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DETERMINING OF FLUORIDE CONTENT IN TEA BY SPECTROPHOTOMETRY AND POTENTIOMETRY

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ABSTRACT

Tea contains a high amount of fluoride and its consumption may affect the whole intake of fluoride. Excessive intake of fluoride over the long term may lead to the development of dental or skeletal fluorosis. In this study, fluoride content of 30 tea samples was determined using potentiometry and spectrophotometry. Results of study revealed that in determination of fluoride content potentiometric method is more precise than spectrophotometry. Further, results of study did not show any significant difference in the fluoride content of black and green tea, but the difference between the fluoride content of black tea bags with black and green tea is significant.

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1 Introduction

Initially tea was used as a medical plant but later on it excessively used as herbal beverage (Kakuzo, 2011). Tea is a Chinese word which is used in China and north of India and it was introduced into Persian language with the same pronunciation. This plant was identified 5000 years ago in China and its medicinal properties were gradually discovered. For many years, it was used only in China; it entered Poland at the beginning of the 18th century and, from there, it was introduced into other European countries.

Tea is classified into three main groups: black, green and oolong, depending on the way of production. Fourth class i.e. white tea which is made in a different way from other categories. After water, tea infusion is the most favorite beverage in the world. It contains caffeine, theophyllin, tannin, catechin, vitamin, flavonoids, antioxidant, calcium, magnesium, sodium and potassium (Chung et al., 2003). It also contains a relatively high amount of fluoride and its consumption can affect total fluoride absorption inside the body. Fluoride is an important anion which is found in foods and water and its small amount is necessary to prevent dental disorders. Also, its average daily intake decreases dental decay in children and adults (Levi et al., 1983; Malinowska et al., 2008).

Drinking tea is one of the most important sources of fluoride intake in Iran and other far-east countries, because tea drinking is part of daily regime in these countries. Although fluoride content of tea is an important factor in the dental skeleton but intake in high amount of fluoride can cause dental and skeletal fluorosis (Shu et al., 2003; Institute of Medicine, 1997). This issue becomes more serious when fluoride intake from tea is added to fluoride intake from other sources like drinking water and food (Webb Peplow & Bradley, 1996). Similarly, Cao et al. (1996) reported that 51.2% of the total dental fluorosis and 32.83% of the total skeletal fluorosis among Tabat children are because of the long term tea consumption.

Present study was conducted to access the fluoride content in different tea samples collected from Tehran market. Further efficacy of two fluoride estimation methods (potentiometry and spectrophotometry) in determination of total fluoride content was also evaluated in present study.

2 Materials and Methods

30 tea samples (including imported and local teas) were collected from Tehran market. These samples included: 15 black tea, 4 green tea, 5 black tea bags and 6 green tea bags. Fluoride content of the collected tea samples were analyzed by potentiometry (fluoride ion-selective electrode) and spectrophotometry methods.

2.1 Black & Green Tea preparation

100g of each sample was equally divided into 4 parts; from this 10g samples were randomly taken and thoroughly mixed. Then, it was dried for 4 h at 60°C and 2g was used for the analysis. Further, four samples were prepared by adding 200 ml of 100°C distilled water to each sample and was put on water bath for 10 minutes (at 100°C). Then, it was cooled down to reach the room temperature, filtered and again make the volume 200 ml.

2.2 Black & Green Tea Bags preparation

Four bags from each black and green tea bag were randomly taken and thoroughly mixed. Then, they were dried for 4 h at 60°C and 2g sample was used for the analysis. Rest of the preparation was according to the method described for the black and green tea preparation.

2.3 Potentiometry

2.3.1 Preparation of Tisab buffer

58.5g NaCl + 66 g sodium acetate + 14.2 ml glacial acetic acid were dissolved in the sufficient amount of distilled water. Then, 1g CDTA (1,2 cyclohexylenedinitrilo tetra acetic acid) was added and completely dissolved and reached to 1 liter with distilled water.

2.3.2 Stock solution

Standard solutions with the concentration of 10 mg/kg and 100 mg/kg were prepared from the standard solution of 1000 mg/kg.

2.3.3 Standard solution:

0.5, 1 and 2 ml of standard solutions (10 mg/kg and 100 mg/kg) were moved to 6 flasks of 50 ml and brought to volume with distilled water and standard solutions with concentrations 0.1, 0.2, 0.4, 1, 2 and 4 mg/kg were prepared, respectively. Potentiometry was calibrated using fluoride electrode. 10 ml of each standard solution was transferred to separate tubes and 10 ml of Tisab buffer was added to each one. The absorption of each solution was determined at the wavelength of 620 nm. Then, calibration curve was plotted (according to log concentration). 10 ml of Tisab buffer was added to the sample solution and its absorption was read at 620 nm wavelength. Then, according to calibration curve, the amount of fluoride was determined.

2.4 Spectrophotometry

2.4.1 Preparing of acetate buffer solution

60g sodium acetate anhydrous was dissolved in 600 ml distilled water. Then, 100 ml glacial acetic acid was added and reached to 1 liter with distilled water.

2.4.2 Alizarin Fluorine

960mg Alizarin Fluorine $[C_{14}H_7O_4CH_2N(CH_2COOH)_2]$ was dissolved in 100 ml distilled water and reached to 250 ml with distilled water in a flask (kept in the refrigerator).

2.4.3 Coloring working solution

300 ml buffer+150 ml acetone+ 50 ml tertiary butanol+ 36 ml alizarin fluorine+40 ml lanthanum nitrate+2 ml polyoxyethylen lauryl ether 23 were mixed and volume were prepared to 1 lit with distilled water.

2.4.4 Stock standard solution:

Standard stock solution 10 ppm was prepared from 1000 ppm absolute solution with the help of distilled water.

2.4.5 Working standard solution:

0.2, 0.4, 0.8, 1.2, 1.6 and 2 ppm standard solutions were prepared from 10 ppm solution. 10 ml of each solution were mixed with 10 ml coloring working solution. After 1h, the absorption of each solution was determined at the wavelength of 620 nm. The calibration curve was plotted. Also, 10 ml of coloring working solution was added to the sample solution and shaken. After 1h, the absorption was determined and the amount of fluoride was specified according to the calibration curve.

2.5 Statistical Analysis

The results of fluoride determinations were presented as means of mean $\pm$ standard deviation ($\bar{x} \pm SD$). Data were analyzed using ANOVA in SPSS15. Significant level was considered $\alpha=0.05$.

3 Results and Discussion

The fluoride levels of black tea, green tea and tea bags are listed in Tables 1–3. Results of study revealed a significant difference in the determination of fluoride content. The accuracy of results was studied by using both spectrophotometry and potentiometry and reported 72.9% and 98% fluoride recovery respectively. So, the results obtained from the potentiometry were analyzed and those of spectrophotometry results were excluded.

In present study, fluoride content was reported 0.48-1.85 mg/L, 0.73-3.39 mg/L, 0.34-4.09 mg/L and 1.3-3.85 mg/L in black teas, green teas, black tea bags and green tea bags, respectively. Results showed that the amount of fluoride in black teas was less than that of green teas and tea bags contained the greatest amount of fluoride. Further, no significant difference was reported between the fluoride content of black and green teas ($p>0.05$), while the difference in fluoride content of green and black tea bags was

Table1 Fluoride content in tea samples by potentiometry

No	Code	Fluoride in dry material (mg/kg)	Fluoride in the tea infusion (mg/lit)
		$\bar{X} \pm SD$	$\bar{X} \pm SD$
1	BT01	5.5 $\pm$ 86.3	0.05 $\pm$ 0.86
2	BT02	3.2 $\pm$ 126.0	0.03 $\pm$ 1.26
3	BT03	1.9 $\pm$ 182.8	0.02 $\pm$ 1.83
4	BT04	2.3 $\pm$ 47.8	0.06 $\pm$ 0.48
5	BT05	2.4 $\pm$ 90.7	0.02 $\pm$ 0.91
6	BT06	5.5 $\pm$ 182.4	0.06 $\pm$ 1.82
7	BT07	3.9 $\pm$ 85.6	0.04 $\pm$ 0.86
8	BT08	4.6 $\pm$ 159.8	0.05 $\pm$ 1.6
9	BT09	5.5 $\pm$ 66.2	0.06 $\pm$ 0.66
10	BT10	2.1 $\pm$ 81.9	0.02 $\pm$ 0.82
11	BT11	3.3 $\pm$ 65.1	0.03 $\pm$ 0.65
12	BT12	2.7 $\pm$ 129.1	0.02 $\pm$ 1.29
13	BT13	3.1 $\pm$ 184.7	0.03 $\pm$ 1.85
14	BT14	4.2 $\pm$ 62.9	0.04 $\pm$ 0.63
15	BT15	1.9 $\pm$ 129.2	0.02 $\pm$ 1.29
16	BTB01	5.5 $\pm$ 337.0	0.06 $\pm$ 0.34
17	BTB02	6.5 $\pm$ 101.3	0.07 $\pm$ 1.01
18	BTB03	14.2 $\pm$ 408.8	0.14 $\pm$ 4.09
19	BTB04	10.3 $\pm$ 133.8	0.1 $\pm$ 1.34
20	BTB05	3.9 $\pm$ 143.9	0.04 $\pm$ 1.44
21	GT01	3.4 $\pm$ 161.7	0.03 $\pm$ 1.62
22	GT02	7.4 $\pm$ 338.5	0.07 $\pm$ 3.39
23	GT03	3.7 $\pm$ 125.6	0.04 $\pm$ 1.26
24	GT04	3.1 $\pm$ 73.0	0.03 $\pm$ 0.73
25	GTB01	3.8 $\pm$ 129.6	0.04 $\pm$ 1.3
26	GTB02	4.0 $\pm$ 307.4	0.04 $\pm$ 3.07
27	GTB03	13.5 $\pm$ 335.5	0.14 $\pm$ 3.36
28	GTB04	7.9 $\pm$ 272.9	0.08 $\pm$ 2.73
29	GTB05	8.6 $\pm$ 384.6	0.09 $\pm$ 3.85
30	GTB06	10.1 $\pm$ 23.05	0.1 $\pm$ 2.31

BT=Black Tea BTB= Black Tea Bag, GT= Green Tea, GTB= Green Tea Bag

significant ($p<0.05$). Regarding the recommendation of daily fluoride intake by FAO and average daily consumption of four cups (1000 ml), it can be concluded that tea bag samples are unsafe for children while black and green tea samples are safe for both children and adults. Out of the 30 analyzed samples, 7 samples (1 green tea with the average fluoride content of 3.39

Table 2 Fluoride content in tea samples with spectrophotometry

No	Code	Fluoride in dry material (mg/kg) X±SD	Fluoride in the tea infusion (mg/lit) X±SD
1	BT01	8.9±3.46	04.47±0.0
2	BT02	2.5±5.63	05.64±0.0
3	BT03	9.4±5.120	06.21±0.1
4	BT04	2.1±3.42	03.42±0.0
5	BT05	7.7±3.46	04.47±0.0
6	BT06	3.2±6.82	06.82±0.0
7	BT07	2.7±3.40	03.41±0.0
8	BT08	4.6±5.80	05.81±0.0
9	BT09	4.5±3.42	03.43±0.0
10	BT10	7.9±2.35	02.36±0.0
11	BT11	1.3±3.35	03.35±0.0
12	BT12	1.6±3.47	03.48±0.0
13	BT13	8.3±4.75	05.75±0.0
14	BT14	7.7±3.69	04.7±0.0
15	BT15	5.7±2.37	03.38±0.0
16	BTB01	4.4±2.41	02.41±0.0
17	BTB02	2.6±3.53	03.54±0.0
18	BTB03	8.4±10.277	11.77±0.2
19	BTB04	1.0±5.67	05.67±0.0
20	BTB05	1.1±4.165	14.65±0.1
21	GT01	5.8±6.82	07.83±0.0
22	GT02	3.207±8	08.07±0.2
23	GT03	0.0±4.59	04.59±0.0
24	GT04	6.0±2.33	03.33±0.0
25	GTB01	4.4±6.76	06.76±0.0
26	GTB02	4.5±6.194	06.95±0.1
27	GTB03	9.6±10.203	11.04±0.2
28	GTB04	3.83±13.172	13.73±0.1
29	GTB05	5.9±30.301	21.02±0.3
30	GTB06	9.7±5.160	06.61±0.1

BT=Black Tea BTB= Black Tea Bag, GT= Green Tea, GTB= Green Tea Bag

mg/L, 1 black tea bag with the average fluoride content of 4.09 mg/L, 5 green tea bags with average fluoride content of 3.07, 3.36, 2.73, 3.85 and 2.31 mg/L) contained higher fluoride amount than the daily intake recommended by WHO (2 mg/L).

Fluoride is an essential element in human diet and it plays important role in teeth and bone mineralization, inhibitory and

Table 3 Comparison of fluoride content in tea samples by potentiometry

Tea	Number of samples	Fluoride in dry material (mg/kg) X±SD	Fluoride in the tea infusion (mg/lit) X±SD
Black tea	15	47.7 ^a ±112.1	0.4 ^a ±1.1
Green tea	4	115.1 ^a ±174.7	1.1 ^a ±1.7
Black and green Tea bag	11	111.2 ^b ±253.3	1.1 ^b ±2.3

BT=Black Tea BTB= Black Tea Bag, GT= Green Tea, GTB= Green Tea Bag;

Values in the same column with different superscripts are significantly different ($p < 0.05$)

stimulatory effects on many enzymes and dental caries resistance. On the other hand excessive fluoride absorption from food and water as well as occupational exposure to fluoride may cause dental or skeletal fluorosis (Wong et al., 2003, Ruan & Wang, 2001). After water, tea infusion is the most famous beverage in the world, which is an important source of fluoride intake. In Iran and middle-eastern countries, in which tea drinking is part of daily diet, tea as a beverage is an important source of fluoride intake. So, quality control of tea as a fluoride source is noticeable.

Koblar et al. (2012) assessed the human exposure to the fluoride from commercial teas. The efficacy of fluoride leaching from the green, oolong and black teas were ranged from 55-90% with continuous and 74-100% with repeated infusions, which were not affected by the type or manufacturing type. This study showed that daily consumption of five cups of tea can lead to 9-101% of adequate intake in a 70kg adult. Consumption of tea with daily diet can represent 25-173% and 35-210% of fluoride adequate intake in non-fluoridated and fluoridated areas respectively. The upper limits of these intakes can be already associated with the risk of developing fluoride-related adverse effects. Further, Karak & Bhagat (2010) reported trace amount of fluoride in tea leaves, tea and tea infusion. The amount of fluoride in 17 commercial teas (green, oolong, black and red) was reported between 170-878 mg/kg. Further, least amount was determined in oolong tea leaves (170-224 mg/kg).

In another study Mlinowska et al. (2008), determined the amount of fluoride in black, green, oolong and pu-erh tea infusions by an ion-selective electrode. Herbal infusions and instant tea were also analyzed. They concluded that brewing time (5, 10, 30 minutes) doesn't increase the fluoride amount. Fluoride content of black tea infusion (5 minutes) was more than the others. Fluoride range was reported 0.32-4.54 mg/L, 0.37-0.54 mg/L, 0.02-0.09 mg/L in black tea, white tea and herbal tea infusions, respectively. Similarly, Cao et al. (2006) assessed 37 different commercial teas

by spectrophotometry and ion-selective electrode method to determine fluoride content and evaluate the safety of different black teas. The result showed great difference in fluoride content. The amount of fluoride was 0.95-1.41 mg/L in black tea leaves, 0.7-2.44 mg/L in granola black tea and 1.15-6.01 mg/L in black tea bags. The highest fluoride content was reported in black tea bags presumably because they are made of older and low-cost leaves. As per the WHO's recommendation 56 and 44% of the tea bags are considered unsafe for children and adults respectively. At the average rates of consumption, 24% of the black tea bags could result in the increased risk of osteofluorosis and fractures. It is concluded that 23% of the analyzed samples were unsafe for children. Regarding the results, it seems that it is necessary to do more research on the evaluation of fluoride content of beverage and food products and also run epidemiologic study on their disadvantages. Furthermore national and international organizations should evaluate developing standards about the fluoride content of tea products.

Conflict of interest

Authors would hereby like to declare that there is no conflict of interests that could possibly arise.

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