ADDIS ABABA UNIVERSITY

COLLEGE OF NATURAL SCIENCES

Department of Chemistry

Analytical Chemistry Stream

MSc Thesis

By

Yohannes Belete

A thesis submitted to the College of Natural Sciences of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Analytical Chemistry.

Advisors: Prof. B.S. Chandravanshi
Dr. Feleke Zewge

June, 2016

Title

Levels of fluoride in traditionally fermented alcoholic beverages commonly consumed in Ethiopia

DECLARATION

I, the undersigned, declare that this thesis is my original work and has been submitted in partial fulfillment of the requirements for the degree of masters of Science at Addis Ababa University. All sources of materials used for this thesis have been duly acknowledged. This paper has never been submitted to and/or presented in any other university, college or institution in candidature of any other degree, diploma, or certificate.

Name: ______________________

Signature: _________

Date: ________________

ACKNOWLEDGEMENTS

Above all, I thank the almighty God for His unlimited blessings.

It gives me great pleasure to express my deepest gratitude to my project advisors Prof. B.S. Chandravanshi and Dr. Feleke Zewge for their interest, guidance, and encouragement, without which all my efforts would be fruitless. I am also indebted to Mrs. Meseret Dessalegne and Ms. Adanech Adera for their kindness in supporting during fluoride analysis in the laboratory.

I am deeply thankful to Water works design and supervision enterprise for giving me the opportunity to join the school of graduate studies. I am also grateful to the Department of Chemistry, Addis Ababa University.

My very sincere thanks to Bewketu Dagne, Abel Adam & Eshetu Nega for their parent-like support, generous care and the home feeling whenever I was in need during my study.

Finally, I take this opportunity to express the profound gratitude from my deep heart to my beloved grandparents, parents, and my siblings for their love and continuous support – both spiritually and materially.

TABLES OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LISTS OF ACRONYMS AND ABBREVIATIONS

ISE	Ion Selective Electrode
ANOVA	Analysis of Variance
WHO	World Health Organization
TISAB	Total Ion Strength Adjustment Buffer
EDTA	Ethylene Diamine Tetra Acetic acid
mV	milli Volt
SD	Standard Deviation
TFI	Total Fluoride Intake
DIF	Daily Intake of Fluoride
RDIF	Relative Daily Fluoride Intake
LOD	Limit of Detection
SPSS	Statistical Program for Social Sciences
Df	Degree of freedom
SCHER	Scientific Committee on Health and Environmental Risks
FAO	Food and Agriculture Organization
USNRC	United States National Research Council
UNESCO	United Nations Education and Scientific Organization
IPCS	International Program on Chemical Safety

ABSTRACT

Fluorine is one of several trace elements receiving much attention owing to its possible harmful effects on health and the environment. Fluorosis is a public health problem in certain part of Ethiopia, especially in rift valley. Beverages are the largest contributors to an individual's total exposure to fluoride. Total daily fluoride exposure can vary markedly from one region to another depending on concentration of fluoride in drinking water and water-based beverages. The objective of this study was to determine levels of fluoride in traditional fermented alcoholic beverages (*tella*, *tej*, *areki*, *shamita*, *borde* and *korefe*) widely consumed in selected areas of Ethiopia. The beverages samples and the water used for their preparation were collected from the rift valley and outside rift valley areas of Ethiopia: Adami Tulu, Meki, Alem Tena, Deguna Fanigo, Debre Birhan, Debre Markos, and Wogera towns. The beverages samples were collected from local markets and house hold levels in the areas where they are prepared and consumed in large amount. Levels of fluoride in the beverages and water samples were determined by fluoride ion selective electrode. The mean fluoride concentration (mg/L) ranged in beverages and corresponding water samples, respectively, were: *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* (5.26, 0.78), *borde* (4.95, 0.92) and *korefe* (1.39, 0.35). Pearson correlation showed that the levels of fluoride in the traditional fermented alcoholic beverages were found to correlate positively with the levels found in the water. The mean daily dietary fluoride intake from the beverages was different with different fluoride concentration of the water used. In this study, the estimated daily intake of fluoride through beverages for the regular drinker ranges from 0.004-3.28 mg/day. The beverages prepared using low concentration of fluoride water is safe for human consumption while beverages prepared using high concentration of fluoride containing water may cause dental as well as skeletal fluorosis.

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

1. INTRODUCTION

1.1. Background

Alcoholic beverages are a part of human dietary culture and have an inseparable relationship with the life of mankind in history. In the world there are a large variety of fermented foods and beverages with traditional and cultural value. The diversity of such fermented products derives from the heterogeneity of traditions found in the world, cultural preference, different geographical areas where they are produced and the staple and/or by-products used for fermentation. In many instances it is highly likely that the methods of production were unknown and came about by chance, and passed down by cultural and traditional values to subsequent generations [1-3]. The making and drinking of alcoholic beverages are ways of enhancing the nutritional significance as well as social relationships for human beings [2].

Fermented products can play an important role contributing to the livelihoods of rural and peri-urban dwellers alike, through enhanced food security, and income generation via a valuable small scale enterprise option. There is such a diversity of fermentable substrate available year round, that the activity can provide a regular income. Although harvesting or substrate may be seasonal, fermentation itself is largely independent of weather, and byproducts can be recycled into livestock fodder [1, 3].

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 usually for local consumption. Among Ethiopian traditional fermented alcoholic beverages are varieties of *Tella*, *tej*, *korefe*, *areki*, *borde*, and *shamita* drinks that each household brews to treat guests [3, 4]. Pictures of selected traditional fermented alcoholic beverages used in this study are shown in Figure 1.

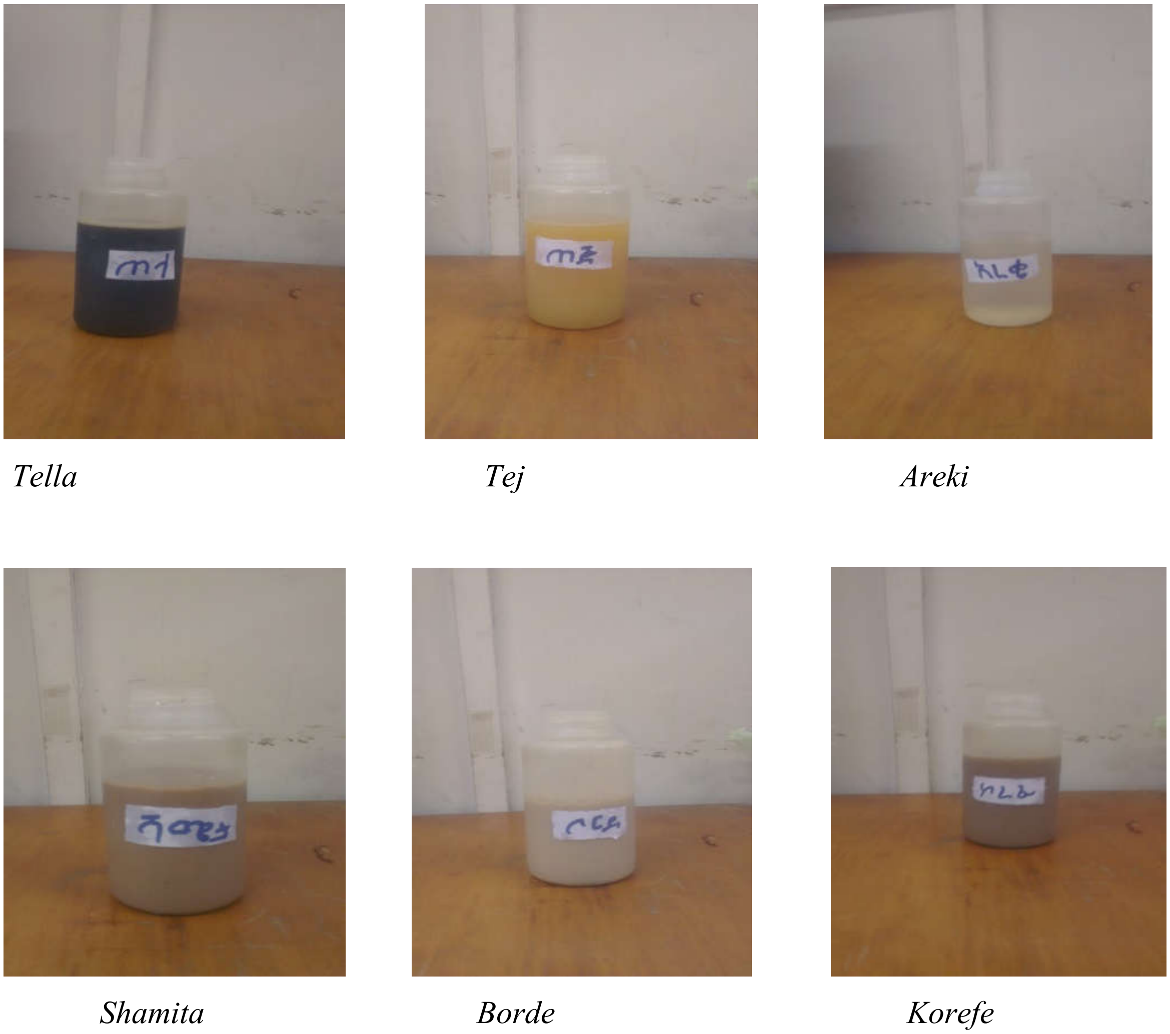

Figure 1. *Tella*, *Tej*, *Areki*, *Borde*, *Shamita* and *Korefe* samples.

There are several reports in the literature on the levels of fluoride in the food, water and beverages samples in Ethiopia [5-14]. However, there is no systematic study conducted to determine the levels of fluoride in traditional fermented alcoholic beverages in Ethiopia. Hence, it is worthwhile to determine the levels of fluoride in traditional fermented alcoholic beverages (*tella*, *tej*, *areki*, *shamita*, *borde* and *korefe*) widely consumed in Ethiopia.

1.2. Objectives of the study

1.2.1. General objective

The main objective of this thesis is to determine the levels of fluoride in traditional fermented alcoholic beverages (*tella*, *tej*, *areki*, *shamita*, *borde* and *korefe*) widely consumed in selected areas of Ethiopia.

1.2.2. Specific objectives

1. To determine the levels of fluoride in traditional fermented alcoholic beverages (*tella*, *tej*, *areki*, *shamita*, *borde* and *korefe*) widely consumed in selected areas of Ethiopia;
2. To compare the levels of fluoride in selected traditional fermented alcoholic beverages analyzed in this study;
3. To calculate total daily fluoride intake of an adult person based on fluoride concentrations of traditional fermented alcoholic beverages;
4. To compare levels fluoride of traditional fermented alcoholic beverages of this study with values reported in other literatures.

1.2.3. Significance of the study

Food sources contain various concentrations of fluoride and are the largest contributor to fluoride exposure. Beverages contribute most of the fluoride intake by the people who regularly drink. However, there is no systematic study conducted to determine the levels of fluoride in traditional fermented alcoholic beverages widely consumed in Ethiopia. This study provides new information on the levels of fluoride in traditional fermented alcoholic beverages widely consumed in selected areas (including the rift valley and non-rift valley regions) of Ethiopia and gives clue for further studies.

2. LITERATURE REVIEW

2.1. Selected traditional fermented alcoholic beverages used in this study

2.1.1. *Tella*

Tella is one of the Ethiopian traditional beverages, 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 part of Ethiopia. It is brewed from different cereals which include; barely, corn, wheat and sorghum and also from teff, maize and millet while *Rhamnus prinioides* is added as a flavoring agent [15]. With regard to the substrate, there is no as such basic difference between *tella* and beer. The alcoholic level of *tella* is between 3.5 to 6.48% (v/v) [16, 17].

The way of preparing *tella* differs among the ethnic groups and depends on traditional and the economic situation. The clay container (*insera*) is washed with *Grawa (Vernonia amygdalina)* and water several times and after that smoked with wood from *Weyra (Oleaeuropaea subsp. Cuspidate)* for about ten minutes, in order to get it as clean as possible. Germinated grain of barley, or corn, or wheat *(bikil*), bought in the local market or prepared at home, are dried and milled. For making *bikil*, the grains are moistened in water and the moist grains are placed between fresh leaves, left to germinate for 3 days and after that dried. The leaves *gesho* (*Rhamnus prenoides*) is dried in the sun for about ½ hour and after that pounded. The leaves are separated from the stems, which need a longer time to dry. The ground *gesho* (*Rhamnus prenoides*) leaves are placed in a clay container with water and left to ferment for 2-3 days. *Gesho* (*Rhamnus prenoides*) is responsible for the bitter taste of *tella* and the bitterness of *tella* is directly related to the amount of *gesho* added during brewing. Some of the grains intended for *tella* preparation are toasted and milled, and then mixed with water and baked on the *mitad* to prepare what is known as *kita* (a thin, 5-10 mm thick, pancake-like bread). This *kita*, broken into small pieces, part of the milled *bikil* and the pounded *gesho* stems are added to the water and allowed to ferment for 1-2 days. The rest of the flour is toasted on *mitad*, sprinkled with water and toasted until dark brown to form what is known as *enkuro*. This mixture *enkuro*, the rest of the germinated grains (*bikil*), some *gesho* (*Rhamnus prenoides*), and water are added to the container. The mixture is kept covered overnight, after which more water is added and the container is kept sealed for 5-7 days, until when the beverage is ready [16-19].

2.1.2. ***Tej***

Tej is a home-processed, but also commercially available honey wine. It is a beverage mainly used for great feasts, such as weddings and the breaking of fasting. It is prepared from honey, water and leaves of *gesho* (*Rhamnus prenoides*). Sometimes, widely for commercial purposes, mixture of honey and sugar could be 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 [20]. Some people also add different concoctions such as barks or roots of some plants or secrete herbal ingredients to improve flavor or potency and to attract customers. Due to concoction, adulteration practices and possibly some other reasons, producers usually are not willing to tell about additives used and their composition [24].

During the preparation of *tej*, the fermentation pot is seasoned by smoking over smoldering *Rhamnus prenoides* stems and olive wood. One part of honey mixed with 2 to 5% (v/v) parts of water is placed in the pot, covered with a cloth for 2 to 3 days to ferment after which wax and top scum is removed. Some portion of the must is boiled with washed and peeled *Rhamnus prenoides* and put back to the fermenting must. The pot is covered and fermented continuously for another 5 days, in warmer weathers, or for 15-20 days, in colder cases. The mixture is stirred daily and finally filtered through cloth to remove sediment and *Rhamnus prenoides*. Good quality *tej* is yellow, sweet, effervescent and cloudy due to the content of yeasts. The flavor of *tej* depends upon the part of the country where the bees have collected the nectar and the climate [3, 16]. The total alcohol levels of *tej* were varied between the values of 8.94 to 13.16% (v/v) [21] and 2.7 to 21.7% (v/v) [22].

2.1.3. ***Areki***

Areki is a distilled beverage. It is a colorless, clear, traditional alcoholic beverage which is distilled from fermentation products prepared in almost the same way as *tella* except that the fermentation mass in this case is more concentrated [20]. *Areki* is usually 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 *areki* are predominantly lower class people or those who have become dependent on alcohol and cannot afford to buy industrially produced alcohol [25]. Traditionally *areki* is classified into two: *Terra-areki* and *Dagim-areki*. The term *dagim* in Amharic refers to 'second time' and, indicates that it is distilled second time, whereas the term *terra* in Amharic refers to 'ordinary'.

Terra-areki is a colorless, clear, local alcoholic beverage, which is distilled from a fermentation product known as *Yereki-tinsis* [23].*Yereki-tensis* is prepared by mixing powdered *gesho* leaves and powdered *bikil* (1:2 ratios) with water to give a mixture of free flowing consistency, and which will be put aside to ferment for about five days. An amount of *Dagussa* (*Elusine coracann*) roughly equivalent to four times that of the *bikil*, is powdered kneaded with water to make a dough and baked into cakes. The hot cakes are broken into pieces, added to the first mixture and with more water, well mixed and again left aside to ferment for about four days. Portions of the second mixture are transferred to the traditional distillation apparatus and distilled to give what is known as *terra-areki*. The alcohol content of *terra-areki* is reported to be 34.09% (v/v) [23], and varies between 22.0 – 28.0% (v/v) [16].

Dagim-areki is a stronger type of *terra-areki*, which is prepared in the same way as *terra-areki*, except that the distillation process is allowed to proceed for a shorter period of time, or three volumes of *terra-areki* are redistilled to give about one volume of *dagim-areki* [23]. The redistilled *areki* will then have higher alcohol content. The average alcohol content of *dagim areki* is around 45% (v/v) [16]. It was also reported to have a mean value of 46.6% (v/v).

2.1.4. *Borde*

Borde is an Ethiopian spontaneously fermented, low or non-alcoholic cereal beverage. It is produced from a variety of locally available cereal ingredients using traditional techniques. The adjunct (all un-malted cereal ingredients) and each malt can be from one type or a mixture of cereals. *Borde* is an opaque, effervescent, whitish-grey to brown colour and has a thick consistency and sweet-sour taste. It must be consumed while actively fermenting within a day due to its poor keeping quality. It is widely consumed by adults and children mainly in southern and western parts of Ethiopia. *Borde* is often consumed as a low-cost meal replacement for many poor people [31]. The production of *borde* is a laborious and complex technology and is exclusively carried out by women. The production of *borde* has four major phases, which are marked by the introduction of fresh ingredients into the fermentation vessel. In Phase I, maize grits are mixed with water enough to immerse them and allow fermenting for 44 to 72 h. The content is apportioned into three parts at different periods (44-48 h and 66-72 h), is cooked with other non-fermented ingredients and is used at Phase II, III and IV of main fermentation. In Phase II, a portion of 44-48 h fermented mass from Phase I is roasted at about 90 ^{o}C for 30-45 min and is cooled and fresh malt flour are blended with water in a cleaned and smoked to a light brown thick mash. The brewers mix the ingredients using their hands and adjust the consistency of the mash. This mixture is called *tinsis* and is allowed to ferment for about 24 h. During preparation of un-malted ingredients for the third phase, a second portion of the fermented mass (now about 66 h old) from Phase I is slightly roasted, cooled, thoroughly kneaded with more flour and water and then is moulded into dough balls. They are then steam-cooked for 1-1.5 h to give *gafuma*. The *gafuma* is broken into pieces, cooled and blended with the fermented *tinsis* and water to a thick brown mash called *difdif*. The *difdif* is then allowed to ferment for 18 h. In Phase IV, the last portion of fermented mass (about 72 h) from Phase I is mixed with a boiling porridge prepared from flour or grain. The content is maintained with continuous stirring at about 90 ^{o}C for 1-1.5 h into a very thick porridge. The cooled thick porridge is blended with the fermented *difdif*, along with some additional malt and water. This mixture is then sieved using a *wonfit* (about 1 mm pore size). The residue is wet-milled using grinding stones and then sieved again by slurring with more water. The wet-milling and -sieving operations are repeated up to 3 times. All the three fractions of filtrate are collected together, poured back into the rinsed main fermentation vessel and the consistency adjusted if necessary. This filtrate is allowed to ferment for a further 4-6 h. The actively fermenting, effervescent *borde* is then ready for consumption [28, 30].

2.1.5. *Shamita*

Shamita is another traditional beverage of Ethiopia, which is low in alcohol content, made by overnight fermentation of mainly roasted barley flour and, consumed as meal-replacement [19]. *Shamita* 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. It is produced by fermenting roasted barely overnight. Barley was soaked in excess water for 15 min, the excess water was then strained off and the soaked barely was dehulled using mortar and pestle. This was allowed to air dry for a short period and the free seed coat was removed. The dehulled barely was then roasted on a flat metal pan until it turned light brown. After a further dry dehulling, the roasted barely was ground finely. Barley flour, salt, ground linseed and spices were mixed which is the stock ingredient used for several *shamita* fermentation. From this ingredient take some amount and mix with water and allow fermenting overnight. However, *shamita* has poor keeping quality because of these high numbers of live microorganisms and becomes too sour about four hours after being ready for consumption [29].

2.1.6. *Korefe*

Korefe is the name of the local beer made in Begemder (Gondar) province among the Koumant ethnic group [16]. It is a beverage prepared for sales, holidays and different ceremonials from *Rhamnus prinioides*, water and malt of barely after processing. The malt barely and leaf of *Rhamnus prinioides* are bought from market, then the barely under goes cleaning by hand peaking, cooking followed by sieving and drying of the barely on the sun; after cuisine on *mithad*, dehusked with *mukecha*, subsequently that toasted and milled then after baked on *mithad* to prepare *derekot*. *Tinsis* is prepared by mixing of pounded malt of barely and leaf of *Rhamnus prinioides* in the ratio of (2:1) with water and withstand for five days in a pot which is seasoned by smoking over smoldering *Rhamnus prinioides* stems, *tinjut* and olive after cleaning by washing. *Kita* is prepared and mixed with the *tinsis* then tolerate for a day or over until foam is observed by properly covering. Here is the time to mix the fermented result with *derekot* which gives *difdif* in the big pot. Finally, after three days the *difdif* is mixed with water and ready for consumption.

2.2. Fluoride

Fluoride is the negatively charged ion of fluorine, which is the most electronegative and the most reactive of all elements [32]. Fluorine is a common element that is widely distributed in the earth's crust and exists in the form of fluorides in a number of minerals, such as fluorite (CaF_2), cryolite (Na_3AlF_6),and fluoroapatite ($Ca_5(PO_4)_3F$) [33]. Fluorine is one of several trace elements receiving much attention owing to its possible harmful effects on health and the environment [34]. The fluoride ion (F^-) is not considered to be essential for human growth and development including for the development of healthy teeth and bones, and it is a potentially toxic ion at higher levels [35-37].

In the world more than 200 million people rely on drinking water with fluoride concentrations exceeding the present World Health Organization (WHO) guideline of 1.5 mg/L [38]. Long-term use of drinking water with fluoride significantly above the WHO guideline value of 1.5 mg/L can have serious effects on health. In 2006, the WHO registered 28 countries where dental fluorosis and skeletal fluorosis were associated with exposure to high fluoride concentration in drinking water. Among these, the most affected were India, Ethiopia, and China [34, 35].

In the Ethiopian rift valley area an estimated of 8 million people are exposed to high levels of naturally occurring fluoride [39]. Daily intake of fluoride (primarily through drinking water and beverages but also via food) put a large part of the rural population in the Ethiopian rift valley at risk of dental and skeletal fluorosis. Water is epidemiologically the most important source of fluoride (75–90% of daily intake) in most areas of the rift valley of Ethiopia. Fluorosis does not only affect people's health. It also has serious economic and social consequences [40, 41].

2.2.1. Environmental occurrence of fluoride

Fluorine is the most electronegative of all the elements which means that it has a strong tendency to acquire a negative charge, and in solution forms fluoride ions. Other oxidation states are not found in natural systems, although uncharged complexes may be. Fluoride ions have the same charge and nearly the same radius as hydroxide ions and may replace each other in mineral structures [42]. Fluoride thus forms mineral complexes with a number of cations and some fairly common mineral species of low solubility contain fluoride. Fluorine in the environment is therefore found as fluorides which together represent about 0.06–0.09 per cent of the earth's crust. The average crustal abundance is 300 mg kg^{-1} [43]. Fluorides are found at significant levels in a wide variety of minerals, including fluorspar, rock phosphate, cryolite, apatite, mica, hornblende and others [44]. Fluorite (CaF_2) is a common fluoride mineral of low solubility occurring in both igneous and sedimentary rocks. Fluoride is commonly associated with volcanic activity and fumarolic gases. Thermal waters, especially those of high pH, are also rich in fluoride [45].

2.2.2. Exposure of human to fluoride

2.2.2.1. Air

Due to dust, industrial production of phosphate fertilizers, coal ash from the burning of coal and volcanic activity, fluorides are widely distributed in the atmosphere. However, air is typically responsible for only a small fraction of total fluoride exposure [46]. In non-industrial areas, the fluoride concentration in air is typically quite low (0.05–1.90 µg m^{-3} fluoride) [44]. In areas where fluoride-containing coal is burned or phosphate fertilizers are produced and used, the fluoride concentration in air is elevated leading to increased exposure by the inhalation route [44].

2.2.2.2. Water

Fluoride is found in all natural waters at some concentration. Seawater typically contains about 1 mg/L while rivers and lakes generally exhibit concentrations of less than 0.5 mg/L. In ground waters, however, low or high concentrations of fluoride can occur, depending on the nature of the rocks and the occurrence of fluoride-bearing minerals. Concentrations in water are limited by fluorite solubility, so that in the presence of 40 mg/L calcium it should be limited to 3.1 mg/L [34, 42]. It is the absence of calcium in solution which allows higher concentrations to be stable [45]. High fluoride concentrations may therefore be expected in ground waters from calcium-poor aquifers and in areas where fluoride-bearing minerals are common. Fluoride concentrations may also increase in ground waters in which cation exchange of sodium for calcium occurs [45]. In Ethiopia, well water, spring water, and tap water are the common water supply source used in both urban and rural areas [47]. The amount of fluoride present naturally in non-fluoridated drinking water is highly variable, being dependent upon the individual geological environment from which the water is obtained [48].

2.2.2.3. Food and beverages

Most foods, aside from tea and marine fish, are relatively low in fluoride (<0.05 mg/100 g) [49], although foods and beverages prepared with fluoridated water can contain appreciable amounts, depending on the fluoride concentration in the water. Tea leaves have high concentrations of fluoride (up to 400 mg/kg dry weight), and individual exposure due to the consumption of tea can range from 0.04 to 2.7 mg/day. High consumption of some types of tea (e.g. 'brick tea' made from older tea leaves) over long periods has been associated with the development of skeletal fluorosis in some developing countries, particularly if the water used for brewing is high in fluoride [50].

2.2.2.4. Dental products

A number of products administered to, or used by, children to reduce dental decay contain fluoride. This includes toothpaste (1.0–1.5 g kg^{-1} fluoride), fluoride solutions and gels for topical treatment (0.25–24.0 g kg^{-1} fluoride) and fluoride tablets (0.25, 0.50 or 1.00 mg fluoride per tablet), among others. These products contribute to total fluoride exposure, albeit to different degrees. It is estimated that the swallowing of toothpaste by some children may contribute about 0.50 or 0.75 mg fluoride per child per day [34, 44].

2.2.3. Metabolism of fluoride

2.2.3.1. Absorption

Approximately 75–90 per cent of ingested fluoride is absorbed. In an acidic stomach, fluoride is converted into hydrogen fluoride (HF) and up to about 40 per cent of the ingested fluoride is absorbed from the stomach as HF. High stomach pH decreases gastric absorption by decreasing the concentration up take of HF. Fluoride not absorbed in the stomach is absorbed in the intestine and is unaffected by pH at this site [52, 53]. Relative to the amount of fluoride ingested, high concentrations of cations that form insoluble complexes with fluoride (e.g. calcium, magnesium and aluminium) can markedly decrease gastrointestinal fluoride absorption [52, 53].

2.2.3.2. Distribution

Once absorbed into the blood, fluoride readily distributes throughout the body, with approximately 99 per cent of the body burden of fluoride retained in calcium rich areas such as bone and teeth (dentine and enamel) where it is incorporated into the crystal lattice. In infants about 80 to 90 per cent of the absorbed fluoride is retained but in adults this level falls to about 60 per cent. Fluoride crosses the placenta and is found in mother's milk at low levels essentially equal to those in blood [51, 52]. Under certain conditions, plasma fluoride levels provide an indication of the level of fluoride in the drinking-water consumed. Levels of fluoride that are found in the bone vary with the part of the bone examined and with the age and sex of the individual. Bone fluoride is considered to be a reflection of long-term exposure to fluoride [52].

2.2.3.3. Excretion

The kidneys play the major role in the removal of fluoride from the body. Normally kidneys are very efficient and excrete fluoride very rapidly. However, decreased fluoride removal may occur among persons with severely impaired renal function who may not be on renal dialysis.
Fluoride is excreted primarily via urine [52].Urinary fluoride clearance increases with urine pH due to a decrease in the concentration of HF. Numerous factors (e.g. diet and drugs) can affect urine pH and thus affect fluoride clearance and retention [56].

2.2.4. Health effects of fluoride

Fluoride has beneficial effects on teeth at low concentrations in drinking-water, but excessive exposure to fluoride in drinking-water, or in combination with exposure to fluoride from other sources, can give rise to a number of adverse effects. These range from mild dental fluorosis to crippling skeletal fluorosis as the level and period of exposure increases. Crippling skeletal fluorosis is a significant cause of morbidity in a number of regions of the world [36, 38].

2.2.4.1. Dental fluorosis

Excessive, chronic ingestion of the fluoride ion during tooth formation can lead to tooth discoloration, which in most cases takes the form of slight, whitish spots that are barely visible and tend to fade over time. This change in the appearance of teeth is known as dental fluorosis. Other etiological factors, such as exposure to amoxicillin at an early age, can have a similar effect on teeth appearance [54]. Fluoride-related fluorosis usually appears in children between birth and age 3, most commonly on the permanent central incisors [55], which are the most important teeth from an esthetic standpoint. With the exception of the third molars, enamel formation on permanent teeth starts around birth and is complete by about age 5. Later, dental enamel is completely mineralized and the risk of developing fluorosis disappears, even when fluoride is ingested in excessive amounts [56]. Dental fluorosis in its very mild, mild or moderate form is not considered to be a toxic effect [57]. Mild or very mild fluorosis is often imperceptible to the untrained eye, but moderate fluorosis, caused by the ingestion of large quantities of fluoride during tooth formation, does affect tooth appearance [57]. Ingestion of very large quantities of fluoride during tooth formation can lead to severe fluorosis and enamel erosion, making teeth more vulnerable to decay. This undesirable effect is generally associated with water fluoride concentrations in excess of 4 mg/L [57].

When fluoride is present in oral fluids (i.e., saliva), fluorapatite, rather than hydroxyapatite, forms during the demineralization process. Fluoride ions (F^-) replace hydroxyl groups (OH^-) in the formation of the apatite crystal lattice [63].

$$Ca_{10}(PO_4)_6(OH)_2 \longrightarrow Ca_{10}(PO_4)_6F_2$$

Figure 2. Dental fluorosis.

2.2.4.2. Skeletal effects

Endemic skeletal fluorosis is well documented and is known to occur with a range of severity in several parts of the world, including India, China and northern, eastern, central and southern Africa. It is primarily associated with the consumption of drinking-water containing elevated levels of fluoride but exposure to additional sources of fluoride such as high fluoride coal is also potentially very important. This is compounded by a number of factors which include climate, related to water consumption, nutritional status and diet, including additional sources of fluoride and exposure to other substances that modify the absorption of fluoride into the body [34]. Fluoride is readily incorporated into the crystalline structure of bone, and will accumulate over time. Concerns about fluoride's effects on the musculoskeletal system are focused on a condition called skeletal fluorosis and also on increased risks of bone fracture. Models that estimate the accumulation of fluoride into bone (pharmacokinetic models) have been developed that are useful in understanding fluoride's effect on bone. Skeletal fluorosis is a bone and joint condition associated with prolonged exposure to high concentrations of fluoride. Fluoride increases bone density and causes changes in the bone that lead to joint stiffness and pain [58].

In bone, fluoride replaces the hydroxyl ion in hydroxyapatite to form fluorapatite, thus changing the physicochemical properties of the bone [63].

$$Ca_{10}(PO_4)_6(OH)_2 \longrightarrow Ca_{10}(PO_4)_6F_2$$

Figure 3. Skeletal fluorosis.

2.2.4.3. Other problems

This aspect of fluorosis is often overlooked because of the notion prevailing that fluoride only affects bones and teeth [59]. Besides skeletal and dental fluorosis, excessive consumption of fluoride may lead to muscle fiber degeneration, low hemoglobin levels, deformities in red blood cells, excessive thirst, headache, skin rashes, nervousness, neurological manifestations (it affects brain tissue similar to the pathological changes found in humans with Alzheimer's disease), depression, gastrointestinal problems, urinary tract malfunctioning, nausea, abdominal pain, tingling sensation in fingers and toes, reduced immunity, repeated abortions or still births, male sterility, etc. It is also responsible for alterations in the functional mechanisms of liver, kidney, digestive system, respiratory system, excretory system, central nervous system and reproductive system, destruction of about 60 enzymes. The effects of fluoride in drinking water on animals are analogous to those on human beings. The continuous use of water having high fluoride concentration also adversely affects the crop growth [60].

2.2.5. Methods of fluoride determination

There are different methods described in the literature for the determination of fluoride contents in the different types of samples. The most commonly used methods are described briefly in the following sub-sections.

2.2.5.1. Colorimetric method

Colorimetric method, the SPADNS {sodium 2-(parasulfophenylazo)-l,8-dihydroxy-3,6-naphthalene disulfonate} method, is based on the reaction between fluoride and a dark red zirconium dye lake, forming a colorless complex anion. This method results in a bleaching of the red color in an amount proportional to the fluoride concentration. As the amount of fluoride increases, the resulting color becomes lighter. Color then is determined photometrically using a filter photometer or spectrophotometer. Colorimetric methods are susceptible to interfering substances. High concentrations of alkalinity ($CaCO_3$), aluminum (Al^{+3}), chloride ($C1^-$), turbidity, color, (Fe^{+3}) and sulfate (SO_4^{2-}) in the sample will result in error in the determination of fluoride content [61, 62].

2.2.5.2. Ion chromatography method

Ion chromatography (IC) utilizes anion exchange resins as a stationary phase to separate fluoride ions from other species. In most cases, conductivity detectors are used to detect the ions in the eluent. Both the stationary phase and the eluent must be chosen to separate fluoride from overlapping ions. This method is generally applied to the separation of weak organic acids and its use for fluoride determinations is based on the fact that fluoride is an anion of a weak acid, hydrogen fluoride, with a pKa of 3.19, similar to that of weak organic acids. The acids elute in order of increasing pKa. At low pH, anions of strong acids remain disassociated and are excluded from the resin and are rapidly eluted. Hydrogen fluoride exists primarily in the molecular form, and interacts with the resin, delaying its elution. In this way, fluoride is sufficiently separated from ionic interferences to be reliably quantified. Interfering anions, such as chloride, emerge as one peak before the fluoride elutes. Resolution can be controlled by adjusting the pH [63].

2.2.5.3. Fluoride ion-specific electrode method

Fluoride ion selective electrode is the most successful ion sensitive electrode, and has received wide acceptance to stage where it is now uncommon to see alternatives reported [76]. The advantage of ion selective electrode usage for determination of fluoride ion is in a wider range of linearity, short time of response, non-destructivity of samples and there is no contamination of samples [64]. Potentiometric analysis of fluoride content (as F^{-}) in solutions by using fluoride ion-selective electrode is simple, reliable and cheap. Very small concentrations of fluoride-ions (to 10^{-6} M) can be determined by fluoride selective electrode, with regulation of ion strength of a solution and control of concentration of hydroxide ions and interfering ions of metals [68]. FISE should be a method of choice for those with little experience in analytical chemistry. It is easy to operate, relatively rapid and require little technical skill [65]. In direct potentiometry method, concentration can be determined with a single measurement [66]. In this research, the levels of fluoride in traditionally fermented alcoholic beverages commonly consumed in Ethiopia were determined by the method of direct potentiometry.

3. EXPERIMENTAL

3.1. Samples and sample collections description

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 (Adami Tulu, Meki, Alem Tena, Deguna Fanigo) and from areas located outside the rift valley (Debre Birhan, Debre Markos, Wogera). All the samples were collected based on universality in consumption from adolescent to agers, widely available in the local market and on household level. *Tella* and *tej* were collected from Adami Tulu, Meki, Alem Tena, Debre Birhan, Debre Markos, and *Areki* from Adami Tulu, Meki and Alem Tena. *Shamita* and *Borde* are familiar in the area of southern region, and five representative samples were purchased from Deguna Fanigo. *Korefe* is familiar in the area of north region, and five representative samples were purchased from Wogera. From each sample area, five representative samples were collected from different vending houses (0.5 L from each) which were selected randomly to prepare a bulk sample by mixing because instead of analyzing separately, analyzing of the mixture reduces the variance and resource consumption and triplicate analysis was performed for each. From each sample area, the water samples were collected and triplicate analysis were performed for each sample. All the samples were collected using polyethylene plastic bottles then the bulk samples were kept in refrigerator at 4 ^{o}C until analysis [67].

Geographical locations of the traditional fermented alcoholic beverages (*tella*, *tej*, *areki*, *shamita*, *borde* and *korefe*) from selected areas of Ethiopia are given in Table 1.

Table 1. Geographical location of the sample areas.

Sample area	Longitude	Latitude	Altitude in m	Distance from Addis Ababa in km
Alem Tena	38.95°E	8.30°N	1,611	112
Meki	38°49′E	8°9′N	1,636	135
Adami Tulu	38°42′E	7°52′N	1,636	168
Diguna Fango	37°45′E	7°00′N	1,600	375
Debre Birhan	39°32′E	9°41′N	2,840	120
Debre Markos	37°43′E	10°20′N	2,446	300
Wogera	37°37′E	12°46′N	2,812	779

(Sourcehttp://www.4shared.com/rar/gy2VBcEV/Google_Earth_Pro_7111871_Final.html)

3.2. Apparatus, instrumentation, chemicals and reagents

3.2.1. Apparatus and instruments

The beverage and water samples were collected with polyethylene plastic bottles and transported to the laboratory. An oven (Digitheat, J.P. Selecta, Spain) was used to dry the semi-solid residue beverage samples. Electronic balance (Adam Equipment, Model WL 3000, UK), with precision of 0.0001 g was used for weighing of semi-solid residue beverage samples and chemicals for solution preparation. Muffle furnace (Audiotronics, Wagtech International Ltd., UK) was used for fusion of samples within nickel crucibles (50 mL). A pH/ISE meter (Orionmodel, EA 940 Expandable Ion Analyzer, USA) equipped with combination fluoride ion selective electrode (Orion Model 96-09, USA) was employed for the determination of fluoride in the samples and standards solutions. A pH meter (HANNA instrument, HI 9025, Singapore) equipped with pH glass electrode was used to measure the pH values of sample solutions. Borosilicate volumetric flasks (50, 100, 500 and 1000 mL) were used for preparation of both 8 M NaOH solution and 1000 mg/L NaF stock solution. Hot plate with magnetic stirrer was used for dissolution of the sample and fusion cake. Measuring cylinders (Duran, Germany), pipettes (Pyrex, USA); micropipettes (Dragonmed, 1–10 μL, 100–1000 μL, Shangai, China) were used during measuring different quantities of volumes of sample solution and fluoride standard solutions.

3.2.2. 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 reagent also includes concentrated hydrochloric acid (36%, Fisher Scientific UK Limited) for neutralization after alkali fusion and dissolution of semi-solid traditional fermented alcoholic beverages samples.

3.3. Sample preparation for fluoride analysis

The determination of fluoride in the filtrate/clear solution traditional fermented alcoholic beverages samples and water were analyzed directly by adding TISAB and taking the reading from calibrated fluoride ion selective electrode but for the semi-solid traditional fermented alcoholic beverages samples using a fluoride ion selective electrode were done by slightly modifying the reported method [68]. A 0.5 g semi-solid residue from each sample was accurately weighed and placed into 50 mL nickel crucibles. 5 mL of 8 M NaOH was added. Then, the samples and sodium hydroxide solutions were carefully mixed. The crucibles were placed on a hot plate for evaporation until the mixed NaOH and sample was solidified. The crucible was then placed in a muffle furnace and set at 200 ^{o}C for 2 h, after which the temperature was increased to 525 ^{o}C and kept there for 3 h in muffle furnace in order to fuse the sample in the crucible. The crucible was then placed in a hood and allowed to cool. 10 mL distilled water was added and placed on hot plate to facilitate the dissolution of the fusion cake. After 2 h, the sample solution was transferred to plastic volumetric flask, made up to volume with distilled water and filtered through a Whatman No. 40 filter paper. The sample solutions were neutralized using concentrated and then diluted hydrochloric acid. Concentrated hydrochloric acid was added drop-wise until the pH decreased from 12.0-13.0 to 8.0-8.5 then to pH of 7.2-7.5 by adding dilute hydrochloric acid [69]. The sample solutions were then transferred to a 50 ml plastic volumetric flask and diluted to volume with de-ionized water. Then, these digested samples were kept in the refrigerator until the analysis.

During the fusion of fluoride containing sample with NaOH, fluoride from the sample gets extracted as NaF. The chemical reaction can be presented as:

$$\text{Sample-F} + \text{NaOH} \longrightarrow \text{NaF} + \text{Sample-OH}$$

3.4. Determination of fluoride in samples

Aliquots of 5 mL were taken out from the ashed residue of semi-solid traditional fermented alcoholic beverages samples for analysis after the ash has settled and the solutions were clear and 5 mL TISAB were added to obtain a pH of 5.2-5.4, which is the optimal pH for fluoride determination. Reagent blanks were prepared together with the samples and were brought through the whole procedure for blank determination. The purpose of reagent blank is to correct for signals arising from sources other than the analyte and the blank contains no deliberately added analyte, but does contain the same solvent, reagents and chemicals as the other test samples, and is subjected to exactly the same sequence of analytical procedures of the samples. The standard solutions and the sample solutions were then analyzed using fluoride ion selective electrode [69]. The filtrate/clear solution of traditional fermented alcoholic beverages and including water were analyzed directly by adding TISAB and taking the reading from calibrated fluoride ion selective electrode. All the analysis was made in triplicate.

3.5. Instrument calibration

3.5.1. Calibration of fluoride electrode

The fluoride selective electrode was used because of its excellent performance, speed, and general convenience and it has become an important method for determining fluoride in a wide variety of environmental and industrial samples [70, 71]. The selectivity of the electrode is based on the properties of a membrane of sparingly soluble single crystals of lanthanum fluoride. It gives an electrochemical response that is proportional to the logarithm of fluoride ion activity in the sample [71].

The basic practice with ion selective electrodes is to measure electrode potentials (in millivolts, mV) and from that to determine analyte concentrations in solution. This involves measuring potentials in millivolts from two electrical contact points. One is a reference electrode whose potential is constant. The other is a sensing electrode, whose potential varies with analyte concentration. $E_{ISE} - E_{Ref}$ is the net potential measured. The fluoride ion-selective electrode is made of a single crystal of LaF_3 doped with a little EuF_2. The crystal has some fluoride vacancy sites (due to doped EuF_2), allowing a response to external fluoride solution [72].

The calibration standard solutions of concentrations 0.3, 1, 5, 10, and 20 mg/L were prepared by serial dilution of the 1000 mg/L fluoride stock solution. Standard solution was prepared by dissolving 2.21 g of NaF in 1000 mL deionized water An aliquot of 5 mL of each of 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. Adding of TISAB to standards and to samples breaks fluoride complexes of Fe and Al, providing a constant ionic strength [56]. When calibrating, it is assumed that the added TISAB has no effect on the standard concentration [73]. The electrode is calibrated using a series of known concentrations of fluoride. The voltage of each solution measured and this voltage plotted as a function of concentration. The slope of the curve was 57.3 mV/decade which was found in the optimum range (57 to 60 mV/decade). This value of slope was nearest to the theoretical value (59.2 mV/decade at room temperature). The calibration curve equation also showed that the correlation coefficient was 0.9996 which indicated very good correlation between electrode potential and concentration of fluoride.

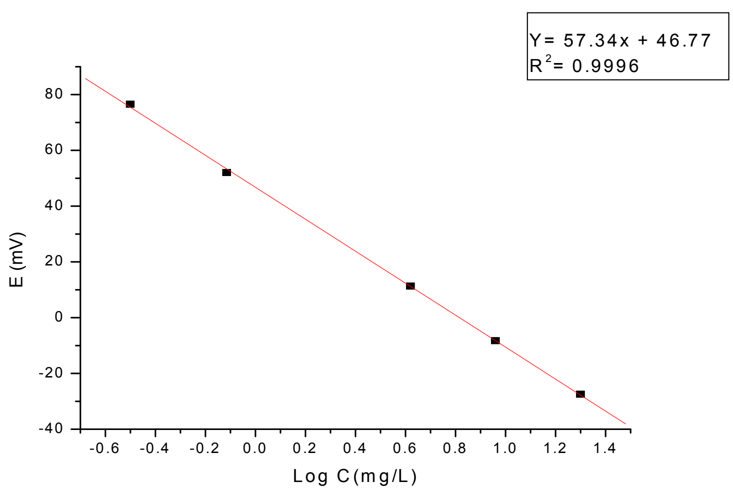

Figure 4. Calibration curve for fluoride determination

The operation of ion selective electrodes is based on the fact that there is a linear relationship between the electrical potential developed between an ISE and a reference electrode immersed in the same solution, and the Logarithm of the concentration (strictly speaking activity or "effective concentration") of the ions in the solution. This relationship is described by the Nernst equation:

$$E = E^0 \pm (2.303RT/ zF) \times \text{LogC}$$

Where: E = the total potential (mv) developed between the sensing and reference electrodes, E^0 = constant characteristic of the particular ISE/reference pair which is the sum of all the liquid junction potentials in the electrochemical cell, 2.303 = the conversion factor from natural to base10 logarithm, R = gas constant (8.314 joules/degree/mole), T = absolute temperature (K), z = absolute value of the charge on the ion, F = Faraday constant (96,500 coulombs per mole), log C = logarithm of the concentration of the measured ion. The slope of electrode response is ± 2.303RT/zF (which shows plot of E (mV) against Log C is a straight line within the concentration range within linear range of the electrode response. This is an important diagnostic characteristic of an ISE. When the ion to which the electrode responds is negative charged the slope becomes negative.

3.6. Method validation

Method validation is the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with what the application requires. The criteria used for evaluating analytical methods are called figures of merit. Based on these characteristics, one can predict whether a method meets the needs of intended purpose. These figures of merit are accuracy, precision, sensitivity, detection limit, and the quantitation limit [75].

3.6.1. Precision and accuracy

Precision is a measure of the random error associated with a series of repeated measurements of the same parameter within a sample. Precision describes the closeness with which multiple analyses of a given sample agree with each other, and is sometimes referred to as reproducibility. Precision is determined by the absolute standard deviation, relative standard deviation, variance, coefficient of variation, relative percent difference, or the absolute range of a series of measurements.

The accuracy of an analytical method is defined as the closeness of agreement between a test result and the accepted reference value. Accuracy may be measured in different ways and the method should be appropriate to the matrix. Accuracy can be expressed as an absolute error or percentages relative error. It can also be expressed as percentage recovery in spiking experiment [76].

3.6.2. Detection limits

Measurements are made at low analyte or property levels, example, in trace analysis, it is important to know what is the lowest concentration of the analyte or property value that can be confidently detected by the method? There are several different types of detection limits depending what is being defined. However, method detection limit (MDL) is the only one designed to be determined in laboratory using chemicals, equipment, and technicians. The other detection limits are either determined by the instrument manufacturer or are calculated from the MDL [77]. Limit of detection (LOD) or detection limit, is the lowest concentration level that can be determined to be statistically different from a blank (99% confidence) [76]. The generally accepted and common definition of method detection limit is the concentration that gives a signal three times the standard deviation of the blank or background signal. In this study, the precision of the results were evaluated by standard deviation of the results of nine measurements triplicate sample preparation and triplicate readings. Instrument detection limit is 0.02 mg/L.

3.6.3. Validation of the procedure

Analytical method can be validated using different techniques: Validation by using alternative method, validation by using proficiency testing schemes or by using certified reference materials [75]. In this work, the method validation was established by spiking experiments. The spiked samples were prepared by adding a small known quantity of fluoride standard solutions. Validation of the method used was checked by performing recovery tests. The percentage recovery of method was evaluated by calculating percentage recovery (% R). Recovery is defined as the ratio of the observed result to the expected result expressed as a percentage [75].
In this study, the procedure was validated by determining fluoride concentration of sample prepared by alkali fusion for semi-solid traditional fermented alcoholic beverages and spiked with known amounts of fluoride. The spiked samples were prepared by adding a known

concentration of the standard fluoride solution to 0.5 g residue of semi-solid traditional fermented alcoholic beverages 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 fluoride standard solution to the clear liquid traditional fermented alcoholic beverages samples by applying similar procedure to prepare the sample and analyzed for the levels of fluoride to calculate the recovery percent. Each traditional fermented alcoholic beverages sample was spiked with fluoride solution of which the fluoride content was equivalent to 25%, 50%, or 100% of the fluoride content of the original (un-spiked) samples. Thus, percent recovery was obtained by comparing the results between the fluoride found and the fluoride added [75]. The recovery tests were also performed for the water samples in triplicates.

4. RESULTS AND DISCUSSION

4.1. Recovery results of fluoride 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 fluoride into the traditional fermented alcoholic beverages samples (Tables 2, 3 and 4).

The percentage recovery of method was evaluated by calculating percentage recovery (% R). Recoveries for traditional fermented alcoholic beverages and water samples were found in the range 91 109% and 90 109% (Tables 2, 3 and 4). These recoveries are within the acceptable range (100±10%) [78].

Table 2. Recovery test results of fluoride determination in the traditional fermented alcoholic beverages of *tella*, *tej* and *areki* samples

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

Table 3. Recovery test results of fluoride ion determination in traditional fermented alcoholic beverages in both the filtrate and the residue of the semi-solid parts of *shamita*, *borde* and *korefe* samples

Sample Site	Types of beverages	Concentration of F^- in un spiked sample (mg/L)	Amount of F^- added (mg/L)	Concentration of F^- in spiked sample (mg/L)	Recovery (%)	Concentration of F^- in un spiked sample (mg/kg)	Amount of F^- added (mg/L)	Concentration of F^- in spiked sample (mg/kg)	Recovery (%)
Deguna Fanigo	*Shamita*	1.13	0.28	1.4 ± 0.05	95 ± 7	7.54	1.89	9.33 ± 0.15	95 ± 8
		1.13	0.57	1.7 ± 0.05	95 ± 9	7.54	3.77	11.4 ± 0.10	102 ±3
		1.13	1.13	2.2 ± 0.02	92 ± 1	7.54	7.54	14.7±0.31	95 ± 4
Deguna Fanigo	*Borde*	1.24	0.31	1.6 ± 0.03	103 ± 9	6.48	1.62	8.07± 0.15	98 ± 9
		1.24	0.62	1.9 ± 0.06	104 ± 9	6.48	3.24	9.50±0.20	93 ± 6
		1.24	1.24	2.6 ±0.02	109 ± 1	6.48	6.48	12.4 ±0.12	92 ± 2
Wogera	*Korefe*	0.41	0.10	0.5 ± 0.01	93 ± 6	3.80	0.95	4.67 ± 0.06	91± 6
		0.41	0.20	0.6 ± 0.02	99 ± 10	3.80	1.90	5.63 ± 0.15	96 ± 8
		0.41	0.41	0.8 ± 0.04	105 ± 9	3.80	3.80	7.33 ± 0.15	93 ± 4

Table 4. Recovery test results of fluoride determination in raw water for preparing traditional fermented beverages samples

Sample site	Types of raw water	Concentration of F^- in un spiked sample (mg/L)	Amount of F^- added (mg/L)	Concentration of F^- in spiked 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
Adami Tulu	*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
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
Wogera	*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

4.2. Levels of fluoride in traditional fermented alcoholic beverages

Fluoride levels found in the traditional fermented alcoholic beverages samples of this study are presented in Tables 5 and 6 and that of the water samples are presented in Table 7. The lowest (0.32 mg/L) and highest (8.19 mg/L) content of fluoride was found the traditional fermented alcoholic beverages of *tella* samples from Debre Markos and from Alem Tena, respectively. The lowest amount (0.30 mg/L) and highest (9.02 mg/L) content of fluoride was found in *tella* water samples from Debre Markos and *areki* water samples from Alem Tena, respectively. The traditional fermented alcoholic beverages sample from Alem Tena, Meki, Adami Tulu and Deguna Fanigo, located within the rift valley, have higher fluoride contents than the sample from Debre Markos, Debre Birhan and Wogera, located within the non-rift valley, which shows the contribution of fluoride from the water. Therefore, fluoride from water and soil contributes to the fluoride content of traditional fermented alcoholic beverages.

Table 5. Average levels (mean ± SD, n = 3, mg/L) of fluoride in traditional fermented alcoholic beverages (*Tella*, *Tej* and *Areki*) from five sample sites

Types of beverages	Sample site		Fluoride level, mean (mg/L) ± SD
	rift valley	Non rift valley	
Tella	Alem Tena		8.19 ± 0.41
	Meki		3.97 ± 0.11
	Adami Tulu		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
	Adami Tulu		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
	Adami Tulu		0.52 ± 0.02

Table 6. Average levels (mean ± SD, n = 9, mg/L and mg/kg) of fluoride in filtrate and residue of semi-solid traditional fermented alcoholic beverages (*shamita*, *borde* and *korefe*) from two sample sites

Types of beverages	Sample site		Filtrate fluoride level, mean (mg/L) ± SD	Residue fluoride level, mean (mg/kg) ± SD	Total fluoride level, (mg/L)
	rift valley	Non rift valley			
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
Korefe		Wogera	0.41 ± 0.01	3.80 ± 0.19	1.39

Table 6 shows the fluoride concentration in beverage as a sum of a "filtrate", i.e. dissolved and "residue", i.e. suspended fluoride. Thus the study shows that the fluoride dissolved in the beverage is only 22-29% of the total fluoride, while the suspended fluoride is the rest, i.e. 78-71 % of the total fluoride. Thus this study clearly showed that the beverage fluoride occurs mainly in the suspended materials of beverage.

This may be due to the fact that fluoride in the beverages varied depending on the type of ingredients used, the amount of ingredients added, fluoride content of ingredients, and fluoride 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 fluoride compared to the fluoride content of residues. Furthermore, the traditional fermented beverages (semi-solid beverages) contain higher concentration of polyphenols[79], most of which are insoluble and remain as suspended fine particles, may adsorb fluoride ions from the soluble parts of the beverages resulting in higher concentration of fluoride in the residues than in the filtrate of the beverages. It should also be noted that suspended fine solid particles have natural tendency to adsorb ions from bulk solution.

Table 7. Average levels (mean ± SD, n = 3, mg/L) of fluoride in raw water (*tella, tej*, *areki*, *shamita*, *borde* and *korefe water*) from seven sample sites

Types of raw water	Sample site		Fluoride level, mean (mg/L) ± SD
	rift valley	Non rift valley	
	Alem Tena		5.06 ± 0.19
	Meki		3.46 ± 0.11
	Adami Tulu		1.56 ± 0.01
Tella water		Debre Birhan	0.31± 0.01
		Debre Markos	0.30 ± 0.01
	Alem Tena		5.06 ± 0.08
	Meki		3.50 ± 0.08
	Adami Tulu		1.78 ± 0.03
Tej water		Debre Birhan	0.37 ± 0.01
		Debre Markos	0.31 ± 0.01
	Alem Tena		9.02 ± 0.15
Areki water	Meki		4.07 ± 0.16
	Adamitulu		2.09 ± 0.04
Shamita water	Deguna		0.78 ± 0.01
	Fanigo		
Borde water	Deguna		0.92 ± 0.03
	Fanigo		
Korefe water		Wogera	0.35 ± 0.03

4.3. 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 sample. ANOVA uses the F statistic to compare whether the differences between sample means are significant or not [81].

In this study, *tella* and *tej* samples were collected from five different areas and *areki* samples were collected from three different areas. The fluoride level of each sample was determined by 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 sample mean of the analyte was tested by using ANOVA, whether the source for variation was from experimental procedure or heterogeneity among the samples (i.e. the difference in topographical location, difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc. or other variations in brewing process between groups of samples). The ANOVA results (Table 8) showed that there exist statistically significant differences at 95% confidence level in mean concentrations of all the three types of traditional fermented alcoholic beverages.

Table 8. Analysis of variance (ANOVA) between and within in traditional fermented alcoholic beverages samples at 95% confidence level

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

Where, df - is degree of freedom between sample and within sample.

4.4. Pearson correlation of fluoride in traditional fermented alcoholic beverages and water sample

In this study, to correlate the fluoride concentration of water sample to that of traditional fermented alcoholic beverages, the Pearson correlation matrices using correlation coefficient (r) for the samples were used.

Table 9. Pearson correlation coefficient of fluoride in traditional fermented alcoholic beverages with water sample (n = 5, for *tella* and *tej* samples and n = 3, for *areki* samples)

Sample type	*Tella*	*Tej*	*Areki*
R	0.9806	0.9955	0.9986

Where, r is the Pearson correlation coefficient between fluoride level in traditional fermented alcoholic beverages and fluoride level in water.

As shown in Table 9, one can see that the higher the levels of fluoride in the water, the higher the levels of corresponding fluoride in the traditional fermented alcoholic beverages. This verifies that the dependence of fluoride concentration in the traditional fermented alcoholic beverages on fluoride concentration of respective water.

4.5. Daily intake of fluoride by an adult person through traditional fermented alcoholic beverages

Total fluoride intake (TFI) is the summation of the daily fluoride intake through entire beverage sources. Daily intake of fluoride (DIF) from the particular beverages was calculated by multiplying the fluoride concentration in a particular beverage item with total quantity of that particular item consumed per person per day. The total fluoride intake was calculated as follows: $TFI = \sum (DIF)$ and $DIF = FC \times QF$. Where: FC is the fluoride concentration in the beverages source; and QF is the quantity of the particular beverages item taken per person per day. The quantity of a particular beverages item taken per person per day has been calculated by multiplying the amount of beverages item taken in a day with frequency of consumption in a week and divided by the number of days in a week.

The percentage of recommended daily intake [81, 82] of fluoride (% RDIF) from a particular beverage was calculated as: % RDIF = (DIF/RDIF) x 100. According to European Food Safety Authority [81] and National Academy of Sciences Institute of Medicine [82], the recommended daily intake of fluoride (RDIF) for adults having 60 70 kg body weight is usually within the range of 3 4 mg/kg [81, 82]. It indicates that the daily intake of 3 4 mg F/kg body weight is tolerable but not necessary, while daily intake of fluoride beyond this limit is harmful to human health. In this study, the average amount of beverages consumed by adults is assumed and daily intake of fluoride by adults from beverages is predicted and presented in Table 10.

In this study, fluoride intake through traditional fermented alcoholic beverages for an adult regular drinker of 60 kg weight was found in the range 0.004 mg/day (*areki*) to 3.28 mg/day (*tella*). The average contribution of *tella*, *tej*, *areki*, *shamita*, and *borde* from rift valley area was 63.0%, 26.8%, 0.21%, 31.0%, and 29.5% and the average contribution *tella*, *tej* and *korefe* from the non-rift valley area was 4.34%, 2.47%, 6.62% fluoride intake of adult regular drinker per day, respectively. For an adult regular drinker who consumes all types of beverages from rift valley area (Table 10) daily, the person may ingest 4.56 mg/day or 150% and from non-rift valley area and the person may ingest 0.405 mg/day or 13.4% of fluoride from beverages alone. An adequate/recommended daily intake level of fluoride was 3 mg/day that means the rift valley area of 4.56 mg/day of fluoride is above the recommended fluoride level and the non-rift valley area of 0.405 mg/day below the recommended fluoride level.

Table 10. Expected daily fluoride intake through consumption of different types of traditional alcoholic beverages

Types of beverages	Sample site		Average amount of traditional fermented alcoholic beverages consumed by an adult (L/week)	Average amount of traditional fermented alcoholic beverages consumed (L/day)	Concentration of fluoride in traditional fermented alcoholic beverages (mg/L)	Fluoride intake through traditional fermented alcoholic beverages (mg/day)	% Relative daily intake of fluoride
	rift valley	Non rift valley					
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
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

4.6. Comparison of fluoride levels of this study with literature values

The comparative study of the fluoride concentration in traditional fermented alcoholic beverages samples determined in this study and those reported in the literature are presented in Table 11. The fluoride levels of Ethiopian traditional fermented alcoholic beverages of *tella* (0.32-8.19

mg/L) samples is a bit lower than that of reported by Macdonald Partners (10 mg/L) [83] and almost comparable to that of reported by Tekle-Haimanot and Haile (1.60-8.50 mg/L) [41]. The level of fluoride in beverages of *tej* (0.35-5.76 mg/L) samples of this study is comparable to that of reported by Tekle-Haimanot and Haile (1.30-1.40 mg/L) [41] but much lower than that of reported by Macdonald Partners (13 mg/L) [83]. The level of fluoride in beverages of *areki* (0.52-0.97 mg/L) samples of this study is comparable to that of reported by Macdonald Partners (0.6 mg/L) [83]. The possible reasons of the different levels of fluoride in the traditional fermented alcoholic beverages samples might be due to the difference in topographical location, difference in mineral contents of soil, water, atmosphere; variation in application of agrochemicals like fertilizers, pesticides, herbicides, etc. or other variations in brewing process between groups of samples. There is no any reported literature values of fluoride in traditional fermented alcoholic beverages (*shamita*, *borde* and *korefe*) samples, therefore, the levels of fluoride in these beverages found in this study were not compared with any reported fluoride levels.

Table 11. Comparison of fluoride levels in traditional fermented alcoholic beverages with previously reported values

Types of beverages	Sample site	Raw water F (mg/L)	Alcoholic drink F (mg/L and mg/kg)	Reference
	Alem Tena	5.06	8.19	Present study
	Meki	3.46	3.97	Present study
	Adami Tulu	1.56	2.02	Present study
	Debre Birhan	0.31	0.33	Present study
	Debre Markos	0.30	0.32	Present study
	Wonji Gefersa	1.20	2.40	[41]
Tella	Wonji/Shoa Alem Tena	1.10	3.00	[41]
	Wonji/Shoa plantation	0.50	1.60	[41]
	Sami Berta	8.00	8.50	[41]
	Wonji Gefersa	9.20	10.0	[83]
	Metehara	1.30	1.50	[41]
	Alem Tena	5.06	5.76	Present study
	Meki	3.50	3.84	Present study
	Adami Tulu	1.78	2.46	Present study
	Debre Birhan	0.37	0.39	Present study
Tej	Debre Markos	0.31	0.35	Present study
	Wonji Gefersa	1.20	1.30	[41]
	Wonji/Shoa Alem Tena	1.10	1.40	[41]
	Wonji Gefersa	9.20	13.0	[83]
	Alem Tena	9.02	0.97	Present study
	Meki	4.07	0.67	Present study
Areki	Adami Tulu	2.09	0.52	Present study
	Wonji Gefersa	9.20	0.60	[83]
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

5. CONCLUSION

In this study the levels of fluoride were determined in the traditional fermented alcoholic beverages which are prepared and consumed in Ethiopia and found in the range of 0.32–8.19 mg/L. Fluoride levels in beverages from rift valley areas were higher than those from outside the rift valley areas. The estimated daily intake of fluoride through beverages by the people who drink regularly was found in the range 0.004–3.28 mg/day. The beverages prepared using low concentration of fluoride water is safe for human consumption while beverages prepared using high concentration of fluoride containing water may cause dental as well as skeletal fluorosis. Analysis of variance showed that there was statistically significant difference at 95% confidence level in the mean levels of fluoride among the beverages samples. Pearson correlation showed that the levels of fluoride in the traditional fermented alcoholic beverages were found to correlate positively with the levels found in the water.

6. REFERENCES

1. Elaine M, Danilo M. Traditional fermented food and beverage for improved livelihoods. Rome: *Food and Agriculture Organization of the United Nations*; **2012**.
2. Alcoholic Beverages. The Free Encyclopedia. Available at: http://www.en.wikipedia. Org/wiki/alcoholic beverages. Accessed 22 April **2016**.
3. Steinkraus KH (Ed.). *Handbook of indigenous fermented foods*. New York: Marcel Dekker, Inc.; **1983**. pp 1-58.
4. Lee M, Regu M, Seleshe S. Uniqueness of Ethiopian traditional alcoholic beverage of plant origin, tella. *J Ethnic Foods*, **2015**; 2(3): 110-114.
5. Malde MK, Zerihun L, Julshamn K, Bjorvatn K. Fluoride, calcium and magnesium intake in children living in a high-fluoride area in Ethiopia. Intake through food. *Int J Paed Dent*, **2004**; 14: 167-174.
6. Zerabruk S, Chandravanshi BS, Zewge F. Fluoride in black and green tea (*Camellia sinensis*) infusions in Ethiopia: measurement and safety evaluation. *Bull Chem Soc Ethiop*, **2010**; 24: 327-338.
7. Malde MK, Scheidegger R, Julshamn K, Bader H-P. Substance flow analysis: a case study for fluoride exposure through food and beverages in young children living in Ethiopia. *Environ Health Perspect*, **2011**; 119: 579-584.
8. Tegegne B, Chandravanshi BS, Zewge F. Fluoride levels in commercially available rice in Ethiopia. *Bull Chem Soc Ethiop*, **2013**; 27: 179-189.
9. Dessalegne M, Zewge F. Daily dietary fluoride intake in rural villages of the Ethiopian Rift Valley. *Toxicol Environ Chem*, **2013**; 95: 1056-1068.
10. Embiale A, Chandravanshi BS, Zewge F. 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*, **2014**; 10: 447-455.
11. Asayehegn G, Chandravanshi BS, Zewge F. Fluoride levels in *tef* [*Eragrostis tef* (Zucc.) Trotter] and *enjera* and health implications. *SINET Ethiop J Sci*, **2014**; 37: 53-62.
12. Mustofa S, Chandravanshi BS, Zewge F. Levels of fluoride in staple cereals and legumes produced in selected areas of Ethiopia. *SINET Ethiop J Sci*, **2014**; 37: 43-52.

13. Syume M, Chandravanshi BS. Levels of fluoride in niger seed (*Guizotia abyssinica*) cultivated in Ethiopia and Eritrea. *Fluoride*, **2015**; 49(3): 259-265.
14. Nigus K, Chandravanshi BS. Levels of fluoride in widely used traditional Ethiopian spices. *Fluoride*, **2016**; 49(2): 165-177.
15. Sokół-Łętowska A, Kucharska AZ, Wińska K, Szumny A, Nawirska-Olszańska A, Mizgier P, Wyspiańska D. Composition and antioxidant activity of red fruit liqueurs. *Food Chem*, **2014**; 157: 533-539.
16. Selinus R. The traditional foods of the central Ethiopian highlands. Scandinavian Institute of African Studies, Research Report No. 7, **1971**. Available at: http://ethnomed.org/ethnomed/cultures/ethiop/ethiop_foods.html, accessed 29 March 2004.
17. Debebe G. Determination of ethanol level in beverages. Master Thesis, Addis Ababa University, Addis Ababa, Ethiopia, **2006**.
18. Berhanu A. Microbial profile of tella and the role of gesho (*Rhamnus prinoides*) as bittering and antimicrobial agent in traditional tella (beer) production. *Int Food Res J*, **2014**; 21(1): 357-365.
19. Sahle S, Gashe BA. The microbiology of *tella* fermentation. SINET: *Ethiop J Sci*, **1991**; 14: 81-92.
20. Fite A, Tadesse A, Urga K, Seyoum E. Methanol, fuel oil and ethanol contents of some Ethiopian traditional alcoholic beverages. SINET: *Ethiop J Sci*, **1991**; 14: 19-27.
21. Yohannes T, Melak F, Siraj K. Preparation and physicochemical analysis of some Ethiopian traditional alcoholic beverages. *Afr J Food Sci*, **2013**; 7: 399-403.
22. Bekele B, Tetemike M, Mogessie A. Chemical and nutritional properties of '*tej*', an indigenous Ethiopian honey wine: variations within and between production units. *J Food Technol Africa*, **2001**; 6(3): 104-108.
23. Desta B. A survey of the alcoholic contents of traditional beverages. *Ethiop Med J*, **1977**; 15: 65-68.
24. Bahiru B. Chemical and nutritional properties of '*tej*', an indigenous Ethiopian honey wine: variations within and between production units. M.Sc. Thesis, Addis Ababa University, Ethiopia, **2000**.
25. World Health Organization: Global Status Report on alcohol. Available ~~from~~ at: ~~(~~www.who.int/substance_abuse/publications/lobal_status_report_2004_overview.pdf.

Accessed 23 May 2006~~)~~.

26. Bahiru B, Mehari T, Ashenafi M. Chemical and nutritional properties of tej, an indigenous Ethiopian honey wine: variations within and between production units. *J Food Technol Africa*, **2001**; 6: 104-108.
27. Ashenafi M. A review on the microbiology of indigenous fermented foods and beverages of Ethiopia. *Ethiop J Biol Sci*, **2006**; 5(2): 189-245.
28. Bacha K, Mehari T, Ashenafi M. Microbial dynamics of 'borde' fermentation, a traditional Ethiopian fermented beverage. SINET: *Ethiop J Sci*, **1998**; 21: 195-205.
29. Bacha K, Mehari T, Ashenafi M. Microbiology of the fermentation of shamita, a traditional Ethiopian fermented beverage. SINET: *Ethiop J Sci*, **1999**; 22: 113-126.
30. Solange A, Georgette K, Gilbert F, Koffi Marcellin D, Bassirou B. Review on African traditional cereal beverages. *Am J Res Commun*, **2014**; 2(5): 103-153.
31. Kebede A. Isolation, characterization and identification of lactic acid bacteria involved in traditional fermentation of *borde*, an Ethiopian cereal beverage. *Afr J Biotechnol*, **2007**; 6(12): 1469-1478.
32. Balcerzak M, Janiszewska J. Fluorides in tea products and analytical problems with their determination. *Crit Rev Anal Chem*, **2013**; 43 (3): 138-147.
33. Housecroft CE, Sharpe AG. Inorganic chemistry. England: *Pearson Prentice Hall*, **2008**.
34. Fawell J, Bailey K, Chilton J, Dahi E, Fewtrell L, Magara Y. *Fluoride in drinking water*. London: World Health Organization and IWA Publishing, Inc.; **2006**. pp. 15-35.
35. Scientific Committee on Health and Environmental Risks (SCHER). Opinion of critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water. Brussels, Belgium: Directorate General for Health and Consumers, European Commission; **2011** May 16. pp. 2-4.
36. World Health Organization. Fluorides: environmental health criteria 227. Geneva: World Health Organization (Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals); **2002**. Available at: http://www.inchem.org/documents/ehc/ehc/ehc227.htm#1.5. Accessed 10 April 2016
37. Emanuel Š, Jiří B. Determination of fluoride in spices using microwave induced oxygen

combustion. *Acta Veterinary Brno*, **2012**; 81: 319-323.

38. World Health Organization. *Guidelines for drinking-water quality: third edition incorporating the first and second agenda*, Volume 1 Recommendations. Geneva: World Health Organization; **2008**.
39. Tekle-Haimanot R. Study of fluoride and fluorosis in Ethiopia with recommendations on appropriate defluoridation technologies. Addis Ababa, Ethiopia: A UNICEF Sponsored Consultancy; **2005**.
40. Tekle-Haimanot R, Melaku Z, Kloos H, Reimann C, Fantaye W, Zerihun L, Bjorvatn K. The geographic distribution of fluoride in surface and groundwater in Ethiopia with an emphasis on the rift valley. *Sci Total Environ*, **2006**; 367: 182-190.
41. Tekle-Haimanot R, Haile G. Chronic alcohol consumption and the development of Skeletal fluorosis in a fluoride endemic area of the Ethiopian Rift Valley. *J Water Resource Protection*, **2014**; 6: 149-155.
42. Hem, JD. Study and interpretation of the chemical characteristics of natural water. Water supply paper 2254, 3rd edition, *US Geological Survey*, Washington,**1989**; pp 263.
43. Tebutt THY. *Relationship between natural water quality and health*. United Nations Educational, Scientific and Cultural organization, Paris, **1983**.
44. Murray JJ. *Appropriate use of fluorides for human health*, Geneva: World Health Organization, **1986**.
45. Edmunds WM, Smedley PL. Ground water geochemistry and health: an overview. In: Appleton JD, Fuge R and McCall GJH (Eds) Environmental geochemistry and health. *Geological Society Special Publication*, **1996**; 113: 91–105.
46. USNRC. Health effects of ingested fluoride. National Research Council, Washington DC: National, *Academy Press*, **1993**.
47. Gebrekidan M, Zerabruk S. Concentration of heavy metals in drinking water from urban areas of the Tigray Region. *MEJS*, Northern Ethiopia, **2011**; 3(1): 105-121.
48. Facts S. Source document Level2-Details on Climate Change, **2007**; 1-40.
49. Taves DR. Dietary intake of fluoride ashed (total fluoride) v. unashed (inorganic fluoride) analysis of individual foods. *Br J Nutr*, **1983**; 49(3): 295-301.
50. Cao J, Zhao Y, Liu J, Xirao R, Danzeng S, Daji D, Yan Y. Brick tea fluoride as a main source of adult fluorosis. *Food Chem Toxicol*, **2003**; 41(4): 535-542.

51. WHO. *Trace elements in human nutrition and health. Geneva*: World Health Organization, **1996**.
52. IPCS. Fluorides. Environmental Health Criteria 227. International Programme on Chemical Safety. Geneva: World Health Organization, **2002**.
53. Whitford GM. The metabolism and toxicity of fluoride. 2nd rev. ed. Monographs in oral science. Basel, Switzerland: Karger; **1996**.
54. Hong L, Levy SM, Warren JJ, Dawson DV, Bergus GR, Wefel JS. Association of amoxicillin use during early childhood with developmental tooth enamel defects. *Arch Pediatr Adolesc Med*, **2005**; 159(10): 943-948.
55. Hong L, Levy SM, Broffitt B, Warren JJ, Kanellis MJ, Wafel JS, Dawson DV. Timing of fluoride intake in relation to development of fluorosis on maxillary central incisors. *Community Dent Oral Epidemiol*, **2006**; 34(4): 299-309.
56. Horowitz HS. Indexes for measuring dental fluorosis. *J Public Health Dent*, **1986**; 46(4): 179 - 183.
57. National Research Council of the National Academies. Fluoride in drinking water: A Scientific Review of EPA's standards. Washington, D.C.: *The National Academy Press*, **2006**.
58. Scientific Committee on Health and Environmental Risks (SCHER) European Commission.*Critical review of any new evidence on the hazard profile, health effects, and human exposure to fluoride and the fluoridating agents of drinking water*, the 7th Plenary, Europe, **2010**.
59. Fluoride Pollution of Groundwater, Discussion Paper, New Delhi, India.
60. Meenakshi, Maheshwar RC. Fluoride in drinking water and its removal. *J Hazard Mater*, **2006**; 137(1): 456–463.
61. Hach, Co. DR 100 Colorimeter Manual. Loveland, Colorado, **1983** pp 3-21.
62. Gall E, Dennis J, Raymond A. The use of a colorimeter in analyzing the fluoride content of public well water. *The American Academy of Pediatric Dentistry*, **1987**, 9(3), 204-207.
63. Carolyn A, Tylenda DMD, Dennis J, Lisa I, Gloria S, Lara C. Toxicological profile for fluorides, hydrogen fluoride, and fluorine. *Public Health Service Agency for Toxic Substances and Disease Registry*, USA, **2003**.
64. Akibode S, Maredia M. Global and Regional Trends in Production, Trade and

Consumption of Food Legume Crops. Department of Agricultural, Food and Resource Economics Michigan State University, Report Submitted to SPIA March 27, **2011**, pp 1-50.

65. Campbell AD. Determination of fluoride in various matrices. *Pure and Applied Chemistry*, **1987**, 59 (5), 695-702.
66. Bratovčić A, Odobašić A, Ćatić S. The advantages of the use of ion-selective potentiometry in relation to UV/VIS Spectroscopy. *Agriculturae Conspectus Scientificus*, **2009**, 74 (3), 139 142.
67. Danielle R, Gary M. Laboratory Procedure Manual. Georgia Regents University, *Fluoride in water*, 2014.
68. Malde MK, MÍge A, Julshamn K, Macha E, Bjorvatn K. Fluoride content in selected food items from five areas in East-Africa. *J Food Comp Anal*, **1997**; 10: 233-45.
69. Malde MK, Bjorvatn K, Julshamn K. Determination of fluoride in food by the use of alkali fusion and fluoride ion-selective electrode. *Food Chem*, **2001**; 73: 373-379.
70. Jacobson JS, Weinstein LH. Sampling and analysis of fluoride: Methods for ambient air, plant and animal tissues, water, soil and foods. *J Occup Med*, **1977**; 19: 79-87.
71. Frant MS, Ross JW. *Electrode for sensing fluoride ion activity in solution.Science,* **1966**; 154: 1553-1554
72. Ghirma M. Manual for fluoride measurements in water, tooth paste and tea infusion, *ChemTest Consulting*, Addis Ababa, **2009**.
73. Buck PR, Cosofret VS. Recommended procedures for calibration of ion selective electrodes (technical report). *Pure Appl Chem*,**1993**; 65(8): 1849-1858.
74. Atlabachew M, Chandravanshi BS, Zewge F, Redi M. Fluoride content of Ethiopian khat (*Catha edulis* Forsk) chewing leaves. *Toxicol Environ Chem*, **2011**; 93: 32-43.
75. Boqué R, Maroto A, Jordi Riu J, Rius FX. *Validation of analytical methods*. Rovirai Virgili University- Tarragona, Spain, **2002**, 53: 128-143.
76. Jeffrey R. Analytical detection limit guidance and laboratory guide for determining method detection limits, Wisconsin: *Wisconsin Department of Natural Resources Laboratory Certification Program*, **1996**.
77. Tim L. Method Detection Limits, Lagoon System in Maine, **2003**. Available at: http://www.lagoonsonline.com/laboratory-articles/mdl.htm. Accessed 22 April 2013.
78. Harris DC. *Quantitative Chemical Analysis*, 4th ed., Freeman and Company: New York;

1982.

79. Debebe A, Chandravanshi BS, Redi-Abshiro M. Total contents of phenolics, flavonoids, tannins and antioxidant capacity of selected traditional Ethiopian alcoholic beverages. *Bull Chem Soc Ethiop*, **2016**;30:27-37.
80. Miller JN, Miller JC. Statistics and chemo metrics for analytical chemistry. 4th ed. Harlow, England: *Prentice Hall, an imprint of Pearson Education Limited*, **2000**. pp 107-147.
81. European Food Safety Authority. Scientific Opinion on Dietary Reference Values for Fluoride. *EFSA Journal,* **2013**;11:3332. doi:10.2903/j.efsa.2013.3332.
82. Academy of Nutrition and Dietetics. The impact of fluoride on health. *J Acad Nutr Diet,* **2012**; 112:1443-53.
83. Macdonald Partners. *Final Report to the Ethiopian Sugar Corporation*, "Supply of Reduced Fluoride Potable Water to Wonji/Shoa and Metehara Sugar Estates,".Wonji/Show, Ethiopia; **1985**.