to be analyzed for their anions. Each sample was analyzed twice. To check the experimental conditions, all the procedures for the samples were repeated for control samples containing no tea samples in them.

A total of 1000 ppm stock solutions of sodium fluoride, sodium nitrate, sodium chloride, sodium sulfate, and sodium phosphate were used to prepare standard solutions of fluoride (0–4 ppm), nitrate (0–10 ppm), chloride (0–10 ppm), sulfate (0–10 ppm) and phosphate (0–10 ppm), respectively. Using these standard solutions for plotting their calibration curves, fluoride, nitrate, chloride, sulfate, and phosphate in tea samples were determined.

Ionic contents were determined by ion chromatography using an 882 compact IC plus (Metrohm AG) made up of an isocratic eluent delivery pump, sample injection port, separation column, suppressor, and conductivity detector. The mobile phase was prepared by preparing the solvent of 1.8 mmol/L sodium carbonate and 1.7 mmol/L sodium hydrogen carbonate. All chromatographic experiments were done at 25 °C. The mobile phase was filtered through 0.45 µm filter and degassed before use. Metrosep A Supp 4-250/4 analytical column, from Metrohm, containing 9 µm polyvinyl alcohol with quaternary ammonium groups anion exchanger was guarded by a Metrosep A Supp 4.4/5 guard. The eluent flow rate was 1 mL/minute, while the chemical suppression was done by MSM suppressor and, also, carbonate elimination was done by a CO_2 suppressor.

Determination of F concentration in the dried tea leaves was performed as follows: 0.1 g of each tea leaf samples was dried for 5 hours at 60 °C and mixed with 3 mL of 0.1 N NaOH in a nickel crucible inside an oven for 2.5 hours at 150 °C. After that, the crucible was put in a muffle furnace for 30 minutes at 300 °C and for another 30 minutes at 600 °C. After cooling, 5 mL of distilled water was added to each sample. Then, the pH of the samples was adjusted to 8–9 using HCl 37%, the samples were added to an equal volume of total ionic strength adjustment buffer (TISAB), and the mixture was diluted to the mark in a 100-mL volumetric flask with distilled water. The solution, then, was filtered through Whatman No. 40 filter paper. Sodium citrate dihydrate (22.05 g) and sodium hydroxide (0.8 g) were dissolved in deionized water and diluted to 100 mL to prepare the TISAB solution. The pH of this solution was adjusted to 5.3 using $HClO_4$.[4]

Daily fluoride intake was also assessed. To do this, daily fluoride intake by consumption of one to four cups of the studied green teas, and also the expected fluoride intake through consumption of four cups of the teas brewed with drinking water containing different fluoride levels (<0.1–7 mg/L) were determined.

Materials. All chemicals were of analytical reagent grade (Merck) and were used without further purification.

Data analysis. All data were analyzed by Statistical Package for the Social Sciences version 16 using analysis of variance (ANOVA; to compare F concentration among the different tea samples and different anions in each tea sample), Tukey's test (to compare F concentration between tea samples and different anions in each tea sample), and one-sample *t*-test (to compare F concentration in the studied tea samples with the World Health Organization [WHO] recommendation for daily fluoride intake thresholds for different age groups).

Results

Fluoride in the tea samples. The fluoride concentrations of 16 different types of plain and flavored leaf and bagged green teas in their dried and liquor samples are tabulated in Table 1. The results showed a wide range of average fluoride concentration, from 15 to 173.1 mg/kg, in the studied dry green teas. The minimum and maximum average fluoride concentrations in tea liquor were seen in cinnamon green tea leaf (CNGTL), in the range of 0.14–0.18 mg/L, and peach green tea bag (PGTB), in the range of 3.24–3.31 mg/L, respectively.

Table 1. Total fluoride content of dry flavored green tea and tea liquor.

TEA FORM	TYPE OF ESSENCE	ABBREVIATIONS	F CONTENT mg/kg DRY TEA LEAF	F CONTENT MG/L TEA LIQUOR				% OF F INFUSED
				MEAN ± SD	95% CONFIDENCE INTERVAL FOR MEAN		RANGE	
					LOWER BOUND	UPPER BOUND		
Bag	Mint	MGTB	116.2	1.85 ± 0.06	1.71	1.99	1.81–1.92	79
Bag	Saffron	SGTB	59.9	0.75 ± 0.13	0.45	1.06	0.64–0.88	62
Bag	Lime	LGTB	66.9	0.88 ± 0.07	0.71	1.05	0.81–0.95	71
Bag	Cinnamon	CNGTB	71.6	1.10 ± 0.03	1.02	1.18	1.06–1.12	74
Bag	Apple	AGTB	74	1.19 ± 0.06	1.04	1.33	1.12–1.23	76
Bag	Ginger	GGTB	72.8	1.18 ± 0.03	1.11	1.25	1.16–1.21	80
Bag	Orange blossom	OGTB	47	0.64 ± 0.02	0.57	0.70	0.61–0.66	65
Bag	Cardamom	CGTB	164.5	2.88 ± 0.11	2.61	3.14	2.77–2.98	88
Bag	Peach	PGTB	184.8	3.29 ± 0.03	3.20	3.36	3.24–3.31	89
Bag	Plain	GTB	80.6	1.35 ± 0.05	1.23	1.48	1.31–1.41	80
Leaf	Mint	MGTL	24.5	0.28 ± 0.06	0.13	0.43	0.22–0.34	56
Leaf	Cinnamon	CNGTL	15.7	0.16 ± 0.02	0.13	0.23	0.14–0.18	55
Leaf	Cardamom	CGTL	23.4	0.54 ± 0.04	0.44	0.63	0.51–0.58	62
Leaf	Orange blossom	OGTL	33.7	0.34 ± 0.02	0.29	0.39	0.32–0.36	54
Leaf	Lemon	LGTL	25.2	0.26 ± 0.01	0.25	0.28	0.26–0.27	52
Leaf	Plain	GTL	15	0.25 ± 0.02	0.20	0.30	0.23–0.27	54

The plain leaf of green tea showed a lower fluoride content than the flavored leaves of green tea. As seen in Table 1, for the flavored green tea leaves, the fluoride content followed the order of OGTL > LGTL > MGTL > CGTL > CNGTL > GTL and for the bagged ones the order was as PGTB > CGTB > MGTB > GTB > AGTB > GGTB > CNGTB > LGTB > SGTB > OGTB. One-way ANOVA results revealed a statistically significant difference among the mean fluoride concentrations of different green tea infusions ($P < 0.001$). Using Tukey's test, it was found that significant differences existed between the mean fluoride concentration in green teas, when they were compared pairwise ($P < 0.05$). However, according to statistical analysis, no significant difference was found in the fluoride levels among the dried tea samples ($P > 0.05$). The extraction of fluoride from tea leaves into tea liquor was also compared for different types of green teas (Table 1). As can be seen from Table 1, the percentage of the total fluoride content of the dried tea released into the tea liquor ranged from about 52% for LGTL to 89% for PGTB. According to statistical analysis, no significant difference was found in the fluoride levels among the dried teas and their infusions ($P > 0.05$). Therefore, it is not expected that such additives affect the amount of fluoride in tea.

Other anions in the tea samples. The concentrations of other anions in different tea infusions are presented in Table 2. The results showed a large variation for all the four anions: chloride (ranging from 0.21 to 2.74 mg/L, with an average of 1.01 mg/L), nitrate (ranging from 0.14 to 3.61 mg/L, with an average of 1.11 mg/L), sulfate (ranging from 0.12 to 2.72 mg/L, with an average of 1.18 mg/L), and phosphate (ranging from 0.23 to 2.92 mg/L, with an average of 0.79 mg/L). Based on the one-way ANOVA results, there were statistically significant differences among the mean concentrations of all the studied anions in green tea infusions ($P < 0.001$).

Tukey's test results are shown in Table 2, with the asterisks on the anions (other than F) showing how these anions have or do not have statistically significant differences with fluoride ion. According to Table 2, no fixed pattern exists between fluoride and other ion concentrations in tea infusions.

Daily fluoride intake by consumption of the studied green teas. Daily fluoride intake by consumption of different amounts of the studied green teas was also determined and presented in Table 3. As can be seen in Table 3, if a person consumes four cups (400 mL) of tea per day and considering 2 g of tea leaves or one tea bag per cup, daily fluoride intake would vary from 0.06 g for GTL to 1.32 g for PGTB.

Effect of drinking water fluoride on fluoride daily intake by tea infusion. Due to the importance of tap water for drinking purpose and tea preparation, and in order to provide a perspective of daily fluoride intake in different parts of the country, the results of daily fluoride intake modified for flavored green tea brewed with drinking water containing various levels of fluoride (Table 4).

Table 2. Anion content in flavored green tea infusions.

TEA TYPE	ANIONS CONCENTRATION IN TEA BREW (mg/l)				
	NITRATE	CHLORIDE	SULFATE	PHOSPHATE	FLUORIDE
MGTB	0.21 ± 0.03***	0.41 ± 0.02***	1.22 ± 0.15***	0.28 ± 0.04***	1.85 ± 0.06
SGTB	0.56 ± 0.05***	0.21 ± 0.04	0.58 ± 0.06	1.34 ± 0.11***	0.75 ± 0.01
LGTB	0.14 ± 0.02***	0.47 ± 0.07***	2.17 ± 0.09***	0.67 ± 0.09***	0.88 ± 0.07
CNGTB	2.10 ± 0.13***	1.76 ± 0.11***	2.5 ± 0.12***	0.23 ± 0.03***	1.10 ± 0.03
AGTB	1.17 ± 0.26	1.60 ± 0.10***	1.17 ± 0.15	1.45 ± 0.32**	1.19 ± 0.06
GGTB	1.52 ± 0.31***	2.6 ± 0.45***	2.3 ± 0.41***	0.24 ± 0.03***	1.18 ± 0.03
OGTB	0.80 ± 0.10*	0.28 ± 0.06***	0.73 ± 0.08	0.66 ± 0.06	0.64 ± 0.02
CGTB	1.32 ± 0.21***	2.74 ± 0.38	2.3 ± 0.26***	2.1 ± 0.23***	2.88 ± 0.11
PGTB	3.61 ± 0.62*	0.23 ± 0.02***	0.83 ± 0.07***	2.92 ± 0.23*	3.29 ± 0.03
GTB	1.88 ± 0.45***	1.93 ± 0.23***	2.72 ± 0.34***	0.27 ± 0.02***	1.35 ± 0.05
MGTL	0.63 ± 0.16	0.46 ± 0.05*	0.53 ± 0.04*	0.36 ± 0.07***	0.28 ± 0.06
CNGTL	0.47 ± 0.05**	0.39 ± 0.02*	0.56 ± 0.02**	0.31 ± 0.02***	0.16 ± 0.02
CGTL	0.63 ± 0.04	0.50 ± 0.06	0.42 ± 0.04	0.62 ± 0.07	0.54 ± 0.04
OGTL	0.48 ± 0.06*	0.38 ± 0.04	0.53 ± 0.07*	0.41 ± 0.05	0.34 ± 0.02
LGTL	0.80 ± 0.07***	0.61 ± 0.08***	0.27 ± 0.05	0.42 ± 0.04*	0.26 ± 0.01
GTL	1.37 ± 0.33***	1.56 ± 0.15***	0.12 ± 0.02**	0.34 ± 0.02*	0.25 ± 0.02

Notes: *$P < 0.05$. **$P < 0.001$. ***$P < 0.0001$.

Discussion

Since all the studied tea samples were obtained from a single manufacturer (placed in the main area of tea cultivation in Iran), the broad variation of the fluoride concentrations in green teas might be due to the differences in the quality of irrigation water, harvesting time, and harvesting system. But, probably it is not related to the type of the processing.[16] Moreover, fluoride concentrations in tea liquor of the bagged green teas were higher than the leaf ones. This might be due to the application of old and very fine-grained tea leaves with a low quality to make the tea bags. It is known that the level of fluoride in the low grade (especially in dust form) and older tea leaves is higher than the young shoots.[16] This finding is in agreement with the result obtained by Fung et al.[17] Also, Tokalioglu et al reported that fluoride accumulation in old leaves was over 2000 mg/kg and in young leaves it ranged from 250 to 360 mg/kg.[18] Comparing the results of the present study with those of others indicated that there is a difference in the amount of fluoride in green tea leaves and their tea liquors,[19–21] which might be due to the origin of green tea, its processing methods, and brewing ways.

Table 3. Expected daily fluoride intake through consumption of different amounts of flavored green teas.

TEA TYPE	DAILY FLUORIDE INTAKE (mg/day)			
	1-CUP	2-CUP	3-CUP	4-CUP
MGTB	0.18	0.37	0.55	0.73
SGTB	0.07	0.15	0.22	0.30
LGTB	0.10	0.19	0.29	0.38
CNGTB	0.11	0.21	0.32	0.42
AGTB	0.11	0.22	0.34	0.45
GGTB	0.12	0.23	0.35	0.47
OGTB	0.06	0.12	0.18	0.25
CGTB	0.30	0.60	0.89	1.19
PGTB	0.33	0.67	0.99	1.32
GTB	0.13	0.26	0.39	0.52
MGTL	0.03	0.06	0.08	0.11
CNGTL	0.02	0.03	0.05	0.07
CGTL	0.03	0.06	0.09	0.12
OGTL	0.04	0.07	0.11	0.15
LGTL	0.03	0.05	0.08	0.10
GTL	0.02	0.03	0.05	0.06

Table 2 shows that tea is an important source of chloride, nitrate, sulfate, and phosphate. The tea plant takes up these anions from soil and accumulates them in its leaves. Therefore, according to Tables 1 and 2, a substantial amount of these anions is released during tea infusion and will be absorbed by consumers. Variation among these anions might be explained similarly to what was expressed for fluoride. In terms of maximum allowed levels of anions in foodstuff, there were no overridden values. Comparing the results presented in Table 3 with the WHO recommendation for daily fluoride intake thresholds[22,23] for different age groups (2 mg/kg for children and 2–4 mg/kg for adults), one-sample *t*-test showed that it is safe for all the age groups to consume four cups of tea prepared from all types of the studied green teas. According to Wong et al, a total daily fluoride intake of over 13.0–14.5 mg/day

Table 4. Expected fluoride intake through consumption of four cups of the teas brewed with drinking water with different fluoride levels.

TEA TYPE	DRINKING WATER FLUORIDE CONCENTRATION (mg/l)				
	<0.1	0.1–0.5	0.5–1.5	1.5–4	4–7
MGTB	<0.77	0.77–0.93	0.93–1.33	1.33–2.33	2.33–3.53
SGTB	<0.34	0.34–0.50	0.50–0.90	0.90–1.90	1.90–3.10
LGTB	<0.42	0.42–0.58	0.58–0.98	0.98–1.98	1.98–3.18
CNGTB	<0.46	0.46–0.62	0.62–1.02	1.02–2.02	2.02–3.22
AGTB	<0.49	0.49–0.65	0.65–1.05	1.05–2.05	2.05–3.25
GGTB	<0.51	0.51–0.67	0.67–1.07	1.07–2.07	2.07–3.27
OGTB	<0.29	0.29–0.49	0.49–0.85	0.85–1.85	1.85–3.05
CGTB	<1.23	1.23–1.39	1.39–1.79	1.79–2.79	2.79–3.99
PGTB	<1.36	1.36–1.52	1.52–1.92	1.92–2.92	2.92–4.12
GTB	<0.56	0.56–0.72	0.72–1.12	1.12–2.12	2.12–3.32
MGTL	<0.15	0.15–0.31	0.31–0.71	0.71–1.71	1.71–2.91
CNGTL	<0.11	0.11–0.27	0.27–0.67	0.67–1.67	1.67–2.87
CGTL	<0.16	0.16–0.32	0.32–0.72	0.72–1.72	1.72–2.92
OGTL	<0.19	0.19–0.35	0.35–0.75	0.75–1.75	1.75–2.95
LGTL	<0.14	0.14–0.30	0.30–0.70	0.70–1.70	1.70–2.90
GTL	<0.11	0.11–0.27	0.27–0.67	0.67–1.67	1.67–2.87

may cause fluorosis in adults.[24] Thus, it can be said that drinking the studied green teas in the mentioned ranges (Tables 3 and 4) cannot make a significant contribution to the daily dietary intake of F for consumers.

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Author Contributions

Designed the study, coordinated all the experiments, participated in manuscript preparation and final approval of the manuscript: AM. Helped in manuscript preparation, data analysis and final approval of the manuscript: HD. Performed laboratory tests, drafting the work, final approval of the manuscript: EM. Performed laboratory tests and data analysis and interpretation, final approval of the manuscript: SZ. Contributed to data analysis and interpretation, critical revision and final approval of the study: PT. Counselor, contributed to manuscript preparation, and final approval of the manuscript: AHM. Statistical analysis, critical revision and final approval of the manuscript: FG. All authors reviewed and approved of the final manuscript.

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