Extractability of Fluoride into Black Tea Liquors, Kenya

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Abstract- **Fluoride in food is currently a subject of immense interest globally as it is directly related to health and disease. Tea leaves are rich in fluoride, part of which is released into the tea liquor during the tea making process. Therefore, the main objective of this study was to determine the levels of fluoride in tea liquors prepared from different grades of black Cut, Tear and Curl (CTC) tea sourced from various tea growing regions in Kenya as well as other tea producing countries in East Africa (Rwanda, Uganda and Tanzania). The fluoride levels in tea liquors (1 g of black tea + 100 ml of boiling distilled water agitated on a mechanical shaker for 10 minutes) were quantified to be in the range between 0.11 and 1.35 µg/ml using a simple, reliable and cheap potentiometric method by a Fluoride Ion Selective Electrode (FISE). These results confirm that tea consumption is indeed an important dietary source of fluoride. Also, these data demonstrate regional variations in the fluoride contents as tea liquors of the black CTC tea samples from different regions varied widely in terms of their fluoride contents.**

Keywords- Fluoride; Black CTC Tea; Tea liquor; Fluoride Ion Selective Electrode

I. INTRODUCTION

Fluorine (F) occurs in a number of minerals in the earth's crust including; cryolite (Na_3AlF_6), fluorspar (CaF_2), chiolite ($Na_5Al_3F_{14}$) and apatite (calcium fluorophosphate) (Wong, Fung, and Carr, 2003; Sucman, and Bednar, 2012). Fluoride (F^-), the anionic form of fluorine (F) is found dissolved in sea water, lakes and rivers. Also, appreciable amounts of fluoride are found in plants, with the actual amounts being dependent on the species of the plant, part and age of the plant. Leone, Bennan, and Daines, (1956) argue that mature plants generally tend to contain higher levels of fluoride than young ones. It has also been reported that fluorides are discharged into the atmosphere by aluminium (Al) and fertilizer production plants (Garrec, Lounowski, and Plebin, 1977).

Studies have demonstrated that the tea plant (*Camellia sinensis*) has the ability to take up fluoride from both the soil and air, accumulating it in its leaves (Hidekazu and Toshiyuki, 1977; Duckworth and Duckworth, 1978; Fung, Zhang, Wong, and Wong 1999; Sha, and Zheng, 1994; Lee, Shyu, and Chiang, 2003; Shyu, Jiann-Hwa, and Yi-Hwar, 2009), which happen to be the raw materials for the production of various types of tea products. The mechanism of uptake of fluoride from the soils on which tea is grown (usually acidic; pH less than 5.5) to the tea plant is well understood (Wong, Fung and Carr, 2003). The mobility of the ion (F^-) in the soil is influenced by a number of factors, most importantly the quantity of the minerals present, soil pH, adsorption of positively charged complexes, concentrations of iron (Fe), calcium (Ca), and aluminium (Al) in the soil among others (Fung, Zhang, Wong and Wong, 1999). Under acidic conditions, the aluminium-fluoride-halide complexes present in the soil decompose into aluminium ions (Al^{3+}) and fluoride ions (F^-) which correspond to high mobility (solubility) and hence the availability of the anion (F^-) for uptake by the tea plant. The free fluoride ion is then absorbed by tea roots and transported to and stored in the tea plant's leaves (Ruan and Wong, 2001), a phenomenon that has led to the tea plant to be referred to as a fluoride accumulator (Cao, Zhao, Liu, Zhan, Wan, and Liu, 1997).

A moderate amount of fluoride has been confirmed to be an effective way of reducing the incidence of dental caries (Levi, Zilberman, and Sarnat, 1983). This ion (F^-) interacts with hydroxyapatite, replacing the hydroxyl ions (OH^-) to form fluoroapatite, a more crystalline phase that is more resistant to erosion by plaque acid and demonstrates a lower surface energy which makes plaque adhesion more difficult. It also increases the rate of enamel remineralization, so that calcium (Ca) and phosphate ions are protected and not lost during demineralization. In addition, fluoride may reduce oral concentration of cariogenic bacteria or reduce the metabolism of bacteria in plaque. Therefore, the intake of fluoride is necessary in the promotion dental and oral health. However, excessive intake of fluoride results in both dental and skeletal fluorosis (WHO, 2002).

Data on the levels of fluoride in Kenyan tea are scarce. Therefore, the purpose of this study was to quantify the levels of fluoride in Kenyan black tea. For comparison purposes, black tea from other tea producing countries in East Africa (Rwanda, Uganda and Tanzania) was also included. Data obtained from this study are handy in keeping a safe threshold for fluoride exposure to avoid probable dental and skeletal fluorosis.

II. MATERIALS AND METHODS

A. Sample Collection

A total of 72 black tea samples of the three main grades (24 BP1, 24 PF1, and 24 PD) from 24 tea factory catchments selected to give a good regional representation were collected in triplicates. Also, 12 black tea samples (3 BP1, 3 PF1, and 3 PD) from the four tea producing countries in East Africa (Tanzania, Rwanda, Uganda and Kenya) were obtained from the Venus Tea Brokers (VTB) and Tea Brokers of East Africa (TBEA) at the Mombasa tea auction.

B. Sample Pre-treatment

The black tea samples were transported to the Tea Research Foundation of Kenya (TRFK), Timbilil Estate, Kericho (latitude 0˚ 22'S, longitude 35˚ 21'E, altitude 2180m asl) and oven dried (Memmert, 854 Schwabach, Germany) to a constant weight for 12 hours at a temperature of 103ºC. The samples were then finely ground using an electric blending device (Moulinex AR1043, China) to reduce the sample particle size and homogenize the sample.

C. Glassware and Reagent Quality

All glassware were soaked in 50% nitric acid (HNO_3) for 24 hours, thoroughly cleaned and subsequently rinsed with distilled water and oven dried before use. All reagents used in this work were of analytical grade (Sigma Aldrich, UK) and distilled water was used for all dilutions.

D. Sample Preparation and Tea Liquor Fluoride Content Analysis

1% w/v tea liquor was prepared by dissolving 1 g of black tea in 100 ml boiling water, agitated for 10 minutes on a mechanical shaker and filtered. This is the liquor ratio that has been commonly suggested by a number of authors (Duckworth and Duckworth, 1978; Fung, Zhang, Wong and Wong, 1999; Shyu, Jiann-Hwa and Yi-Hwar, 2009) and used in the preparation of commercial tea drinks. The filtrate obtained was allowed to cool to room temperature before analysis. In addition, a series of twelve (12) blank solutions were prepared alongside the tea liquors. For quality control, a standard addition method was used on two control distilled water samples A and B spiked with known analyte concentrations and extracted and analysed alongside the samples.

Fluoride working standards, with concentrations ten-fold apart, were prepared by serially diluting a commercial stock (1000ppm F^-) standard solution. 25 ml of a Total Ionic Strength Adjustment Buffer (TISAB; pH 5.30-5.35) solution was prepared by dissolving 300 g of sodium citrate dehydrate ($C_{14}H_{22}N_2O_8.2H_2O$), 22 g of cyclohexylenediamine tetra acetic acid (CDTA), and 60 g of sodium chloride (NaCl) in a litre of distilled water (Colina, Arias, and Rodriguez, 1990; Shyu, Jiann-Hwa and Yi-Hwar, 2009) was then added to an equal volume of the standards and samples. The purpose of the buffer was to reduce and stabilize the total ionic strength of the solutions, to adjust and maintain a relatively constant pH (between 5.0-5.5) and to chelate various polyvalent cations such as Al (III), Si (IV), Fe (III) among others which interfere with the analysis of fluoride (Kauranen, 1977; Nicholson and Duff, 1981; Colina, Arias, and Rodriguez, 1990). CDTA has six complexing groups and preferentially complexes with the metal cations in solution forming more stable complexes than metal-fluoride complexes (AlF_6^{3-}, FeF_6^{3-} etc).

A potentiometric fluoride specific electrode method was applied in this work. A flow plus fluoride ion selective electrode (EDT, direct-ion 5221) was then immersed in the standard series and tea liquor samples and readings (potential) on the ion analyzer (EDT, direct ion, DR359TX) in millivolts were recorded. The fluoride standards responses were used to check the instrument slope as well as prepare the calibration curve from which the blank analyses as well as the sample analyte concentrations were computed. The limits of detection (LOD) and quantification (LOQ) of the current method were estimated using the non-zero standard deviation of the twelve blank analyses and the slope of the calibration curve.

E. Statistical Analysis

All the fluoride determinations were carried out in triplicates, and the data were subjected to analysis of variance (ANOVA) using GraphPad Prism version 5.0 statistical analysis package for windows. Statistically significant differences were evaluated at $p \le 0.05$. Graphical representation of these data was done using the same software.

III. RESULTS AND DISCUSSION

A. Calibration and Method Validation

1) Linearity of the Calibration Curve:

A plot of the machine response (electrode potential in millivolts, mV) against the logarithm of the concentration of the fluoride working solutions (log F) gave a calibration curve for fluoride determination. The calibration curves obtained for each replicate demonstrated adequate linearity, with all having r^2 values greater than 0.999. This demonstrates that the response obtained (electrode potential, mV) was directly proportional to the logarithmic ionic activity of the fluoride ions in the working

solutions.

The measured potential (mV) corresponds to the amount of fluoride in solution and can be described by the Nernst Equation;

$$E = S \log F + B \quad \text{(Nernst Equation)}$$

Where, F is the concentration of (µg/ml) of fluoride yielding a millivolt potential E, S is the slope of the calibration curve obtained and B is the reference potential. The mean S value obtained from the calibration curves was -59.4 which is in good agreement with the theoretical Nerstian slope for monovalent anions whereas the mean B value obtained was 69.7 mV.

2) Limit of Detection (LOD) and Limit of Quantitation (LOQ):

Being an essential part of a comprehensive quality assurance system in analytical chemistry, LOD and LOQ are fundamental elements of method validation, which is necessary in ensuring the reliability of analytical procedures. LOD, is the smallest mass of analyte that can be distinguished from the statistical fluctuations in a blank (Butcher, and Sneddon, 1998; Armbruster, and Pry, 2008) and is expressed as a concentration "derived" from the smallest measure that can be detected with reasonable certainty for a given analytical procedure (Thomsen, Schatzlein, and Mercuro, 2003). On the other hand, LOQ is the lowest concentration of an analyte in a sample that can be "determined" with acceptable precision and accuracy under the stated conditions of test. The LOD ($3_{sb/b}$, n=12) and LOQ ($10_{sb/b}$, n=12) of the method used in the present study were 0.02 and 0.07 µg/ml respectively where sb is the standard deviation of the blank analyses, b is the slope of the calibration curve and n is the number of replicate analyses.

3) *Accuracy and Precision*:

The two control distilled water samples A (high concentration control sample) and B (low concentration control sample) were spiked with known concentrations of fluoride drawn from a commercial fluoride stock solution of 1000 µg/ml and taken through the extraction and analysis to assess recovery for every batch of samples analysed. Accuracy expressed as the average percent recovery of 96.70% and 102.0% were obtained for controls A and B respectively as shown in Table 1. The precision of the method expressed as relative standard deviations are also tabulated. Based on these data, it is evident that the method used in this study demonstrated good accuracy and precision.

TABLE I ACCURACY AND PRECISION

Control	*Fluoride Added (µg/ml)	*Added Fluoride Quantified (µg/ml)	Recovery (%)	Precision (RSD)
A	2.0	1.934	96.70	0.67
B	0.1	0.102	102.00	2.59

*n= 8

B. Extractable Fluoride Content of Tea

The 72 Kenyan tea samples had a mean fluoride concentration of 0.37 µg/ml for the 1% tea liquors (Fig. 1). The mean fluoride levels for each of the three grades of tea considered were 0.40 µg/ml (BP1), 0.37 µg/ml (PF1) and 0.33 µg/ml (PD). The differences in the levels of fluoride in the three grades of tea (BP1, PF1 and PD) considered from the same tea factory catchments (region) were not statistically significant ($p \leq 0.05$) as clearly depicted in Fig. 1.
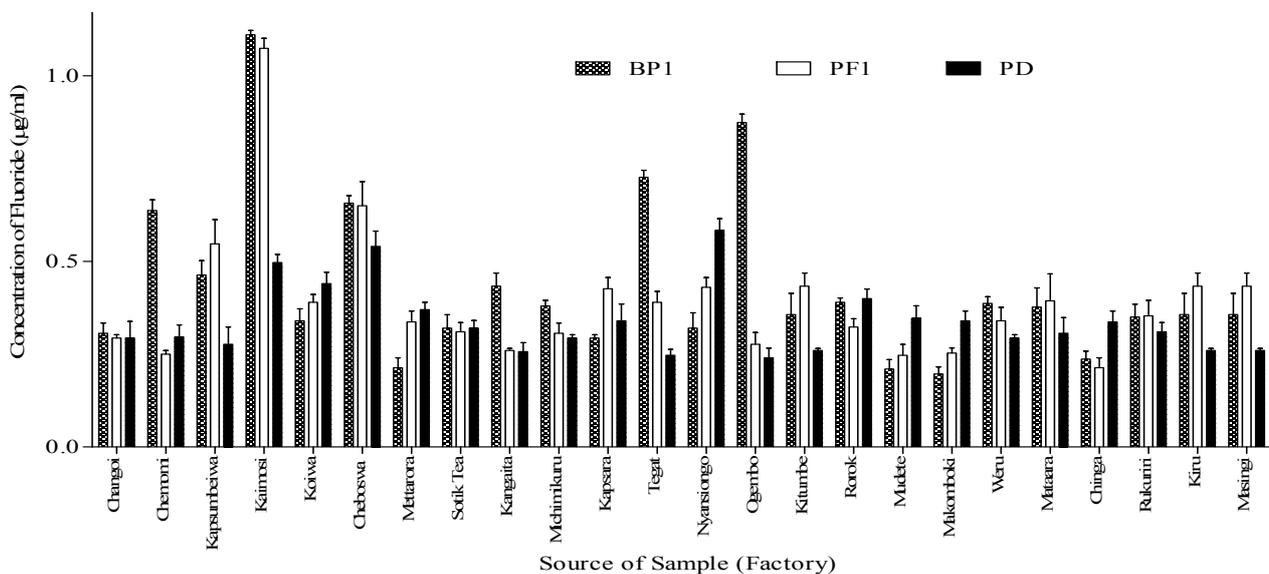

Fig. 1 Fluoride levels in tea from 24 factory catchments in Kenya

This data clearly demonstrate that tea samples from the same region (factory), regardless of the grade have the same fluoride levels. However, a few exceptions were observed as is evident for the case of Ogembo, Tegat and Kaimosi tea factories. For instance, The BP1 sample from Ogembo had mean fluoride levels that were statistically different ($p \leq 0.05$) from the mean fluoride levels obtained for the other two grades of tea (PF1 and PD).

According to the Tea Board of Kenya, TBK, (2007), tea production in Kenya is characterized by two sub-sectors; the large-scale sub-sector (estate plantations) with production units larger than 20 ha, and the small-scale sub-sector with smaller production units averaging 0.25 ha per farmer. Due to the differences in agronomic practices in the large and small-scale tea sub-sectors, an attempt was made to find any effects, if any, of these would have on the extractable levels of fluoride in the tea samples from the two sub-sectors. Table II gives the 24 tea factories grouped into sub-sectors.

TABLE II TEA FACTORIES FROM THE TWO TEA SUBSECTORS IN KENYA

Sub-sector	Tea Factory
Large Scale	Masingi, Changoi, Chemomi, Kapsumbeiwa, Kaimosi, Koiwa, Cheboswa, Mettarora, Sotik Tea, and Kitumbe,
Small Scale	Kangaita, Michimikuru, Kapsara, Tegat, Nyansiongo, Ogembo, Rorok, Mudete, Makomboki, Weru, Mataara, Chinga, Rukuriri, and Kiru

The levels of fluoride for the two tea sub-sectors were not statistically different ($p \leq 0.05$), indicating that the differences in the agronomic practices did not affect the levels of fluoride in tea. The levels obtained were as given in Fig. 2 below.
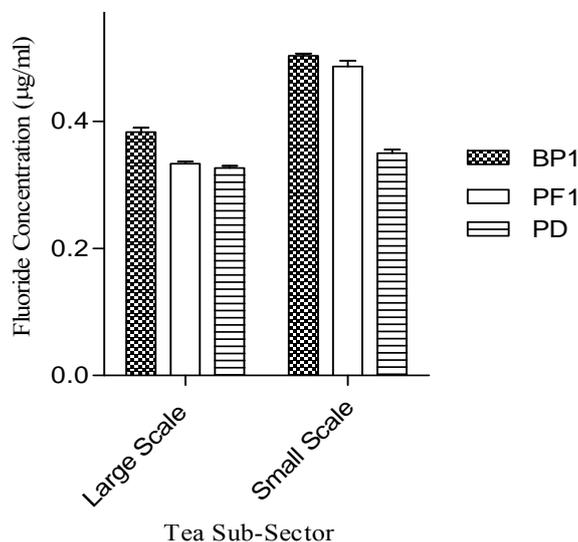

Fig. 2 Fluoride levels in tea samples from the large scale and small scale tea subsectors in Kenya

The liquors of the samples from Tanzania, Uganda, Rwanda and Kenya obtained from the Mombasa tea auction gave fluoride levels presented in Fig. 3. The mean fluoride levels for each of the three grades were 0.36, 0.54 and 0.42 µg/ml for BP1, PF1 and PD respectively. The samples from Uganda and Kenya had the highest mean fluoride concentration (0.66 µg/ml) while the lowest concentration was obtained in the samples from Rwanda (0.25 µg/ml of fluoride).
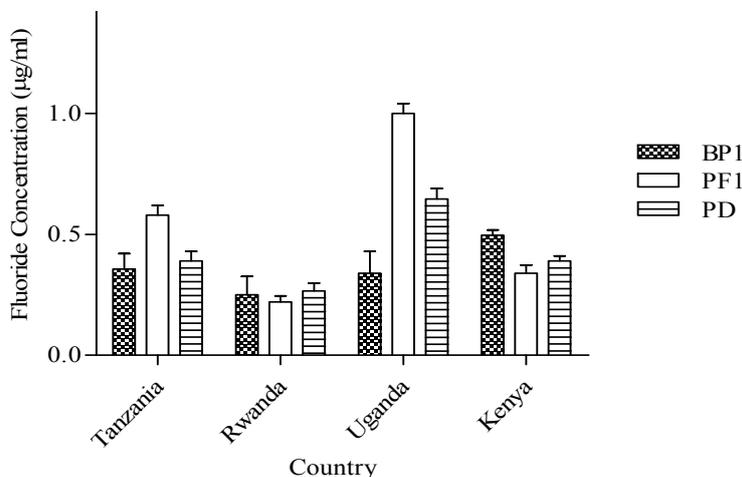

Fig. 3 Fluoride levels in tea liquors of the black tea samples from Tanzania, Rwanda, Uganda and Kenya

The current data are well in agreement with reports on similar work globally. For instance, the water soluble fluoride concentration of Turkish teas was found to range between 55 and 127 µg/g (Tokalioglu, Sahin and Kartal, 2001) which correspond to 0.6 to 1.3 µg/ml when converted into liquor concentrations (2 g tea infused in 200 mL of H_2O). Kalayci and Somer (2003) reported concentrations of 2.6 and 3.9 µg/ml of fluoride in black tea liquors extracted for 5 and 20 minutes respectively. Hudaykuliev, Tastekin, Poyrazoglu, Baspinar, and Velioglu, (2005) reported a range of fluoride concentrations for Turkish teas that corresponded to between 0.8 and 2.6 µg/ml liquor concentrations. Further work that corroborates with the findings of the current study include; Tokalioglu, Kartal, and Sahin, 2004 (0.64-3.55 µg/ml F^-), Yuwono, 2005 (0.95-4.73 µg/ml F^- for black teas; from 0.70-1.00 µg/ml F^- for green teas, and from 0.26-0.27 µg/ml F^- for herbal teas.), Mahvi, Zazoli, Younecian, and Esfandiari, 2006 (0.53±0.07 to 2.60±0.18 µg/ml F^-), and Li et. al., 2009 (mean, 1.81±1.09 µg/ml F^-) among others.

The fluoride contents released into the tea liquor depend on a number of factors including the duration of tea infusion, the temperature at which the infusion process is carried out and the type of tea (Yuwono, 2005; Giljanovic, Prkic, Bralic, and Brkljaca, 2012). There were significant differences in the fluoride contents released into the tea liquors in both the locally grown and processed black tea and tea from the market (Mombasa tea auction). Since the infusion method used for all the groups of the samples was the same, then these data demonstrate that tea samples from different regions actually contain different levels of fluoride (Rajkovic, and Novakovic, 2007). These differences are attributable to the geological, chemical and physical characteristics of the area of production (Hudaykuliyev, Tastekin, Poyrazoglu, Baspinar, and Velioglua, 2005). Other factors that may influence the fluoride content in tea liquor include the leaf age, maturity (Fung, Zhang, Wong, and Wong, 1999; Shu, Zhang, Lan, and Wong, 2003) and genetics of the plant among others.

Tea is consumed by over two thirds of the world's population owing to its medicinal, refreshing and mild stimulating effects (Karak, and Bhagat, 2010). Therefore, based on our findings and those reported elsewhere by researchers globally, it is evident that tea consumption is indeed an important additional dietary source for fluoride, besides water and other fluoride containing feedstuffs world over. However, chronic consumption of large volumes of brewed tea has been reported to result in skeletal fluorosis (Shu, Zhang, Lan, and Wong, 2003; Izuora, Twombly, Whitford, Demertzis, Pacifici, and Whyte, 2011; Kakumanu, and Rao, 2013), hence the need for a safe threshold for fluoride exposure. Unfortunately, there is no guideline for the levels of fluoride in tea in the Kenyan tea standard KS 65: 2009. However, according to the World Health Organization, WHO, (2011), the guideline for fluoride in drinking water is 1.5 µg/ml. Based on these, and the findings of the current study, Kenyan teas as well as those from other tea producing countries in the region can be regarded to be safe for consumption with respect to their fluoride content.

IV. CONCLUSIONS

Despite the regional variations observed, the levels of fluoride in tea liquors of Kenyan tea compare well to tea grown in other East African countries (Rwanda, Uganda and Tanzania) as well as those reported by other researcher's world over. However, this study recommends the inclusion of a guideline for the maximum permissible concentration (MPC) for fluoride in tea by the Kenya Bureau of Standards (KEBS) in the Kenyan tea standard, KS 65: 2009. The standard should also be based on tea liquor, since the highest potential risk is due to the tea liquor consumed rather than the total fluoride content in processed black tea.

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