LEVELS OF THE FLUORIDE ION IN SIX TRADITIONAL ALCOHOLIC FERMENTED BEVERAGES COMMONLY CONSUMED IN ETHIOPIA

Yohannes Belete,[a] Bhagwan Singh Chandravanshi,[a,*] Feleke Zewge[a]

Addis Ababa, Ethiopia

ABSTRACT: The main objective of this study was to determine the levels of the fluoride ion (F) in six traditional fermented alcoholic beverages (tella, tej, areki, shamita, borde, and korefe) which are widely consumed in selected areas of Ethiopia. The samples of the beverages and the raw water used for their preparation were collected from towns both in (Alem Tena, Meki, Adamitulu, and Deguna Fanigo) and outside (Debre-Birhan, Debre-Markos, and Wogera) the Rift Valley of Ethiopia. The beverage samples were collected from local markets and at the household level in the areas where they are prepared and consumed in large amounts. The levels of F in the beverages and water samples were determined by a fluoride ion selective electrode. The mean F concentration (mg/L) ranges, or values when only one town was tested, in the beverages and the corresponding raw water samples were, respectively: tella (0.32–8.19, 0.30–5.06), tej (0.35–5.76, 0.31–5.06), areki (0.52–0.97, 2.09–9.02), shamita (5.21, 0.78), borde (4.95, 0.92) and korefe (1.39, 0.35). Testing with the Pearson correlation coefficient showed that the levels of F in the traditional fermented alcoholic beverages correlated positively with the levels found in the raw water used for their preparation. The mean daily dietary F intake from the beverages varied in line with the different F concentrations of the water used for their preparation. The range of the estimated daily adult intake of F from beverages was 0.004–3.28 mg/day with the corresponding F concentrations in the raw water used for their preparation being 2.09 mg/L (for areki prepared in Adamitulu) and 5.05 mg/L (for tella prepared in Alem Tena).

Keywords: Alcoholic beverages; Fermented beverages; Food; Fluoride; Ethiopia.

INTRODUCTION

Fluorine is one of several elements receiving much attention owing to its possible harmful effects on health and the environment.[1] The fluoride ion (F) is not considered to be essential for human growth and development,[2] including for the development of healthy teeth and bones. F is a potentially toxic element and, at higher levels, is able to cause adverse health effects such as dental and skeletal fluorosis.[1-4] There is suggestive evidence of an increased risk of effects on the skeleton at total F intakes above about 6 mg/day.[3] Total exposure to F depends on contributions from different sources. In general, drinking water, beverages, food, dentifrices and other dental agents are regarded as the main dietary contributors to human F intake.[3-8] Several studies have been carried out on the dietary intake of F in Ethiopia.[9-19] The F intake in Ethiopia from chewing the leaves of khat, a psychoactive substance, has also been reported in the literature.[20]

World-wide, more than 200 million people rely on drinking water with F concentrations exceeding the present World Health Organization (WHO) "desirable" upper limit guideline of 1.5 mg/L.[21] The long-term use of drinking

[a]Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia; *For correspondence: Professor Chandravanshi, Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia; E-mail: bscv2006@yahoo.com

water with F significantly above 1.5 mg/L can have serious effects on health.[1,2] In 2006, the WHO registered 28 countries where dental fluorosis and skeletal fluorosis were associated with exposure to a high F concentration in drinking water. Among these, the most affected were India, Ethiopia, and China.[1,2]

In the Ethiopian Rift Valley area an estimated 8 million people are exposed to high levels of naturally occurring fluoride.[22] The daily intake of F (primarily through drinking water and beverages but also via food) puts a large part of the rural population in the Ethiopian Rift Valley at risk of fluorosis, including dental and skeletal fluorosis. Water is epidemiologically the most important source of F (75–90% of daily intake) in most areas of the Rift Valley of Ethiopia. Fluorosis not only affects people's health but also has serious economic and social consequences.[23,24]

In the world there are a large variety of fermented foods and beverages with traditional and cultural values. The diversity of such fermented products derives from the heterogeneity of traditions found in the world, cultural preference, the different geographical areas where they are produced, and the staple foods and/or by-products used for fermentation. In many instances it is highly likely that the methods of production were unknown, came about by chance, and were then passed down as cultural and traditional values to subsequent generations.[25,26]

Alcoholic beverages are a part of human dietary culture and have an inseparable relationship with the life of mankind in history. The making and drinking of alcoholic beverages has nutritional significance as well as enhancing social relationships. Ethiopia is a country rich in cultural diversity. The variety of foods and beverages processed and consumed among the various ethnic groups are manifestations of this diversity. Ethiopia is one of the countries where a wide variety of traditional fermented alcoholic beverages are prepared and consumed. The various traditional fermented beverages are produced on a fairly small scale and are usually for local consumption. Among the Ethiopian traditional fermented alcoholic beverages are varieties of tella, tej, areki, shamita, borde, and korefe drinks that each household may brew to treat guests.[27,28]

Tella is a Ethiopian traditional beverage which is prepared from different ingredients. It is, by far, the most commonly consumed alcoholic beverage in Ethiopia. Tella is widely brewed and consumed in both rural and urban parts of Ethiopia. It is brewed from different cereals, including barley, corn, wheat, and sorghum, and also from teff, maize, and millet. *Rhamnus prinioides* is added as a flavoring agent.[29] With regard to the substrate, there is no basic difference between tella and beer. The alcoholic level of tella is between 3.5 to 6.48% (v/v).[30-33]

Tej (indigenous honey wine) is usually a home-processed honey wine but is also available commercially. It is prepared from honey, water, and the leaves of *Rhamnus prinioides*. Sometimes, often for commercial purposes, a mixture of honey and sugar is used for its preparation. In cases where sugar is used as part of the substrate, natural food coloring is added so that the beverage attains a yellow color similar to that made from honey. Good quality tej is yellow, sweet, effervescent, and, due to the content of yeasts, cloudy. The alcohol content of tej is between 6.98% and 10.9% (v/v).[31,34,35]

Araki is a distilled beverage. Ground *Rhamnus prinioides* leaves and water are kept for three to four days and after that a kita made of teff or other cereals and germinated barley or wheat is added. (A kita is a thin, 5–10 mm thick, pancake-like bread prepared by mixing teff, wheat, maize, sorghum, or barley flour with water and baking on a hot plate). The mixture is allowed to ferment for five to six days and then distilled. In the villages distillation is carried out with primitive equipment made of gourds and wood. The local beer tella can also be distilled to produce araki. The araki can be redistilled and it will then have higher alcohol content. The average alcohol content of dagim araki is around 45%. The term dagim in Amharic refers to “second time” and indicates that it was distilled a second time. Araki is brewed in rural and semi-urban areas and is used more commonly by farmers and semi-urban dwellers than by people who live in the cities. In cities, those who drink araki are predominantly lower class people or those who have become dependent upon alcohol and cannot afford to buy industrially produced alcohol. Since the government has no control over production of locally brewed alcoholic drinks, it is difficult to estimate the amount of alcohol production and consumption in Ethiopia.[31,35]

Shamita is another traditional beverage of Ethiopia, which is low in alcohol content and made by overnight fermentation of mainly roasted barley flour with ground linseed, salt, and spices being added for flavor.[36] It is a widely consumed beverage in different regions of Ethiopia. It has a thick consistency and most people who cannot afford a reasonable meal consume it as meal replacement.

Borde is made from maize or wheat. A thick coarse paste of wheat or maize flour is roasted on a hot flat metal pan and cooled for one hour. Then it is thoroughly mixed with ground malt. The whole mixture is put into a jar and further blended with water and allowed to ferment at ambient temperature for 24 hr.[37]

Korefe is the name of the local beer made in Begemder Province among the Koumant ethnic group. Dehusked barley is left in water overnight, and then toasted and milled. After mixing with water and dried *Rhamnus prinioides* leaves, it is fermented in a clay container for two to three months. When the beverage is needed, a small quantity of the mixture is taken, more water is added, and after a further day's fermentation the beverage is ready for consumption.[31,38]

Food sources contain various concentrations of F and are the largest contributor to F exposure. Beverages contribute most of the F intake by the people who regularly drink. However, no systematic study has been conducted to determine the levels of F in the traditional fermented alcoholic beverages which are widely consumed in Ethiopia. The objective of this study was to determine the F levels in six traditional fermented alcoholic beverages (tella, tej, areki, shamita, borde, and korefe) which are widely consumed in selected areas of Ethiopia, including areas in the Rift Valley and in non-Rift Valley regions.

MATERIALS AND METHODS

Sample collection: For this study, six types of traditional fermented alcoholic beverages (tella, tej, areki, shamita, borde and korefe) from selected areas of Ethiopia were collected. The samples were collected from localities within the Ethiopian Rift Valley (Adamitulu, Meki, Alem Tena, and Deguna Fanigo) and from areas located outside the Rift Valley (Debre-Birhan, Debre-Markos, and Wogera). All the samples were collected based on universality of consumption, from adolescents to the elderly, and were widely available at both the local market and the household levels. Tella and tej were collected from Adamitulu, Meki, Alem Tena, Debre-Birhan, and Debre-Markos, and areki from Adamitulu, Meki, and Alem Tena. Shamita and borde are familiar drinks in the southern region, and five representative samples were purchased from Deguna Fanigo. Korefe is a familiar beverage in the northern region, and five representative samples were purchased from Wogera. From each sample area, five representative samples, each of 0.5 L, were collected from different vending houses which were selected randomly to prepare a bulk sample by mixing, instead of analyzing separately, because analyzing the mixture reduces the variance and resource consumption. Triplicate analysis was performed for each. Water samples were also collected from each sample area and triplicate analysis performed for each sample. All the samples were collected using polyethylene plastic bottles and the bulk samples were kept in refrigerator at 4ºC until analysis.

Instrumentation: A pH/ISE meter (Orion model, EA 940 Expandable Ion Analyzer, USA) equipped with a combination fluoride selective electrode (Orion Model 96-09, USA) was employed for the determination of F in the samples and standard solutions. The pH was measured with a pH/ION meter (HANNA instruments HI 9025, Malaysia) using a pH glass electrode. A muffle furnace (Audiotronics, Wagtech International Ltd., UK) was used to ash the samples in nickel crucibles.

Chemicals and reagents: The reagents that were used in the analysis were all of analytical grade. Fluoride stock solution was prepared from anhydrous sodium fluoride (99.0% NaF, BDH Chemicals Ltd, England) and standard solutions of the required concentration were then prepared by appropriate dilution from fluoride stock solution with de-ionized water. Glacial acetic acid (100%, Sigma-Aldrich Laborchemikalien, Germany), sodium chloride (Oxford Laboratory, Mumbai, India), trisodium citrate (BDH Chemicals, England), ethylene di-amine tetra acetic acid (EDTA) (Scharlau Chemie S.A., Barcelona, Spain), and sodium hydroxide (Scharlau Chemie S.A., Sentmenat, Spain) were used to prepare the total ionic strength adjustment buffer (TISAB). The TISAB II was prepared by dissolving 58.5 g sodium chloride, 57.0 mL glacial acetic acid, 7 g of trisodium citrate, and 2 g EDTA in 500 mL distilled water. The pH of this solution was adjusted to 5.0–5.5 with 5 M sodium hydroxide and finally diluted to 1000 mL in a volumetric flask with deionized water. The reagents also included concentrated hydrochloric acid (36%, Fisher Scientific UK Limited) for neutralization after alkali fusion and dissolution of semi-solid traditional fermented alcoholic samples.

F determination in the traditional fermented alcoholic beverage samples: The F concentration in the clear liquid traditional fermented alcoholic beverages samples and the water samples were determined directly by adding TISAB and taking the reading from calibrated fluoride ion selective electrode. The F concentration in the semi-solid traditional fermented alcoholic beverages samples was determined using a fluoride ion selective electrode by slightly modifying the reported method.[9] The semi-solid samples were filtered and the F concentration in the filtrate was determined directly while the F concentration in the residue was determined after alkali fusion.[9] The F content in the residue of the semi-solid traditional fermented alcoholic beverages samples was then determined based on the calibration curve plotted using standard solutions. All the analyses were made in triplicate.

The calibration standard solutions of concentrations 0.3, 1, 5, 10, and 20 mg F/L were prepared by serial dilution of the 1000 mg/L fluoride stock solution. An aliquot of 5 mL of each standard solution was poured into a 50 mL plastic beaker separately and 5 mL of TISAB II solution was mixed with it to calibrate the fluoride ion selective ion electrode. The calibration curve of five series points was constructed as electrode potentials versus log of standard F concentrations. The slope of the calibration curve was –57.2 mV per decade of concentration change which showed a good precision of measurement.

Validation of the procedure: In this study, the procedure was validated by determining the F concentration of a sample prepared by alkali fusion for the semi-solid traditional fermented alcoholic beverages and spiked with known amounts of F. The spiked samples were prepared by adding a known concentration of the standard F solution to 0.5 g of the residue of a semi-solid traditional fermented alcoholic beverage and 5 mL of the filtrate separately. Validation of the method used was checked by performing recovery tests. The spiked samples were prepared by adding a known quantity of F standard solution to the clear liquid traditional fermented alcoholic beverage samples by applying a similar procedure to prepare the sample and analyzed for the levels of F to calculate the recovery percentage. Each traditional fermented alcoholic beverage sample was spiked with a F solution of which the F content was equivalent to 25%, 50%, or 100% of the F content of the original (unspiked) samples. Thus, the percent recovery was obtained by comparing the results between the F found and the F added.[39] The recovery tests were also performed for the water samples in triplicate.

Statistical analysis: The analysis of variance for the presence of a significant difference at the 95% confidence level ($p<0.05$) between the mean F levels in the traditional fermented alcoholic beverage samples and the Pearson correlation coefficient between the F level in the traditional fermented alcoholic beverages and the F levels in raw water samples were done using the SPSS 20.0.[40]

RESULTS AND DISCUSSION

Recovery results of F determination: The recovery test was done for all the samples since all the samples had different matrices and the percentage recoveries were calculated by spiking known amounts of F into the traditional fermented alcoholic beverages samples (Tables 1–5).

Table 1. Recovery test results of the fluoride ion (F) determination in samples of the traditional fermented alcoholic beverages of tella, areki, and tej

Sample site	Type of beverage	Concentration of F in unspiked beverage sample (mg/L)	Amount of F added (mg/L)	Concentration of F in spiked beverage sample (mg/L)	Recovery (%)
Alem Tena	Tella	8.19	2.05	10.2±0.10	98±5
		8.19	4.10	12.6±0.15	107±4
		8.19	8.20	17.1±0.25	108±3
Meki	Areki	0.67	0.17	0.9±0.01	107±3
		0.67	0.34	1.0±0.01	103±3
		0.67	0.67	1.4±0.04	103±5
Adamitulu	Tej	2.46	0.60	3.0±0.05	97±8
		2.46	1.23	3.8±0.02	105±1
		2.46	2.46	5.1±0.03	109±1

Table 2. Recovery test results of the fluoride ion (F) determination in the filtrates of samples of the traditional fermented alcoholic beverages of shamita, borde, and korefe

Sample site	Type of beverage	Concentration of F in unspiked beverage filtrate sample (mg/L)	Amount of F added (mg/L)	Concentration of F in spiked beverage filtrate sample (mg/L)	Recovery (%)
Deguna Fanigo	Shamita	1.13	0.28	1.4±0.05	95±7
		1.13	0.57	1.7±0.05	95±9
		1.13	1.13	2.2±0.02	92±1
Deguna Fanigo	Borde	1.24	0.31	1.6±0.03	103±9
		1.24	0.62	1.9±0.06	104±9
		1.24	1.24	2.6±0.02	109±1
Wogera	Korefe	0.41	0.10	0.5±0.01	93±6
		0.41	0.20	0.6±0.02	99±10
		0.41	0.41	0.8±0.04	105±9

Table 3. Recovery test results of the fluoride ion (F) determination in the residues of the semi-solid parts of samples of the traditional fermented alcoholic beverages of shamita, borde, and korefe

Sample site	Type of beverage	Concentration of F in unspiked beverage semi-solid residue sample (mg/L)	Amount of F added (mg/L)	Concentration of F in spiked beverage semi-solid residue sample (mg/L)	Recovery (%)
Deguna Fanigo	Shamita	7.54	1.89	9.33±0.15	95±8
		7.54	3.77	11.4±0.10	102±3
		7.54	7.54	14.7±0.31	95±4
Deguna Fanigo	Borde	6.48	1.62	8.07±0.15	98±9
		6.48	3.24	9.50±0.20	93±6
		6.48	6.48	12.4±0.12	92±2
Wogera	Korefe	3.80	0.95	4.67±0.06	91±6
		3.80	1.90	5.63±0.15	96±8
		3.80	3.80	7.33±0.15	93±4

Table 4. Recovery test results of the fluoride ion (F) determination in the raw water used for preparing samples of the traditional fermented alcoholic beverages of tella, areki, and tej

Sample site	Type of beverage prepared with the raw water	Concentration of F in unspiked raw water sample (mg/L)	Amount of F added (mg/L)	Concentration of F in spiked raw water sample (mg/L)	Recovery (%)
Alem Tena	Tella	5.06	1.25	6.42±0.25	109±2
		5.06	2.50	7.85±0.05	108±1
		5.06	5.06	10.43±0.25	106±5
Meki	Areki	4.07	1.02	5.17±0.02	108±1
		4.07	2.03	6.32±0.04	108±2
		4.07	4.07	8.40±0.06	106±1
Adamitulu	Tej	1.78	0.45	2.19±0.04	92±8
		1.78	0.89	2.76±0.05	104±3
		1.78	1.78	3.42±0.04	92±2

Table 5. Recovery test results of the fluoride ion (F) determination in the raw water used for preparing samples of the traditional fermented alcoholic beverages of shamita, borde, and korefe

Sample site	Type of beverage prepared with the raw water	Concentration of F in unspiked raw water sample (mg/L)	Amount of F added (mg/L)	Concentration of F in spiked raw water sample (mg/L)	Recovery (%)
Deguna Fanigo	Shamita	0.78	0.20	4.67±0.06	93±3
		0.78	0.40	5.63±0.15	98±6
		0.78	0.78	7.33±0.15	90±3
Deguna Fanigo	Borde	0.92	0.23	1.14±0.02	97±7
		0.92	0.46	1.40±0.03	105±7
		0.92	0.92	1.94±0.05	108±2
Wogeria	Korefe	0.35	0.09	0.44±0.01	96±7
		0.35	0.18	0.54±0.01	105±4
		0.35	0.35	0.71±0.01	104±2

The percentage recovery of method was evaluated by calculating the percentage recovery (% R). Recoveries for the traditional fermented alcoholic beverages and the water samples were found to be in the range 91–109% and 90–109% (Tables 1–5). These recoveries were within the acceptable range (100±10%).

Levels of F in traditional fermented alcoholic beverages: The F levels found in the traditional fermented alcoholic beverage samples of this study are presented in Tables 6 and 7 and that of the water samples are presented in Table 8. For the traditional fermented alcoholic beverages, the lowest (0.32 mg/L) and highest (8.19 mg/L) F contents were found in tella samples from Debre-Markos and from Alem Tena, respectively. For the raw water samples used for preparing the beverages, the lowest (0.30 mg/L) and highest (9.02 mg/L) F contents were found in tella water samples from Debre-Markos and areki water samples from Alem Tena, respectively. The traditional fermented alcoholic beverage samples from towns located within the Rift Valley, (Alem Tena, Meki, Adamitulu, and Deguna Fanigo), had higher F contents than the samples from the non-Rift Valley towns (Debre-Markos, Debre-Birhan and Wogera), which shows the contribution of the raw water F to the F content of the beverages. Therefore, F from water and soil contributes to the F content of traditional fermented alcoholic beverages.

Table 6. Average levels of the fluoride ion (F) (mean±SD, mg/L, n=3) in samples of the traditional fermented alcoholic beverages of tella, tej, and areki from three sample sites in the Rift Valley (Alem Tena, Meki, and Adamitulu) and two sample sites which were not in the Rift Valley (Debre-Birhan and Debre-Markos)

Type of beverage	Sample site		Fluoride level (mean±SD, mg/L)
	Rift Valley sites	Non-Rift Valley sites	
Tella	Alem Tena		8.19±0.41
	Meki		3.97±0.11
	Adamitulu		2.02±0.05
		Debre-Birhan	0.33±0.02
		Debre-Markos	0.32±0.01
Tej	Alem Tena		5.76±0.09
	Meki		3.84±0.10
	Adamitulu		2.46±0.06
		Debre-Birhan	0.39±0.01
		Debre-Markos	0.35±0.01
Areki	Alem Tena		0.97±0.03
	Meki		0.67±0.04
	Adamitulu		0.52±0.02

Table 7. Average levels of the fluoride ion (F) (mean±SD, mg/L or mg/kg, n=9) in samples of the filtrate and the semi-solid residue of the traditional fermented alcoholic beverages of shamita, borde, and korefe from a sample site in the Rift Valley (Deguna Fanigo) and a sample site which was not in the Rift Valley (Wogera)

Type of beverage	Sample site		Fluoride level in filtrate (mean±SD, mg/L)	Fluoride level in semi-solid residue (mean±SD, mg/kg)	Total fluoride level (mean±SD, mg/L)
	Rift Valley sites	Non-Rift Valley sites			
Shamita	Deguna Fanigo		1.13±0.05	7.54±0.27	5.21
Borde	Deguna Fanigo		1.24±0.04	6.48±0.22	4.95
Korfe		Wogera	0.41±0.01	3.80±0.19	1.39

Table 8. Average levels of the fluoride ion (F) (mean±SD, mg/L, n=3) in samples of the raw water used for the preparation of the traditional fermented alcoholic beverages of tella, tej, areki, shamita, borde, and korefe from four sample sites in the Rift Valley (Alem Tena, Meki, Adamitulu, and Deguna Fanigo) and three sample sites which were not in the Rift Valley (Debre-Birhan, Debre-Markos, and Wogera)

Type of beverage prepared with the raw water	Sample site		Fluoride level (mean±SD, mg/L)
	Rift Valley sites	Non-Rift Valley sites	
Tella	Alem Tena		5.06±0.19
	Meki		3.46±0.11
	Adamitulu		1.56±0.01
		Debre-Birhan	0.31±0.01
		Debre-Markos	0.30±0.01
Tej	Alem Tena		5.06±0.08
	Meki		3.50±0.08
	Adamitulu		1.78±0.03
		Debre-Birhan	0.37±0.01
		Debre-Markos	0.31±0.01
Areki	Alem Tena		9.02±0.15
	Meki		4.07±0.16
	Adamitulu		2.09±0.04
Shamita	Deguna Fanigo		0.78±0.01
Borde	Deguna Fanigo		0.92±0.03
Korefe		Wogera	0.35±0.03

Table 7 shows the F concentration in a beverage as the sum of a “filtrate”, i.e., dissolved F, and a “residue”, i.e., suspended F. The study shows that the F dissolved in the beverage is only 22–29% of the total F, while the suspended F is the rest, i.e., 78–71% of the total F. Thus, this study clearly showed that the beverage F occurs mainly in the suspended materials of the beverage.

This may be due to the fact that the F in the beverages varied depending on the type of ingredients used, the amount of ingredients added, the F content of the ingredients, and the F concentration of the water used for preparation. The amount of water used for the preparation of semi-solid beverages is relatively small and hence the filtrate and clear solution contains smaller amounts of F compared to the

F content of the residues. Furthermore, the traditional fermented beverages (semi-solid beverages) contain higher concentration of polyphenols,[41] most of which are insoluble, remain as suspended fine particles, and may adsorb fluoride ions from the soluble parts of the beverages resulting in a higher concentration of F in the residues than in the filtrate of the beverages. It should also be noted that suspended fine solid particles have a natural tendency to adsorb ions from a bulk solution.

Analysis of variance: Analysis of variance (ANOVA) is an extremely powerful statistical technique which can be used to separate and estimate the different causes of variation. The one-way ANOVA can compare the mean of more than two groups of samples. ANOVA uses the F statistic to compare whether the differences between sample means are significant or not.[40]

In this study, the tella and tej samples were collected from five different areas and the areki samples were collected from three different areas. The F level of each sample was determined by a fluoride ion selective electrode. During the processes of sample preparation and analysis, a number of random errors may be introduced in each aliquot and in each replicate measurement. The variation in the sample mean of the analyte was tested by using ANOVA to see whether the source of the variation was from the experimental procedure or heterogeneity among the samples (i.e., differences in topographical location; differences in the mineral contents of the soil, water, and atmosphere; variation in the application of agrochemicals like fertilizers, pesticides, herbicides, etc.; or other variations in the brewing process between the groups of samples). The ANOVA results for tella, tej, and areki showed that statistically significant differences existed at the 95% confidence level in the mean fluoride ion concentrations of all three types of traditional fermented alcoholic beverages (Table 9)

Table 9. Analysis of variance (ANOVA) for the fluoride ion concentrations between and within the samples of the traditional fermented alcoholic beverages of tella, tej, and areki at the 95% confidence level

Sample type	Comparison	Df*	F calculated	F critical	Remark
Tella	Between groups	4	878	3.48	Significant difference between sample means
	Within groups	10			
Tej	Between groups	4	3645	3.48	Significant difference between sample means
	Within groups	10			
Areki	Between groups	2	190	5.14	Significant difference between sample means
	Within groups	6			

*Df is the degrees of freedom between the samples and within the samples.

Pearson correlation of F in the traditional fermented alcoholic beverage and water samples: The Pearson correlation matrices, using the correlation coefficient (r), were used to correlate the F concentration of the water samples to that of the traditional fermented alcoholic beverages,.

As shown by the high values for r in Table 10, the higher the levels of F in the water, the higher the levels of corresponding F in the traditional fermented alcoholic beverages. This verifies that the dependence of the F concentration in a traditional fermented alcoholic beverage on the F concentration of the water used in the preparation of the beverage.

Table 10. Pearson correlation for the levels of the fluoride ion (F) in the samples of the traditional fermented alcoholic beverages of tella (n=5), tej (n=5) and areki (n=3), and in the raw water used for the preparation of the beverages (raw water for tella: n=5, raw water for tej: n=5, and raw water for areki: n=3)

Pearson correlation coefficient r*	Type of beverage		
	Tella	Tej	Areki
r	0.9806	0.9955	0.9986

*r is the Pearson correlation coefficient between the fluoride ion (F) levels in traditional fermented alcoholic beverages of tella, tej, and areki, and the fluoride ion levels in the raw water used for their preparation.

Daily intake of F by an adult person through traditional fermented alcoholic beverages: The total F intake (TFI) is the summation of the daily F intake from all the beverage sources. The daily intake of F (DIF) from a particular beverage was calculated by multiplying the F concentration in a particular beverage item with total quantity of that particular item consumed per person per day. The daily intake of F and total F intake were calculated as follows:

$$\text{DIF (mg/day)} = \text{FC (mg/L)} \times \text{QF (L/day)}$$

$$\text{TFI (mg/day)} = \Sigma\text{DIF (mg/day)}$$

where DIF = daily intake of fluoride ion in mg/day, FC = fluoride concentration in the beverage source in mg/L, QF = quantity of the particular beverage taken per person per day in L/day, TFI = total fluoride ion intake in mg/day, and DIF = daily intake of fluoride ion in mg/day.

The quantity of a particular beverage item taken per person per day was calculated by multiplying the amount of the beverages item taken in a day by frequency of consumption in a week and divided by the number of days in a week.

The percentage of the recommended daily adequate intake[42,43] of F (% AI) from a particular beverage was calculated as follows:

$$\% \text{ AI} = \frac{\text{DIF (mg/kg)}}{\text{RDAIF (mg/kg)}} \times 100$$

where % adequate intake = the percentage of the adequate intake of the fluoride ion, DIF = daily intake of the fluoride ion in mg/kg, and RDAIF = the recommended daily adequate intake of the fluoride ion in mg/kg.

According to the European Food Safety Authority (EFSA)[42] and the Institute of Medicine of the National Academy of Sciences,[43] an adequate intake (AI) for female adults having body weights of 58.5 and 61 kg, respectively, are 2.9 and 3.0 mg/day, respectively.[42,43] For adult males, with body weights of 68.1 and 76 kg, respectively, the AIs are given as 3.4 and 4.0 mg/day, respectively.[42,43] The EFSA notes that F is not an essential nutrient and therefore no average requirement for the performance of essential physiological functions can be defined.[42] An AI is given by the EFSA because, based on epidemiological studies performed before the 1970s, they consider that dietary F has beneficial effects in preventing dental caries.[42] A daily intake of 3–4 mg F/day can be considered to be tolerable but not necessary, while a daily intake of F beyond this limit may be harmful to human health. In the present study, assuming that the adults consumed an average amount of beverages, we calculated the expected the daily F intake by adults from fermented traditional alcoholic beverages (Tables 11–12).

Table 11. The expected daily adult fluoride ion (F) intake from the consumption of the traditional fermented alcoholic beverage of tella from three sample sites in the Rift Valley (Alem Tena, Meki, and Adamitulu) and two sample sites which were not in the Rift Valley (Debre-Birhan and Debre-Markos)

Sample	Sample site: Rift Valley	Sample site: Non-Rift Valley	Mean weekly adult alcoholic beverage intake (L/week)	Mean daily adult alcoholic beverage intake (L/day)	Conc. of F in alcoholic beverage (mg/L)	Mean daily adult F intake from alcoholic beverage (mg/day)	Relative daily intake of F from alcoholic beverage (%)
Tella	Alem Tena		2.80	0.40	8.19	3.28	109
	Meki		2.80	0.40	3.97	1.59	52.9
	Adami-tulu		2.80	0.40	2.02	0.81	26.9
		Debre-Birhan	2.80	0.40	0.33	0.13	4.40
		Debre-Markos	2.80	0.40	0.32	0.13	4.27

Table 12. The expected daily adult fluoride ion (F) intake from the consumption of the traditional fermented alcoholic beverages of tej, areki, tej, shamita, borde, and korefe from three sample sites in the Rift Valley (Alem Tena, Meki, and Adamitulu) and three sample sites which were not in the Rift Valley (Debre-Birhan, Debre-Markos, and Wogera)

Sample	Sample site		Mean weekly adult alcoholic beverage intake (L/week)	Mean daily adult alcoholic beverage intake (L/day)	Conc. of F in alcoholic beverage (mg/L)	Mean daily adult F intake from alcoholic beverage (mg/day)	% of adequate intake of F from alcoholic beverage (%)
	Rift Valley	Non-Rift Valley					
Tej	Alem Tena		1.40	0.20	5.76	1.15	38.4
	Meki		1.40	0.20	3.84	0.77	25.6
	Adami-tulu		1.40	0.20	2.46	0.49	16.4
		Debre-Birhan	1.40	0.20	0.39	0.08	2.60
		Debre-Markos	1.40	0.20	0.35	0.07	2.33
Areki	Alem Tena		0.06	0.01	0.97	0.008	0.28
	Meki		0.06	0.01	0.67	0.006	0.19
	Adami-tulu		0.06	0.01	0.52	0.004	0.15
Shamita	Deguna Fanigo		1.25	0.18	5.21	0.93	31.0
Borde	Deguna Fanigo		1.25	0.18	4.95	0.88	29.5
Korefe		Wogera	1.00	0.14	1.39	0.20	6.62

In the present study, the F intake from traditional fermented alcoholic beverages for an adult regular drinker of 60 kg weight was found to be in the range 0.004 mg/day (areki) to 3.28 mg/day (tella). Assuming a adult body weight of 60 kg and a recommended adequate intake (AI) of 3 mg/day, the average % contributions to the AI coming from traditional alcoholic fermented beverages from the Rift Valley area, for tella, tej, areki, shamita, and borde were 63.0%, 26.8%, 0.21%, 31.0%, and 29.5%, respectively, and for the non-Rift Valley area the average AI contributions for tella, tej, and korefe were 4.34%, 2.47%, and 6.62%, respectively. For a regular adult drinker in the Rift Valley area who consumes all the types of locally available beverages daily, the person may ingest up to 4.56 mg/day or 150% of the AI, e.g., tella, tej, and areki produced in Alem Tena (Tables 11 and 12). For a regular adult drinker in the non-Rift Valley area who consumes all the types of locally available beverages daily, the person may ingest up to 0.405 mg/day or 13.4%% of the AI, e.g., tella and tej produced in Debre-Birhan or Debre-Markos, and korefe produced in Wogera (Tables 11 and 12). The daily intakes of 4.56 and 0.405 mg/day are, respectively, above and below the AI of 3 mg/day.

Comparison of the F levels found in this study with the values in the literature: Comparisons of the F concentrations in the traditional fermented alcoholic beverages samples determined in this study and those reported in the literature are presented in Tables 13 and 14.

Table 13. Comparison of the fluoride ion (F) levels in the traditional fermented alcoholic beverage of tella found in the present study with those previously reported in the literature

Type of beverage	Sample site	F level in the raw water used to prepare the alcoholic beverage (mg/L)	F level in the alcoholic beverage (mg/L and mg/kg)	Reference
Tella	Alem Tena	5.06	8.19	Present study
	Meki	3.46	3.97	Present study
	Adamitulu	1.56	2.02	Present study
	Debre-Birhan	0.31	0.33	Present study
	Debre-Markos	0.30	0.32	Present study
	Wongi Gefersa (i)	1.20	2.40	24
	Wonji/Shoa Alem Tena	1.10	3.00	24
	Wonji/Shoa plantation	0.50	1.60	24
	Sami Berta	8.00	8.50	24
	Wonji Gefersa (ii)	9.20	10.0	44
	Metehara	1.30	1.50	24

The F levels found in the present study in the samples of the Ethiopian traditional fermented alcoholic beverage of tella (0.32–8.19 mg/L) were a little lower than that reported by Macdonald Partners (10 mg/L)[44] and almost comparable to that of reported by Tekle-Haimanot and Haile (1.60-8.50 mg/L).[24] The F levels we found in the samples of tej (0.35–5.76 mg/L) were comparable to those of reported by Tekle-Haimanot and Haile (1.30–1.40 mg/L)[24] but much lower than that reported by Macdonald Partners (13 mg/L).[44] The F levels found in our study for areki (0.52–0.97 mg/L) were comparable to that reported by Macdonald Partners (0.6 mg/L).[44]

Table 14. Comparison of the fluoride ion (F) levels in the traditional fermented alcoholic beverages of tej, areki, tej, shamita, borde, and korefe found in the present study with those previously reported in the literature

Type of beverage	Sample site	F level in the raw water used to prepare the alcoholic beverage (mg/L)	F level in the alcoholic beverage (mg/L and mg/kg)	Reference
Tej	Alem Tena	5.06	5.76	Present study
	Meki	3.50	3.84	Present study
	Adamitulu	1.78	2.46	Present study
	Debre-Birhan	0.37	0.39	Present study
	Debre-Markos	0.31	0.35	Present study
	Wonji Gefersa (i)	1.20	1.30	24
	Wonji/Shoa Alem Tena	1.10	1.40	24
	Wonji Gefersa (ii)	9.20	13.0	44
Areki	Alem Tena	9.02	0.97	Present study
	Meki	4.07	0.67	Present study
	Adamitulu	2.09	0.52	Present study
	Wonji Gefersa	9.20	0.60	44
Shamita	Deguna Fanigo	0.78	5.21	Present study
Borde	Deguna Fanigo	0.92	4.95	Present study
Korefe	Wogera	0.35	1.39	Present study

The possible reasons for the differences in F levels in the traditional fermented alcoholic beverages samples, between the three studies that were compared and the various regions include: (i) differences in topographical location; (ii) differences in the mineral contents of the soil, water, and atmosphere; (iii) variations in the application of agrochemicals, e.g., fertilizers, pesticides, herbicides, etc.; and (iv) variations in the brewing processes between the groups of samples. There are no values reported in the literature for the F concentrations in shamita, borde, and korefe so comparisons could not be made for these beverages.

CONCLUSION

In the present study, the range for the F levels in six Ethiopian traditional fermented alcoholic beverages was 0.32–8.19 mg/L. The F levels in the beverages from the Rift Valley areas were higher than those from outside the Rift Valley. The estimated range for daily F intake of F from beverages by regular drinkers of the beverages was 0.004–3.28 mg/day. The beverages prepared using water with a low concentration of F were considered safe for human consumption. The consumption of beverages prepared using water with a high concentration of F may cause adverse health effects such as dental and skeletal fluorosis. The analysis of variance showed that there was a statistically significant difference at the 95% confidence level in the mean levels of F among the beverage samples. Analysis with the Pearson correlation coefficient showed that the levels of F in the traditional fermented alcoholic beverages correlated positively with the levels present in the raw water used to prepare the beverages.

ACKNOWLEDGMENTS

The authors express their gratitude to the Department of Chemistry, Addis Ababa University, Ethiopia, for providing the laboratory facilities. Yohannes Belete is thankful to the Water Works Design and Supervision Enterprise, Addis Ababa, Ethiopia, for sponsoring his study.

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