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FLUORIDE LEVELS IN *TEF* [*ERAGROSTIS TEF* (ZUCC.) TROTTER] AND *ENJERA* AND HEALTH IMPLICATIONS

Gebreegziabher Asayehegn, Bhagwan Singh Chandravanshi * and Feleke Zewge

Department of Chemistry, College of Natural Sciences, Addis Ababa University, PO Box 1176, Addis Ababa, Ethiopia. E-mail: bscv2006@yahoo.com

ABSTRACT: The main objective of this study was to estimate the fluoride levels in tef grain and in *enjera*, its fermentation product in different regions of Ethiopia, especially in the regions where tef is dominantly cultivated. The effect of water used to bake the *enjera* was assessed by preparing fluoride-containing water in the laboratory. The daily dietary fluoride intake through *enjera* was assessed in Wonji Shoa by recipe questionnaires and field observations in ten households. The fluoride contents of both tef grain and *enjera* were higher in the Rift-Valley region of Ethiopia than in the highlands. Of the different varieties of tef, the mixed tef grain was higher (8.74 mg/kg) in its fluoride content and the brown tef grain was the least (1.89 mg/kg). *Enjera* from mixed tef grain baked using 10 mg/L fluoride water was found to have high (26.82 mg/kg) fluoride contents and the least (2.63 mg/kg) was for *enjera* processed from brown tef grain baked using tap water. Daily dietary fluoride intake was different with different fluoride concentration of the water used. In this study, the fluoride content of *enjera* ranges from 2.63–26.82 mg/kg and the daily-recommended human intake of fluoride ranges from 4.8-14.6 mg/kg/day. The fluoride contents of the different tef varieties vary depending on the soil type, soil pH and the type of minerals in the soil. The value of fluoride in *enjera* baked using tap water is safe for human consumption while *enjera* baked using 5 and 10 mg/L fluoride containing water may cause dental as well as skeletal fluorosis.

Keywords/phrases: ***Enjera***, ***Eragrostis tef***, **Ethiopia, flourosis, fluoride**

INTRODUCTION

Tef [*Eragrostis tef* (Zucc.) Trotter] is a C_4* tropical cereal (Geremew Bultosa, 2007; Seyfu Ketema, 1997) and is a major food crop grown in Ethiopia. Tef belongs to the grass family poecae (Gramineae), genus *Eragrostis*. Tef originated in Ethiopia but is also produced in other countries. Countries such as USA, Canada, Australia, South Africa and Kenya produce tef for different purposes such as forage crop and thickener for soups, stews and gravies (Geremew Bultosa *et al.*, 2002). The crop exhibits high variability within regions of cultivation and between plants of the same accession (Dawit Tadesse, 1993).

In Ethiopia, the primary use of tef grain is for grinding into flour to make *enjera* (a fermented, pancake-like, soft, sour, circular flatbread), a major food staple. Nutritionally, 100 g edible portion of tef grain contains: water 11.0 g, protein 9.6 g, carbohydrate 74.6 g, fat 2.53 g, fibber 2.63 g, thiamine 0.3 mg, riboflavin 0.2 mg, niacin 2.5 mg and ascorbic acid 88 mg (Abraham Besrat *et al.*, 1980). It is an excellent source of essential amino acids, especially lysine. Lysine is the amino acid that is most often deficient in other grain foods such as barley, millet and wheat (Jansen *et al.*, 1962; Admassu Mamo, 2008; Gamboa and van Ekris, 2008). Tef is also an excellent source of fiber and contains higher amounts of calcium, potassium, magnesium and iron found in an equal amount of other grains such as rice, wheat and maize (Abraham Besrat *et al.*, 1980; Malde *et al.*, 2004).

There are several varieties of tef, each with characteristics best suited to specific conditions. Although there is no unifying classification of the tef types throughout the country, in general, there are three main types: white (*nech* tef), brown (*tikur* tef) and mixed (*sergegna*). White tef is the preferred type but only grows in certain regions of Ethiopia. White tef is the most expensive type of tef. The prestige associated with consuming white tef contributes to its

* Author to whom all correspondence should be addressed.

increased cost. The shelf life of *enjera* is extended with the use of white tef (Mesfin Haile *et al.*, 2004). Brown tef grain is the least expensive form and the least preferred type and has the highest iron content (Deckers *et al.*, 1998). In persons living in areas of the country where consumption of brown tef grain is most prevalent, haemoglobin levels were found to be higher with a decreased risk of anaemia related to parasitic infection (Jansen *et al.*, 1962). According to the studies, due to the increased health benefits associated with high iron contents in brown tef grain, there is more acceptance of this grain type in the society currently.

Tef flour can be used as a substitute for part of the flour in baked foods, or the grains added uncooked or substituted for part of the seeds, nuts, or other small grains. It is a good thickener for soups, stews, gravies, and puddings and can also be used in stir-fry dishes, and casserole dishes. Cooked tef can be mixed with herbs, seeds, beans or tofu, garlic, and onions to make grain burgers. The seeds can also be sprouted and the sprouts used in salads and in sandwiches. Tef flour is also used for making traditional alcoholic drinks like *tella* (local opaque beer) and *katikalla* (local spirit). It is also used to prepare *kitta* (sweet dry unleavened bread), *muk* (gruel) (Zewdu Abdi Debele, 2007; Gamboa and van Ekris, 2008).

Tef grain is well known by Ethiopians for its superior nutritional quality. It provides about two-thirds of the daily dietary protein intake of most Ethiopians (Ashenafi Mengistu *et al.*, 2007). Tef grain contains more lysine than barley, millet, and wheat and slightly less than rice or oats and it is gluten free. Besides providing protein and calories, tef grain is a good source of minerals, particularly iron (Jansen *et al.*, 1962). Compared with other cereals, tef grain is reported to have higher iron content.

Fluoride is a necessary element to human health; a moderate amount of fluoride intake is confirmed that it is the effective way of reducing dental caries among children and adults. However, excessive fluoride intake through drinking water or food results in dental fluorosis and skeletal fluorosis (Liub *et al.*, 2009).

There are many studies reported in the literature about the dietary intake of fluoride (Malde *et al.*, 1997; Zohouri and Rugg-Gunn, 1999; 2000; Malde *et al.*, 2004; 2006; 2011; Samuel Zerabruk *et al.*, 2010; Battaleb-Looie *et al.*, 2013; Bisratewongel Tegegne *et al.*, 2013; Meseret Dessalegne and Feleke Zewge, 2013; Asamene Embiale *et al.*, 2014). Among these are studies of fluoride content in water, beverages and foods. Staple foods are prepared from different cereals including tef (Malde *et al.*, 1997; Meseret Dessalegne and Feleke Zewge, 2013), wheat (Sunitha and Reddy, 2008; Bhargava and Bhardwaj, 2009; Yadav *et al.*, 2012), corn (Sunitha and Reddy, 2008; Gautam *et al.*, 2010; Meseret Dessalegne and Feleke Zewge, 2013), barley (Bhargava and Bhardwaj, 2009; Gautam *et al.*, 2010), rice (Bisratewongel Tegegne *et al.*, 2013) and legumes including pea (Malde *et al.*, 1997; Gautam *et al.*, 2010), beans (Malde *et al.*, 1997; Sunitha and Reddy, 2008), chickpea or gram (Sunitha and Reddy, 2008).

Drinking water is traditionally considered to be the main source of fluoride and, therefore, artificial fluoridation is common in many water supplies (Chandrajith *et al.*, 2007). Virtually all foodstuffs contain at least traces of fluorine. All vegetation contains some fluoride, which is absorbed from soil and water (WHO, 2004). Intake of fluoride is variable and is derived from a number of sources in the diet and the environment (Simposom *et al.*, 2001).

There are several reports on the fluoride concentration of ground water sources in different sectors of the Ethiopian Rift Valley. However, studies regarding the fluoride concentration of different foodstuffs are very limited. Hence, the objective of this study was to determine the fluoride concentration of tef grain and *enjera*, its fermentation products, sampled from different regions of Ethiopia. In addition, contribution of the water used for cooking to the fluoride concentration of *enjera* was estimated. Finally, daily intake of fluoride per adult person was estimated based on the fluoride concentrations of *enjera* including drinking water.

MATERIALS AND METHODS

Instrumentation

Orion F^- ion selective electrode was used for the routine determination of F^- ion in tef grain and *enjera* samples. A pH/ISE meter (Orion Model, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride-selective electrode (Orion Model 96-09, USA) was employed. The pH was measured with pH/ion meter (WTW Inolab pH/ION Level 2, Germany) using unfilled pH glass electrode.

Chemicals and reagents

Chemicals and reagents that were used in the analysis were all analytical grade. Fluoride stock solution (1000 mgL^{-1}) was prepared from sodium fluoride (99.0% NaF, BDH Chemicals Ltd, Poole, England) and stored in 1 L volumetric flask. Total ionic strength adjustment buffer (TISAB) solution containing 58 g of sodium chloride (Scharlau, European Union), 57 mL of glacial acetic acid, 2 g of ethylene diamine tetra-acetate (EDTA, Spain), 7 g of sodium citrate (Research-Lab Fine Chem. Industries Mumbai 400 002, India) and approximately 100 mL of 6 M NaOH (Scharlau, European Union) in a volume of 1000 mL (to adjust pH, 5.0-5.5). 37% HCl (Riedel-de-Haen, Germany) were also used for neutralization of fusion cake in the fluoride determination. The TISAB solutions regulate the ionic strength of samples, standard solutions and adjust the pH, and also avoid interferences by polyvalent cations such as Al(III), Fe(III) and Si(IV); which are able to complex or precipitate with fluoride and reduce the free fluoride concentration in the solution.

Sample pre-treatment

The tef grain samples were collected from four-sampling areas (Wonji Shoa, Arsi Negelle, Debre Markos and Jimma) were washed with distilled and deionised water to remove soil and dust particles and dried using sun light to constant weight. The tef grain samples were ground using electronic blending device (Moulinex, France) and the flour was sieved using a 0.425 mm polyethylene sieves. The part of the sample that passes through the sieve was returned to the original sample bag and stored ready for analysis. 0.5 g of the tef grain powder was taken and used for fluoride analysis. The rest of the samples were used for baking *enjera* in the traditional way. Tap water, 5 mg/L and 10 mg/L fluoride containing water was used for baking *enjera*. The 5 mg/L and 10 mg/L fluoride containing water was prepared in the laboratory.

Procedures for baking enjera

A known weight (450 g) of tef grain flour was taken into a container called *buhaka* that is large enough to hold the entire recipe and about 600 mL of water was added and mixed the dough well. Water (80 mL) was added gradually with frequent stirring by hand until it was mixed well. The dough was covered and allowed to ferment for four days. To bake the *enjera*, in the traditional manner, the supernatant water on top of the dough was poured off and a small amount of the dough was added to 320 mL of water and then it was boiled (called *absit*). Then the *absit* was mixed with the remaining dough. The hot mixture was well stirred and allowed to stand for 30 min. After fermentation, the batter was thinned again by adding a small amount of water. The fermented batter was then taken in a small can, or a gourd, called *mazoria* or *masfiya* from the *buhaka* and poured and spread on to the *metad* (stove) with a circular motion beginning at the outer perimeter and working towards the centre. It is then covered with a *metad* cover called *akambalo*. The edges of the *akambalo* rest on the *metad*, on the outer circle of the *enjera*. Cracks or the openings between the lid and the griddle were covered with a strip of moist cloth. After 3 minutes, the *enjera* was fully baked. The baked *enjera* lifted up by hand and placed on a *sefed* which is a kind of basket made of grass stems (ENI, 1980). The *enjera* dried to constant weight using sun light, ground using pestle and mortal and made ready for analysis.

Preparation of the sample for fluoride determination

The fluoride content of tef grain and *enjera* was determined using the alkaline fusion by slightly modifying the methods of Mcquaker and Gurney (1977).

Calibration for fluoride determination

Calibration of the fluoride ion selective electrode was made by using a series of 0.5, 1, 5, 10 and 20 mg/L fluoride standards solutions from stock solution of 1000 mg/L fluoride, then 5 mL of standard solution from each series was taken and mixed to 5 mL of TISAB to each of 50 mL plastic beaker, the mixture were stirred (uniformly) thoroughly using the magnetic stirrer, the fluoride selective electrode was immersed in each beaker and the calibration was done. The concentration range, slope and correlation coefficients of the calibration curves for the determination of total fluoride in tef grain and *enjera* are given in Table 1.

Table 1. Calibration of fluoride ion selective electrode and correlation coefficients of the calibration curves for total fluoride in tef grain and *enjera* and recovery test for total fluoride.

No.	Purpose of calibration	Concentration of working standards (mg/L)	Slope (mV/dec)	Correlation coefficient of the calibration curve	Equation of the calibration curve
1	Determination of total fluoride in tef grain sample	0.5, 1.0, 5.0, 10, 20	-58.3	0.9996	Y = 111.3 – 58.33*X
2	Determination of total fluoride in *enjera* sample	0.5, 1.0, 5.0, 10, 20	-57.0	0.9999	Y = 105.8 – 57.00*X
3	Recovery test for total fluoride in tef grain sample	0.5, 1.0, 5.0, 10, 20	-58.9	-0.9995	Y = 116.3 – 58.9*X
4	Recovery test for total fluoride in *enjera* sample	0.5, 1.0, 5.0, 10, 20	-60.3	-0.9995	Y = 114.8 – 60.3*X
5	Mean of the two measurements of fluoride in tef grain and *enjera* analysis	0.5, 1.0, 5.0, 10, 20	-58.8±1.5	-0.9999	Y =119.7 – 58.8*X

After calibration of the fluoride ion selective electrode within the selected working range (0.5 to 20 mg/L), the fluoride concentrations of tef grain and *enjera* were determined. Thus, F^- concentrations in tef grain and *enjera* were measured by mixing equal volumes (5 mL) of tef grain and *enjera* sample solutions and 5 mL of TISAB in a 50 mL plastic beaker and the mixture was stirred (uniformly) thoroughly using the magnetic stirrer. The combination fluoride selective electrode was immersed in the solution and the fluoride concentration of the tef grain and *enjera* was determined from a direct readout. All measurements were made in triplicate.

Recovery test for fluoride determination

The efficiency of the optimized procedure is checked by various methods. These are certified standard reference material analyzing and spiking sample with known concentration of the analyte. In this work, the method validation was established by spiking experiments (recovery test). The spiked samples were prepared by adding, for Wonji Shoa white tef grain, 0.05 mL, 0.1 mL and 0.2 mL; for brown tef grain 0.04 mL, 0.08 mL and 0.2 mL; for mixed tef grain 0.05 mL, 0.11 mL and 0.22 mL; for Debre Markos white tef grain, 0.03 mL, 0.06 mL and 0.12 mL; for brown tef grain 0.02 mL, 0.05 mL and 0.1 mL; for mixed tef grain 0.03 mL, 0.06 mL and 0.12 mL of 20 mg/L standard fluoride solution. Samples of *enjera* prepared from Wonji and Arsi Negelle tef grain were spiked and analyzed. 0.5 g of different *enjera* samples were spiked with following amounts of 20 mg/L of standard fluoride solution: *enjera* from Wonji white tef grain 0.07 mL, 0.14 mL and 0.3 mL; from brown tef grain 0.06 mL, 0.12 mL and 0.24 mL; from mixed tef grain 0.08 mL, 0.2 mL and 0.33 mL; from Arsi Negelle white tef grain 0.06 mL, 0.12 mL and 0.2 mL; from brown tef grain 0.05 mL, 0.1 mL and 0.2 mL; from mixed tef grain 0.06 mL, 0.13 mL and 0.3 mL, respectively. The spiked and un-spiked tef grain and *enjera* samples were prepared and analyzed in similar conditions.

RESULTS AND DISCUSSION

Method of validation for fluoride determination

A 0.5 g tef grain flour and *enjera* samples each were spiked with fluoride solution of which the fluoride content was equivalent to 25%, 50%, or 100% of the fluoride content of the original (unspiked) tef grain and *enjera* samples. After spiking the determination of fluoride was made in triplicate. The percentage recoveries were in the range 90 to 107% (Table 2).

Table 2. Recovery test results of tef grain flour and *enjera* samples prepared using water containing 5 mg/L fluoride.

Sample site	Types of tef grain	Concentration of F^- in unspiked sample (µg/g)	Amou-nt of F^- added (µg/g)	Concentration of F^- in spiked sample (µg/g)	Recovery (%)	Sample site	Type of *enjera* made from	Concentration of F^- in un spiked sample (µg/g)	Amount of F^- added (µg/g)	Concentration of F^- in spiked sample (µg/g)	Recovery (%)
Wonji Shoa	White tef grain	7.9	1.98	9.74±1.2	91±1	Wonji Shoa	White tef grain	11.6	2.90	14.4±2.1	99±3
		7.9	3.97	10.7±0.8	96±3			11.6	5.79	17.0±3.2	94±3
		7.9	7.93	15.9±1.4	101±2			11.6	11.60	23.8±3.2	105±5
	Brown tef grain	6.6	1.64	8.13±1.3	95±4		Brown tef grain	9.45	2.36	11.7±1.9	97±2
		6.6	3.29	9.56±1.6	90.9±2			9.45	4.73	13.9±2.1	95±3
		6.6	6.57	13.6±2.1	104±4			9.45	9.45	19.0±3.3	101±4
	Sergegna (mixed) tef grain	8.7	2.19	10.7±3.2	90±1		*Sergegna* (mixed) tef grain	13.3	3.31	16.4±2.6	93±2
		8.7	4.37	12.8±1.6	94±3			13.3	6.65	19.9±3.9	101±5
		8.7	8.73	17.7±3.4	102±4			13.3	13.3	26.9±2.3	103±3
Debre Markos	White tef grain	4.6	1.15	5.65±2.3	91±1	Arsi Negelle	White tef grain	9.21	2.30	11.4±4.0	94±4
		4.6	2.30	6.85±2.2	98±3			9.21	4.61	13.4±4.2	91±2
		4.6	4.60	9.21±2.2	100±2			9.21	9.21	18.9±3.6	105±6
	Brown tef grain	3.9	0.99	4.92±1.9	98±4		Brown tef grain	8.34	2.09	10.3±5.1	93±4
		3.9	1.98	5.91±1.9	99±5			8.34	4.17	12.4±3.2	97±5
		3.9	3.95	8.01±2.0	103±4			8.34	8.34	17.2±3.3	107±3
	Sergegna (mixed) tef grain	4.7	1.18	5.90±3.0	100±3		*Sergegna* (mixed) tef grain	10.2	2.55	12.7±3.4	96±2
		4.7	2.36	6.97±2.6	96±4			10.2	5.11	14.9±2.6	93±2
		4.7	4.71	9.49±3.2	101±5			10.2	10.2	20.7±4.1	102±4

Fluoride contents of tef grain and enjera

The lowest (1.89 mg/kg) and highest (8.74 mg/kg) content of fluoride was found in brown tef grain from Jimma and mixed tef grain from Wonji, respectively. The lowest amount (2.63 mg/kg) of fluoride was found in *enjera* made from brown tef grain from Jimma using tap water for baking and the highest amount (26.82 mg/kg) of fluoride was found in *enjera* made from mixed tef grain from Wonji. The results are given in Table 3. The *enjera* sample from Wonji Shoa and Arsi Negelle, located within the Rift Valley, have higher fluoride contents than the sample from Debre Markos and Jimma, which shows the contribution of fluoride from the soils. Therefore, fluoride from soil and water contributes to the total fluoride content of *enjera* in both cases.

Table 3. Fluoride content (X ± SD, n = 3, µg/g dry weight) of tef grain flour and *enjera* samples (prepared using tap water and 5 and 10 mg/L F^- containing water) from four different areas (Wonji, Arsi Negelle, Debre Markos and Jimma) of Ethiopia.

Sample site	Type of tef grain	Average concentration of F^- (mg/kg)			
		in tef grain	in *enjera* made by using tap water	in *enjera* made by using 5 mg/L F^- containing water	in *enjera* made by using 10 mg/L F^- containing water
Wonji Shoa	White tef grain	7.93±0.67	8.44±0.06	12.0±0.22	25.6±0.21
	Brown tef grain	6.57±0.36	7.78±0.40	11.5±0.12	21.9±0.06
	Mixed tef grain	8.74±0.07	9.56±0.21	15.8±0.04	26.8±0.02
Arsi Negelle	White tef grain	5.62±0.09	7.24±0.05	9.21±0.15	19.3±0.26
	Brown tef grain	5.12±0.27	6.44±0.23	8.34±0.02	16.8±0.02
	Mixed tef grain	6.03±0.13	7.67±0.09	10.2±0.03	17.6±0.01
Debre Markos	White tef grain	4.60±0.09	4.92±0.43	7.40±0.24	15.5±0.06
	Brown tef grain	3.95±0.31	4.63±0.02	6.76±0.08	15.3±0.07
	Mixed tef grain	4.71±0.11	5.44±0.03	7.64±0.03	16.4±0.04
Jimma	White tef grain	4.12±0.16	4.33±0.03	5.15±0.08	10.5±0.01
	Brown tef grain	1.89±0.09	2.63±0.06	4.74±0.02	9.17±0.04
	Mixed tef grain	5.53±0.19	5.88±0.05	8.64±0.02	16.7±0.04
	Dark brown (tikur) tef grain	3.06±0.18	3.64±0.04	5.46±0.06	10.3±0.02

Fluoride content of enjera

The fluoride concentration of *enjera* varied depending up on several factors such as: fluoride concentration of ingredients used for cooking, amount of each ingredient used, fluoride concentration of the water used for cooking and type of cooking material. In this study, the *enjera* were prepared using three different water sources. From the results obtained in this study, it was possible to evaluate the influence of the water used for cooking. The different samples of *enjera* showed increasing fluoride concentration with increasing fluoride concentration of the water used for baking.

The fermentation products of tef from Wonji Shoa have higher fluoride content than the other sites. This may be due to presence of fluoridated soil at Wonji Shoa. Dabeka *et al.* (1982) has studied the fluoride concentration of dry cereals. According to this study, fluoride contents 90–200 µg/L and 4,000–6,000 µg/L were found in dry cereals produced with non-fluoridated and fluoridated water, respectively.

Daily intake of fluoride through enjera consumption

Daily intake of fluoride was calculated using three different types of water separately.

i.e., $$X_T = \sum_{j=1}^{3} x_j \qquad \text{.......} (1)$$

where,
X_T is total daily intake (mg/person/day),
x_j is daily intake through *enjera*, and
j_1, j_2, j_3 is prepared *enjera*.

$$x_j = \text{consumption of } j * c_j \qquad \text{.......} (2)$$

where,
consumption of j is the daily consumption of j (kg/person/day),
c_j is concentration of fluoride in *enjera* (mg/kg).

The fluoride intake through *enjera* was calculated to assess the influence of *enjera* on the total intake.

$$\text{Consumption of } j = \frac{\text{amountof } j \text{ consumed (kg)} * \text{Frequency of consumption in a week}}{\text{No. of days in a week}} \qquad \text{.......} (3)$$

where,
j is *enjera*.

The average amount of *enjera* consumed by one adult person was calculated from the results of recipe questionnaires by taking the average of all the ten households in Wonji Shoa. The information of frequency of the *enjera* to be consumed within a week was obtained from the field observation and results of the questionnaires. Mass of one *enjera* was found to be 400 g and the average amount consumed per person as well as average amount consumed in (kg/person/day) was calculated using equations 2 and 3 and the results are given in Table 4.

Malde *et al.* (2004) studied total daily fluoride intake of children living in two villages A and K (with fluoride concentration in the water about 2 mg/L and 14 mg/L, respectively), in Wonji Shoa

Table 4. Fluoride intake of an adult person by consuming *enjera*.

Items	Average amount consumed per person (kg)	Frequency of consumption in a week	Average amount consumed (kg/person/day)	Average concentration of F^- (mg/kg)	F^- intake (mg/person/day)
-*Enjera* made from white tef grain using tap water	0.8	5	0.57	8.44	4.8
-*Enjera* made from white tef grain using 5 mg/L F^- containing water	0.8	5	0.57	11.58	6.6
-*Enjera* made from white tef grain using 10 mg/L F^- containing water	0.8	5	0.57	25.62	14.6

Sugar Estate, located in the Ethiopian Rift Valley. According to this study, the daily fluoride intake of children in village A, was derived 63% from food while children in high-fluoride village K, got most of their fluoride through drinking water (60%). Anasuya *et al.* (1996) studied range of intakes based on consumed foodstuffs and local supplies of drinking water (normal and fluorotic) by adults living in rural areas of India. The study found a daily fluoride intake of 0.84-4.69 mg/day in normal water and 3.40-27.1 mg/day in fluorotic water areas. The contribution of fluoride in water used for preparation of *enjera* showed an increasing pattern with the increasing fluoride concentration in the water.

Contribution of fluoride in water to the total daily fluoride intake

A **c**onsiderable amount of fluoride from water used for the preparation of *enjera* is, as shown in the present study, retained in the *enjera*. This is also clearly demonstrated in the daily fluoride consumption in the three different types of water. Baking *enjera* using water with fluoride concentration of 0.147, 5 and 10 mg/L, gives a daily fluoride intake of 4.8, 6.6 and 14.6 mg/day, respectively. The *enjera* baked using tap water gives a daily fluoride intake of 4.8 mg/day. This shows that the contribution of tap water to the daily fluoride intake is higher than the other two types of water as compared to their fluoride concentration. This may be attributed to the presence of interferences ions in the tap water. However, the other two were prepared in the laboratory with de-ionized water.

Comparison of fluoride content in tef grain with other cereals

Determination of fluoride concentration in food has received considerable attention due to its effect on health. Many researchers have reported the concentration of fluoride in different cereals and infant foods. The fluoride content in tef grain (1.9 mg/kg) found in this study is comparable to barley (2.0 mg/kg), rice (2.1 mg/kg) and oat meal (1.0 mg/kg) cultivated in non-fluoridated areas in other countries while fluoride content in tef grain (8.7 mg/kg) cultivated in the Rift Valley is higher than in the cereals cultivated in fluoridated areas in other countries (Singer and Ophaug, 1979). Shredded wheat (9.6 mg/kg) (PFPC, 2001) contain much higher amounts of fluoride than tef grain (8.7 mg/kg) cultivated in non-fluoridated area but are comparable to that cultivated in the Rift Valley. The fluoride content in tef grain (8.7 mg/kg) found in this study is much lower than the fluoride content reported in brown tef grain powder (15.1 mg/kg) and corn powder (12.2 mg/kg) (Meseret Dessalegne and Feleke Zewge, 2013), lower than wheat (3.24 -14.3) (Yadav *et al.*, 2012) but comparable with that in wheat (7.0 mg/kg) and corn (5.1 mg/kg) (Gautam *et al.*, 2010) from different origins. The fluoride content of tef grains found in the present study is comparable to the values reported in barley (0.45-3.65 mg/kg) (Bhargava and Bhardwaj, 2009), wheat (4.6 mg/kg) and corn (5.9 mg/kg) (Sunitha and Reddy, 2008) from India. The level of fluoride in tef grain found in this study (1.9-8.7 mg/kg) is higher than the levels of fluoride in Basmati rice (5.2 mg/kg), Jasmine rice (2.2 mg/kg), Royal rice (0.43 mg/kg), Ethiopian white rice (0.1 mg/kg, Ethiopian red rice 4.4 mg/kg, and NERICA (2.5 mg/kg) (Bisratewongel Tegegne *et al.*, 2013).

Fluoride intake by humans and safety evaluation of food (enjera)

Fluoride is a recognized substance used world-wide to control dental caries. However, ingestion of high levels of fluoride during tooth formation and mineralization is responsible for dental fluorosis. Prolonged ingestion of high levels of fluoride, leads to corroded, pitted rusty brown teeth. The upper limit for clinically acceptable dental fluorosis is not well known, but the value of 0.05-0.07 mg F/kg/day is generally accepted as a reference (Lu *et al.*, 2004).

Surplus of fluorides in organisms can provoke teeth and skeleton fluorosis. Fluorides inhibit many enzymes. Affected enzymes contain metal ion which unites with fluoride and creates metal-fluoride complex. Fluoride in organisms has its optimal, security-tolerant and toxic dose, which depends on person’s age, weight and health. On the first year of life the optimal content of fluoride is 0.045 mg/kg of body mass, tolerant at 0.073 mg/kg, and chronically toxic at 0.150 mg/kg. Optimal dosage of fluoride for adults is 0.020-0.025 mg/kg of body mass (Crosby *et al.*, 1968).

As taking of fluorides, according to World Health Organization recommendation (WHO), is limited in the range from 2 to 4 mg per day, it is necessary to know the content of fluoride in all products that are used in human consumption (Crosby *et al*., 1968).

The Food and Nutrition Board of the Institute of Medicine (USA) has developed adequate intakes (AIs) for fluoride. The AI is the "estimated fluoride intake that has been shown to reduce the occurrence of dental caries maximally in a population without causing unwanted side effects, including moderate dental fluorosis." The AIs for each age group are presented in Table 5 (Malde *et al*., 2006).

As shown in this study, the fluoride content of *enjera* ranges from 2.63–26.82 mg/kg (Table 3) and the daily dietary intake of fluoride in Ethiopia ranges from 4.8–14.6 mg/kg/day (Table 4). The fluoride contents of the different tef varieties vary depending on the soil type, soil pH and the type of minerals in the soil. If the soil contains high amount of minerals such as Al, Mg and Fe, the bioavailability of fluoride in the plant increases. Therefore, we expect that the Ethiopian Rift Valley areas have optimum conditions for the bioavailability of fluoride as compared with the highlands. From the calculation of daily dietary intake of fluoride, the value of fluoride in *enjera* baked using tap water is safe for consumption while *enjera* baked using 5 and 10 mg/L fluoride containing water may cause dental as well as skeletal fluorosis as this value is above the recommended value, therefore unsafe for human consumption.

Table 5. Adequate/recommended daily intake levels for fluoride (Malde *et al*., 2006).

Age range	Adequate intake level (mg/day)	Adequate intake level (mg/kg/day)
0 – 6 months	0.01	0.0014
7 – 12 months	0.5	0.056
1 – 3 years	0.7	0.054
4 – 8 years	1	0.045
9 – 13 years (females or males)	2	0.05
14 – 18 years (males)	3	0.046
14 -18 years (females)	3	0.053
>18 years (males)	4	0.052
>18 years (Females)	3	0.049

Comparison of fluoride content in different tef varieties

From the sample sites in this study, the tef from Wonji Shoa has higher fluoride content, as expected, because the area is found within the Ethiopian Rift Valley. The least fluoride content was found in Jimma, located on the highlands of Ethiopia. Among the tef varieties, the mixed tef grain has high fluoride content, which is a combined effect of both the white and the brown tef grains in both sample sites. The brown tef grain has the least while the white tef grain is intermediate between the two. This difference in accumulation of fluoride may result in the nature the tef plant to take up nutrients from the soil. The order from higher to lower in the fluoride levels of the different tef varieties is: mixed tef grain from Wonji Shoa < white tef grain from Wonji Shoa < brown tef grain from Wonji Shoa < mixed tef grain from Arsi Negelle < white tef grain from Arsi Negelle < mixed tef grain from Jimma < brown tef grain from Arsi Negelle < mixed tef grain from Debre Markos < white tef grain from Debre Markos < white tef grain from Jimma < brown tef grain from Debre Markos < dark brown tef grain from Jimma < brown tef grain from Jimma. The order from higher to lower in the fluoride levels of *enjera* baked using tap water is: mixed tef grain from Wonji Shoa < white tef grain from Wonji Shoa < brown tef grain from Wonji Shoa < mixed tef grain from Arsi Negelle < white tef grain from Arsi Negelle < brown tef grain from Arsi Negelle < mixed tef grain from Jimma < mixed tef grain from Debre Markos < white tef grain from Debre Markos < brown tef grain from Debre Markos < white tef grain from Jimma < dark brown tef grain from Jimma < brown tef grain from Jimma. The same trend exists in the case of *enjera* in all sample sites baked by using 5 and 10 mg/L fluoride containing water. *Sergegna* (mixed) tef grain contains more minerals as compared to the white and brown tef grains. The presence of high content of minerals may contribute to the higher fluoride content of mixed tef grain.

CONCLUSION

The fluoride concentration of tef and *enjera* using different water sources for baking *enjera* were assessed in this study. The sample preparation and efficiency of the instrument were tested by assessing relative standard deviation and conducting recovery tests. The fluoride concentration in tef ranged from 1.889 mg/kg (Jimma) to 8.738 mg/L (Wonji Shoa). The fluoride concentration of *enjera* ranged from 2.63 mg/kg (tap water in brown tef grain from Jimma) to 26.82 mg/kg (using 10 mg/L F^- containing water in mixed tef grain from Wonji Shoa).

The fluoride content of *enjera* ranges from 2.63-26.82 mg/kg and the human daily dietary intake of fluoride ranges from 4.8-14.6 mg/kg/day. The fluoride contents of the different tef varieties vary depending on the soil type, soil pH and the type of minerals in the soil. If the soil contains high amounts of minerals such as aluminium, magnesium and iron, the bioavailability of fluoride in the plant increases. Therefore, we expect that the Rift Valley areas have optimum conditions for the bioavailability of fluoride as compared with the highlands. From the calculation of daily dietary intake of fluoride, the value of fluoride in *enjera* baked using tap water is safe for human consumption while *enjera* baked using 5 and 10 mg/L fluoride containing water may cause dental as well as skeletal fluorosis as this value is above the recommended amount, therefore, unsafe for human consumption.

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REFERENCES

1. Abraham Besrat, Atnafseged Admasu and Mebrahtu Ogbai (1980). Critical study of the iron content of tef (*Eragrostis tef*). *Ethiop. Med. J.* **8**:45–52.
2. Admassu Mamo (2008). Tef, Amazing Grain! Lissan Magazine, Powered by Word Press. Available at: http://lissanonline.com/ blog Accessed on March 22, 2012.
3. Anasuya, A., Bapurao, S. and Paranjape, P. (1996). Fluoride and silicon intake in normal and endemic fluorotic areas. *J. Trace Elem. Med. Biol.* **10**:149–155.
4. Asamene Embiale, Chandravanshi, B.S. and Feleke Zewge (2014). Levels of fluoride in the Ethiopian and imported black tea (*Camellia sinensis*) infusions prepared in tap and fluoride-rich natural waters. *Int. J. Food Eng.* **10:**447–455.
5. Ashenafi Mengstu, Abule Ebro, Tadesse Assefa, Adane Hirpa and Belete Shenkute (2007). Effect of supplementation of tef (*Eragrostis tef*) straw with different levels of noug (*Guizotia abyssinica*) meal on worked Arsi oxen (*Bos indicus*). *Trop. Sci.* **47:**49–51.
6. Battaleb-Looie, S., Moore, F., Malde, M.K. and Jacks, G. (2013). Fluoride in groundwater, dates and wheat: Estimated exposure dose in the population of Bushehr, Iran. *J. Food Comp. Anal.* **29**:94–99.
7. Bhargava, D. and Bhardwaj, N. (2009). Study of fluoride contribution through water and food to human population in fluorosis endemic villages of North-Eastern Rajasthan. *Afr. J. Basic Appl. Sci.* **1**:55–58.
8. Bisratewongel Tegegne, Chandravanshi, B.S. and Feleke Zewge (2013). Fluoride levels in commercially available rice in Ethiopia. *Bull. Chem. Soc. Ethiop.* **27**:179–189.
9. Chandrajith, R., Abeypala, U. and Dissanayake, B.C. (2007). Fluoride in Ceylon tea and its implications to dental health. *J. Environ. Health* **29**:429–434.
10. Crosby, N.T., Dennis, A.L. and Stevens, J.G. (1968). An evaluation of some methods for the determination of fluoride in potable waters and other aqueous solutions. *Analyst* **93**:643–652.
11. Dabeka, R.W., McKenzie, A.D., Conacher, H.B.S. and Kirkpatrick, D.C. (1982). Determination of fluoride in Canadian infant foods and calculation of fluoride intake by infants. *Can. J. Pub. Health* **73**:188–191.
12. Dawit Tadesse (1993). Study on genetic variation of landraces of tef *(Eragrostis tef* (Zucc.) Trotter) in Ethiopia. *Gen. Resour. Crop Evol.* **40**:101–104.
13. Deckers, J., Nachtergaele, F. and Spaargaren, O. (1998). World Reference Base for Soil Resource: Belgium; Agricultural Cooperative Council of Oregon, Leuven/Amersfoort.
14. ENI (1980). *Ethiopian Traditional Recipes*. ENI (Ethiopian Nutrition Institute), Addis Ababa, Ethiopia, 41 pp.
15. Gamboa, P.A. and van Ekris, L. (2008). Tef "Survey on the nutritional and health aspects of tef (*Eragrostis tef*)". Memorias, Red-alfa Lagrotech, Comunidad Europea, Cartagena, pp. 319–367.
16. Gautam, R., Bhardwaj, N. and Saini, Y. (2010). Fluoride accumulation by vegetables and crops grown in Nawa Tehsil of Nagaur District (Rajasthan, India). *J. Phytol.* **2**:80–85.

17. Geremew Bultosa (2007). Physicochemical characteristics of grain and flour in 13 tef [*Eragrostis tef* (Zucc.) Trotter] grain varieties. *J. Appl. Sci. Res.* **3(12)**:2042–2051.

18. Geremew Bultosa, Hall, A.N. and Taylor, J.R.N. (2002). Physico-chemical characterization of grain tef [*Eragrostis tef* (Zucc.) Trotter] starch. *Starch/Stärke* **54**:461–468.

19. Jansen, G.R., DiMaio, L.R. and Hause, N.L. (1962). Amino acid composition and lysine supplementation of tef. *J. Agric. Food Chem.* **10**:62-64.

20. Liub, Q., Wanga, W.;,Lin-sheng Yanga, L., Fenga, F. and Zhaoa, X. (2009). Fluoride in drinking water, brick tea infusion and human urine in two counties in Inner Mongolia, China. *J. Hazard. Mater.* **167**:892–895.

21. Lu, Y., Guo, W.F. and Yang, X.Q. (2004). Fluoride content in tea and its relationship with tea quality. *J. Agric. Food Chem.* **52**:4472–4476.

22. Malde, M.K., Greiner-Simonsen, R., Julshamn, K. and Bjorvatn, K. (2006). Tealeaves may release or absorb fluoride, depending on the fluoride content of water. *Sci. Total Environ.* **366**:915–917.

23. Malde, M.K., Måge, A., Julshamn, K., Macha, E. and Bjorvatn, K. (1997). Fluoride content in selected food items from five areas in East-Africa. *J. Food Comp. Anal.* **10**:233–245.

24. Malde, M.K., Scheidegger, R., Julshamn, K. and Bader, H.-P. (2011). Substance flow analysis – a case study for fluoride exposure through food and beverages in young children living in Ethiopia. *Environ. Health Perspect.* **119**:579–584.

25. Malde, M.K., Legesse Zerihun, Julshamn, K. and Bjorvatn, K. (2004). Fluoride, calcium and magnesium intake in children living in a high-fluoride area in Ethiopia. Intake through food. *Int. J. Paed. Dent.* **14**:167–174.

26. Mcquaker, N.R. and Gurney, M. (1977). Determination of total fluoride in soil and vegetation using an alkali fusion–selective ion electrode technique. *Anal. Chem.* **49**:53–56.

27. Meseret Dessalegne and Feleke Zewge (2013). Daily dietary fluoride intake in rural villages of the Ethiopian Rift Valley. *Toxicol. Environ. Chem.* **95**:1056–1068.

28. Mesfin Haile, Agajie Tesfaye, Lemlem Aregu and Eyob Mulat (2004). Market access versus productivity: The case of tef. Paper presented at the Ethiopian Economic Association, Conference on Ethiopian Economy, held in Addis Ababa, June 3–5, 2004.

29. PFPC (2001). Fluoride in cereals. Parents of Fluoride Poisoned Children (PFPC). Available at http://www.bruha.com/pfpc/ Accessed on March 28, 2012.

30. Samuel Zerabruk, Chandravanshi, B.S. and Feleke Zewge (2010). Fluoride in black and green tea (*Camellia sinensis*) infusions in Ethiopia: measurement and safety evaluation. *Bull. Chem. Soc. Ethiop.* **24**:327–338.

31. Seyfu Ketema (1997). Tef [*Eragrostis tef* (Zucc.) Trotter]. *Promoting the Conservation and Use of Underutilized and Neglected Crops* 12. Institute of Plant Genetics and Crop Plant Research, Gatersleben/International Plant Genetic Resources Institute, Rome, Italy, 52 pp.

32. Simposom, A., Shaw, L. and Smith, A.J. (2001). The bioavailability of fluoride from black tea. *J. Dent.* **29**:15–21.

33. Singer, L. and Ophaug, R. (1979). Total fluoride intake of infants. *Pediatrics* **63**:460.

34. Sunitha, V. and Reddy, M.R. (2008). Fluoride in ground water and fluorosis. *e-J. Earth Sci. India* Popular Issue, October, **2008**. Available at: http://www.earthscienceindia.info/pop_-archiv.php . Accessed on February 22, 2012.

35. WHO (2004). Fluoride in drinking water. Background document for development of WHO Guidelines for Drinking-water Quality World Health Organization, Geneva, 9 pp.

36. Yadav, R.K., Sharma, S., Bansal, M., Singh, A., Panday, V. and Maheshwari, R. (2012). Effects of fluoride accumulation on growth of vegetables and crops in Dausa District, Rajasthan, India. *Adv. Biores.* **3**:14–16.

37. Zedwu Abdi (2007). Aerodynamic properties of tef grain and straw material. *Biosys. Engin.* **98**:304–309.

38. Zohouri, F.V. and Rugg-Gunn, A.J. (1999). Fluoride concentration in foods from Iran. *Int. J. Food Sci. Nutr.* **50**:265–274.

39. Zohouri, F.V. and Rugg-Gunn, A.J. (2000). Sources of dietary fluoride intake in 4-year-old children residing in low, medium and high fluoride areas in Iran. *Int. J. Food Sci. Nutr.* **51**:317–326.