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Determination of Fluoride in Tea Leaves and Tea Infusions by Ion Selective Electrode

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ABSTRACT

A fluoride ion selective electrode method for the determination of fluoride contents in tea leaves and tea infusions was set up. Using commercial timothy grass powder and tea leaf powder as standard materials, this method demonstrated linearity between 0.02 and 2.00 mg/L of fluoride with R^2 greater than 0.999. Both materials had coefficient of variations (CV, %) less than 3%, indicating good reproducibility in the determinations. The recovery tests of tea infusions spiked with different amounts of sodium fluoride demonstrated 94-107% of recovery with CV less than 1.2%, indicating that the method is very accurate. With 1% of tea leaves to boiling water ratio, up to 63-78% of fluoride in tea leaves could be released into tea infusion by repeated infusions and 82-83% by continuous infusion. By this method, total fluoride contents of 12 brands of tea leaves purchased from Nantou County were determined to be 100-451 mg/kg dry weight, and those of their infusions in boiling water for a 5-min period were determined to be 0.39-1.21 mg/L. Assuming that daily tea infusions drinking for an adult is 2 L, fluoride intake from tea infusions of these tea leaves is far below the daily fluoride tolerable upper limit 10 mg.

Key words: fluoride, tea, tea infusion, fluoride ion selective electrode

INTRODUCTION

Tea (*Camellia sinensis*) is a perennial plant capable of taking up fluoride from soil and accumulating it in leaves[(1-4)]. Excess fluoride in tea leaves causes necrosis in leaf margins and tips[(3)]. Fluoride in tea leaves may be released into tea infusions and contributes to total fluoride intake in human[(1,4)]. Tea drinks have been the most popular beverages in Taiwan in recent years and the average tea consumption rate was 0.27 and 1.56 kg/person/year in 1971[(5)] and 2002, respectively[(6)]. Tea drinks are one of the main fluoride sources from ingestion. Adequate fluoride ingestion is helpful to avoid caries, but over ingestion induces dental and skeletal fluorosis, which may result in malfunction of the bone and joint system[(7-8)]. It has been suggested that daily fluoride intake should not exceed 10 mg per person[(7)].

Fluoride ion selective electrode is a fast, economical, and precise tool to determine fluoride contents in plant, liquid, food, and soil samples[(9-10)]. The purpose of this work is to determine the fluoride contents in commercial tea leaves by using fluoride ion selective electrode. Fluoride contents in tea infusions prepared from tea leaves by two different infusion methods were also determined and compared.

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MATERIALS AND METHODS

I. *Samples*

Two standard reference materials (SRM) were used for the quality assurance of fluoride content determination in tea materials. SRM 2695, consisting of powdered timothy grass of high and low fluoride levels, was purchased from the National Institute of Standards and Technology of America. GBW 08516 is reference tea leaf powder and was purchased from the Academiae Medicine ZheJiang (approved by the National Research Center for CRM, P. R. China).

Tea leaves from 12 brands, i.e., four brands of Chin-Shin Oolong tea, three brands of TTES No.12 (Chinhsuan) tea, two brands of TTES No.13 (Tzuiyu) tea, two brands of Shy-Jih-Chuen tea, and one brand of Jasmine green tea were purchased from tea stores in Minjian Township of Nantou County in Taiwan. For each band, tea leaves were grounded into fine pieces and passed through a 0.15 mm sieve to collect tea leaf powder. The tea leaf powder samples were dried at 105°C for 2 hrs and stored in a desiccator at room temperature before experiments.

II. *Reagents and Chemicals*

Sodium fluoride, hydrogen chloride, sodium hydrox-

ide, sodium chloride, glacial acetic acid, sodium acetate trihydrate, and sodium citrate dehydrate, all of reagent grade, were purchased from MERCK & Co., Inc.

III. *Sample Preparation for Total Fluoride Content Analysis*

For total fluoride content analysis, 0.25 g of either the standard reference materials or the tea leaf powder was transferred to a 150 mL nickel crucible and moistened with a small amount of de-ionized water. Six mL of 16.75 N NaOH was added and the crucible was placed in an oven (150°C) for 1.5-2.0 hr until NaOH was solidified. The crucible was placed in a muffle furnace set at 300°C, then raised to 600°C and kept at 600°C for 30 min in order to fuse the sample in the crucible. The crucible was placed in a hood and allowed to cool, and 10 mL distilled water was added. Then, 37% HCl solution (about 8 mL) was added slowly to adjust the pH to 8-9. The sample solution was transferred to a 100 mL plastic volumetric flask, diluted with distilled water to the volume, and filtered through a Whatman No. 40 filter paper[9]. The filtrate was used for the fluoride content determination. A filtrate was prepared in parallel from a 150 mL nickel crucible containing no sample to serve as a control.

IV. *Fluoride Content Analysis*

Fluoride ion selective electrode method was used to assay the fluoride content of plant samples, tea leaves and tea infusions. Serial concentrations of sodium fluoride solution were prepared, and 25 mL of each concentration was added to equal volume of TISAB solution (total ionic strength adjustment buffer; 58.5 g/L NaCl, 15 mL/L glacial acetic acid, 102 g/L sodium acetate trihydrate, 30 g/L sodium citrate dehydrate, pH 5.30-5.35)[11]. TISAB was used to stabilize ionic strength condition and to chelate various cations such as aluminum which interfere with the analysis of fluoride[11-13]. Fluoride ion selective electrode (ISE, ORION Model 96-09) was immersed in the solutions and readings on the ion analyzer (Mettler-Toledo Ltd, METTLER DELTA 350) were recorded. A standard curve for fluoride concentrations determination was made. Fluoride concentration in unknown solution was determined by adding 25 mL of TISAB solution to 25 mL of the unknown solution. The fluoride concentration could be determined using the standard curve.

V. *Tea infusion and Recovery Test*

Unless otherwise specified, tea infusions were prepared using 1% tea to boiling water ratio, since this is the infusion ratio commonly used for making of commercial tea drinks, suggested for uses of many commercial tea bags, and used in other reports[1-2].

1% tea infusion was prepared by immersing 5 grams of tea leaves in 500 mL of boiling de-ionized water for 5 min, and filtered through a Whatman No.1 filter paper. The filtrate was collected as tea infusion. Twenty-five millliter of tea infusion was then added to 25 mL of TISAB solution and fluoride content was determined as described above. The % of fluoride infused is defined as the percent of fluoride in leave released into infusion and is calculated as following.

% of fluoride infused = fluoride content determined (mg/L) × D × V (L) ÷ W (kg) ÷ total fluoride content (mg/kg) of tea leaf × 100%

D: dilution factor; 2 in this case.
V: volume of boiling de-ionized water for preparing tea infusion; 0.5 L in this case.
W: weight of tea leaves for preparing tea infusion; 0.005 kg in this case.

For the recovery, 100 mL of tea infusion was spiked with sodium fluoride solution of which the fluoride content was equivalent to 50%, 100% or 200% of the fluoride content of the tea infusion. Fluoride content in the spiked samples was determined as described above.

VI. *Repeated Infusion*

Five grams tea leaves were immersed into 500 mL of boiling de-ionized water for 5 min and filtered through a Whatman No. 1 filter paper. The filtrate was collected as the first tea infusion, and fluoride content was determined. The tea leaves was then again immersed into 500 mL of boiling de-ionized water for 5 min, filtered and the filtrate was collected as the second tea infusion. The third, fourth, and fifth tea infusions were collected by the same way. Fluoride contents in these tea infusions were also determined. Three replicates of each tea infusion were taken for the determination, and boiled de-ionized water was used as blank.

VII. *Continuous Infusion*

In order to know whether the amount of fluoride released into the tea infusion correlates to the time of infusion, 5 grams of tea leaves were immersed into 500 mL of boiling de-ionized water for 5, 10, 15, 30, 60, 120, 180 or 480 min, and tea infusions were obtained by filtration through a Whatman No. 1 filter paper. Fluoride contents of these tea infusions were determined as described above. Three replicates of each tea infusion were taken for the determination, and boiled de-ionized water was used as blank.

VIII. *Tea Infusions in 2% or 20% Tea to Boiling Water Ratio*

In some cases, 3 or 30 grams of tea leaves were immersed into 150 mL of boiling water for 5 min and filtered through a Waterman No. 1 filter paper. These resulted in tea infusions of 2% or 20% tea to boiling water ratio, respectively.

RESULTS AND DISCUSSION

I. *Linearity of the Standard Curve*

Using fluoride ion selective electrode and serial concentrations of sodium fluoride solutions, a standard curve for the fluoride concentration was obtained (Figure 1). A respectable linearity was achieved in the range of 0.02-2.00 mg/L of fluoride. The measured potential from fluoride ion selective electrode corresponding to the level of fluoride ion in solution is described by the Nernst equation: $E = S \log C + B$. Where C is the mg/L fluoride yielding a millivolt potential of E, S is the slope, and B is reference potential. Three measurements were carried out at different times with three replicates for each measurement. A calibration curve was generated for each measurement with R^2 greater than 0.999. Three S and B values obtained from the three calibration curves were used to calculate the final S and B values, which was -59.4 ± 0.7 and 69.7 ± 0.6 mV, respectively, with coefficient of variation (CV, %) less than 1.5% for both values.

II. *Accuracy and Reproducibility of the Method*

Using commercial timothy grass powder and tea leaf powder with known fluoride contents as reference materials, the accuracy and reproducibility of the fluoride ion selective electrode method were evaluated. The fluoride contents of the two reference materials were determined intra-daily (five times within a 24-hr period) and inter-daily (three times at successive 10 days with at least 24-hr intervals) with five and three replicates, respectively. As shown in Table 1, the fluoride contents in the two reference materials were consistent with those described in the certificates of the two materials. The CV of both materials are all less than 3%, indicating high degrees of reproducibility of the method.

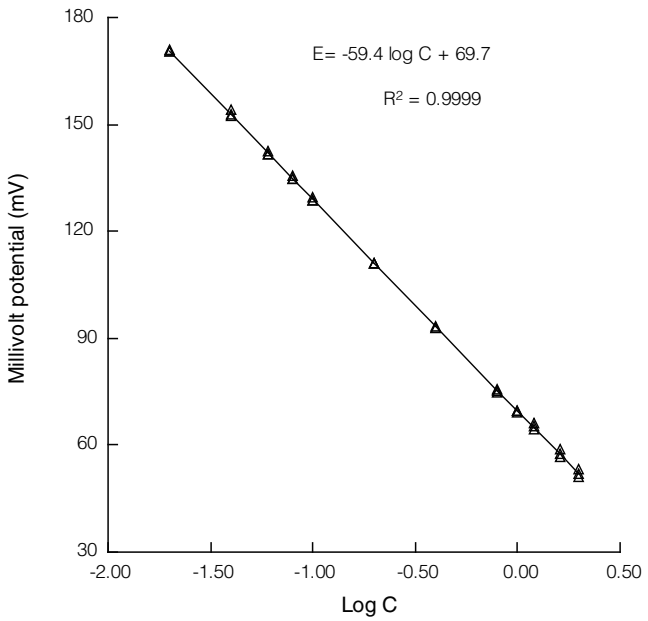

Figure 1. Standard calibration curves of fluoride content determination. The linear regression equations for fluoride was calculated as E= -59.4 log C + 69.7, where C is the concentration of various fluoride standard solutions and E is the yielding millivolt potential of each standard solutions using the fluoride ion selective electrode. The regression line has p value <0.05 (p value = 4.02E-23).

III. *Recovery Test*

The accuracy of the fluoride ion selective electrode method was also evaluated by the recovery test. Recoveries of fluoride from three kinds of tea infusions spiked with three different amounts of sodium fluoride were tested. As shown in Table 2, recoveries were 103-105%

Table 1. Intra-day and inter-day analyses of total fluoride contents of two reference materials

Standard reference materials	Mean ± SD (CV, %) (mg/kg d.w.)	
	Intra-day (n=5)	Inter-day (n=3)
Timothy grass powder		
high level	278.3 ± 6.1 (2.2)	276.7 ± 7.8 (2.8)
low level	65.9 ± 1.5 (2.3)	66.3 ± 1.4 (2.1)
Tea leaf powder	62.8 ± 1.2 (1.9)	62.2 ± 1.2 (2.0)

The fluoride contents of the standard reference materials are 277 ± 27 mg/kg (timothy grass powder, high level), 64.0 ± 8.4 mg/kg (timothy grass powder, low level) and 64.3 ± 5.4 mg/kg (tea leaf powder). (Data from certificates of the standard reference materials.)

Table 2. The recovery test of fluoride content in tea infusions (n=4)

Tea infusion	Sodium fluoride spiked level	Fluoride content determined	Recovery % (CV, %)
	(mg/L)		
Chin-Shin Oolong	-	0.84	-
	0.40	1.27	105 (0.9)
	0.79	1.68	104 (1.2)
	1.58	2.47	103 (0.6)
TTES No.12	-	0.42	-
	0.20	0.63	106 (0.5)
	0.40	0.84	104 (0.3)
	0.79	1.19	97 (0.3)
TTES No.13	-	1.26	-
	0.60	1.90	107 (0.5)
	1.19	2.44	99 (0.7)
	2.40	3.51	94 (0.2)

from spiked Chin-Shin Oolong tea, 97-106% from spiked TTES No.12 tea and 94-107% from spiked TTES No.13 tea with CV of 0.6-1.2%, 0.3-0.5%, and 0.2-0.7%, respectively. This method showed a very good accuracy due to the high recovery.

IV. *Fluoride Content of Commercial Tea Leaves and Their Infusions*

Since 1% tea to water ratio was the ratio used for makings of commercial tea drinks, suggested for many commercial tea bags to make tea infusions, and used in other reports[1-2], we prepared tea infusions in 1% tea to boiling water ratio for this study. Total fluoride contents of tea leaves and tea infusions from 12 brands of five different tea types were determined and the results are shown in Table 3. Total fluoride contents of tea leaves were 133-376 mg/kg d.w. for Chin-Shin Oolong teas, 100-193 mg/kg d.w. for TTES No.12 tea, 178-451 mg/kg d.w. for TTES No.13 tea, 184-196 mg/kg d.w. for Shy-Jih-Chue tea, and 225 mg/kg d.w. for Jasmine green tea, whereas fluoride contents of tea infusions were 0.41-0.84 mg/L for Oolong tea, 0.39-0.73 mg/L for TTES No.12 tea, 0.57-1.21 mg/L for TTES No.13 tea, 0.74-0.80 mg/L for Shy-Jih-Chue tea, and 1.14 mg/L for Jasmine green tea. Brand No.1 of tea type TTES No. 13 had the highest fluoride contents in both tea leave and tea infusion, whereas brand No. 2 of tea type TTES No. 12 had the lowest fluoride contents in both tea leave and tea infusion. Since fluoride in tea infusions is the one actually intaked by human, fluoride content in tea infusion, rather than in tea leave, is particularly concerned. It appears that Chin-Shin Oolong tea and TTES No.12 tea, in general, were the two teas that contained least fluoride in the tea infusions, despite that variation existed among brands of the same tea type. The percentage of fluoride infused from 12 brands of tea leaves ranged from 22 to 51%, indicating about two-fold differences in infusion efficiencies among different tea types. In general, Chin-Shin Oolong tea had the lowest infusion percentage and Jasmine green tea had the highest.

In some cases, people in Taiwan make tea infusions by immersing 3 g or 30 g of tea leaves into 150 mL of boiling water for 5 minutes. Tea infusions in 2% tea to boiling water ratio were prepared from the same 12 tea samples and the fluoride contents were determined. As shown in Table 3, fluoride contents of these particular tea infusions were ranged from 0.65 to 2.53 mg/L, and the percentage of fluoride infused ranged from 20 to 47%. As

Table 3. Total fluoride contents and fluoride contents in 1% and 2% (w/v) tea infusions of commercial tea leaves (n = 3)

Tea leaf	Total fluoride content (mg/kg d.w.)	Fluoride content in tea infusion (mg/L)		% of fluoride infused	
		1%	2%	1%	2%
Chin-Shin Oolong					
brand 1	376 ± 1*	0.84 ± 0.02	1.54 ± 0.06	22	20
brand 2	185 ± 2	0.59 ± 0.02	1.00 ± 0.03	32	27
brand 3	133 ± 2	0.41 ± 0.02	0.90 ± 0.04	31	34
brand 4	215 ± 1	0.65 ± 0.03	1.12 ± 0.04	30	26
TTES No.12					
brand 1	193 ± 2	0.73 ± 0.02	1.48 ± 0.05	37	38
brand 2	100 ± 3	0.39 ± 0.01	0.65 ± 0.01	39	33
brand 3	103 ± 3	0.45 ± 0.02	0.79 ± 0.02	43	38
TTES No.13					
brand 1	451 ± 5	1.21 ± 0.02	2.53 ± 0.08	27	28
brand 2	178 ± 2	0.57 ± 0.00	0.98 ± 0.03	32	28
Shy-Jih-Chue					
brand 1	184 ± 2	0.74 ± 0.03	1.37 ± 0.05	40	37
brand 2	196 ± 2	0.80 ± 0.02	1.50 ± 0.01	40	38
Jasmine green					
brand 1	225 ± 9	1.14 ± 0.03	2.10 ± 0.04	51	47

*Mean ± SD

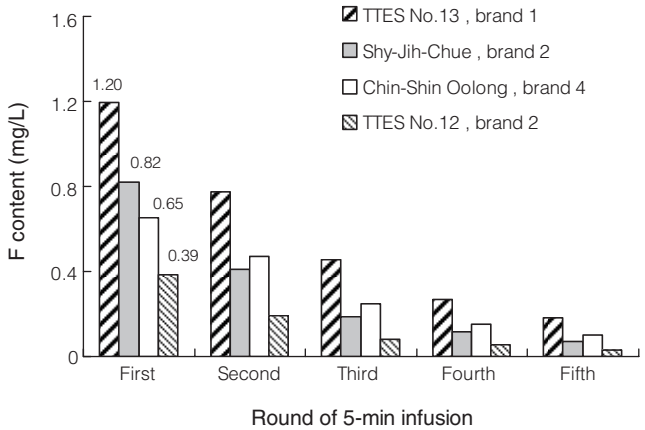

Figure 2. Fluoride contents in 1% (w/v) tea infusion prepared by repeated 5-min infusion.

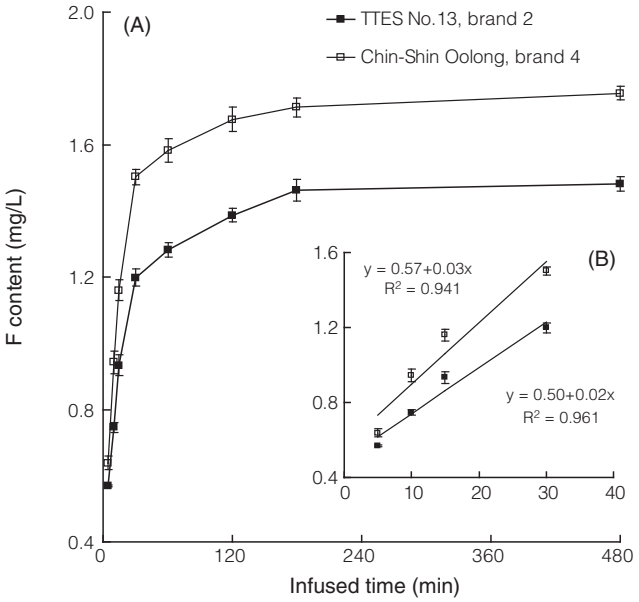

Figure 3. Fluoride contents in 1% (w/v) tea infusions prepared by continuous infusion, (A) Infusions for 5, 10, 15, 30, 60, 120, 180, and 480 min, (B) Infusions for 5, 10, 15, and 30 min. Both of the regression lines have p value <0.05.

compared to tea infusions in 1% tea to boiling water ratio, a 0.7 to 1.2-fold increase in fluoride content was observed with tea infusions in 2% tea to boiling water ratio.

Tea infusions of 20% tea to boiling ratio was prepared from brand No. 4 of Chin-Shin Oolong tea type and brand No. 2 of TTES No. 13 tea type. The fluoride contents were determined to be 6.94 ± 0.30 and 5.64 ± 0.19 mg/L, and the percentage of fluoride infused were 16 and 16%, respectively.

V. *Repeated Infusion*

Figure 2 illustrates the fluoride contents of five repeated infusions from tea leaves of four types. Fluoride content in the fifth infusion was 0.03-0.18 mg/L, with TTES No.12 tea being the lowest and TTES No.13 tea the highest. Totally, percentages of fluoride released into the five repeated infusions were 76% for Chin-Shin Oolong tea, 71% for TTES No.12 tea, 63% for TTES No.13 tea, and 78% for Shy-Jih-Chue tea. Fluoride in the first three repeated infusions accounts for 53-69% of the total fluoride in the tea leaves (84-89% of the fluoride contents of the five repeated infusions).

VI. *Continuous Infusion*

In order to know the relationship between the amount of fluoride released into the tea infusions and the duration of infusion time, tea leaves of brand No. 2 of TTES No. 13 tea and brand No. 4 of Chin-Shin Oolong tea were chosen for infusions for 5, 10, 15, 30, 60, 120, 180 or 480 min with 1% tea to water ratio, and fluoride contents in the infusions were determined. As shown in Figure 3A, fluoride content increased as the infusion time increased for the first 120 min of infusion. After 120 min of infusion, the fluoride content reached a plateau. It is shown, in figure 3B, that fluoride content in tea infusions increases linearly between 5 and 30 min of infusion, with R^2 of 0.941 for brand No. 4 of Chin-Shin Oolong tea and 0.961 for brand No. 2 of TTES No.13 tea. Based on the results of the fluoride contents in tea leaves and in the corresponding tea infusions, percentage of fluoride released from tea leaves after 5 min, 10 min, 15 min, 30 min, 60 min, 120 min, 180 min and 480 min infusions were calculated to be 30, 44, 54, 70, 74, 78, 80 and 82% for brand No. 4 of Chin-Shin Oolong tea and 32, 42, 52, 67, 72, 78, 83 and 83% for brand No. 2 of TTES No.13 tea. After infusion for 30 minutes, about 70% of fluoride in tea leaves was released into tea infusions.

CONCLUSIONS

By fluoride content analyses of the reference materials and the recovery tests of tea infusions, this study demonstrated that fluoride ion selective electrode is a simple but very accurate, sensitive, and reproducible tool for the determination of the fluoride content in tea leaves and tea infusions. With the 12 brands of commercial tea leaves purchased in Minjian Township of Nantou County in Taiwan, the total fluoride contents were determined to be 100-451 mg/kg dry weight in tea leaves and 0.39-1.21 mg/L in tea infusions which generally is prepared by infusion of tea leaves in boiling water for 5 minutes with 1% of tea leaves to boiling water ratio. Percent of fluoride infused from tea leaves depends on methods of infusion and can be up to 83% of total fluoride contents of tea leaves. If we assume that adequate daily water consumption for an adult is about 2 L and tea infusions are the only water source, daily fluoride intake from tea infusions of these tea leaves is

below the daily fluoride intake upper limit (10 mg per person). Thus, there is no harm to drink tea infused from these commercial Taiwan tea leaves in the 1% tea to boiling water ratio.

ACKNOWLEDGEMENTS

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