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Potentiometric Determination of Fluoride Release from Three Types of Tea Leaves

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The purpose of this study is to investigate the fluoride release properties from three types of tea leaves which are commonly consumed in Hong Kong and Hangzhou. Totally 72 drinking water samples of 50 ml each were collected from Hong Kong Island and Hangzhou. The drinking water collected and deionized (D.I.) water were used to brew puerh tea leaves, black tea bags, and green tea leaves by two infusion methods (repeated and continuous). Fluoride concentration of drinking and D.I. water and tea infusion samples taken at specific time intervals was measured by fluoride-ion selective electrode. In tea infusion samples, pH value was recorded as well. Fluoride concentration (ppm) of drinking water samples collected from Hong Kong (95% CI = 0.48-0.54) was significantly higher than Hangzhou (95% CI= 0.19-0.20). Black tea bags (about 10 ppm) released significantly higher fluoride content than puerh tea leaves (about 5 ppm), which was significantly higher than green tea leaves (about 2 ppm) ($p<0.05$). Prepared by repeated infusion method, original fluoride level in brewing water (D.I. water<drinking water) and round of infusion ($1^{st}<2^{nd}<3^{rd}$) also statistically related to fluoride content in tea infusions. The pH values of all tea infusion samples were below 7.0 and had no significant relation with fluoride release. Fluoride concentration in tea infusion could saturate within several minutes after brewing. It seems the dynamics of fluoride release from tea leaves may be affected by original ionic level of brewing water, brewing time, and round of infusion.

Keywords: fluoride, tea leaves, drinking water

1. INTRODUCTION

The impact of fluoride on enamel structure was evident. It was first noted as a side-effect of fluoride in early 1900's as "enamel fluorosis". High-level water-borne fluoride ingestion during

enamel formation period was recognized as the major cause to this defect [1]. At the same time, the low prevalence of caries on fluorosed teeth was also concluded by Black and McKay [2]. Since then, the dual role of fluoride to teeth has been substantially studied to maximize the caries-preventive effect with minimum occurrence of side-effect [3, 4]. Other than a variety of applications and formulae of fluoride advocated during past decades, food consumption, including tea-drinking, is still the major regular source of daily fluoride intake.

Tea leaves are rich in fluoride and increasingly popular worldwide. This healthy drink contains various bioactive components, e. g. tannin, catechin, caffeine etc. Fluoride is the best documented one among them which benefits dental health via both systemic and topical ways [5]. Fluoride in tea infusion absorbed through oral and gastro-intestinal ingestion may incorporate into enamel structure and enhance acid-resistance of teeth. It is believed that fluoride retained on tooth surface could locally remineralize enamel, dilute acid and offer anti-bacterial properties [4, 5]. Moderate ingestion of tea could provide protection against dental caries [6]. Nevertheless, potential risk of overexposure to fluoride exists because of uncontrolled drinking of some type of tea containing extremely high fluoride level [3, 7]. It is hoped that the understanding of the dynamics of fluoride release from tea leaves could give some insight in avoiding this problem.

United Nations reported that China nowadays is the largest tea producing and consumption country in the world [8]. Hong Kong and Hangzhou are two cities where different tea cultures are deep-rooted in peoples' lifestyle. In Hong Kong, where a unique tea culture has flourished as a combination of Southern Chinese culture and the colonial historical background, puerh tea and black tea bags are most popular types of tea consumed daily. In addition, drinking water of Hong Kong which is used for brewing tea was artificially-fluoridated to around 0.5 ppm (ranged from 0.2 ppm to 0.7 ppm) based on the officially issued report [9]. Unlike Hong Kong, a famous green tea "Longjing" was best produced and popularly consumed in Hangzhou. Drinking water in Hangzhou was not artificially fluoridated according to personal communication with the government. It is also of public health interest to obtain information about the properties of fluoride release from tea leaves and tea-bags based on two geographical distinct tea cultures.

In this study, puerh tea leaves, black tea bags, and green tea leaves were infused by drinking water collected from Hong Kong Island and Hangzhou. Long-term continuous infusion method was used in some tea houses. The other repeated infusion method was ordinarily used in households and restaurants. These two common infusion methods were adopted to test all three types of tea. The purpose of this study is to investigate the dynamics of fluoride release from tea leaves by using fluoride-ion selective electrode, which is an easy and accurate electrochemical method [10], and pH measurement.

2. MATERIALS AND METHODS

2.1. Drinking water and de-ionized water collection

Drinking water samples were collected from most densely populated areas of Hong Kong Island, Hong Kong SAR, China and Hangzhou, Zhejiang province, China. Six households from four

geographical districts of Hong Kong Island (Central and Western, Wan Chai, Southern, and Eastern) and eighteen households from six main urban districts of Hangzhou (Xiacheng, Shangcheng, Binjiang, Gongshu, Xihu and Jianggan) were randomly selected. From each household, three samples of 50 ml each of tap water were collected with polypropylene centrifugal capped tubes. Drinking water samples from Hangzhou were refrigerated and shipped at 4 ^{o}C. The technique used is adopted from WHO report [11] and China National Standard [12].

Three de-ionized (D.I.) water samples of 50 ml each were obtained from the laboratory under the same procedure as drinking water. All samples were analyzed within 14 days after collection.

2.2. Preparation of tea infusions by repeated and continuous methods

Three types of commercially packed tea, puerh tea leaves (Yunnan, China), black tea bag (Lipton®, blended and packed in India, distributed by Unilever Hong Kong Ltd., Hong Kong SAR), and green tea leaves (Luzhenghao® Tea Co. Ltd., Zhejiang, China) were purchased. To simulate the daily situation of tea preparation, puerh tea leaves and black tea bags were brewed with drinking water from Hong Kong Island only and green tea leaves was brewed with drinking water collected from Hangzhou only. D.I. water was used as control groups of all three types of tea leaves. Repeated and continuous infusion methods were applied. In both methods, tea infusions were prepared with 2.0 grams (electronic balance EB200HZY-S, A-Tech Global Science Limited, HK) of tea leaves and 100 ml of hot water at 80^{o}C±5^{o}C. Triplicate was made for each preparation protocol.

For continuous infusion method, the temperature was kept by a thermal controlled water bath. At 5, 10, 20, 40, 60, 80, 100 and 120 minutes of brewing, 5.00 ml tea liquor sample was taken to determine the pH value and fluoride concentration. For repeated infusion method, 5.00 ml tea liquor samples were taken by pipette (Finnpipette, Thermo Labsystems, USA) at 5, 10, 15, 20, 25 and 30 minutes of brewing to test pH values and fluoride concentration. After that, the tea liquor was discarded and the same volume (100 ml) of hot water (80^{o}C±5^{o}C) was refilled. The same procedure was repeated another two times to collect tea infusion samples from the second and third rounds.

2.3 Measurement of the fluoride concentration in water and tea infusions

Fluoride concentration was measured with fluoride-ion selective electrode (Fluoride electrode Orion 9609BNWP, Thermo Scientific, MA, USA). The electrode was calibrated with four standard solutions (1, 2, 10 and 100 ppm) according to manufacturer's instructions before test. Calibration curve was obtained for further analysis.

For drinking water and D.I. water samples, low-level fluoride measurement method was performed. Water samples were buffered and tested with TISAB II (Cat. No.: 940909, Thermo Fisher Scientific Inc., USA) at the volume ratio of 1:1. While for tea liquor, the sample was analyzed after buffering with TISAB III (Cat. No.: 940911, Thermo Fisher Scientific Inc., USA) at the volume ratio of 1 (TISAB III):10 (sample). When a stable voltage reading was displayed, the value was recorded for evaluation. Fluoride concentration was determined corresponding to the calibration curve.

2.4 Measurement of the pH value

The pH value was determined with pH electrode (STH-E301, Ruosull, Shanghai, China) and pH meter (CyberScan 500 pH meter, Eutech Cybernetics, Singapore). Prior to measurement, the electrode and meter were calibrated with standard buffer solutions (pH=4, Cat. No.: Orion910104; pH=10, Cat. No.: Orion910110, Thermo Electron Co., Beverly, USA) according to manufacturer's instructions at $80^{o}C \pm 5^{o}C$.

2.5 Statistical analysis

For the analysis of fluoride concentration in drinking water, mean value, standard deviation, and 95% confidence interval were calculated. For tea infusion samples, descriptive statistics of pH value and fluoride concentration were presented. Fluoride release curves were constructed. Repeated measures analysis of variance (ANOVA) was performed to analyze the factors related to fluoride release from tea leaves. Statistical analysis was performed by IBM SPSS Statistics software version 20.0. In all cases, the statistical significance was set at $\alpha = 0.05$.

3. RESULTS

3.1 Fluoride concentration in drinking water and D.I. water

Table 1. Fluoride concentration in drinking water collected from Hong Kong and Hangzhou.

City	District	No. of Samples	Fluoride concentration (ppm, mg/L)		95% Confidence Interval
			mean	SD	
Hong Kong Island	Central, Western, Wan Chai	9	0.53	0.03	
	Southern, Eastern	9	0.48	0.06	0.48, 0.54
Hangzhou	Bin Jiang	9	0.16	0.01	
	Gong Shu	9	0.20	0.01	
	Jiang Gan	9	0.20	0.01	
	Shang Cheng	9	0.21	0.02	
	Xia Cheng	9	0.21	0.01	
	Xi Hu	9	0.18	0.00	0.19, 0.20

Totally 72 drinking water samples, 54 from Hangzhou and 18 from Hong Kong, were obtained. Mean value, standard deviation (SD) and 95% confidence interval (CI) of fluoride concentration in drinking water was showed in Table 1. After comparing 95% CI, fluoride content in drinking water collected from Hong Kong Island (95% CI = 0.48-0.54) was significantly higher than samples from Hangzhou (95% CI = 0.19-0.20). The mean (SD) value of fluoride concentration in D.I. water was 0.0020 (0.0002) ppm which means the fluoride content in D.I. water was negligible.

3.2 Fluoride concentration in tea infusions

Table 2. Water-soluble fluoride concentration (mg/L, mean±SD) in three types of tea infusions prepared by continuous method.

Type of Tea	Water	Brewing Time (min)							
		5	10	20	40	60	80	100	120
Puerh tea	D.I. water	2.62±0.34	3.33±0.10	4.55±0.30	4.94±0.16	5.59±0.03	5.84±0.29	6.08±0.35	6.45±0.60
	Drinking water (HK)	3.00±0.17	3.79±0.08	4.80±0.29	5.62±0.35	5.87±0.59	6.20±0.56	6.20±0.43	6.68±0.38
Black tea bag	D.I. water	9.68±0.71	10.18±0.48	10.40±0.42	10.37±0.27	10.51±0.34	10.53±0.20	10.65±0.20	10.61±0.26
	Drinking water (HK)	7.72±0.39	9.85±0.13	10.20±0.05	10.29±0.03	10.61±0.42	10.60±0.29	10.67±0.23	11.05±0.25
Green tea	D.I. water	1.85±0.07	1.94±0.11	2.44±0.11	2.45±0.11	2.53±0.33	2.70±0.07	2.78±0.21	2.55±0.12
	Drinking water (HZ)	2.13±0.18	2.29±0.22	2.38±0.18	2.42±0.18	2.49±0.21	2.59±0.32	2.67±0.30	2.76±0.21

Table 3. Water-soluble fluoride concentration (mg/L, mean±SD) in tea infusions prepared by repeated method.

Type of Tea	Water	Infusion Round	Brewing Time (min)					
			5	10	15	20	25	30
Puerh tea	D.I. water	1	2.21±0.30	3.00±0.25	3.39±0.16	3.62±0.14	3.70±0.09	3.82±0.04
		2	0.93±0.05	1.32±0.05	1.67±0.03	1.89±0.11	2.07±0.10	2.21±0.11
		3	0.60±0.06	0.84±0.07	1.01±0.12	1.20±0.12	1.32±0.13	1.47±0.11
	Drinking	1	3.05±0.07	3.61±0.28	4.01±0.35	4.26±0.33	4.46±0.26	4.50±0.16
	water	2	1.98±0.12	2.24±0.08	2.53±0.11	2.78±0.18	3.02±0.13	3.16±0.18
	(Hong Kong)	3	1.72±0.09	1.93±0.18	2.13±0.15	2.32±0.14	2.51±0.09	2.59±0.09
Black tea	D.I. water	1	9.97±0.99	10.42±0.20	10.86±0.71	10.56±0.23	10.60±0.22	10.74±0.19
bag		2	2.58±0.08	2.94±0.16	3.08±0.18	3.33±0.24	3.47±0.21	3.58±0.25
		3	0.73±0.04	0.84±0.04	0.97±0.09	1.01±0.05	1.11±0.01	1.18±0.00
	Drinking	1	9.68±0.47	10.36±0.26	10.51±0.41	10.74±0.17	10.79±0.16	10.93±0.47
	water	2	3.46±0.06	3.68±0.11	3.98±0.18	4.13±0.25	4.23±0.34	4.27±0.24
	(Hong Kong)	3	1.85±0.14	2.00±0.09	2.15±0.10	2.25±0.13	2.35±0.14	2.53±0.05
Green tea	D.I. water	1	1.90±0.11	2.25±0.13	2.46±0.09	2.60±0.16	2.77±0.11	2.96±0.20
		2	0.49±0.06	0.52±0.06	0.56±0.05	0.61±0.05	0.63±0.06	0.68±0.07
		3	0.17±0.01	0.18±0.02	0.20±0.03	0.22±0.02	0.24±0.03	0.26±0.03
	Drinking	1	2.18±0.06	2.39±0.24	2.84±0.20	3.00±0.25	3.16±0.21	3.36±0.22
	water	2	1.15±0.04	1.30±0.10	1.38±0.07	1.48±0.05	1.57±0.08	1.67±0.10
	(Hangzhou)	3	0.91±0.03	1.01±0.05	1.09±0.04	1.19±0.07	1.25±0.07	1.31±0.07

Fluoride concentration in tea infusions prepared by two infusion methods was showed in Table 2 (continuous) and Table 3 (repeated). And fluoride release curves were illustrated in Fig. 1 (continuous) and Fig. 2 (repeated). In both preparation methods, black tea bag released significantly higher fluoride level than puerh tea leaves, which was significantly higher than green tea leaves ($p<0.05$). Brewing time had statistical significant ($p<0.05$) impact on fluoride concentration of all types of tea infusions.

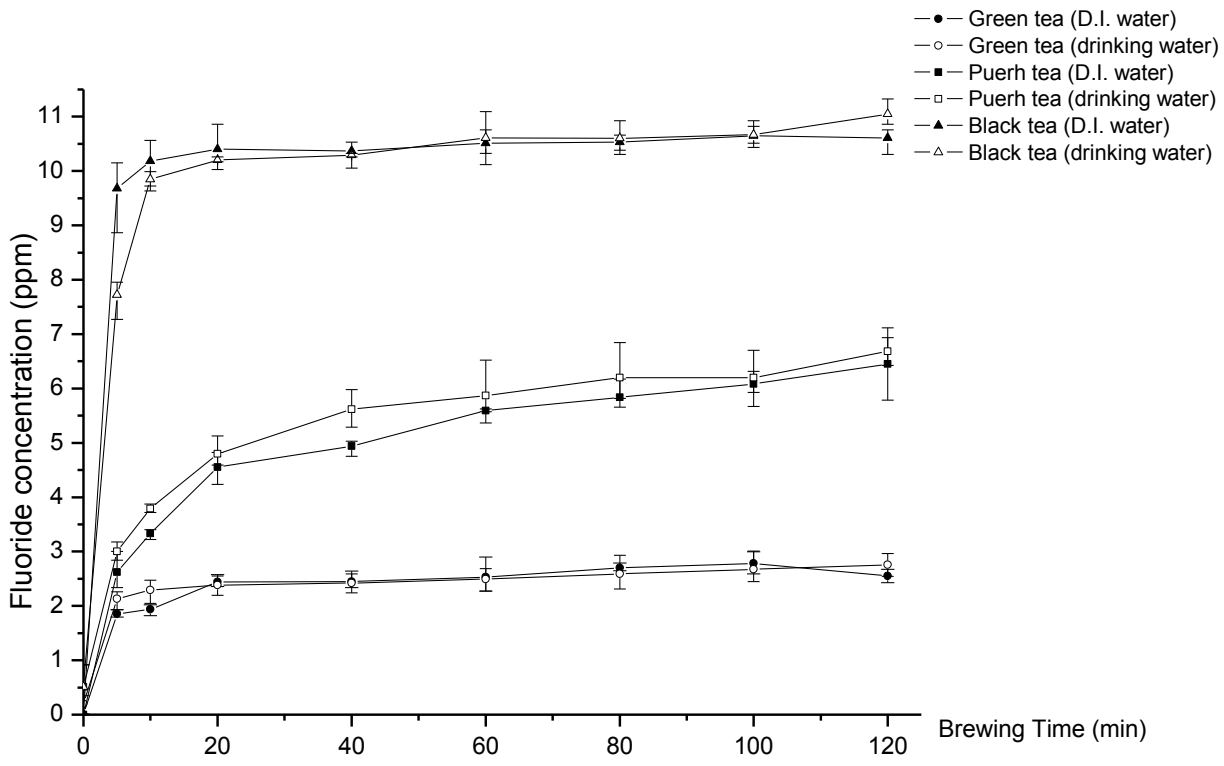

Figure 1. Fluoride release curves from tea leaves during 120-minute continuous infusion. The symbols on each curve showed the mean values of fluoride concentration. Error bars indicated the maximum and minimum value.

During 120-minute continuous infusion, fluoride release dynamics can be easier observed and described. The fluoride release curves showed sharp rises in first five minutes and then slowly tended to be stable (Fig. 1). In the initial 20 minutes, for green tea leaves and 60 minutes for puerh tea leaves, tea infusions prepared with drinking water released more fluoride content than samples prepared with D.I. water (Fig. 1). The difference was narrowing with time and even reversed for green tea leaves at the 120-minute test point. On the other hand, black tea bag had a distinctive mode which also proved in Fig.2 (Round 1 of repeated infusion method only). In the first approximate 20 minutes, fluoride release from tea bag into drinking water was less than to D.I. water. But this phenomenon reversed when brewing time increased (Fig.1) or disappeared in the later round of infusion (Round 2 and 3 in Fig. 2).

Prepared by repeated method, tea infusions of the 1st round contained the significant highest fluoride content, and the 2nd round were significantly higher than the 3rd round ($p<0.05$). Furthermore, fluoride released to D.I. water by repeated tea infusion method was significantly less than to drinking water ($p<0.05$). However, the influence of original ionic concentration in brewing water was not statistical significant throughout the 120-minute continuous infusion.

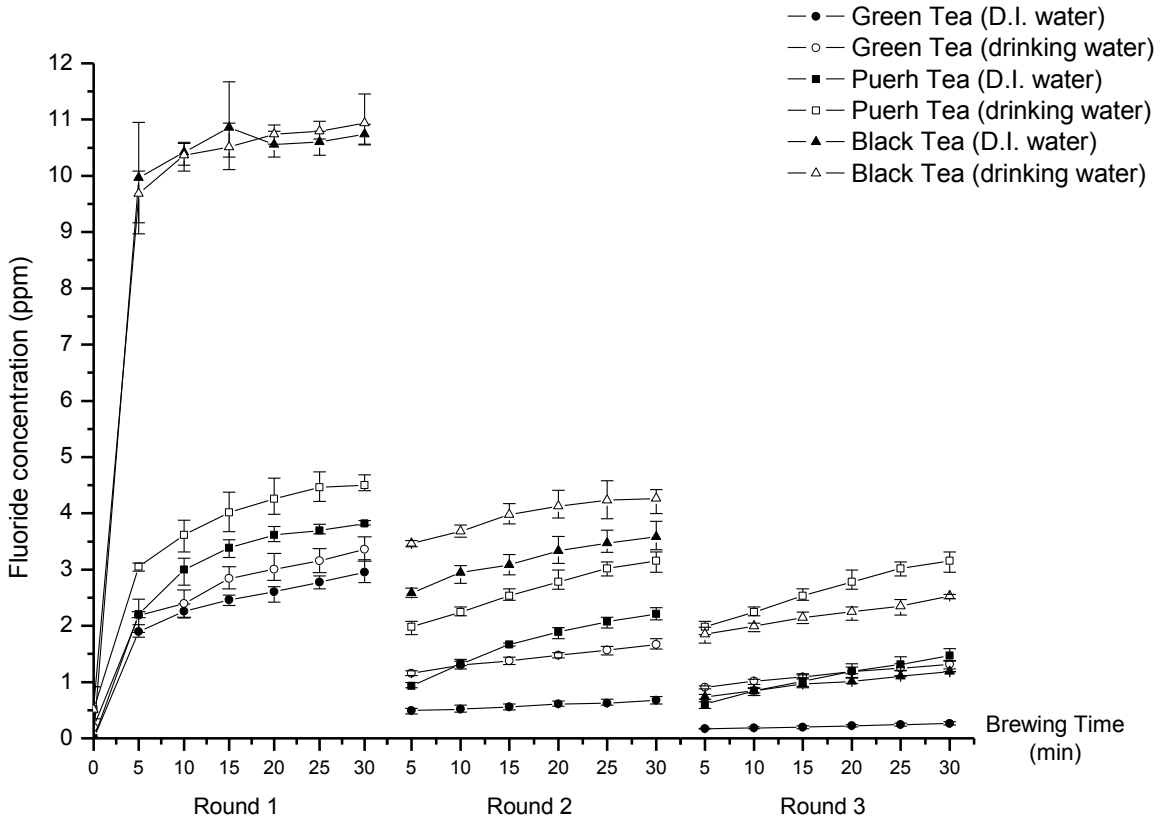

Figure 2. Fluoride release curves from tea leaves in three rounds of repeated infusion. The symbols on each curve showed the mean values of fluoride concentration. Error bars indicated the maximum and minimum value.

3.3 The pH value of tea infusions

Table 4. Range of pH values in tea infusions.

Tea	Repeated Infusion			Continuous Infusion
	1st Round	2nd Round	3rd Round	
Puerh tea (D.I. water)	5.23-5.33	5.40-5.50	5.43-5.49	5.06-5.20
Puerh tea (HK drinking water)	5.29-5.40	5.66-5.76	5.72-5.78	5.04-5.30
Black tea (D.I. water)	4.85-4.99	5.08-5.18	5.22-5.26	4.83-4.96
Black tea (HK drinking water)	4.96-5.07	5.37-5.59	5.62-6.15	4.84-5.21
Green tea (D.I. water)	5.82-5.97	6.13-6.21	6.23-6.29	5.52-5.83
Green tea (HZ drinking water)	6.07-6.18	6.47-6.60	6.69-6.54	5.60-6.10

Table 4 exhibited the range of pH values of tea infusion samples. All values were below 7.00. Green tea leaves reached higher pH level than puerh tea leaves, which was higher than black tea bag. Generally, pH value would slightly decrease with increasing brewing time. But the change is not obvious and no unified model can be achieved.

4. DISCUSSION

Tea infusions of puerh tea leaves, black tea bag, and green tea leaves prepared in native manners of Hong Kong and Hangzhou were able to provide different levels of fluoride. Fluoride-ion selective electrode was used to determine the fluoride concentration. This method has been widely applied in similar studies [10, 13]. Interference induced by hydroxide ion was reported as the major shortcoming of this technique and can be eliminated when pH value of test sample is below 7.0 [14]. In order to overcome the limitation and enhance the precision of results, TISAB (Total Ionic Strength Adjustment Buffer) was designed to adjust the pH value of test samples. In this study, pH values of all tea infusion samples were already below 7.0 before the adjustment of TISAB (Table 4). Therefore, it is rational to consider that the interference was minimal.

With the limitation of this study, only concentration of free fluoride as ionic form was detected. Because all chemical reactions are in equilibrium, the free fluoride content in tea leaves may be in equilibrium with organic and inorganic forms. This study represented the active fluoride level before human consumption. If the equilibrium was shifted to higher or lower, fluoride level is not certain after human consumption. More studies are needed in this aspect of fluoride chemistry and metabolism.

Fluoride concentration of tea infusion can be affected by several factors. Type of tea seems to be the most basic one. Soil conditions, maturity of tea leaves, part of tea bushes are all correlated to fluoride content in tea leaves [15]. Green tea leaves, which were usually selected from young leaves, released significantly lower fluoride than puerh tea leaves and black tea bag. The results agreed with other publications [16, 17]. Furthermore, infusion of black tea bag showed extremely high fluoride level compare to optimal water fluoride concentration and it was also several times higher than the other two types of tea. Other than the influencing factors mentioned above, the notable fluoride concentration released from black tea bag might relate to package as well. It has been reported that bagged tea tended to release more fluoride than leaf, stick and granular tea [16, 17]. According to current results, drinking more than 400 ml per day of the 1st round infusion of black tea bag can already reach the fluoride tolerable upper limit of 3.0 to 4.0 mg/day for adults [4]. Hence, there exists a risk of excessive fluoride intake as a result of regular and customary drinking of black tea bag infusion.

Besides the type and package of tea, prolonged brewing time also affect the fluoride level in tea infusion. The pattern of the dynamics of fluoride release from three types of tea is somehow similar. Fluoride concentration increased rapidly within several minutes of brewing and then gradually achieved saturation (Fig. 1). To control the amount of fluoride ingestion, discarding the tea liquor of initial period of brewing and having second or more rounds of infusion is viable to decrease the ingestion of fluoride (Fig.2).

The overall statistic analysis of 120-minute continuous infusion didn't show any significant differences in fluoride concentration between tea infusions prepared with drinking water and D.I. water. However, the original fluoride level in brewing water might have an impact on the dynamics of fluoride release from tea leaves in repeated infusion method. This effect seems to quite depend on the infusion method and the type of tea. Based on the features of the two infusion methods, it can be deduced that the influence of fluoride level in brewing water may be masked during long-term brewing. Unfortunately, there is little publication to support this hypothesis. Moreover, black tea bag

exhibited a characterized release mode different from the other two types of tea leaves. Fluoride release curves (Fig. 1 and Fig. 2) indicated that higher fluoride level in drinking water inhibited fluoride releasing from black tea bag at the early stage of brewing process. It is reported that fluoride in tea leaves would be easier to release into lower-fluoride water [18]. However, our study showed that this influence was not lasting and related to type of tea. Hence, further investigations are needed.

In conclusion, the dynamics of fluoride release indicated fluoride levels of tea infusions may reach 2 to 10 ppm, depending on the types of tea, within 5 to 20 minutes of brewing. Fluoride concentration in tea infusions could be affected by original ionic level of brewing water, brewing time, and the round of tea infusion. From the public health perspective, children at the age of tooth formation should avoid tea to prevent dental fluorosis. Alternatively, shorter brewing time, or consumption second or third round of tea infusion may reduce systemic fluoride intake.

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