ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

LEVELS OF ESSENTIAL ELEMENTS IN THREE TEF

[*ERAGROSTIS TEF(ZUCC.*)TROTTER] VARIETIES.

A GRADUATE PROJECT SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

Zeleke Kebede

July 2009

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Advisor: Merid Tessema (Ph.D)

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Dedicated

To

My mother- Bijige Waqjira

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List of Abbreviations

C_4-plants	Plants whose primary product is a 4-carbon molecule
DZARC	Debrezeit Agricultural Research Center
EHNRI	Ethiopian Health and Nutrition Research Institute
%RSD	percentage relative standard deviation
MDL	Method Detection Limit
MQL	Method Quantitation Limit
PMT	Photomultiplier tube
RDA	Recommended dietary Allowance
$\mu g / g$	Micro gram per gram
mg/L	milligram per liter
ppm	Parts Per million .
S_{blank}	Standard deviation of the blank
CSA	Central Statistics Authority
%R	Percentage of Recovery
Psi	pound per square inch
SD	Standard deviation
DL	Instrument Detection Limit
Lc	lamp current
CL	Confidence Level
nm	Nanometer
λ	Wave length
R	Correlation coefficient

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LEVELS OF ESSENTIAL METALS IN THREE TEF [*ERAGROSTIES TEF* (ZUCC.) TROTTER] VARIETIES.

By: Zeleke Kebede

Advisor: Merid Tessema (Ph.D)

Abstract.

Tef, [*Eragrostis tef*(zucc.)Trotter] is an indigenous crop plant in Ethiopia. It provides the most staple food-*Enjera* for most Ethiopians. An efficient wet digestion procedure with the percentage recovery ranges from 85.66 to 105.22% (%R $\pm$ SD) was developed for tef flour sample using standard addition (spiking) method. The optimal digestion procedure allowed the use of acids with minimum volume leading to reduced blank contamination, and lower detection limit. The levels of nine essential elements (Na, K, Mg, Ca, Mn, Co, Cu, Zn &Fe) were determined by FAAS applying four point calibration curve in the three most common and commercially available tef types (Red, Mixed and White tef) collected from four mill houses in Arada sub city of Addis Ababa. The observed average metal concentration in red, mixed and white tef respectively are:Na(22.06 $\pm$ 1.46,25.68 $\pm$ 2.05,22.89 $\pm$ 1.42), K (215.81 $\pm$ 8.13, 219.23 $\pm$ 8.35, 205.59 $\pm$ 7.17), Mg (161.91 $\pm$ 7.76, 173.7 $\pm$ 8.89, 153.16 $\pm$ 9.45), Ca (178.54 $\pm$ 9.5, 168.64 $\pm$ 11.03, 180.7 $\pm$ 14.65), Mn (22.36 $\pm$ 0.15, 13.26 $\pm$ 0.029, 4.84 $\pm$ 0.044), Co (6.20 $\pm$ 5.38, 5.21 $\pm$ 0.29, 7.89 $\pm$ 0.75), Cu (2.51 $\pm$ 0.30, 3.79 $\pm$ 0.1, 1.08 $\pm$ 0.075), Zn (4.79 $\pm$ 10.7, 3.79 $\pm$ 0.1, 2.98 $\pm$ 0.12), and Fe (24.62 $\pm$ 1.07,20.05 $\pm$ 1.4, 15.95 $\pm$ 1.7) mg/100g of dry weight. A statistical analysis of variance (ANOVA) using excel and SPSS soft ware revealed that there is a significant difference between the mineral content of the three tef types at 95% ($p \leq 0.05$) CL. Tukey pair wise comparisons also confirmed this difference in mineral contents of the samples. Red tef has higher essential metal contents followed by mixed tef. Further more, all the three-tef types considered are rich in their mineral contents compared to maize, barely, sorghum, wheat, kocho and bulla, hence tef could be suitable sources of dietary minerals.

Key words: Tef, *Eragrostis tef,* red tef, mixed tef , white tef, essential elements, Flame Atomic Absorption spectrophotometer.

1. Introduction

In Ethiopia, Agriculture is characterized by diverse farming practices. Farmers with various ethnic background and cultural diversity living in the country's diverse agro-ecological zones have developed farming systems, characterized by a high degree of species diversity of crops, like tef, maize, Sorghum, and other cereal.

Tef, [*Eragrostis tef* (Zucc.) Trotter] is a C_4^* tropical cereal [1, 2] and is a major food grown in Ethiopia Tef belongs to the grass family poecae (Gramineae), genus *Eragrostis*. It is indigenous to Ethiopia in its origin. Tef is believed to have originated in Ethiopia between 4000 and 1000 BC Vavilov (1951) in [1]. It is a grain crop solely produced in this country for human consumption purpose. There are several varieties of Tef, each with characteristics best suited to specific conditions. In general, there are three main types of Tef: white, red and brown (mixed). Tef is a fine stemmed, tufted annual grass characterized by a large crown, many shoots, and a shallow diverse root system (Figure 1.)

Figure 1: Tef plant

Tef is grown in almost all regions of Ethiopia since it is the preferred grain for local consumption and for market because it fetches the highest grain price compared with other cereals [1]. Nationally, tef ranks first in total cropland and quantity of produce among other cereals. The area devoted to tef cultivation and its productivity is increasing from year to year. In 2003-2004, it occupied about 2 million hectare (ha) which accounts for 28.5% of the total cereal crops grown in the country. However, in 2006/07 main cropping season, the total land allocated for tef production and yield obtained per hectare was 2.41 million hectare of land and 1.0414 ton/ha of grain yield respectively [3]. Amhara and Oromia have the largest acreage of tef followed by Southern Nations and nationalities and peoples region and Tigray [3]. The primary use of tef grain is for grinding into flour to make Enjera (a fermented, pancake-like, soft, sour, circular flatbread), a major food staple in Ethiopia.

Nutritionally tef contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine. Lysine is the amino acid that is most often deficient in other grain foods [4]. Tef contains more lysine than barley, millet, and wheat and slightly less than rice or oats. Tef has higher iron content compared with other cereals [5]. Tef is also an excellent source of fiber and iron, and has many times the amount of calcium, potassium and other essential minerals found in an equal amount of other grains.

Marketwise, urban dwellers have very high demand to consume tef. These demand made its price level higher than other cereals. As a result, tef price is reasonably stable (no price depression). Moreover, tef can be stored for long without quality deterioration if a holder expects upward price movement.

Tef grain, owing to its high mineral content, has started to be used in mixtures with soyabean, chickpea and other grains in the baby food industry [6]. Non-tef consumers have a lower level of hemoglobin, and hookworm anemia develops in non-tef eaters if they are infested with hookworm. On the other hand, since tef eaters have higher levels of hemoglobin in their blood, they do not suffer from hookworm anemia even when infested. Malaria is also frequently ‘found in the groups with lower hemoglobin levels. Moreover, consuming tef prevents the anemia related to pregnancy [12].

Minerals are inorganic substances found in the body that function in conjunctions with enzymes, hormones, vitamins and other compounds. For this reason, to establish recent data on its essential mineral contents, the investigation of essential elements in tef is necessary. Different researchers [5, 7-14] determined the mineral content of tef using different methods. Most of them [5, 7, 9-12] reported the iron content of tef where as others [5, 7, 8, 13] reported Iron, Calcium and Zinc content of tef (cf.section 1.11].

Since no mineral can function singly, the presence or absence of other mineral can influence the bioavailability of iron. That is, minerals have synergistic effect up on their function in the body. Therefore, the present investigation was designed to assess the level of nine essential elements including the already reported ones.

1.1 Origin and Distribution of Tef

Tef, [*Eragrostis tef* (Zucc.) Trotter] is a C_4 tropical cereal [1] and is a major food grain in Ethiopia but is a minor cereal crop worldwide. It is indigenous to Ethiopia in its origin. Teff is endemic to Ethiopia and its major diversity is found only in this country. As with several other crops, the exact date and location for the domestication of teff is unknown. However, there is no doubt that it is a very ancient crop in Ethiopia, where domestication took place far before the birth of Christ [1]. It is believed to have originated in Ethiopia between 4000 and 1000 BC, Vovilov (1951) in [1]. Haudricourt (1941) in [1] suggested that the word tef might have been derived from the Semitic *thaf* applied in Yemen to a wild harvested cereal. Nowadays, tef is grown in almost all regions of Ethiopia since it is the preferred grain for local consumption and for market because it fetches the highest grain price compared with other cereals [1].

Nationally, tef ranks first in total cropland and quantity of produce among other cereals. The area devoted to tef cultivation and its productivity is increasing from year to year. In 2003-2004, it occupied about 2 million hectares (ha) which accounts for 28.5% of the total cereal crops grown in the country. But in 2006/07 main cropping season, the total land allocated for tef production and yield obtained per hectare was 2.41 million hectare of land and 1.0414 ton/ha of grain yield respectively [3].

Regionally, Amhara (778,202 ha) and Oromia (762,119.72 ha) have the largest acreage of tef followed by Southern Nations and nationalities and peoples region (133,882 ha) and Tigray (124,698.64 ha) [3, 15]. The primary use of tef grain is for grinding into flour to make Enjera (a fermented, pancake-like, soft, sour, circular flatbread), a major food staple in Ethiopia.

Tef has been introduced to different parts of the world through various institutions and individuals. The Royal Botanic Gardens, Kew, imported seed from Ethiopia in 1866 and distributed it to India, Australia, the USA and South Africa. According to Tadesse [12] Burt Davy in 1916 introduced tef to California (USA), Malawi, Zaire, India, Sri Lanka, Australia, New Zealand and Argentina; Skyes in 1911 introduced it to Zimbabwe, Mozambique, Kenya, Uganda, Tanzania; Horuitz in 1940 to Palestine.

1.2 Major Climatic conditions for Tef growth in Ethiopia

Tef is adapted to a wide range of environments and is presently cultivated under diverse agro-ecological conditions. It can be grown from sea level up to 2800 meters above sea level under various rainfalls, temperatures, and soil regimes. However, the most suitable condition for excellent tef grow is at an altitude of 1800 to 2100 m, annual rain fall of 750 to 850 mm, growing season rain fall of 450 to 550 mm, and the temperature range of 10 to 27^0C. This almost matches most closely with altitudes in the highland areas of Ethiopia [1]. The ability of tef to perform well on both water logged Vertisols in the highlands as well as water-stressed areas in the semi-arid regions throughout the country is one of the reasons for which tef is preferred over other grain crops such as maize or barley [16]. In addition, the plants germinate quickly and are adapted to environments ranging from drought stress to water logged soil conditions. Tef generally suffers less from biotic stresses compared to most other cereal crops grown in Ethiopia [17]. It is a reliable low risk crop

1.3 Taxonomy and Vernacular Name of Tef

Taxonomically, tef *[Eragrostis tef* (Zucc.) Trotter], belongs to the Kingdom –*plantae*, order – *Poales*, grass family- *poaceae* subfamily – *eragrostidae*, tribe- *eragrosteae*, genus - *eragrostis* and species - *E.tef*. The genus *Eragrostis* includes about 300 species but only a few are of significant agricultural value [18, 19]. The common vernacular name of the crop in Ethiopia is 'tef' in Amharic,*'taafi'* in Afaan Oromoo and '*taf'* in Tigrigna.

1.4 Types of Tef

There are several varieties of Tef, each with characteristics best suited to specific conditions. It is not in the scope of this paper to discuss the details about all the different varieties of this grain. Although there is no unifying classification of the tef type through out the country, in general, there are three main types of tef: white, red and mixed(sergegna). Tef is the smallest grain in the world (Figure 2).

White tef is the preferred type but only grows in certain regions of Ethiopia. White tef grows only in the Highlands of Ethiopia, requires the most rigorous growing conditions, and is the most expensive form of tef. The prestige associated with consuming white tef, as well as its more stringent growing conditions, contributes to the increased cost of white tef. The shelf life of Enjera is extended with the use of white tef [20].

Red tef is the least expensive form and the least preferred type, has the highest iron content [5]. In persons living in areas of the country where consumption of red tef is most prevalent, hemoglobin levels were found to be higher with a decreased risk of anemia related to parasitic infection [5]. According to the studies, due to the increased health benefits associated with high iron contents in red tef, there is more acceptance of this grain in the society. Today in Ethiopia, red tef is becoming more popular related to its increased iron content. However, few researchers indicate that the level of iron in the tef is related to the threshing of the grain on the soil.

The third main type of tef, brown (mixed) tef is a mixture of both red and white tef without any standard proportions from each and locally it is called “mixed” tef [20].

Wholesalers in the major markets divide tef into four, namely; magna (very white), nech (white), mixed (mixed between white and red) and key. Four of the grades indicate the color of the tef variety. The market value gives magna the first while key the last grade position [21]. Poor farmers usually sell the white tef at reasonably higher prices, while buying either the mixed or red tef for their own consumption. White tef is more expensive than the red tef. People who can afford the price usually consume white tef or Magna tef, while the medium and poor families consume the mixed and/or red tef. Farmers who grow white tef often do not consume it. Instead, they sell the white tef at reasonably higher prices and buy either the red or the mixed tef for home consumption

According to the pilot study made by [22], the classification of tef is not uniform, what is referred to as *magna tef* (top quality) in some area of Ethiopia is sometimes sold as *mixed tef* (medium quality) in Addis Ababa commodity market. Quality attributes required in tef appear to be poorly understood with little documentation of quality requirements for products. This introduces some arbitrariness in the comparison of prices at different locations.

(a) (b) (c)

Figure 2: The extremely small size of Tef seed and their colour variation (a) Red, (b) Mixed and (c) White

1.5 Uses of Tef as Food

Tef is produced only in Ethiopia as a grain crop; hence, no studies will be referred from other countries experience. In Ethiopia, it is traditionally grown as a cereal crop. It provides over two-thirds of the human nutrition in Ethiopia [23]. The grain is ground into flour that is mainly used for making popular pancake-like local bread possibly with many eyes called *Enjera* (Figure 3) and sometimes for making porridge. Mixed with water and 'Ersho', the flour is allowed to ferment for a few days. 'Ersho' is starter, which is dough, saved from a previous fermentation and used as a starting fermentation in every new dough preparation. *Enjera* is then, fried into large flat

Figure 3: Enjera prepared from tef flour

pancakes, done on a specialized electric stove or 'mitad' or 'Eele' on fire. *Enjera* made from tef is traditionally consumed with a variety of stews such as *wot,* a sauce made of meat or ground pulses like lentil, faba bean, field pea, broad bean and chickpea. Since wot, supplements the lysine deficit in tef and provides a better balanced diet,the traditional way of consuming tef with *wot* is wise (Figure 4). Because of its high mineral content, the need for tef grain is increasing to be used in mixtures with soybean, chickpea and other grains in the baby food industry [15].

Figure 4: Traditional Ethiopian food with Enjera

The grain is also used to make local alcoholic drinks, called *tela or farsso* and *katikala.* Nowadays, efforts are being made to discover and introduce other methods of utilizing tef. Cooked tef is a good thickener for soups, stews, and gravies. Its mild, slightly molasses-like sweetness makes tef easy to include [24]. It is also grown on a limited basis for livestock forage in other parts of Africa, India, Australia and USA. In the U.S. small acreages of tef are grown for grain production and sold to Ethiopian restaurants or utilized as a late planted livestock forage.

1.6 Nutritional status of Tef

Tef is well known by Ethiopians for its superior nutritional quality. It provides about two-thirds of the daily dietary protein intake of most Ethiopians [13]Nutritionally, whole grain tef per 100 gm edible portion contains water 11.0 gm, Energy 357.6 kcal, Protein 9.6 gm, Carbohydrate 74.6 gm , fat 2.53 gm ,fiber 2.63.0 gm, Thiamine 0.3 mg, riboflavin 0.2 mg Niacin 2.5 mg and ascorbic acid 88 mg [25]. It is an excellent source of essential amino acids-lysine, and the amino acid that is most often deficient in grain foods [4]. Tef contains more lysine than barley, millet, and wheat and slightly less than rice or oats (Table 1) and it is gluten free.

Beside providing protein and Calories, tef is a good source of minerals particularly Iron [26]. Compared with other cereals, tef is reported to have higher iron content [5]. Tef is also an excellent source of fiber, and has many times amount of calcium, potassium and other essential minerals found in an equal amount of other grains. The iron content of tef has been controversial. The relatively high values of iron reported in [5], according to some studies [11, 13], is attributed to extraneous contaminants it receives from soil during threshing and not due to high intrinsic grain contents. Whether derived from contamination or from inherent grain contents, however, the higher iron intake from consumption of tef than non-tef consumers is unequivocal [7]. This can be confirmed from the fact that non-tef consumers have a lower level of hemoglobin, and hookworm anemia develops in non-tef eaters if they are infested with hookworm. On the other hand, since tef eaters have higher levels of hemoglobin in their blood, they do not suffer from hookworm anemia even when infested. Malaria is also frequently 'found in the groups with lower hemoglobin levels. Moreover, consuming tef is reported to prevent the anemia related to pregnancy [12]. When tef is used to make Enjera, a short fermentation process allows the yeast to generate more vitamins [7]. Fermentation of tef flour decreases the pH of the dough with partial breakdown of soluble starch. This also increases the dialyzable portions of its Fe, P and Zn contents. The increase in dialyzable Fe results in chance for the bioavailability [22].The fermentation process increases the bioavailability of metals. Hence, reduces iron-deficiency anemia, among tef-*Enjera* consuming peoples. However, a longer duration of fermentation decreases the protein quality of Enjera [7].

In some regions of Ethiopia, women usually prepare *Enjera* by adding some fenugreek to tef to improve its baking quality. Because of this, the *Enjera* becomes softer and has a shiny appearance. Thus, women should be encouraged to continue this traditional practice and be made aware that their practice not only has the benefit of improving the baking quality of the *Enjera* but also of supplementing its protein content, especially lysine [1].

Table 1: Amino acid content of tef (g/16 g N) compared with other cereals

Aminoacid	Tef	Barley	Maize	Oats	Rice	Sorghum	Wheat	Millet
Lysine	3.68	3.46	2.67	3.71	3.79	2.02	2.08	2.89
Isoleucine	4	3.58	3.68	3.78	3.81	3.92	3.68	3.09
Leucine	8.53	6.67	12.5	7.26	8.22	13.3	7.04	7.29
Valine	5.46	5.04	4.85	5.1	5.5	5.01	4.13	4.49
Phenylalanine	5.69	5.14	4.88	5	5.15	4.9	4.86	3.46
Tyrosin	3.84	3.1	3.82	3.3	3.49	2.67	2.32	1.41
Tryptophan	1.3	1.54	0.7	1.26	1.25	1.22	1.07	1.62
Threonine	4.32	3.31	3.6	3.31	3.9	3.02	2.69	2.5
Histidine	3.21	2.11	2.72	2.1	2.5	2.14	2.08	2.08
Argrinine	5.15	4.72	4.19	6.29	8.26	3.07	3.54	3.48
Methionine	4.06	1.66	1.92	1.68	2.32	1.39	1.46	1.35
Cystine	2.5	-	-	-	-	-	-	3.19

Source: [5]

Tef also have applications for persons with Celiac Disease. It is nearly gluten-free, and because of this gaining popularity in the whole food and Health food industry in Netherlands as an alternative grain for persons with gluten sensitivity [27]. Celiac disease (CD) is a multifactorial disease with a strong genetic association. It is caused by a T-cell mediated immune response to wheat gluten. The treatment is a strict gluten-free diet (GFD). Symptoms include chronic diarhoea, failure to thrive (in children), and fatigue. Celiac (Greek, *coeliac* -abdominal) disease is caused by a reaction to gliadin a gluten protein found in wheat (and similar proteins of such as barley and rye). Upon exposure to gliadin, the enzyme tissue trans- gltaminase modifies the protein, and the immune system cross-reacts with the bowel tissue, causing an inflammatory reaction. That leads to flattening of the lining of the small intestine. This interferes with the absorption of nutrients because the intestinal villi are responsible for absorption. The only effective treatment is a life long gluten-free diet and tef is one of or may be the only such diet [27].

Tef has also another health benefits related to its high fiber content. This is particularly important in the treatment of diabetes and assisting with blood sugar control.

1.7 Economic importance of Tef

Tef contributes to the country's economic input. Now days both the grain and the straw have increasing demand. Thus, their prices are increasing from time to time. According to Debre Zeit Agricultural Research Centre (DZARC) price data, (Unpublished), at the harvesting time, average prices of the three classes of tef rose from 200 Birr /Q in 1999/2000 to 1000 Birr/Q in 2006/07. Tef straw, besides being the most appreciated feed for cattle, also used to reinforce mud and plaster the walls of tukuls and used to fetch income. Based on an average of the last four years the DZARC price data the grain of tef contributed 5.3 billion Birr and the straw 1.7 billion Birr to the Gross Domestic Product (GDP) of the country. The total contribution is estimated to reach eight billion Birr [15]. A small amount of tef produced for local markets is also exported.

Recently official Figures on the export status of tef have appeared during the past few years. Almost equal amounts (18 thousand quintals) of tef grain were exported in 2004/05 and 2006/07, but the value obtained differs remarkably. About nine million birr was obtained in 2004/05 (493.37 Birr/Q) whilst 14.6 million Birr was obtained in 2006/07 (791.83 Birr/Q). The trend indicates that there is a good export market for this crop in the Middle East, North America and Europe mainly for the immigrant Ethiopians [15]. Pure white seeds are preferred for export more than the mixed or the pure brown tef. No specific production areas are targeted for export.

1.8 Mineral Nutrition

Minerals are the nutrients that exist in the body, and are as essential as our need for oxygen to sustain life. In the body, only 5% of the human body weight is mineral matter, vital to all mental & physical processes & for total well being. They are most important factors in maintaining all physiological processes, are constituents of the teeth, bones, tissues, blood, muscle, and nerve cells [27].

Acting as catalysts for many biological reactions within the human body, they are necessary for transmission of messages through the nervous system, digestion, & metabolism or utilization of all nutrients in foods. Vitamins cannot be properly assimilated without the correct balance of minerals. For example; calcium is needed for vitamin "C" utilization, zinc for vitamin "A", magnesium for "B" complex vitamins [27, 28].

Minerals are very important in keeping the blood and tissue fluids from either becoming too acid or too alkaline and they allow other nutrients to pass into the bloodstream, and aid in transporting nutrients to the cells. They also draw chemicals in & out of the cells. A slight change in the blood concentration of important minerals can rapidly endanger life [27]. No single mineral can function without the others, since they are synergistically related [27, 28]. Having the proper balance of minerals in the body can make the difference between disease or sickness and optimum health.

Plant-derived foods have the potential to serve as dietary sources of minerals for all human [28, 29]. Evidence of mineral malnutrition is various; minor and serious health conditions such as energy loss, premature aging, diminished senses and degenerative diseases like heart disease and cancer. Many individuals both in developed and developing countries are failing to attain recommended mineral intakes [30]. In most cases, these could be prevented with proper mineral supplementation. In this line, an increased consumption of plant and plant product food would be beneficial. However, only few individual plant foods are able to supply the daily-recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains tuber crops and root crops, which make up a large proportion of daily food intake in the developing world [31-33]. Thus, as an alternative strategy and efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals [32, 33].

The nutritional value of a diet in terms of macronutrients and trace nutrients is dependent on the bioavailability of the mineral for physiological processes in the organism much more than its contents in the diet. The amount of elements present in a plant is determined by their presence in the soil and air. The proportion depends on many factors: species, root distribution of the plant, physical and chemical nature of the soil, proportion and distribution of the element, method of cultivation, and general climatic condition. In an animal, however, the most important single factor is the *food*, which the animal consumes. For some elements present in very small quantities are as essential to growth as those that compose the greater portion of the plant, or animal, the amount of an element in a plant or animal must not be considered a criterion of its relative value [31, 35].

1.9 Classification and Importance of Minerals in Food

1.9.1 Classification of Minerals in Food.

The elements found in/necessary for living organisms can be classified either based on their importance or amount needed by the body. Based on their importance, they can be essential (like K, Mg, Ca, Mn, Fe, Co, Cu, and Zn) and they are very important for growth and health, or they may be non-essential (like Cd and Pb). Nutritionally (based on the amount needed), minerals are grouped into Bulk or (macro- minerals) and trace (micro- minerals). Elements, such as Mn, Cr, Fe, Co, Cu, Zn, Se, Mo, F and I are essential trace elements, while elements like Ca, Mg and K are grouped under essential macro elements[28].

Many mineral elements occur in living tissues, foods and diets in such small amounts that they are frequently described as traces [31]. In recent years the term trace element is commonly used for elements that are typically occur at a level of less than 10 mg/ 100g in biological system[36]. There is growing interest in the trace elements in the area of medicinal science. It is believed that the great majority of these elements act as key components of essential enzyme systems or other protein [37].

All trace elements are toxic if consumed at sufficiently high levels for long enough periods. The difference between toxic intakes and optimal intakes to meet physiological needs for essential trace elements is great for some elements but is much smaller for others [37].Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [31, 37-40].

The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential elements cannot be wholly replaced by any other elements [31, 40]. However, the bioavailability of essential elements depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition [31, 40].

1.9.2 Importance of Minerals in Food

Minerals are inorganic substances found in the body that function in conjunctions with enzymes, hormones, vitamins and other compounds. They play important roles in nerve transmission, muscle contraction, blood formation and metabolism of macronutrients and energy production. Some minerals can either block or enhance absorption of other nutrients, including other minerals and some vitamins. They are present in the skeletal system and other hard tissues and constitute approximately 3% of the body's weight [38-45]

Minerals in a food; become part of tissue structure, like in bone and teeth, help maintain acid-base balance, to keep the body pH neutral, regulate body processes, such as in enzyme systems, function in nerve impulse transmission and muscle contraction and help release energy from food[28].

The increasing use of highly refined foods, which are low in minerals, vitamins, etc., contributes to health problems. Nutritional deficiency may lead to diseases and disease may lead to nutritional deficiencies. Many times, minerals are discussed separately, but it is important to note that their actions within the body are interrelated; no single mineral can function without the others, since they are synergistically related. The physiological role of K, Ca, Fe, Zn, Cu, Na, Mg, Co, and Mn are briefly described below

1.9.3 Physiological Role of Some Essential Minerals

1. Potassium (K)

Potassium is the most abundant mineral found in the human body next to P and Ca. It is a major electrolyte of intracellular fluid. It is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. It is obviously essential for plants and can not be entirely replaced by any other elements. Deficiency symptom includes irregular heart beat, loss of appetite and muscle cramps [28, 43].

2. Sodium (Na)

Sodium is an essential mineral or micronutrient, which along with potassium helps to regulate the body's fluid balance. Sodium deficiency is not common, and according to some experts the average Western diet provides more than 5 times the recommended daily allowance of sodium. Excess sodium intake is linked with high blood pressure and heart disease. The current recommendation is to consume less than 2,400 milligrams (mg) of sodium a day. This is about 1 teaspoon of table salt per day. It includes all salt and sodium consumed, including sodium used in cooking and at the table [28, 44].

3. Calcium (Ca)

Calcium forms a vital part of bone and tooth structure, and is important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism [22, 32, 38]. Inadequate intake of calcium increases the risk of osteoporosis (bone loss with no apparent cause). Excess intake of calcium may cause kidney stones and reduces mineral absorption in general and Recommended Dietary Allowance (RDA) of Calcium is for adult 800mg pregnant and young adults 1200 mg [44].

4. Iron (Fe)

Iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Iron deficiency is common only among children and pre-menopausal women. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health [28, 46, 47]. Iron absorption can be influenced by calcium, magnesium, manganese, zinc, anti-acids and tetracycline (a common antibiotic) [48].

Deficiency of iron deprives body tissues of oxygen and results in anemia which is recognized by its symptom such as low blood iron level, small red blood cells and low blood hemoglobin values ,fatigue, paleness, dizziness, sensitivity to cold, irritability, poor concentration and heart palpitation[48]. RDA of iron depending on age level and health condition is 10 to 30 mg. Recommended daily intake is 15 mg.

5. Copper (Cu)

The essential role of copper in maintaining normal health in both animals and humans has been recognized for many years. The average daily dietary requirement for copper has been reported by many scholars to be 1.5 to 3 mg while As little as 10 mg of copper can have a toxic effect [49]. Copper is required with iron for synthesis of hemoglobin. It works with many enzymes such as those involved in protein metabolism and hormone synthesis [49, 50]. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder [49].

6. Zinc (Zn)

Zinc is an essential micronutrient for animals, plants and microorganisms. They accumulate considerable amount of Zn in their system without any damaging effect [50]. It is essential to carbohydrate metabolism; protein synthesis and inter nodal elongation (stem growth). Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs with zinc mal-absorption occurs [51]. Zinc deficiency leads to iron deficiency causing similar symptoms. Deficiency of zinc causes loss of appetite, growth retardation and immunological abnormalities [48, 52-53].

The RDA of Zn is 15 milligrams per day for men and 12 milligrams per day for women. Recent research suggests that men have a higher need for zinc than do women. Thus, it is appropriate that the RDA is sex-specific for zinc.

7. Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for the proper formation and maintenance of bones, cartilage connective tissue and important enzyme reactions. It is an important cofactor in the key enzyme of glucose metabolism. It also helps to maintain normal nerve, brain and thyroid function [48] and it is often lost in processed foods [53. 54]. A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest.

The RDA levels and the recommended "not to exceed" daily maximums (tolerable upper intake levels) for manganese is 1.2 and 11 mg respectively [56]. Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements [55, 56]

8. Cobalt (Co)

The only known animal requirement for cobalt is as a constituent of cobalamine commonly recognized as vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin B_{12} deficiency. Cobalt deficiency symptoms include a loss of appetite, emaciation, weakness, anemia, and decreased production. RDA value for cobalt is 0.3mg/day [28]. Inorganic cobalt has no nutritional value but some times added to beer as anti-foaming agent.To be biologically useful, cobalt must be obtained from foods.

9. Magnesium (Mg)

Magnesium is essential for plants and animals. It is relatively more abundant in the parts of plants concerned with vital process, such as seeds and foliages than in storage parts such as stems and roots [58]. It is a critical structural component of the chlorophyll molecule and is necessary for functioning of plant enzymes to produce carbohydrates, sugars and fats. It is used for fruit and nut formation and essential for germination of seeds. Deficient plants appear chlorotic, show yellowing between veins of older leaves; leaves may drop. In animals, magnesium is the essential composition of bone and teeth with RDA for adults of 350 milligrams [59]. It play important role in metabolism of phosphorus, starch and sugars. Many biochemical and physiological processes require magnesium. It is necessary for vitamin C and calcium metabolism. It keeps teeth healthy, brings relief from indigestion and can aid in fighting depression. More than 300 enzymes are known to be activated by magnesium. The best natural sources are whole seeds, nuts, legumes, unmilled grains, green vegetables and bananas. Phytate or fiber may reduce magnesium absorption [58, 59].

1.10 Analysis of Metals in Cereals

Some transition metals at trace level in our metabolism play effective roles for healthy life. Heavy metals normally occurring in nature are not harmful, because they are only present in very small amounts. However, if the levels of these metals are elevated, then they can show negative effects. The essential metals can also produce toxic effects when the metal intake is excessively elevated. Due to the importance of trace elements on human metabolism, their analysis is an important part of public health studies [60]. Therefore, in order to be aware about the levels of metals in food, especially in staple foods, their analysis is mandatory.

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for macro and trace elements determination in plants or biological materials. The different techniques so far reported for the determination of metals in plant products are: Direct current argon plasma optical emission spectroscopy (DCP-OES) [63], Flame atomic absorption spectrometry(FAAS), Hydride generation (HGAAS) or electrotermal atomization (ETAAS) [61-65], graphite furnace atomic absorption(GFAA) [65], inductively coupled argon plasma optical emission spectrometry (ICP-OES) [65, 66], and inductively coupled plasma mass spectrometry (ICP-MS) [65, 67]. These methods are most commonly used for the determination of metals in plant materials because of their inherent selectivity, sensitivity, precision and accuracy.

1.11. Review of Some Methods used for Metal Analysis in Tef

There are a few reports on the mineral composition of tef done on unidentified and mixed samples. According to Areda [11], there are controversies among these few reported findings with respect to mineral composition of the species of tef in general and that of iron in particular.

The interdepartmental committee on the Nutrition for National Defense, Washington D.C.,in its Ethiopian Nutrition Survey in 1959, reported that the iron and calcium content of tef was much higher than those of wheat barley and sorghum. The values given for iron and calcium were 105 and 110 mg per 100 g of tef seeds respectively.

This finding of high iron content in tef was challenged by Almgard [8]. He suggested that the unusually high values are due to soil and species contamination of the sample used by survey team. He reported that the iron content of acid washed red and white tef to be 5.2 and 5.5 mg per 100 g of seeds respectively.

According to mengesha [5], pure strain of purple tef contains 12.7 mg; white tef pure strain contains 10.6 mg; while purple mixed and white mixed strains contain 19.6 and 11.5 mg of iron per 100 g of seeds respectively. These values for iron are almost three times that of Almgard's but still very much lower than that of the survey team. Moreover, Mengesha has also determined the levels of Na, K, Mg Co, Cu, Zn, Mn, Ca, Ba,Mo and Al in these tef types and found values for Na (22,21.2), K (36,20), Mg (18,19),Ca(18,17),Mn(2.12,3.0),Co(0.052,0.064),Cu(5.3,3.6),Zn(6.7,6.8),Fe(19.6,11.5), Al (8.3,0.012),Mo (0.078,0.074) and Ba (1.9,2.35)mg/100g respectively for 12 purple tef and 12 white tef colleted from Ethiopia and grown out in the field at Purdue University's Agronomy farm West lafayetta, Indiana. .

Hofvander[68] also reported the iron content of tef from 20 times dilute acid washed tef sample to be 24.4 and 21.5 mg per 100 g for red and white tef respectively.

Besrat *et al* [13] has made a critical study on the iron content of tef and determined the iron content of 35 cultivars and market samples after washing 20 times with 2% HCl. He used both Calorimetric and atomic absorption methods for determination of iron. In Calorimetric method iron was determined by reducing with hydroxylamine and then reacting with O-phenanthroline and read the colour formed at 510 nm. The iron content of uncleaned red and white tef determined by calorimetric method was 9.31 and 12.14 mg/100 g whereas, that determined by AAS method was 9.57 and 13.98

mg/100 g. The amount of iron found from the same tef types but acid washed 20 times was 5.49, 5.81for red and 5.49, 5.77 mg/100 g respectively for both methods.

Urga [9] has determined the Ca, Fe and Zn content of tef flour from dry ashed sample using AAS method for iron and zinc but titration method for calcium and reported as 197.09±7.63, 2.30 ±0.02 and 9.31±0.19 mg/100g with no acid treatment respectively. The same researcher [10] determined the content of these elements from wet digested red tef: 197.32±4.13, 2.30 ±0.27 and 9.31±2.97 and 205.61±7.32, 2.39 ±0.71 and 9.15±3.17 mg/100g for white tef purchased from bishoftu town.

Abebe & *et al* [14] determined the Ca, Zn and Fe content of red, white and mixed tef flour (‘duqyet’) by AAS method and reported that Ca (155, 124,147), Fe(>150, >150, 37.7) and Zn (4.02,3.86, 2.86) mg/100g respectively in red, mixed and white flour.

In the present investigation, three tef types as purchased from four mill houses found in Arada sub city of Addis Ababa were included. The the two extreme types, red and white tef were pure form from any mixture. The Na, K, Mg, Ca, Mn, Co, Cu, Zn and Fe contents of water washed tef samples were determined using FAAS method.

Most determination methods require pre treatment of samples to be analysed. One of these samples pretreatment involve sample matrix break down or sample decomposition

1.12 Sample Decomposition

Sample dissolution is an integral part of sample treatment due to the intrinsic requirement of most quantitative analytical techniques for sample to be in liquid form. The majority of methods for mineral constituents require the organic matrix of the foods to be removed, or extraction and concentration, before they can be applied. The various techniques for elemental determination do not all requires the same degree of sample matrix breakdown. Sample decomposition is useful for ;converting all the species in which a given element is present in such a way that it becomes present in one defined form, eliminating interfering substances from the matrix and obtaining the element in a homogeneous and easily accessible matrix[61]. The choice of decomposition techniques should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [61]. The different decomposition methods could be classified in to dry ashing, Wet digestion and microwave digestion [73].

1.12.1 Dry Ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. After decomposition, the residue is dissolved in acid and transferred to a volumetric flask prior to analysis. Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50-100°C under reduced pressure in an oxygen plasma discharge. The major drawbacks of the method are the possible loss of some elements by volatilization, contamination of the sample by airborne dust, as it must be left open to the atmosphere and irreversible sorption of analyte into the walls of the vessel [73-76].

1.12.2 Wet Digestion

Flame and electrothermal atomization require that the sample be in a liquid or solution form. Samples in solid form are prepared for analysis by dissolving in an appropriate solvent [55]. The majority of wet digestion methods involve different combinations of five acids (HNO_3, H_2SO_4, $HClO_4$, HCl, and HF) and H_2O_2). The choice of an individual acid or combinations of acids depends upon the nature of the matrix to be decomposed. The mildest solution that will digest the sample is preferred, as stronger acids are more likely to add to the blank, attack digestion vessels, and generally require more care in the laboratory. Nevertheless, this procedure minimizes losses during the oxidation and avoids any reaction between the inorganic constituents and the vessel used for dry incinerations. Concentrated acids may be used individually, in mixtures, or in sequence [61, 65, and 69]. The procedure could take place either in open system or closed system. This digestions procedure involves heating the sample and acid mixture in round bottom or Kjeldahl flask on a Kjeldahl apparatus adjusted with condenser(s).

1.12.3 Microwave Digestion

To perform an atomic absorption or atomic emission measurement, the sample must be dissolved prior to analysis. It is often the case that the sample is not easily dissolved. In such situations, acid digestion in a microwave oven is mandatory.

Microwave ovens began to find widespread use in chemical laboratories in the late 1980's. It consists of a microwave oven, a rotating carousel holding several sample digestion bombs, and a system for venting these in a controlled fashion. It may also provide monitoring and recording of both temperature and pressure in the containers. The sample containers are relatively high-pressure containers, usually made of strong, high-temperature-resistant polymers, often polycarbonate for strength or PTFE for chemical resistance. Modern microwave digestion systems monitor both pressure and temperature in the containers. The containers for sample digestion are commercially available which can be used for ashing samples at temperatures up to 300^{o}C or pressures to 800 psi, under controlled pressure and temperature. Under these conditions, even refractory samples can be digested successfully [65].

2. Objective of the Study

Since Teff (*Eragrosties Tef)* serve as the staple food for many peoples of Ethiopia, the knowledge of its mineral nutrition is of particular interest. Although little information is available on the levels of all essential elements content in tef in the literature, several studies have been carried out on determinations of the level of Iron in red and white tef, using different methods such as, colorimetric [13] and FAAS [5,7-11,13,13] employing dry ashing or wet digestion techniques. Recently the concentrations of three elements (Ca, Fe, Zn,) were also reported in tef samples collected from sidama area [14]. Since no single mineral can function independently, the presence or absence of other essential elements in one way or another can affect the activity of the other element in the living body. Therefore, determination of the levels of other minerals which are potentially related is necessary.

2.1 General objective

The purpose of this study is to determine the levels of nine selected essential elements in red, mixed and white tef grains purchased from four mill houses in Arada sub city of Addis Ababa.

2. 2 Specific Objectives

The specific objectives of this study are to:

- Develop suitable method of tef sample digestion.
- Determine the level and distribution of Na, K, Mg, Ca, Mn, Co Cu Zn and Fe in the three-tef varieties.
- Compare the level of essential metals in the three-tef types.
- Compare the levels of metals determined in tef with other similar food crops.
- Generate up to date base line data on mineral content of tef.

3. Experimental

3.1 Equipment and Reagents

3.1.1 Equipments

A drying oven (DIGITHEAT, J.P.SELECTA,S.a, Spain) was used to dry the washed tef grain samples. Mortar and pestle was used to grind and powder the dried tef sample. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh tef samples. A 100 ml round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered tef samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample till analysis. BUCK SCIENTIFIC MODEL 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium arc back ground correctors was used for analysis of the analyte metals (Na, K,Mg, Mn, K, Fe, Zn, Cu and Co) using air-C_2H_2 flame.

3.1.2 Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69-72 %) and $HClO_4$ (70%) [RESEARCH- LAB FINE CHEM INDUSTRIES MUMBAI 400 002 (INDIA)] were used for digestion of tef samples. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for releasing calcium and magnesium from their phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Na, Mg, Mn, K, Ca, Fe, Zn, Cu and Co (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and spiking experiments. Throughout the experiment, for sample preparation, dilution and rinsing apparatus prior to analysis and during analysis, deionized water was used.

3.2 Procedures

3.2.1 Cleaning Apparatus

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks and all the necessary materials used for the experiment were washed with detergents and tap water, rinsed with deionised water, soaked in hydrochloric acid for 24 hrs, rinsed with deionised water many times and kept in dust free place until analysis.

3.2.2 Collection and Preparation of Tef samples

3.2.2.1 Collection of Samples

Four mill houses from Arada sub city of Addis Ababa were selected. This area is selected for two reasons; its proximity and the other is purpose of this project is to optimize a working procedure and determine the intended metals with out considering the geographical origin of the sample. Addis Ababa is metropolitan of Ethiopia, has high intensive population, and found at 9.03^0N and 38.74^0E. The city has 10 sub cities with a total population of 2,738,248 according to the 2007 population census report. The city is supplied with different raw materials used for food from other regions through different direction. Tef is one of those food crops commercially supplied for the people of Addis Ababa from different regions in the country. Dwellers of the city purchase tef from either 'Ihil berenda' or the available mill houses. There are a number of mill houses in the sub city. For the reason mentioned above, each sampling sites were randomly selected. Tef grain was purchased from only four mill houses where tef is commercially available. About 50 gm of each sample was collected from tef bag randomly totaling four bags per sample then, pooled together and well mixed. Per each sample, a total of 200 gms was collected from one mill house. A total of 800g was collected for each sample from the four mill houses. Again, the collected tef grain was pooled together and mixed well. The sample size was reduced to about 200 gm by coning and quartering process for each tef types. About 50 gm of the sample was used for analysis.

3.2.2.2 Sample Preparation

Tef samples collected from the mill houses were kept in a polyethylene bags. The husk and other light and heavy contaminants were removed from the grain by siftings, winnowing and sieving to ensure it is free from chaffs, dust and other impurities. The sample was washed in a plastic bag by vigorous up and down shaking first with distilled water as many times until all dust is removed and then three times with deionized water. Then, to have constant mass, the washed tef sample was oven dried 105^0C for 5 hours prior to digestion so as to express the result in terms of dry mass basis. Drying at this temperature will not affect the mineral content, *Horwitz* in [14]. The dried tef grain was powdered using mortar and pestle until it feels smooth to touch. Three with 0.5 g aliquot (three from each bulk sample) were taken for final digestion.

3.2.4 Digestion of Tef Samples

For tef samples, in most cases dry ashing [11, 14,] were commonly used than wet digestion for analysis by FAAS. Different combinations of mineral acids have been employed in other studies for the decomposition of tef flour by wet digestion. Urga [9,10], used a procedure involving 5 ml of HNO_3 and 1 ml of HCLO4 to digest tef for digestion time of 1 hour and used this combination of volume for repeated 1 hour until he found colour less solution at a temperature of 350^0c.

In this study, for the digestion of tef flour sample, different conditions such as: digestion time, reagent volume, volume ratio of reagents and digestion temperature were tried to obtain optimum conditions as indicated in (table 3). The optimum conditions for tef sample digestion are Nitric acid-perchloric acid mixture with (5:1 v/v), digestion temperature 300^oc and digestion duration of 3:00 hour. Applying the optimized procedure, 0.5 g of dried and homogenized Tef sample was transferred into a 100 ml round bottomed flask. Then 6 ml mixture of HNO_3 (69- 72%) and HClO4 (70%) (5:1 v/v) was added and the mixture was digested on the Kjeldahl digestion apparatus by setting the temperature first at 120^0c for 30 minutes and then, increased to 300^0c for the remaining 2:30 hours. Then after, the digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 5 min after removing the condenser. To the cooled solution, deionized water was added to dissolve the precipitate formed on cooling. Then the solution is filtered into 50 ml volumetric flask with 125mm diameter filter paper. Subsequently, the round bottom flask was rinsed with some deionized water and filtered into the volumetric flask. After adding 1% lanthanum nitrate solution, the volumetric flask was filled to the mark with deionised water. Blank solutions were prepared following the same digestion procedure as the sample. Triplicate for each bulk sample and sextet for the blank were digested. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS.

3.2.5 Determinations of the Essential Metals

Secondary standard solutions containing 10 mg/L were prepared in 100 ml volumetric flask from the atomic absorption spectroscopy standard stock solutions that contained 1000 mg/L (BUCK SCIENTIFIC). Four working standards for each metal of interest were prepared from these secondary standards. These working standards were prepared freshly for each element from the secondary standards for by appropriately diluting with deionized water for calibration purpose (table 4). Then, Na, K, Ca, Mg, Mn, Co, Zn, Fe, and Cu were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength) were optimized for maximum signal intensity of the instrument. For each elements, their respective hallow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. The acetylene and air flow rates were managed to ensure suitable flame conditions. Three replicate determinations were carried out on each sample. The elements were determined by absorption/concentration mode and then, the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in the six digested blank solutions. The instrument operating conditions for FAAS employed for each analyte are given in Table 2.

Table 2: Instrument operating conditions for the determination of metals in tef samples using flame atomic absorption spectrophotometer.

Element	$\lambda(nm)$	SW(nm)	DL(mg/L)	Lc(mA)	PMT(V)	Energy(erg)
Na	589	0.2	0.002	2	278.6	4.012
K	767	0.7	0.01	2	297.2	3.799
Mg	285	0.7	0.001	1	285.3	3.934
Ca	423	0.7	0.01	2	304.2	3.722
Mn	280	0.7	0.01	3	287.1	3.926
Co	241	0.2	0.05	4.5	333.7	3.42
Cu	325	0.7	0.02	1.5	295.8	3.818
Zn	214	0.7	0.005	2	378.8	3.1
Fe	248	0.2	0.03	7	310.4	3.662

3.2.6 Recovery Test

The efficiency of the optimized procedure was checked by adding known concentration of each metal in 0.5 g sample. For the recovery analysis, 100, 230,170, 170, 100, 50, 50, 50 and 100 μg of Na, K, Mg, Ca, Mn, Co, Cu, Zn and Fe respectively were spiked at once in to 0.5 g of tef sample. Then the same digestion process was followed as for the samples simultaneously. Each recovery test for the sample was performed in triplicates.

3.2.7 Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [69]. It is the concentration that gives a signal three times the standard deviation of the blank or background signal [10,70]. Six blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, K, Ca, Mg, Mn, Co, Zn, Fe, and Cu) by atomic absorption spectrophotometer-meter. The standard deviation for each element was calculated from the six blank triplicate measurements to determine method detection limit. In this study the detection limit of each element was calculated as three times the standard deviation of the blank (3σblank, n = 3), which is summarized in Table 5.

3.2.8 Determination of Limits of Quantitation

Limit of detection and Limit of quantification are two important characteristics in method validation. Limit of quantification (or limit of determination) is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. It is the same as a concentration, which gives a signal 10 times the standard deviation of the blank [10]. Limit of quantification is the lowest limit for precise quantitative measurements [70]. The quantification limit of each element was calculated as ten times the standard deviation of the blank ($10S_{blank}$, n = 9). The results are given in Table 5.

4. Results and Discussions

4.1 Optimization of Digestion of Tef Samples

A series of procedures involving some changes in reagent volume, reagent composition, digestion temperature and time were tested. Accordingly, eleven procedures were tested for digestion of the tef samples. This procedure was developed with some modification of the procedure in literature used to determine Iron content of tef samples from market by FAAS [9, 10]. The optimized procedure and conditions indicated under number 10 (Table 3) were used through out the analysis.

Table 3: Attempted digestion procedure for 0.5 gm of tef flour samples

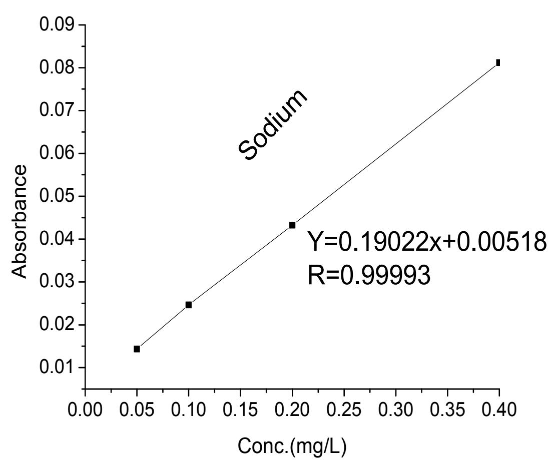
Trial No.	Reagents used	Volume Ratio (ml)	Temp (^oC)	Digestion Time (hrs)	Observations
1	HNO_3 : $HClO_4$	5:10	180	4:40	Clear Light yellow
2	HNO_3 : $HClO_4$	4:10	180	4:40	Clear Light yellow
3	HNO3:HCLO4	5:2	210	4:45	Deep yellow with suspension
4	HNO_3 : $HClO_4$	3:2	210	3:00	Deep Yellow
5	HNO_3 :H_2O_2	10:1	210	3:15	Clear yellow
6	HNO_3 : $HClO_4$	4:10	240	4:00	Clear Colour less
7	HNO_3 : $HClO_4$	5:10	240	4:10	Clear Light yellow
8	HNO_3:$HClO_4$:H_2O_2	3:2:1.5	240	4:00	Yellow
9	HNO3:HClO4:H_2O_2	3:2:1	240	4:45	Clear light yellow
10	*HNO3:HClO4*	*5:1*	*300*	*3:00*	*Clear & colourless (optimized*
11	HNO_3 : $HClO_4$	2.5:2	300	3:15	Cloudy suspension

The optimized procedure was selected depending upon: clarity of digests, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested tef samples, simplicity and acceptable use of masses of tef samples.

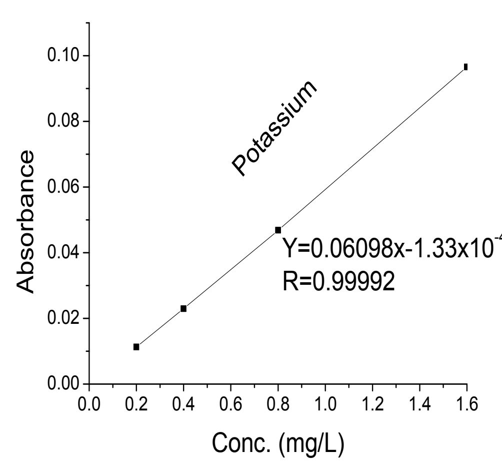
Based upon these criteria, the optimal digestion procedure chosen was the one that requires 3 h for complete digestion of 0.50 g of tef powders, with 5 ml HNO_3 (69-72 %) and 1 ml $HClO_4$ (70 %) (Table 3). However, the other tested procedures have some limitation. They require higher reagent volume, longer digestion time. In addition, they result in the formation of turbid digests and coloured digest solutions. Since acid digestion is used, it is necessary to prepare reagent blanks for each digestion employed. Reagent blanks were also prepared and digested with the same procedure as the sample and used to correct for impurities present in the acid and water.

4.2 Instrument Calibration

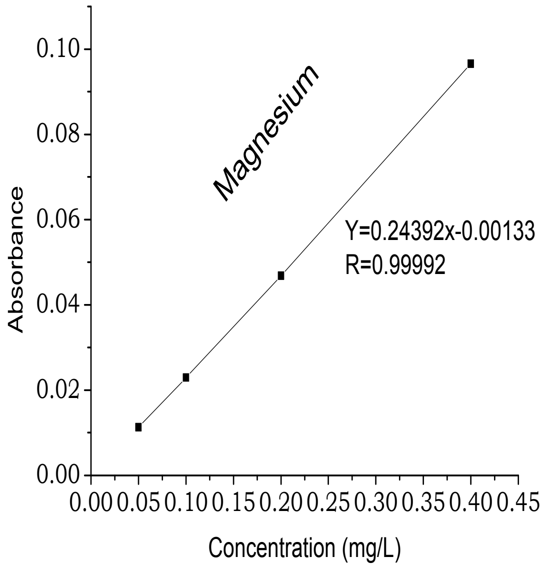
The qualities of results obtained for essential metals analysis using FAAS are seriously affected by the calibration and standard solution preparation procedures. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared fresh by diluting the intermediate standard solutions mentioned under section 2.2.5. Concentrations of the working standards and value of correlation coefficient of the calibration graph for each metal is shown as tabulated in table 4. The calibration graph of each of metals of interest is shown in Figure 5(a – i)



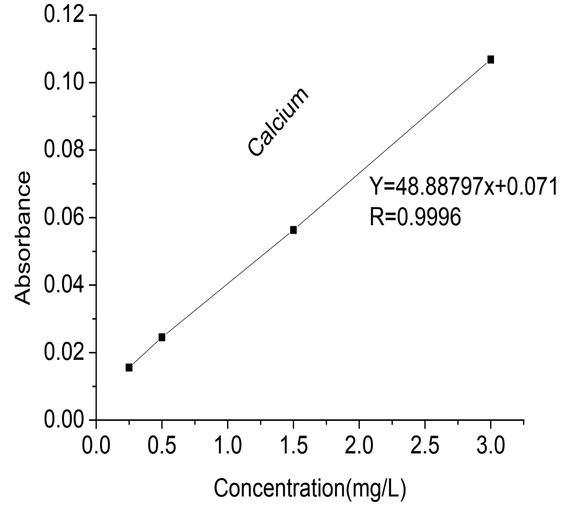
(a)



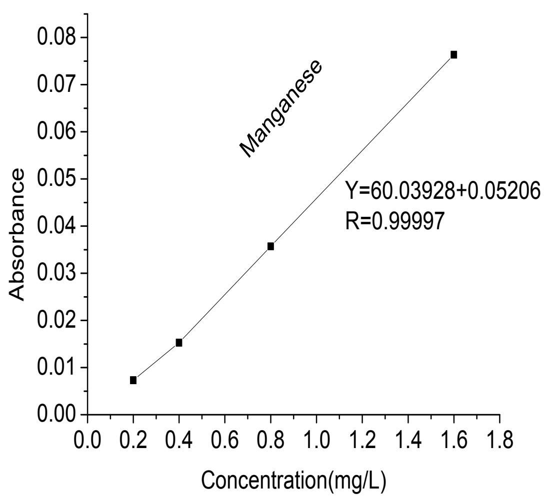
(b)


Magnesium
Y=0.24392x-0.00133
R=0.99992
Absorbance
Concentration (mg/L)
0.00
0.02
0.04
0.06
0.08
0.10
0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45

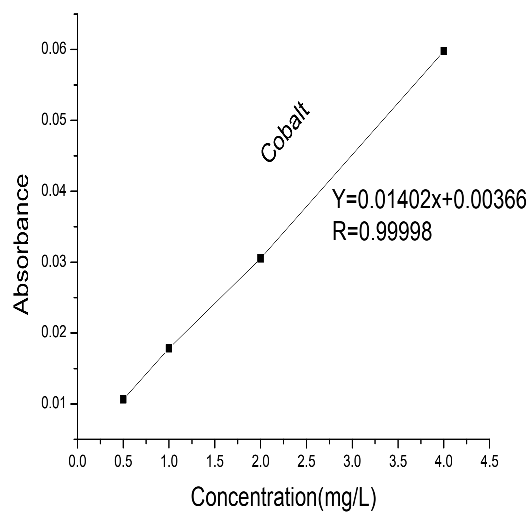
(c)


Calcium
Y=48.88797x+0.0711
R=0.9996
Absorbance
Concentration(mg/L)
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.0 0.5 1.0 1.5 2.0 2.5 3.0

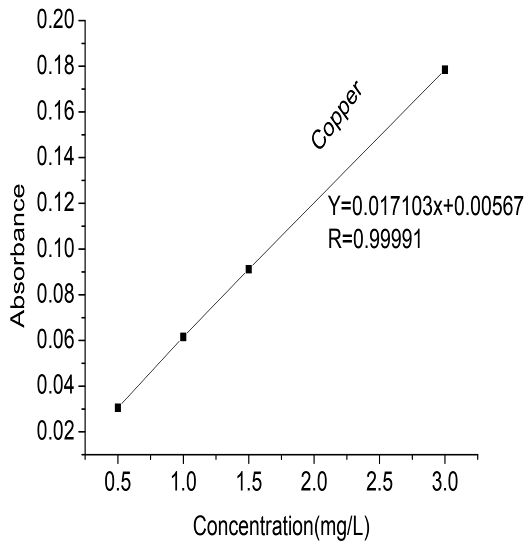
(d)


Manganese
Y=60.03928+0.05206
R=0.99997
Absorbance
Concentration(mg/L)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8

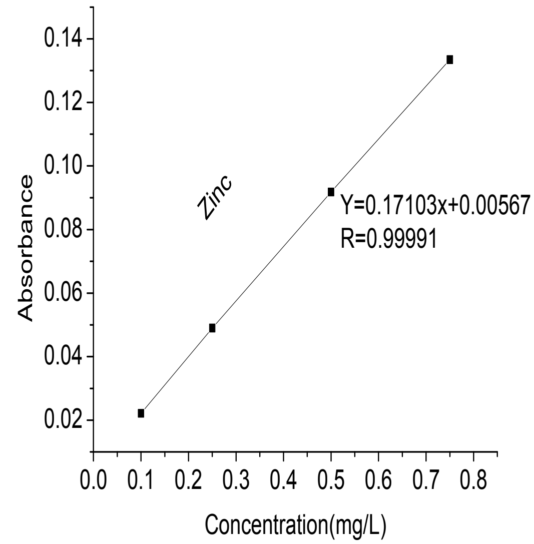
(e)


Cobalt
Y=0.01402x+0.00366
R=0.99998
Absorbance
Concentration(mg/L)
0.01
0.02
0.03
0.04
0.05
0.06
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5

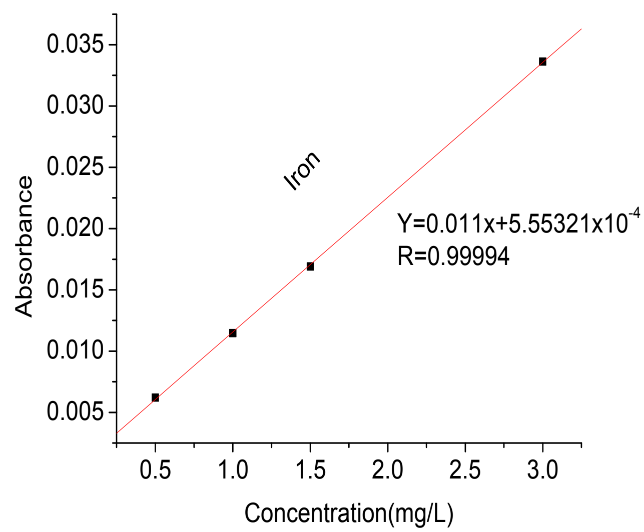
(f)



(g)



(h)



(i)

Figure 5: Calibration graph of metal standard solutions(a-i).

Table 4: Series of working standards and correlation coefficients of the calibration curves for determination of metals in the tef varieties using FAAS.

Metal	Conc. of standards(mg/L)	R
Na	0.05, 0.1, 0.2, 0.4	0.99993
K	0.2, 0.4 , 0.8, 1.6	0.99992
Mg	0.05, 0.1, 0.2, 0.4	0.99992
Ca	0.25, 0.5, 1.5, 3	0.99996
Mn	0.2, 0.4, 0.8, 1.6	0.99997
Co	0.5, 1, 2, 4	0.99998
Cu	0.5, 1, 1.5, 3	0.99991
Zn	0.1, 0.25, 0.5, 0.75	0.99991
Fe	0.5, 1, 1.5, 3	0.99994

4.3 Evaluation of Analytical Results

4.3.1 Precision

The precision of a method is the degree of closeness of the results and is usually reported as percentage of relative standard deviation. It is often subdivided into repeatability and reproducibility. Repeatability expresses the closeness or agreement between a series of measurements obtained from multiple sampling and sample analysis. It is often expressed as the standard deviation or relative standard deviation of replicate measurements.

For the percentage RSD Figure obtained to be ideal in terms of statistical significance, it would be necessary to make quite a large number (in the order of 20) of measurements. However, as compromise to save time, it is quite usual to make 6-10 repeated measurements. Similarly, there are pragmatic compromises that may be made assessing reproducibility. What is ideally required is to carry out replicates of the complete assy at different laboratories or at different days of experiment and find the percentage RSD on the result [74]

In this study, the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of three samples (n = 3) and triplicate readings for each sample meaning, nine measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk sample and triplicate reading per sample and relative standard deviation (Table 6).

4.3.2 Method Detection Limit

Triplicate analyses for six blank samples for all elements were performed and the pooled standard deviation of the Six blank reagents with triplicate measurement was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. As can be seen from Table 5, column two, the method detection limit of each element is above the instrument detection limit.

Table 5: Method detection and Quantitation limit (n = 18, DLM = $3S_{blank}$ and MQL= $10S_{blank}$ in mg/100g) for all metals determined in tef samples.

Metal	MDL (mg/100g)	MQL (mg/100g)	DL (mg/100g)
Na	0.0102	0.0341	0.0002
K	0.0153	0.051	0.001
Mg	0.0195	0.0649	0.0001
Ca	0.0039	0.013	0.001
Mn	0.0039	0.0133	0.001
Co	0.0086	0.0287	0.005
Cu	0.0049	0.0163	0.002
Zn	0.0176	0.0587	0.0005
Fe	0.0737	0.2457	0.003

4.3.3 Method Limits of Quantitation

The quantification limit of each element was calculated as ten times the standard deviation of the blank ($10S_{blank}$, n = 18). The results are given in Table 5, column three. As can be seen from the table, both the MDL and MQL are greater than the instrument DL, hence, the result of the analysis could be reliable.

4.4 Recovery Test for the Optimized Procedure

Method validation is a way of testing a particular analytical method to see if it is suitable for its intended purpose. The validation process begins in method development in that the documentation, reporting the validation data must include a record of the method development process, giving details of the conditions explored and the rationale of the progression of the process.

Since there is no certified reference tef sample to compare with, the performance of the digestion procedure was estimated using lower level of traceability, recovery test, such as spiked samples. The efficiency of the method was assessed by spiking the tef flour samples with known amounts of metals, and each of the metals were analyzed in triplicate. Standard metals solutions were used to fortify the sample to the specified level given in Table 6 and the percentage recovery was calculated using equation (1).

$$\% R = \frac{C_M \text{ in the spiked sample} - C_M \text{ in the non-spiked sample}}{C_A} x100\,\% \qquad [1]$$

Where, %R = percentage of recovered amount.

C_M = Concentration of the metal

C_A = Concentration of metal added for spiking.

The recoveries of the metals in the spiked tef sample are between 85.66 to 105.22%, (%R $\pm$ SD). Table 6 depicts the result of this recovery test. The mean percentage recoveries for all analytes were within an acceptable range (75-125%), thus the laboratory performance for each analyte is in control [71]. Recovery values in the above range are acceptable for both bulk and trace analysis and the digestion procedure is believed to remove metal fractions associated with organic matter..

Table 6: Analytical results for Recovery test of the optimized procedure for tef sample.

Metal	Amount added(μg/g)	Concentration found (μg/g)[a]	% Recovery[b]
Na	100	90.46 ± 4.5	90.46 ± 5.3
K	230	217.94 ± 10.3	94.78 ± 8.3
Mg	170	154.22 ± 4.6	90.72 ± 6.6
Ca	170	162.61 ± 9.6	95.65 ± 9.2
Mn	100	91.14 ± 2.6	91.14 ± 7.6
Co	50	45.68 ± 4.4	91.36 ± 6.4
Cu	50	46.39 ± 2.6	93.52 ± 7.6
Zn	50	46.39 ± 2.9	92.78 ± 2.9
Fe	100	96.42 ± 6.7	96.42 ± 8.8

[a] Values are mean ± SD of triplicate readings of triplicate analyses.
[b] Values are mean ± SD of triplicate percentage recovery values of triplicate analyses

4.5 Determination of the Essential Metals in Tef Samples

The concentration of nine essential elements (K, Ca, Fe, Zn, Mn, Mg, Co, Cu and Na) in the digested and diluted solutions of tef samples were identified with FAAS. All have been identified and shown in Table 7 with their respective percentage RSD. The most abundant metal among the macroelements is K, followed by Mg and Ca whereas Fe and Mn contents of red tef is the predominant among the tested essential nutrient trace metals. In mixed and white tef ,the content of Na, micronutrient [44] for human but non-essential alkali metal in plant nutrition, was found to be high next to Mg. The quantity of each metal was determined using equation (2) and is shown in table 7. As can be seen from the table, and Figure 6(a-c), there is a variation in concentration of Essential elements within tef type.

$$C_M = \frac{C.V.D}{m_s} \qquad [2]$$

Where, C_M = Concentration of metal (mg/g)

C = the Concentration of metal read from the spectrophotometer (mg/l) or ppm

V = the volume of the solution (L)

m_S = the mass of the sample analyzed (gm);

D = the dilution factor (if any)

Table 7: Elemental concentrations* (mg/100g) and (%RSD) of essential elements in the tef samples.

	Red Tef		Mixed Tef		White Tef	
Metal	Mean ± SD	%RSD	Mean ± SD	%RSD	Mean ± SD	%RSD
Na	22.06 ± 1.46	6.64	25.68 ± 2.05	7.97	22.89 ± 1.42	6.21
K	215.81 ± 8.13	3.77	219.23 ± 8.35	3.81	205.59 ± 7.17	3.49
Mg	161.91 ± 7.76	4.79	173.7 ± 8.89	5.12	153.16 ± 9.45	6.17
Ca	178.54 ± 9.5	5.33	168.64 ± 11.03	6.54	180.7 ± 14.65	8.11
Mn	22.36 ± 0.15	0.67	13.26 ± 0.029	0.22	4.84 ± 0.044	0.9
Co	6.20 ± .36	5.73	5.21 ± 0.29	5.62	7.89 ± 0.75	9.54
Cu	2.51 ± 0.30	11.8	1.6 ± 0.07	4.54	1.08 ± 0.075	6.97
Zn	4.79 ± 0.32	6.6	3.79 ± 0.1	2.5	2.98 ± 0.12	4.12
Fe	24.62 ± 1.1	4.36	20.05 ± 1.4	6.97	15.95 ± 1.75	10.98

* Average of nine determinations and their standard deviation.

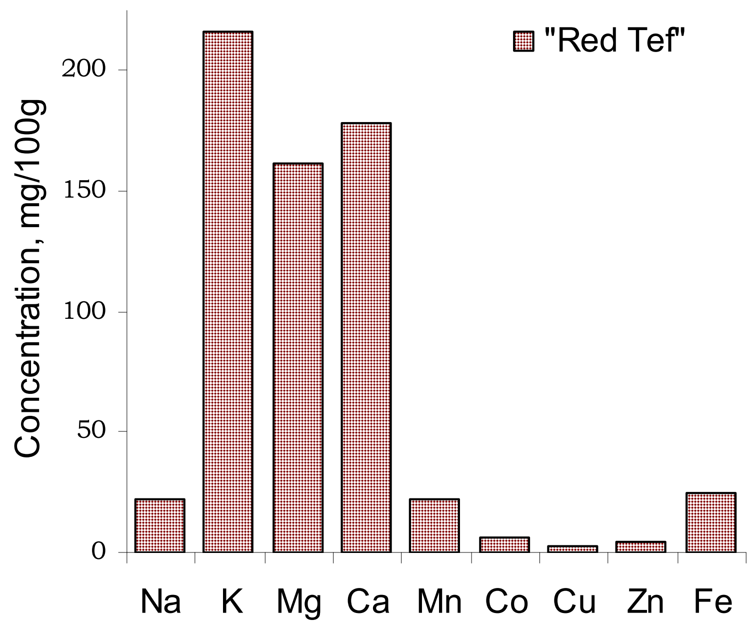


Figure 6a: Distribution of metal concentration in red tef

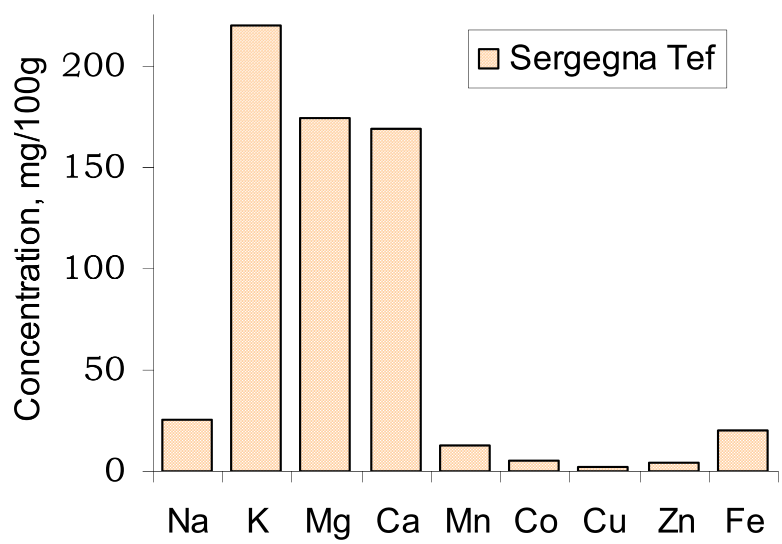


Figure 6b: Distribution of metal concentration in mixed tef

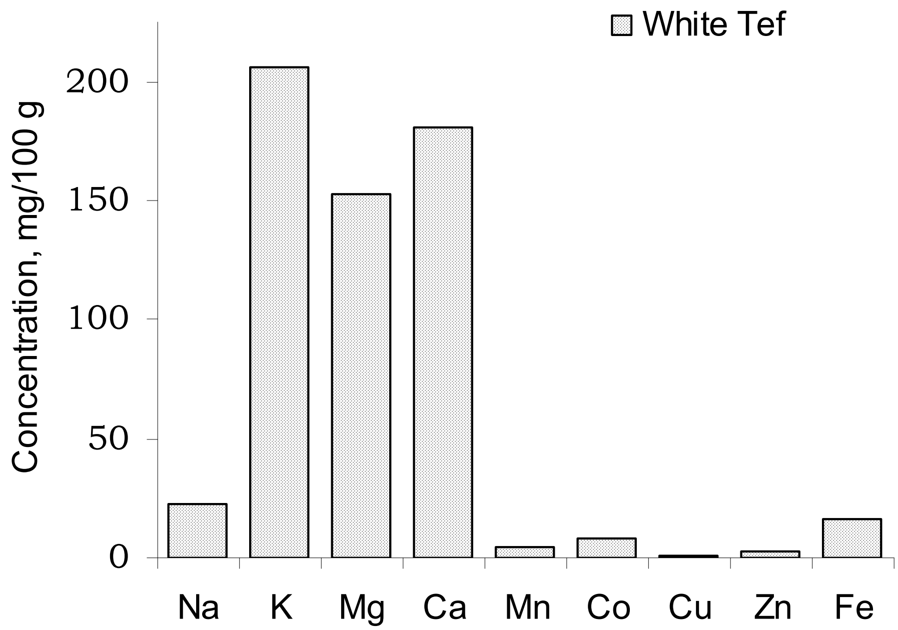


Figure 6c: Distribution of metal concentration in white tef

4.6 Distribution Pattern of the Metals in different Tef Samples

The results of this study showed that the metal contents of tef grains varied between the tef varieties for some metals (cf. section 4.9). This variation is probably attributed to mineral composition of the soil on which the tef has been grown and mineral anchorage capacity of tef variety. According to Helberg (1981) in [10], genetic and environmental factors influence the mineral content of cereals. The highest K content was observed for all tef types, this is probably related to the contribution from potassium containing fertilizer(s) if any. Nevertheless, its concentration is not high in comparison to results from other studies [5]. Similarly, all tef types have higher Mg, Ca and Fe contents. The variation in concentration of the metals between the different tef types studied lies within the over all range summarized in Table 8.

Table 8: Range of metal concentration in tef sample studied (mg/100g) at 95% CL.

Metal	Range of Metal concentration (mg/100g)		
	Red tef	Mixed tef	White tef
Na	20.5 – 23.7	24.5 – 26.9	22.5 – 23.2
K	207.7-222.8	209.7-230.8	198.7- 211.6
Mg	158.3-165.6	171.7-175.7	151.3 – 155.0
Ca	169.9-187.1	154.6-183.2	177.7- 183.3
Mn	21.7-22.9	12.6-13.9	4.3 - 5.4
Co	5.2 -7.2	4.6 – 6.0	7.2 - 8.4
Cu	2.04-3.0	1.5 -1.6	1.0 - 1.3
Zn	4.5 - 5.1	2.9 - 4.6	2.7 - 3.3
Fe	22.14 -27.1	16.8 – 23.3	14.2 – 17.7

4.7 Comparison of Metals within Tef Samples

When the concentration of metals in the tef grains were compared, the red tef has relatively higher concentrations of Fe, Mn, Co, Cu and Zn than the other two varieties except Co which is higher in white tef. Whereas, mixed (mixed) tef has relatively higher contents of K, Mg, and Na while white tef has higher concentration of Ca and Co than red and mixed tef [Figure 6d].

The pattern of concentration of elements in the three varieties of tef studied decreases in the following order for red tef : K > Mg > Ca > Fe > Mn > Na > Co > Zn > Cu for mixed Tef: K > Mg > Ca > Na> Fe> Mn > Co > Zn > Cu and for white tef: K > Ca > Mg> Na> Fe > Co > Mn > Zn > Cu for white tef .

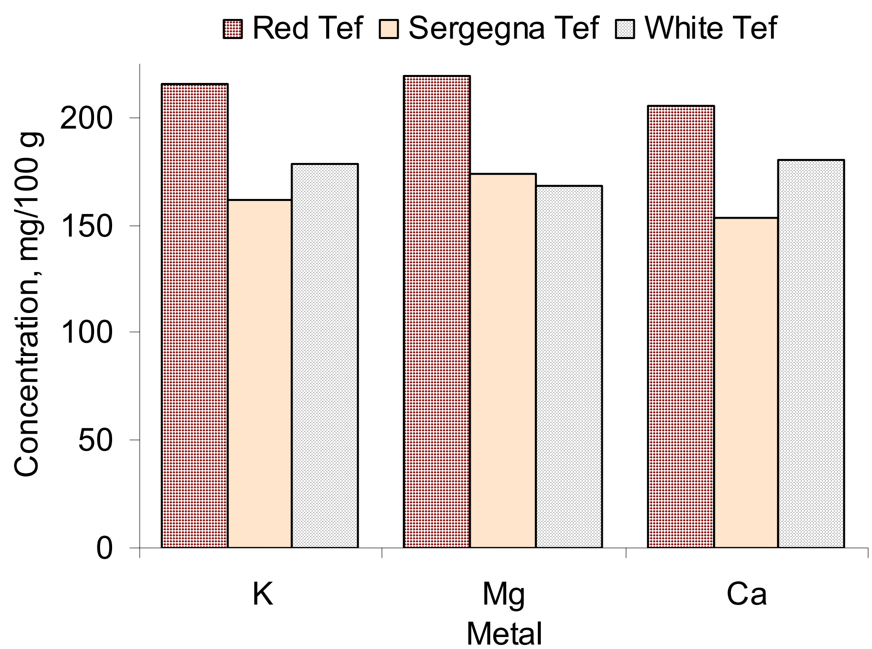


Figure 6d: Average concentrations for essential bulk metals in the three tef types

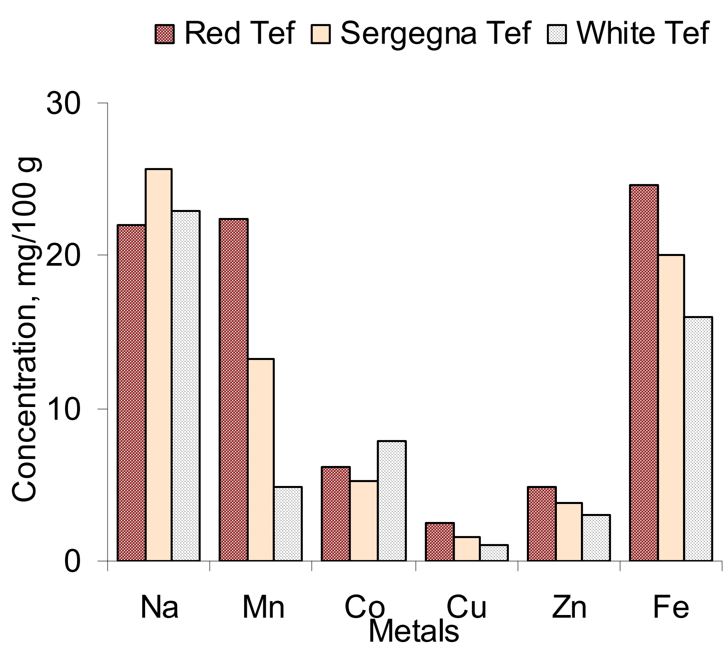


Figure 6e: Average concentrations for essential trace metals in the three tef types

As can be seen from the trends, in table 8 and Figure (6) above, tef serves as a good source of essential metals to human. All tef types contain higher amount of the macronutrients (K, Ca, and Mg) and trace nutrient (Fe). The small amount of cobalt and copper found in tef does not contradict with the requirement of the metal for proper functioning of the body, because these metals are required in small amount (Co = 0.3 mg/day) as a constituent of vitamin B_{12} [69] and Cu = 3.5 mg/day [75].

As can be seen from Figure 6e, Iron is the most concentrated heavy metals in all tef types with values of (24.62 ± 1.07,20.05 ± 1.4 and 15.95 ± 1.8) mg/100g while copper is the lowest (2.51+0.30,1.6+0.07 and 1.08+0.075) mg/100g respectively in red, mixed and white tef. From this, we can say that the iron content of red tef is superior to the other tef varieties and this seems to support the values reported in [5, 68].

4.8 Comparison of Determined Metals Concentration in Tef with Literature Values

4.8.1 Comparison with reported Metal Concentration in Tef.

Since Tef is endemic to Ethiopia, no reference can be made from other countries work on tef. Because of this, there is no detailed analysis report from other countries. Nevertheless, many Ethiopians with foreign co- researchers have reported the concentration of Iron [2, 5, 9-11, 14, 25] and calcium and zinc (5, 9, 10 14) in tef varieties. Although the origin, types and pre-analysis treatment methods used by the researchers were different and dos not seem reasonable to compare, it is important to see the results from this study with the previous studies for qualitative purpose regardless of all the above differences. The comparison of the metal concentrations determined in this study and the reported values from these researchers are presented in table 9 for all tef types.

From the table, one can see that the concentration of calcium determined in this study is almost the same as the values reported in [9, 10, 14] for red and white tef. The cobalt content reported in [5] is less than the present result. This may be due to contamination for hand threshed and contamination free sample was used by [5]. The zinc content of tef included in this study are of relatively equal amount with those values reported in [9,10,14] except for white tef for which Zinc content is is not reported by [9] and mixed tef for which zinc value is not reported in [14]. The Iron content determined in this study is also comparable with those values reported in [5, 68].This is in a good agreement with [5] in which tef's exceedingly high iron and calcium content was confirmed by analyzing the mineral composition of tef from uncontaminated seeds. The result from the acid washed tef reported in [8] for iron is much lower than the result from [5, 80] and the present determination. The metal high iron content reported by [14] could be attributed to contamination during milling. Tef by its nature, because of its seed size, is not suitable to wash and use for making *Enjera*. Therefore, consumers grind tef only by removing loosely held husk and other separable contaminants either by sieving or by winnowing or both. This is an advisable practice, for many studies have shown that efforts should be made to replace refined-grain with whole grain foods. Increased intake of whole grains may reduce disease risk by means of favourable effects on metabolic risk factor [76].

Therefore, the minerals content of cereals should be expressed as a whole grain and as eaten. For tef, the whole seed is ground into flour and most of the metals found in the seed must be found in the flour and subsequently, in the food. Therefore, it should be reasonably safe to predict a positive correlation between tef seed and Enjera. Such correlation can not be predicted in other cereals like wheat since only 60 to 80% of the wheat is used in bread making [5].

The result of this study agrees with result reported in [5, 68]. Therefore, Almgard's [7] statement that the iron content of tef is of the same amount as in other cereals after analyzing acid washed tef sample, can not be accepted on the basis of the present study. Since Almgrad's acid washing may deteriorate the natural mineral content of the cereal, his result may not be convincing, as the mineral content of tef must be expressed as eaten.

Moreover, Areda [11] found that after ten times washing with dilute HCl, the iron content decreased by 51% of the water washed tef. Then, he confirmed that, acid washing of cereal seeds could not be taken only as surface cleaning, because it also extracts some chemical components. This was also further confirmed in [5] by analyzing tef and other cereals (barely, wheat, sorghum, millet and maize) all free of

Table 9: Comparison of observed metals concentration, (mg/100g dry mass), tef flour with reported values.

Metal	Red tef	White tef	Mixed tef	Reference(s)
Na	22	21.2	-	Mengesha [5]
	22.061 ± 1.46	22.89 ± 1.42	25.68 ± 2.05	This study
K	36	20	-	Mengesha [5]
	215.81 ± 8.13	205.59 ± 7.17	219.23 ± 8.35	This study
Mg	18	19	-	[5]
	161.91 ± 7.7	153.16 ± 9.45	173.7 ± 8.89	This study
Ca	18	17	-	Mengesha [5]
	197.32 ± 4.13	205.61 ± 7.32	-	Urga [10] Titration method
	155	124	147	Abebe& *et al*[14] 'Duqyet' FAAS
	178.54 ± 9.52	180.7 ± 14.65	168.64 ± 11.03	This study
Mn	2.12	3.0	-	Mengesha [5]
	22.36 ± 0.15	4.84 ± 0.044	13.26 ± 0.03	This study
Co	0.52	0.64	-	Mengesha [5]
	6.20 ± .36	7.89 ± 0.75	5.21 ± 0.29	This study
Cu	5.3	3.6	-	Mengesha [5]
	2.51 ± 0.30	1.08 ± 0.075	1.6 ± 0.07	This study
Zn	6.7	6.8	-	Mengesha [5]
	2.3 ± 0.03	-	3.86	Urga [9]-FAAS
	2.3 ± 0.27	2.39 ± 0.71	3.79 ± 0.1	Urga [10]-FAAS
	4.02	2.86	-	Abebe& *et al*[14]
	4.79 ± 10.7	2.98 ± 0.12	3.79 ± 0.1	This study
Fe	11.6 – 24.1	9.5 – 13.2	-	Mengesha [5]
	105	110	-	Ethiop.Nutri. Survey [7]
	(5.2)	(5.5)	(11.5)	Almgard [8]-FAAS
	9.31 ± 2.97	9.15 ± 3.17	-	Urga [10]- FAAS

13.98 (5.81*)	9.57(5.77)	-	Besrat[13]-FAAS and Calorimetric
>150	37.7	>150	Abebe & *et al*[14] FAAS
(20.0 – 24.8)	(13.0 – 36.9)	-	Hofvander[68] - FAAS
22.14 – 27.1	14.2 – 17.7	16.8 – 23.3	This study

* Results in bracket are from acid washed tef

contamination in which all seeds were ground with chromium knives and cleaned with chromium screens to avoid iron chips, which might enter into the samples and influence the results.

However, tef seeds analysed by Almgard [8] were collected from ordinary market places, hence, there is a good possibility that their seeds were contaminated with soil. In addition, he has also stated that soil was seen on the surface of the seeds even after they had been washed. Thus, his contaminated and cleaned tef seeds should have given higher iron content than the result reported from uncontaminated tef in [5].

The level Ca determined in the this study is almost similar to the value reported in [10] but some what higher than that of [14] which is expressed on fresh weight basis, whereas, the Zn content of tef determined in this study is seems to be equal to the result reported in [10,14]. The level of Mn, and Co obtained in this study is higher than the values reported in [5] for red and white tef while that of Cu is lower than values in the same report.However, these metals were not in the other researchers' work. The presence of these metal together with Zn, assists the the physiological role of iron (they are important in maintaining DNA stability) [81]

The close relationship between the results from the two studies, however, indicates the reliability of the present analysis on tef. Moreover, tef by its nature, because of its seed size, is not suitable to wash and ground into flour for making *Enjera*. Therefore, its mineral contents should be expressed as a whole grain and as eaten by consumers.

4.8.2 Comparison of Metal Concentration in Tef with other Food Cereals

Wheat, Sorghum, Barley, Tef, Maize, Kocho and Bulla are basic food for people of Ethiopia based on geographical location. Their contents of essential elements depend on plant needs as well as amounts available from the soil. In most part of Ethiopia, where tef is a staple food, people prepare meal from tef only. However, in some areas where tef is co-staple food and because of its high price, people some times use mixture of tef with maize or Sorghum. Although the proportion being dependent upon the individual interest, this mixed use of cereals boosts the nutritional status of the food and may complement each other. Therefore, comparison of the mineral contents of tef with other cereal flours is crucial to know the dietary mineral intake of those individuals who rely on tef as a staple and co-staple food.

Many authors reported the concentration of metals in cereal flours and vegetables. A lot of researchers have reported on the level of mineral nutrients in the major food types such as maize, barley, tef, sorghum, wheat, kocho and bulla [5, 14,30, 75,78] (Table 10). From the table, it is possible to see that the concentration range of some metals is higher in tef as compared to some cereal flours and vegetables. For example, the concentration range of Ca in wheat flour, barely flour, maize flour, sorghum flour, kocho, bulla and tef flour are 11-196, 39.5 – 42.1, 1.3 – 12, 0.18 ,

49.8–58.4, 38.5–44.6 and 143.2–200.5 mg/100 g respectively. The concentration of Ca in tef is as good as Wheat flour with its maximum of the range. Compared to all cereals, kocho and kulla, tef has higher calcium content while sorghum has the least amount of the mineral. However, Na contents of kocho and Bulla and K content of kocho are higher than tef.

Red tef has the highest amount of Manganese content than all the other tef and cereals and generally, all types of tef have higher content of the metal. Manganese can be lost in processed foods [64, 65], but since no processing is involved in making Enjera from tef, there will be greater chance to obtain the metal from tef than other cereals like wheat. According to Keen [54], up to 22 mg per day, dietary Mn has no health hazard. Compared to the other cereals, all types of tef contain higher amount of Fe followed by barley white flour while whole wheat flour, sorghum, barely and maize have almost similar content of the metal. All cereal types have almost comparable amount of Cu and Zn. The iron content of tef is much higher than the iron content of all the cereal grains tabulated in table 10. In fact, this shows that the mineral content of tef is much superior to that of wheat, barley and grain sorghum.

From all the above facts, we can conclude that all tef types considered in this study, are rich in K, Mg, Ca and Fe contents. Cobalt is highest in tef where as, Barely and Sorghum has the least content of the metal. Since no single mineral can function without the others, the presence of high amount of cobalt in tef together with Fe synergistically contributes for the low anemia occurrence in tef consumers [28]. The same effects do occur due to the presence of Cu and Zn in tef along with Fe. Red and Mixed tef have higher content of Mn compared to the other cereals and vegetables except whole wheat, which has higher content of the metal.

Table 10: Comparison of observed metal concentration (mg/100g dry mass) in tef with some reported Values in Barley,Wheat , maize, Kocho and Bulla.

	Metals found and their concentration									
Cereal Type	Na	K	Mg	Ca	Mn	Co	Cu	Zn	Fe	Ref
Barley white flour				45.	NR	NR	NR	3.57	8.4	[14]
	39.2	0.44	0.18	< 0.1	1.2	0.03	1.4	4.5	3.5	[5]
Maize White flour				12	-	-	-	2.15	4.9	[14]
Whole wheat	19.5	0.037	0.015	0.01	5.3	6.0	2.0	6.0	7.9	[5]
				NR	46		5	35	43	[75]
White wheat flour		76-316	19-51	11-196	0.39 – 1.47	0.1-0.28	-	-	1.05-14.7	[78]
Sorghum	14.15	0.044	0.013	0.018	2.9	0.03	2.35	4.4	6.65	[5]
Kocho	46.2 – 68.8	275.3 - 438	18.0 – 29.0	49.8 - 58.4	0.86 – 1.01	0.55- 0.6	0.29–0.38	3.1–3.21	-	[30]
Bulla	40.2 – 44.2	70.8 - 87.5	5.84 – 8.95	38.5 – 44.6	0.1 – 0.496	0.5–0.51	0.20–0.35	2.2–4.43	-	[30]
Red tef	18.7-25-4	197.0-234.6	158.3 – 165.6	156.6-200.5	22.0 - 22.7	5.4-7.0	1.83 - 3.2	4.1 – 5.5	22.1 – 27.1	Ps*
Mixed tef	21.0-30.4	199.9-238.5	1171.7 – 175.7	143.2-194.1	13.2 - 13.3	4.5-5.9	1.4 - 1.8	3.6 – 4.0	16.8– 23.3	Ps
White tef	21.5-24.3	198.4-212.8	151.3 – 155.0	166.0-195.4	4.8 – 4.9	7.1-8.6	1.0 - 1.2	2.9 – 3.1	14.2-17.7	Ps

*Ps = Present study

4.9 Statistical Analysis

In this study, samples were collected from the same area from randomly selected mill houses where they are commercially available. Each sample was mixed thoroughly and one representative bulk sample was taken for each tef type. During this processes a number of random errors may be introduced in each aliquots and in each replicate measurements. Therefore, depending upon the type and nature of results at hand, a statistical method is used to check whether there is contribution from this random errors for difference in results of analysis or not. If there are differences, statistical analysis will tell us whether the differences are significant or not at a specified confidence level.

One –way Analysis of Variance (ANOVA) is used to perform the statistical analysis with tef as independent and concentration of the metals as dependent variable to test whether there are significant differences between means of each tef types at the stated confidence level. Excel and SPSS computer soft wares were used to compare the statistical parameters and the result of the analysis are depicted as in table 11 and 12

Table 11: Analysis of variance (ANOVA) between samples of the three-tef types

Parameters	Metals Compared at 95% confidence level								
	Na	K	Mg	Ca	Mn	Co	Cu	Zn	Fe
$Fe_{2,6}$(***5.143***)	6.28	15.7	28.51	7.45	38.7	49.55	15.19	52.53	10.75
P(sig)	0.034	0.004	0.001	0.023	0.002	0.003	0.004	0.001	0.01

Thus, from the result depicted in table 11, under F value, there exist statistically significant differences between the means of the different samples at 95 percent confidence level. Moreover, we can see that the variation among samples due to analysis procedures has very less contribution to the over all variation as compared to the variation due to inherent difference between the three tef types. However, this F statistic indicates qualitatively there is difference between the means of the three tef types, but does not pinpoint where the differences are. To be more specific and conclusive a tukey pair wise comparison between the mean of the tef types necessitate for clear and indicative result. The result of this multiple comparison is tabulated as in table 12. The table shows the significance (p) values for each pair compared and the following statistical inferences are made from the table.

Table 12: Results of significance test for Pair wise mean comparison between the three tef types

Tef types compared		Metals Compared at 95% confidence level and their p-value								
		Na	K	Mg	Ca	Mn	Co	Cu	Zn	Fe
R	W	0.761	0.01	0.001	0.549	0.002	0.001	0.003	0.001	0.004
	M	0.027	0.118	0.001	0.024	0.002	0.013	0.002	0.001	0.084
M	W	0.018	0.002	0.001	0.011	0.002	0.003	0.004	0.004	0.043

* R = Red tef, M = Mixed tef, W = White tef

No significant difference in the levels of Na, Ca (p = 0.761, 0.549) and K, Fe (p = 0.118, 0.084) between red and white, red and mixed tef, respectively at 95% ($p \leq 0.05$) confidence level. However Significant differences are observed between Red and Mixed tef in the level of all metals. More specifically, the levels of Ca, Mn, Co, and Zn are higher in red tef, whereas, mixed tef contains higher amount of Na, K, and Mg metals. Significant differences are also revealed between Red and white tef in the level of all metals except Na and Ca. That is only the level of cobalt is higher in white tef whereas the reverse is true for the rest. Significant difference is inferred between Mixed and white tef in their contents of all metals at this level. Here, Except Ca and Co all the rest meals are found in a greater amount in mixed tef.

There is also a significant difference in the level of Mg and Mn between the three tef types at ($p \geq 0.05$) CL and a similar difference is observed in the level of Zn between red and white tef at this level. That is Red tef has higher contents of Mg, Mn and Fe than white tef whereas mixed tef contains higher level of Mg than both red and white and less Mn than red tef.

From table 11 and 12, the difference in the level of respective metal content of the three-tef types could be attributed to the chemical composition of each tef type, genetic factors and the specific environmental conditions in which the tef was cultivated. According to Helberg (1981) in [10], genetic and environmental factors influence the mineral content of cereals

5. Conclusions and Recommendations

An efficient digestion procedure for tef flour sample was developed and validated through standard addition (spiking) method. The optimal digestion procedure allowed the use of acids with minimum volume leading to reduced blank contamination, and lower detection limit.

The levels of nine essential elements were determined by FAAS in the three most common and commercially available tef types. A statistical analysis at 95% ($p = 0.05$) confidence level revealed that there is a significant difference in the mineral content of the three-tef types.

The higher Iron content of tef, which was assumed to be due to contamination [8, 11, 13], is not convincing according to this and other research reports by Mengesha [5] and Hofvander [68]. According to Areda [11], the acid washing process for analysis of cereal seeds could not be taken only as surface cleaning, because it also extracts some chemical components and may deteriorate the natural mineral contents of the cereals. Therefore, the mineral contents of tef should be expressed with out acid wash.

Although the origin, types and pretreatment methods used by each researches were different in one way or an other, in case of iron content, the value obtained in this study seems to support reports by Mengesha [5] and Hofvander [68] rather than that of Almgard [8] and the 'as eaten' analysed tef sample by Abebe [14].

Compared to Maize, Barely, Sorghum, Wheat, Kocho and Bulla, Tef is superior in its mineral contents and could be suitable source of dietary minerals for those people using tef as staple and co-staple food source.

Although the data obtained is relatively small to draw authoritative conclusion about the mineral contents of tef, nevertheless, the present analysis on tef will give base line for comparison for young researchers and good awareness for tef users and those who intend to use the cereal.

Therefore, to draw strong and ruling conclusion about the mineral content of tef, further investigations are needed parallel to the study of mineral content of the tef cereal on the: physical and chemical property and mineral contents of the soil, the genetic dependence of the mineral content of the tef, and other artificial chemicals used for tef growth.

Moreover, use of representative number of samples from different geographical sources of tef has not been made so far. Thus, the upcoming researchers are recommended to use the results from this and previous researches as a stepping ladder for further investigation and more elaborative mineral analysis and other related factors on the tef grain.

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[d] Journal found from EHNRI Library, AA

[e] Journal obtained from friends

Declaration

I, the undersigned, declare that this project work is my original work under the supervision of my advisor in Faculty of Science Department of Chemistry AAU in the academic year 2008/09 and it has not been submitted in this or any other university. All sources of ideas and materials used for the project work are honestly acknowledged.

Name : Zeleke Kebede

Signature: ___________________

This project work has been submitted for examination with my approval as university advisor.

Advisor Name: ______________________________

Signature :______________________________

Place and date of submission: School of Graduate Studies

Addis Ababa University

July 2009