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Some Selected Metals in Wheat and Barley Flour and Their Processed Food Consumed in Guna Begemidr Woreda, South Gonder, Ethiopia

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MSc THESIS ON:

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A THESIS SUBMITTED TO THE DEPARTEMENT OF CHEMISTRY PRESENTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CHEMISTRY

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THESIS OF APPROVAL

The thesis entitled "SOME SELECTED METALS IN WHEAT AND BARLEY FLOUR AND THEIR PROCESSED FOOD CONSUMED IN GUNA BEGEMIDR WOREDA, SOUTH GONDER, ETHIOPIA" presented by Getasew Mekuriaw has been accepted for the partial fulfillment for the degree of Master of Science in Chemistry.

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DECLARATION

I declare that this thesis entitled "Mineral Contents of Common Cereals And Its Processed Food Consumed In Guna Begemidr Woreda, South Gonder, Ethiopia" is my own original work done under supervision of **Bisratewongel Tegegne (Ph.D)** at the Chemistry Department of Bahir Dar University under Analytical chemistry stream; and I have not previously submitted it entirely or in part for obtaining any qualification at any other university.

Name: Getasew Mekuriaw   Signature_____ Date_____

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ABBRIVATIONS

AAS   Atomic absorption spectrophotometer

LOD   Limit of detection

LOQ   Limit of quantification

SD   Standard deviation

RSD   Relative standard deviation

C_m Concentration of metal.

WHO   World health organization

FAO   Food and Agriculture Organization

ICP   Inductive coupled plasma

R^2 Determination coefficient

SD   Standard deviation

%RSD   Percent of relative standard deviation

%R   Percentage recovery

WHO   World Health Organization

ABSTRACT

Barley (Hordeum vulgare L.) and Wheat (Triticum aestivum L.) are the two most widely cultivated stable food cereal crops in the world. Barley and Wheat grain samples were collected and homogenized each from three selected kebeles in Guna Begemidr woreda, South Gonder Zone, Ethiopia and its processed food (bread) were prepared from it. The levels of essential metals (Mg, Fe, Mn and Zn) in barley, wheat grains and its processed food were determined by Atomic Absorption Spectrophotometer after 0.5 g barely, wheat and its processed food (bread) samples were digested using mixtures of 4 mL of HNO_3, and 2 mL of $HClO_4$ (4:2, v/v) at 200^oC for 1:30 and at 180^0c for 1:10 hour respectively based on the optimized wet digestion procedure. The optimized wet digestion procedure was evaluated using spiking method and an acceptable percentage recovery was obtained. The mean metal concentration (mg/kg dry weight) in wheat and barley flour was: Mn (5.7), Fe (43.8), Zn (28), Mg (243.4) and Mn (5.6), Fe (44.2), Zn (10.4) and Mg (284.4) respectively. The levels of metal in wheat and barley flour samples determined in this study were found in the following decreasing order: Mg > Fe > Zn > Mn. Among the studied metals, Mg was the highest and Mn was the smallest in all samples. The levels of Fe, Zn, Mn and Mg in wheat and barley samples collected from three kebeles in Guna Begemidr Woreda were found to be less than the level approved by FAO/WHO.

Keywords: *Barley, Wheat, Processed Foods, Essential Metals, Atomic Absorption*

Spectrophotometry.

1. INTRODUCTION

1.1. Background of the study

A cereal is any grass cultivated for the edible components of its grain (botanically, a type of fruit called a caryopsis), composed of the endosperm, germ, and bran. Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop; they are therefore staple crop. The contribution of cereals to the national income is also large. According to the available estimates, cereal contribution to agricultural gross domestic product (GDP) is about 65% (1). Cereals are the principal crops in Ethiopia in terms of both the area coverage and volume of production. Of the total arable land cultivated annually, cereals occupy the greatest proportion of area coverage accounting for about 81.27 % of the total acreage of all grain crops (cereals and oilseeds) (2). Cereals, with the largest distribution and huge production potential across the country, have engaged majority of small-scale farmers for their livelihood. In terms of farm households involved in field crop production, cereals make up the highest proportion. Nearly, more than 90% of the total 14 million farm households engaged in grain crops are involved in cereal production. The cereals most commonly cultivated in Ethiopia are wheat, maize, barley, sorghum and rice (3). Cereals are the important crops that serve as stable food for most people in Ethiopia. Cereals are common food crops of the world. They provide food calories and proteins to human. They are staple foods for most of the population. They are the main source of food in many countries.

Generally, cereals are necessary for a healthy diet and nowadays a daily consumption of between 4 and 6 portions of cereals derived products is recommended due to their content in fiber, trace minerals and vitamins, which are supposed to prevent various and vitamins, which are supposed to prevent various diseases. All cereal derived products are rich in carbohydrates, and therefore, the base of a well balanced and healthy diet. Ethiopia is the second-largest producer of wheat in Sub-Saharan Africa (4). Wheat is the universal cereal of the old world agriculture and the world's foremost consumed crop plant followed by rice and maize. Wheat is one of the major cereal crops in Ethiopia, which ground constitutes roughly 10% of the annual cereal production and plays an appreciable role in supplying the population with carbohydrates, proteins, and minerals (6). Barley is a short-season, early maturing crop with high yield potential and a wide range of adaptation. It is an important cereal crop and cultivated over broad environmental conditions in the world (7). It ranks fourth in the world in after wheat, maize and rice (8).Barley is used commercially for animal

feed, to produce malt, and for human unit area food applications as it is rich in protein, carbohydrates, dietary fibers, minerals, vitamins and antioxidants (9). Barley is also an important crop in Ethiopia (10). It is ranked fifth among the cereals on the basis of area of cultivation while third on the basis of production per in Ethiopia. Cereal processing is a complex. The cereal grains are processed to extract oils that are rich in vitamin E. Rice bran oil has more concentrated amounts of vitamin E than other oils available on the market. The principal procedure is milling. That is, the grinding of the grain so that it can easily cooked and rendered into an attractive foodstuff. The degree of milling, polishing and refining to some extent decides the nutrition content of cereals. Some nutrients are lost during food preparation, especially vigorous washing, soaking and cooking methods, which results in the depletion of the nutrients on the skin of the grains. Heavy metal accumulation may pose a direct threat to human health (11), and are potential environmental contaminants with the capability of causing human health problems if present in excess in the food we eat. They are given special attention throughout the world due to their toxic effects even at very low concentration (12).

Heavy metals are persistent and non- biodegradable, have long biological half-lives and they lives and they can be bioaccumulated through the biologic chains: soil plant-food and seawater-marine organism-food leading to unwanted side effects. So, the presence in high amount of heavy metals in environment represents a potential danger for human health and for environment due to their extreme toxicity. Heavy metal contamination may be occurred due to irrigation with contaminated water, the addition of fertilizers and metal-based pesticides, industrial emissions, transportation, harvesting process, storage and/or sale. Crops and vegetables grown in soils contaminated with heavy metals have greater accumulation of heavy metals than those grown in uncontaminated soil (13). Toxic effects of heavy metals have been widely described by many workers. Elements such as Cd, Cr and As are considered carcinogenic, while Fe, Cu, Zn, Ni and Mn are considered as essential metals, however, if the concentrations of the later elements are higher than their permissible limits they may create toxic effects in human(14) .

1.2. Statement of the Problems

The quality of life in a given society depends on the chemical composition of the food, the biosphere and physical environment in its surroundings (15).The human body needs 51 essential nutrients, and a short supply or lack of even one of these can cause metabolic problems resulting in poor health, sickness, and economic and social costs to the community (16). These nutrients are supplied only from agricultural products. The green revolution has boosted crop yield, and has prevented people from starving in many countries through the high production of cereal crops (wheat, maize and rice (17). Even though there are some reports about the metallic contents of cereals (wheat and barley) from many countries in the world, no detail investigations were made before and after processing for cereals in Ethiopia in this selected sampling area. In addition the optimized condition for sample preparation from cereals and processed food was not examined and reported. Since the availability of the metals in cereals depends on the accumulation of such metals in the soil and the use of synthetic fertilizers and natural compost. Therefore the objective of this study is to determine the mineral content of common cereals (wheat and barley) in Guna Begemidr woreda (Ata gura, Arga Didim and Wukrotadomender kebeles).

1.3. Objective of the Study

1.3.1. General Objective.

The general objective of this study is to determine the concentrations of selected metals (Fe, Zn, Mn and Mg) in cereals before and after processing from Guna Begemidr woreda (Ata gura, Arga Didim and Wukrotadomender kebeles).

1.3.2. Specific Objective.

✓ To optimize the sample digestion parameters for selected cereals before and after processing for metal analysis.

✓ To determine the levels of selected metals (Fe Zn, Mn and Mg) in cereals (wheat and barley) flour before processing collected from Guna Begemidr woreda, south Gonder zone.

✓ To determine the levels of selected metals (Fe Zn, Mn and Mg) in cereals (wheat and barley) flour in the processed food collected from Guna Begemidr woreda, south Gonder zone.

✓ To compare the levels of selected metals in cereals before and after processing in the study area and with the WHO/FAO standard and reported values.

1.4. Significance of the Study

The result of this study will help us to understand the current level of metals (Fe Zn, Mn and Mg) in common cereals produced and consumed in Guna Begemidr Woreda, South Gonder zone. It will have also significant to evaluate the level of selected metals in common cereals before and after processing. In addition the result will give an idea to the consumers to use specific variety of cereals and their processed food to have specific required nutrient values for healthy life.

1.5. Scope of the Study

The study was restricted to the Guna Begemidr Woreda, South Gonder zone on which wheat and barley are the most common cereals produced and consumed widely. This study will be focused on the determination of the level of selected mineral metals (Fe Zn, Mn and Mg) in the two selected cereals before and after processing for their common food item.

1.6. Limitation of the Study

Due to financial scarcity this study did not cover more study sites and more essential and non essential elements found in wheat, barley and its processed food samples.

2. LITERATURE REVIEW

2.1. History of Cereals

The first cereal grains domesticated by early primitive humans about 8,000 years ago, they ware domesticated by ancient farming communities in the fertile Crescent region. Emmer wheat, einkon wheat and barley were three of the so called esticated Neolithic founder crops in the development of agriculture. Sorghum and millets were also being domesticated in sub-Saharan West Africa, which were both used primarily as feed for livestock (18).

2.2. Composition of Cereals

Chemical composition and physical characteristics of cereal grains used in human and livestock feeding have been reported in many studies (19). Variations in the chemical composition and physical characteristics of cereal grains results from changes in the environmental factors, such as rainfall, temperature, soil conditions, fertilization and genetic factors (20). Most of physiologically active proteins (enzymes) in wheat grains are found in the albumin and globulin groups. The albumin and globulin fraction cover about 25% of the total grain proteins (21). Generally, the minerals help in the maintenance of acid-base balance, the response of nerves to physiological stimulation, blood clotting, structural, physiological, catalytic, and regulatory (22). Today, deficiencies of iron and iodine are of most concern to the nutrition community and health care officials although other nutrient deficiencies, including zinc, selenium, calcium and magnesium may be prevalent in some global regions. The consequences of malnutrition create immense economic and societal costs to nations. Micronutrient malnutrition greatly increases mortality and morbidity rates. It diminishes cognitive abilities of children and lowers their educational attainment. It reduces labor productivity. It stagnates national development efforts, contributes to continued high population growth rates and reduces the livelihood and quality of life for all those affected (23).

2.3. Essential Metals

Iron (Fe)-is a component of hemoglobin, myoglobin, the cytochromes, and in other enzymes (24). It serves important functions in oxygen transport and respiration. Approximately to 5mg of iron is lost from the body each day in feces, urine and sweat and twice of this value is lost during menstruation (25). Iron deficiency is the most common nutritional problem in the world today,

which leads to anemia. Iron deficiency in infancy and childhood, can impair learning and the ability to resist diseases (26)

Manganese (Mn): is an essential element, is crucial for a number of biological and physiological processes in the body, including immune function, regulation of cellular energy, reproduction, digestion, bone and connective tissue growth, and blood clotting. Mn also plays an important role as a cofactor for many enzymic reactions including amino acid, lipid, protein, and carbohydrate metabolism (27). Signs of manganese deficiency include impaired growth, skeletal abnormalities, disturbed or depressed reproductive function, ataxia of the newborn, neurotoxic effects, defects in lipid and carbohydrate metabolism (28).

Zinc (Zn): The role of zinc in relation of healing has been known for long time. More than 50 zinc activated enzymes have so far been identified. The more import ants are carbonic anhydrase, alkaline phosphates, pancreatic and car boxy peptidase. Other zinc containing proteins are the neural growth factor (NGF), the biosynthesis of ACTH (Aderenocortico Trophic Hormone), insulin utility and testosterone are influenced by zinc (29). The adult human body contains about 2gm of zinc an amount considerably larger than that of the other trace elements except iron. Tissue concentration varies from10-200mg/gram, the large quantities have been found in the corneal epithelium, the iris, retina, lens and prostate. Dietary requirements for zinc are related to the need of growth, tissue repair. With zinc deficiency, immune response is impaired, hair loss can result, night vision is lost, think less clearly, wound healing is slowed and protein metabolism impaired, reduction in sense of taste and level of testosterone drop. This essential trace element deficiency restricts growth and normal development (30).

Magnesium (Mg): It is a component of many coenzymes that regulate sugar metabolism, energy production, cell membrane permeability, and muscle and nerve conduction, Involved in thyroid/parathyroid production. It is an important factor of bone formation. As calcium is needed for muscle contraction, magnesium is needed for muscle relaxation. Magnesium activates an enzyme located in all cell membranes. This enzyme controls the balance of sodium and potassium, keeping sodium in the fluid outside the cells and potassium inside the cells. Such a balance is essential for normal water balance, nerve cell activity and cellular energy production. Without sufficient cellular magnesium, potassium will be rapidly excreted from the body resulting in

fatigue, heat exhaustion and weakness. Severe magnesium deficiency may cause muscle weakness, fatigue, abnormal heart rhythms, and depression (31).

2.4. Sample Decomposition Techniques

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 from eliminating interfering substances from the matrix and obtaining the element in a homogeneous and easily accessible matrix. The choice of decomposition techniques should take into account the objective of the final determination and factors such as the matrix composition, elemental contents, the possible interferences, the risk of loses and contaminations the practicality and possible safety hazards in the laboratory (32).

2.4.1. Wet digestion techniques

Wet digestion methods involve the use of both heat and mineral acid/s. Acids that have been used in this procedure include H_2SO_4, HNO_3 and $HClO_4$, either in combination or alone (33). Hydrogen peroxide (34) is also used to enhance the reaction speed and to ensure complete digestion. Most laboratories have eliminated the use of $HClO_4$ due to risk of explosion. Wet digestion can be carried out in open vessels, in tubes, on a hot plate or in aluminum heating block or in closed vessels at elevated pressure (digestion bombs) with thermal or microwave heating. Microwave-assisted digestion is an attractive method, especially for small samples. The applicability of this technique is strictly depends on the type of food. Carbohydrates are easily mineralized with nitric acid at 180oC, while fats, proteins and amino acids cause incomplete digestion due to the relative low oxidation potential of nitric acid at $20^{o}C$, these materials require the addition of sulfuric and/or perchloric acid with all the problems related to their use at high temperature and pressure. The type of acid/s used can have important consequences in the measurement step. It is commonly known that in all atomic spectrometric techniques nitric acid is the most desirable reagent. In spite of occasionally observed signal suppression in its presence(e.g.in ICP-OES), no sever analytical problems are encountered in practice with nitric acid at concentration up to 10% ,sometimes higher, in all atomic spectrometric techniques as long as its concentration is similar in calibration and sample solutions. Hydrogen peroxide added in most mineralization procedure is also rarely responsible for analytical problems (35).

The main advantages of Wet ashing are prevents elemental loss by volatilization because (the digestion takes place at a low temperature) & it is faster than dry ashing. However, the limitations

are including more labor intensive, reagent contamination, tedious & require operator attention & low sample throughout than the microwave digestion procedures (36).

2.4.2. 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 main advantages of dry ashing are safety, few reagents are required, large amounts of samples can be analyzed at the same time. It is more precision, simple, rapid digestion method. It is also relatively free from reagent contamination. However, its disadvantage are, requires relatively expensive apparatus (platinum crucibles & muffle furnace) & it cannot be used for the determination of elements that are volatile below the ashing temperature (36) K, P, S, As, Se, & Hg. 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 (37).

2.4.3. 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. 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°C or pressures to 800 psi, under controlled pressure and temperature. Under these conditions, even refractory samples can be digested successfully (38). The advantages of microwave digestion for heavy metal analysis in plants are shorter acid digestion time, better recovery of volatile elements,

lower contamination levels, low volumes of reagents are required & relatively safe to use. So, from the three sample preparation procedures, microwave digestion method is the choice for heavy metal analysis in samples. However, it is more expensive & overall analysis time is not much longer, due to the sample vessel handling (36). In general, all three digestion methods give similar results for the elements studying. The only differences are microwave is fast, simple, & safe to available (39).

2.5. Analytical instruments for metal detection

2.5.1. Atomic Absorption Spectrophotometer (AAS)

AAS is a mature analytical method, which is present in almost any analytical laboratory as a working horse for elemental determinations of metals (39). Aqueous samples can be generally introduced for analysis directly and without any prior treatment. The only major problem associated with working with solutions is their collection and storage. Concerning atomic spectroscopic analysis itself, no particular precautions have to be taken. Non-aqueous samples can sometimes be run directly, but this depends significantly on their viscosity. In AAS analysis, the viscosity should be similar to that of water for which most nebulizers are designed. Only some organic solvents, such as ethanol or methyl isobutyl ketone, are often used for dilution of organic liquids, the major drawback, encountered with these techniques is the dilution factor, which reduces the metal content per unit volume (40).

2.6. Instrumentation

Instrumentation used to carry out atomic absorption spectrophotometry requires a source of light that matches the arrow bands of light that a particular atom absorbs (a hollow cathode lamp), a flame or graphite furnace to heat the sample, a monochromator to select the wave length of light, and a photo detector. Schematic diagram of atomic absorption spectrometry system is shown in figure 1. It consists of light source, an atomizer, detection device and data processor (41)
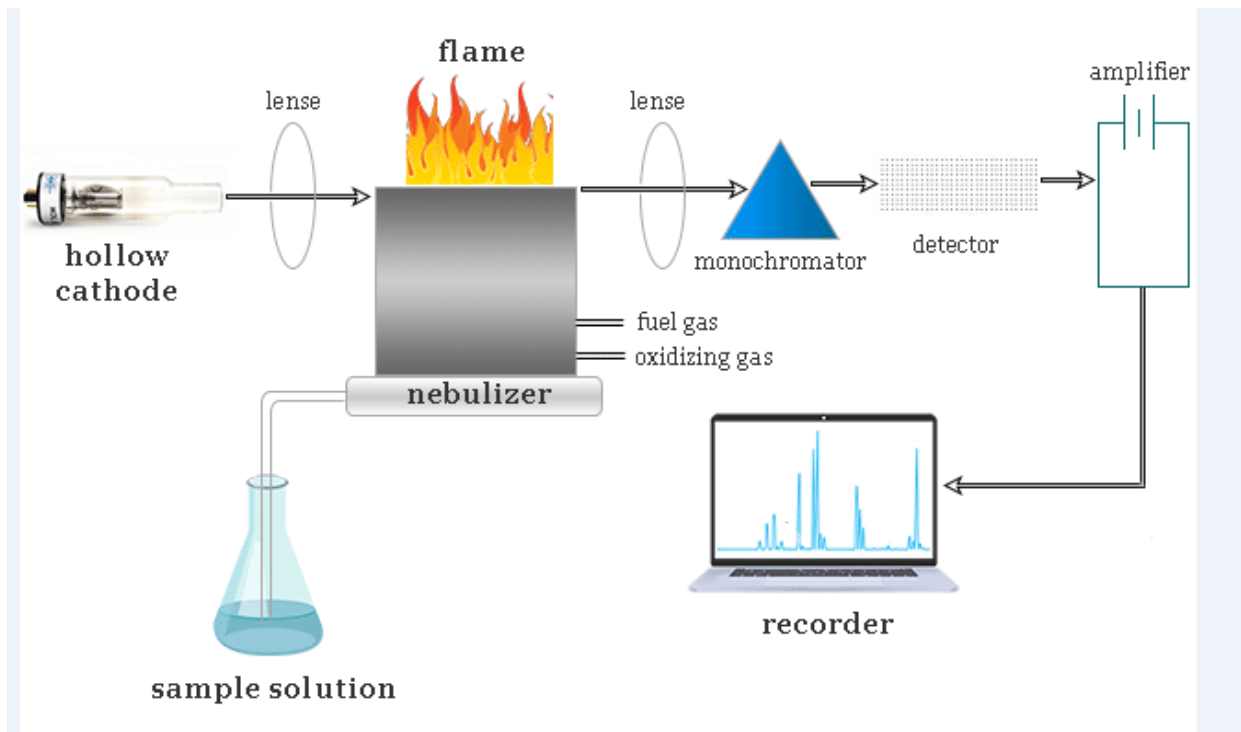
Figure 1: Schematic diagram of atomic absorption spectrometry system. (41)

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This research will be conducted in Guna Begemidr woreda (Ata gura, Arga Didim and Wukrotadomender kebeles), south Gonder zone, Amhara region, Ethiopia. The administrative center of this Woreda is kmirdingay, which is located 29 km from Debretabor, 131 km from Bahir Dar, and 697 km north from Addis Ababa, the capital city of Ethiopia. This is located in $11°49'N$ latitude and $38°13'E$ longitudes. It has an elevation of 2556 to 2971 meters above sea levels.

Figure 2: Maps of wheat and barley sample collection area.

Table1: List of crops commonly used by community of Guna Begemidr woreda.

NO	Common name	Scientific name
1	Barley	Hordeum vulgare L.
2	Wheat	Triticum aestivum L.

3.2. Sample Collection

One kilogram of each cereal (wheat and barley) samples were collected randomly from three selected kebeles Ata gura, Arga Didim and Wukrotadomender kebeles) and mixed together. Then, they washed with tap water to remove the adsorbed soil and other particulate matters. From the mixed composite samples 500g wheat and barley samples were dried and kept for their selected metal analysis and transported to laboratory and the rest samples were processed as show the figure 3:

Figure 3: Sample of wheat grains (A, B) and barley grains(C, D) respectively

3.3. Sample preparation

The bread was prepared according to the traditional procedure used in Ethiopia. From the raw wheat and barley flour samples bread was made using clay pans. The flour was mixed well with tap water. The clay pan was heated and then the prepared dough was placed on the pan. The pan with the dough was covered with lid, and after 10 min the bread was inverted upside down for uniform heat distribution. Then the bread made was taken off from the pan and allowed to cool and was cut in to pieces and exposed to sundry to complete dryness, i.e. removed the water added while baking repeatedly weighed until a constant weight was obtained. Then the dried bread sample was crushed in to powder form and made ready in the clean and dry glass bottle for digestion for analysis.

A   B

Figure 4: Bread prepared from wheat (A) and barley (B).

3.4. Instrumentation and Apparatus

Atomic Absorption Spectrophotometer (AAS) model 210 VGP instrument was used for determination of metal concentrations. For this work different types of apparatus were used, like different sizes of measuring cylinders, (100 ml) conical flask, (50 ml) volumetric flask, beakers (100 ml, 50 ml), Funnel, hot palate, thermometer, micropipette, digital balance, Electrical blender device, fume hood, filtered paper Whatman (42) and A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested samples until analysis.

3.5. Chemicals and Reagents

Reagents that were used in the analysis all analytical grades. HNO_3 acid (69%), per-chloric acid /$HClO_4$ (70%), (Extra pure AR, Research laboratory Fine Chemistry, India), deionized water, tape water and stock solution of the selected metals (Mg, Fe, Zn and Mn standards were used.

3.6. Optimization parameters

The basic requirement for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which required minimum reagent volume consumption, minimum digestion temperature, minimum digestion time reflection and clear colorless digestion solution, ease of simplicity and absence of undigested powder cereal crops sample. In this study, to prepare a clear colorless sample solution that is suitable for the analysis using AAS different digestions, were carried out using HNO_3 and $HClO_4$ acid mixtures by varying parameters such as volume of the acid mixtures, digestion time and digestion temperature.

3.7. Sample Digestion Procedure

The appropriate choice of acid for the wet digestion process of food sample was made (42). In this study, 0.5 gram (g) wheat, barley and its processed food samples were taken in to 100mL round bottom flask. The cereal samples (wheat, barley and its processed food) were digested on a hot plate with 4 mL of concentrated nitric acid (69% HNO_3) and 2 ml of perchloric acid (70% $HClO_4$) (4:2) a colorless solution was obtained for 1:30 hour (hr) at $200^O C$ and for 1:10 hour at $180^0 c$ respectively. The sample solution was allowed to cool for 10 minutes at room temperature. The cooled digested sample was then filtered into a 100 mL standard volumetric flask with Whatman filter paper in order to remove any suspended or turbid matter. To use the portion of the samples remaining in the beaker, the beaker was rinsed with small portion of deionized water and then filtered in to the flask. The samples were stored in 100 mL volume plastics and placed in a freezer to avoid any decomposition until analysis.

3.7. 1. Digestion of the Blank samples

Estimation of the metal concentration of the blank is important for the determination of the detection limit of the analytical method used during the study (43). Thus, one reagent blanks was digested for each sample following the same procedure used for the wheat, barley flour and their processed food flours. For blank samples, 4ml HNO_3 (69%) and 2ml $HClO_4$ (70%) (4:2) were digested without Sample. The digested blank samples were diluted by deionized water to 100 ml volumetric flask for metal analysis.

3.7.2. Optimization of digestion procedure

In any scientific experiments especially in analytical chemistry creating an optimum working condition before starting analysis of the actual samples is a common practice. That means before preparing the samples for analysis the temperature, the volumes of reagents to be used and the duration of the preparation should be optimized. Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form from complex organic matrix based on changing different digestion parameters like volume ratio of reagents to be added, digestion temperature and duration of time. Hence the optimization procedures for the sample preparation for the determination of metal contents were made as shown in Table 2-6**.**

The optimization of reagent volume, temperature and time for digestion of 0.5 gram of wheat flour and wheat bread flour were presented below.

Table 2: Optimization of reagent volume for digestion of the 0.5 g Wheat flour samples at a temperature of 200°C and for 1:30 hour.

Trial No	Optimization volume ratio (ml) HNO_3:$HClO_4$	Total volume	Observation
1	5:1	6	Pale yellow solutions
2	5:2	7	Yellow solution
3	5:3	8	yellow solutions
4	**4:2**	**6**	**Clear and colorless solutions**
6	6:2	8	Yellow solution

❖ The bold font shows the optimized volume ratio.

Table 3: Optimization of time for digestion of 0.5 g of wheat flour samples using optimized reagent volume

Trial No	Optimization volume ratio (ml) HNO$_3$:HClO$_4$	Time (hour)	Temp. (^{0}C)	Observation
1	4:2	1:40	200	Cloud white solution
2	4:2	2:00	200	Clear and colorless solution
3	4:2	2:10	200	Clear and colorless solution
4	4:2	**1:30**	200	**Clear and colorless solutions**
5	4:2	1:45	200	Cloud white solution
6	4:2	2:00	200	Clear with residue solution

❖ The bold font shows the optimized time.

Table 4: Optimization of reagent volume for digestion of the Wheat Bread (0.5 g of cereal Samples, at a temperature of 180°C and fot1:10 hour)

Trial No	Optimization volume ratio (ml) HNO$_3$:HClO$_4$	Total Volume	Observation
1	5:1	6	Clear with residue solutions
2	5:2	7	Pale yellow solutions
3	5:3	8	yellow solution
4	4:2	6	**Clear and colorless solutions**
6	6:2	8	yellow solutions

❖ The bold font shows the optimized reagent volume ratio.

Table 5: Optimization of time for digestion of 0.5 g of wheat bread samples

Trial No	Optimization volume ratio (ml) $HNO_3:HClO_4$	Time (hour)	Temp. ^0C)	Observation
1	4:2	1:35	180	Clear and colorless solution
2	4:2	1:30	180	Clear and colorless solutions
3	4:2	1:35	180	Clear with residue solutions
4	**4:2**	**1:10**	**180**	**Clear and colorless solutions**
5	4:2	1:15	180	Light yellow
6	4:2	1:30	180	Clear and colorless solutions

❖ The bold font shows the optimized time.

Table 6: Optimization of temperature for digestion of 0.5 g of wheat bread samples.

Trial No	Optimization Volume ratio (ml)$HNO_3:HClO_4$	Time (hour)	Temp. (^0C)	Observation
1	4:2	1:10	200	yellow solutions
2	4:2	1:10	190	Cloud white solution
3	**4:2**	**1:10**	**180**	**Clear and colorless solutions**
4	4:2	1:10	170	Light yellow solutions
5	4:2	1:10	160	Cloud white solution

❖ The bold font shows the optimized of temperature.

3.8. Method Verification

Method Verification is a process that is used to demonstrate the suitability of an analytical method for an intended purpose. It is an important requirement in the practice of chemical analysis. Verification procedures will be developed by variety of industrial committees, regulatory agencies, and standards organizations for purpose of quality control and regulatory compliances. Method verification is an important analytical Tool to ensure the accuracy and specificity of the analytical procedures. It is an important parameter in reporting of analytical results (44).

3.8.1. Limit of Detection (LOD or MOD)

Method of detection limit is the lowest concentration (the smallest amount) of analyte that can be detected by using a given analytical procedure. The limit of detection (LOD) is the concentration at which we can decide whether an element was present or not. The method detection limit (MDL) was determined as three times the standard deviation of the blank solution (45).

Limit of detection $= \text{LOD} = 3 \times \text{SD}_{\text{blank}}$

Where: SD_{blank} = standard deviation the blank solution.

3.8.2. Limit of Quantification (LOQ)

The limit of quantification, LOQ, is the lowest amount or concentration of analyte that can be determined with an acceptable level of precision and accuracy (46). Method quantification limits (MQL) were determined as ten times the standard deviation of the blank solution (45).

Limit of Quantization $= \text{LOQ} = 10 \times \text{SD}_{\text{blank}}$

3.8.3. Accuracy (Recovery Test) and Precision

Accuracy is a degree to which the determine value of analyte corresponds to the true value. It is determined by analyzing a sample of known concentration and comparing with the true value, spiking a blank (sample having all components except the analyte) and comparing with expected results and standard addition method in which the sample concentration is determined. The difference of the two concentration values is compared with the actual value of added analyte. Recovery studies involve the addition of known amount of analyte to a sample and then determining what percent of the amount added is detected. The percentage recovery is calculated by using the formula:

%Recovery $= C_m$ in spiked sample $- C_m$ in non-spiked sample $\times 100 / C_m$ added for spiking

Where: C_m is the concentration of metals.

Precision expresses closeness of a series of measurements of the same sample under identical condition, and is expressed as a standard deviation or relative standard deviation. High degree of precision does not necessarily means a high degree of accuracy. Precision is expressed as variance, standard devotion or as coefficient of variation of a series of measurements and shows how close results are to one another (47).

3.9. T-test for sample Analysis

T-test Analysis was used to evaluate the difference in concentration of metals between the samples were significant or not. Under this study, the significance of variation between samples has been studied using independent t- test & SPSS version 20 was applied for all calculations, the results of which were utilized to indicate the presence or absence of significant differences in mean concentration of each metal between the experimental cereal samples.

4. RESULT AND DISCUSSION

4.1. Instrument Operating Condition of AAS for the Determination of Metals in Cereals Samples

The atomic absorption spectrophotometer (AAS) working conditions were adjusted in accordance with the manual for the respective element. Before analyses of elements in samples, it is required to adjust the various instrumental operating conditions for the instruments were prepared for each essential metal at appropriate wavelength, slit width, photo multiplayer tube, lamp current and energy were used for maximum of signal intensity of the instruments based on the instrument instruction (Table 7).

Table 7: Instrumental operation condition for determination of essential metals in cereal samples by using atomic absorption Spectrophotometer (AAS).

Parameter	Mn	Fe	Zn	Mg
Wavelength(nm)	279.5	248.3	213.9	285.2
Band(Slit) width (nm)	0.7	0.2	0.7	0.7
Lamp current(m A)	3.0	7.0	2	1
photo multiplayer tube(pmt)(V)	257.0	323.5	272.9	257.0
Energy(J)	3.86	3.0	0.3	3.8
Flame type	Air acetylene	Air acetylene	Air acetylene	Air acetylene

4.2. Method validation procedure

In order to validate the analytical method, limit of detection, limit of quantification, linearity, precision and accuracy studies were carried out.

After digestion of all samples for the analysis of Mn, Fe, Zn and Mg metals using the same digestion procedure by using AAS. Moreover, method of validation parameters like LOD, LOQ, and Recovery Was determined using the spiked digested samples.

4.2.1. Limit of Detection (LOD or MDL)

Limit of Detection: is the lowest concentration of analyte that can be detected and reliably distinguished from zero (or the noise level of the system), but not necessarily quantified; Limit of Detection (LOD or MDL)

The concentration at which a measured value is larger than the uncertainty associated with it. MDL can be expressed in response units and is taken typically as three times the noise level for techniques or three times the standard deviation (SD) of the sample blank (48).The standard deviation for each element was calculated from the blank measurements to determine method detection limit (MDL) or limit of detection and the value is shown in Table 8 below.

LOD = 3×SD Blank.

4.2.2. Limit of Quantification (LOQ)

Limit of Quantification (LOQ) is lowest concentration at which the performance of a method or a measurement system is acceptable for specific use and this concentration is reported. It is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. It can be calculated as LOQ = 10 times SD of blank reagent (48).

Table 8: Summary of the instrument detection limit, limit of detection and limit of quantification of an elements determined using the blank solution of samples in mg/L.

Metals	Mn	Fe	Zn	Mg
LOD	0.0021	0.0063	0.008	0.0021
LOQ	0.007	0.021	0.026	0.007
IDL	0.002	0.0045	0.003	0.001
Blank of SD	0.0007	0.0021	0.026	0.0007

LOD= Limit of detection, LOQ= limit of quantification; and IDL= Instrumental detection limit

From this study, method detection limit lies in between 0.0021-0.008 mg/L and limit of quantification lies between 0.007- 0.026mg /L. Both the LOD and LOQ are higher value than IDL

4.2.3. Recovery Test

Recovery shows that, the accuracy of the analytical procedure, by spiking a suitable known amount of the analyte metals into a test portion of the sample having a known concentration of the analyte. The percentage recovery was calculated by using the following formula given below and the value is shown in Table 8.

$$\% \text{ Recovery} = \frac{\text{CM in spiked sample} - \text{CM in non spiked sample}}{\text{CM of spike added}} \times 100$$

Where: CM is the concentration of metals.

Table 9: Recovery test results of metals for the analysis of wheat bread samples.

Metals	concentration in the samples (mg/L)	Amount added (mg/L)	Concentration in the spiked sample(mg/L)	Recovery (%)
Mn	0.036	0.1	0.13	94
Fe	0.233	1	1.20	96.7
Zn	0.148	0.5	0.638	98
Mg	1.581	1	2.641	106

The concentration of metals was determined in mg/L. For the sake of simplicity, the concentration in mg/L converted to mg/kg by the following formula: to express the concentration of metals in dry seed in mg/kg. C=A ×V\W

Where: C= total metal concentration (mg/Kg)

A=mg/L of metal in the digested sample

W=weight of the digested sample (g)

V=final volume of digested sample solution (mL)

4.3. Instrument Calibration

The qualities of results obtained for essential metals analysis using AAS are seriously affected by calibration and standard solution preparation procedures. The concentrations of metals (Mg, Fe, Zn and Mn) were determined by atomic absorption spectrophotometer (AAS) in the wheat, barley and its processed food samples after digested and diluted with deionizer water. To calibrate the instrument using the calibration curve, four series of standard solutions were prepared. The standards were prepared fresh by diluting the intermediate metal solution (mg/L) that prepared from the stock solution (1000 mg/L) of each metal. The correlation coefficient (R2) of each element was determined by plotting calibration curves of absorbance versus concentration of the prepared standards. The linear correlation coefficients obtained were within the range: 0.9989-0.9997.

Table 10: Concentration of working solution (mg/L), correlation coefficients of the calibration curves and equation of the calibration curve.

Metal	Concentration of working sample (mg/L)	R-Square (R^2)	Equation for correlation of calibration curve
Mg	0.1, 1, 2, 3	0.9989	Y=0.0019X+0.0012
Fe	0.1, 1, 2, 4	0.9991	Y=0.0037X+0.0006
Zn	0.5, 1, 2, 4	0.9993	Y=0.9997X+0.0563
Mn	0.1, 2, 4, 5	0.9997	Y=0.0265X+0.0186

4.4. Levels of Metals in wheat, barley and its processed food Samples

Each sample was analyzed in triplicate. The mean values were determined from the results of triplicate analysis of each sample for each metal and the results are reported in terms of mean values ± SD (Table 10). The concentrations of metals (Fe, Zn, Mn and Mg) were determined by AAS in the wheat, barley and it process food samples after the digested and diluted with deionizer water. The levels of metals were different in wheat, barley flour and their processed food samples. Due to food processing practices, the levels of metals in wheat and barley flour lower than their processed food samples. The results were summarized below in the table.

Table 11: Concentration of metals (Mean ±SD, n=3, mg/kg dry weight, and %RSD) samples.

Metals	Cereal Samples							
	Wheat flour Mean±SD	%RSD	Wheat bread Mean ±SD	% RSD	Barley flour Mean ±SD	% RSD	Barley bread Mean ±SD	% RSD
Mn	5.7 ± 0.022	2.53	7.2 ± 0.017	5	5.6±0.0017	6.5	6.7±0.0016	4.85
Fe	43.8 ± 0.001	0.46	46.6± 0.003	1.16	44.2±0.0018	0.81	45.4 ± 0.002	0.71
Zn	28 ± 0.001	0.71	29.5± 002	1.42	10.4± 0.002	3.85	16.3 ± 0.003	3.38
Mg	243.4±0.005	0.43	316.4 ± 0.005	0.37	284.4±0.006	0.39	329.86±0.003	0.19

(SD=Standard deviation, %RSD = relative standard deviation)

Based on the above table, the result of the relative standard deviation of the measurements were found in the acceptable range i.e. % RSD ≤ 10. Therefore, all values were found in the recommended limit ≤ 15 %RSD.
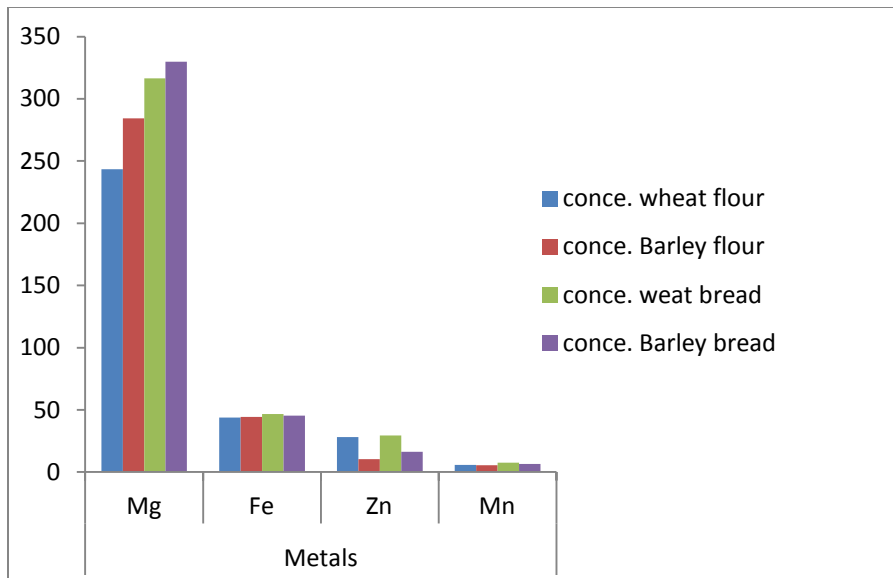
Figure 5: Concentrations of metals in wheat, barley flour and their processed food samples.

4.5. Distribution of patterns of metals in wheat and barley flour samples

The plants uptake metals by different and complex biochemical processes. The accumulation of metals depends on the ability of particular plant to absorb metals from the soil and the availability of the minerals in the soluble forms in the particular areas. The variation in the level of metals in soil depends on the degree of pollution of the biosphere from the rapid industrialization and modern large scale agricultural activities (49). The use of sewage sludge, pesticides, herbicides and fertilizers on agricultural lands highly affect the quality of food products for humans and animals (50).The distribution and accumulation of metals in wheat and barley grains are the reflections of the mineral composition of the soil and the degree of mineral pollution of the environment in which the wheat and barley plant grows(51).The average concentration of metals in studied sites expressed in mg/kg of dry matter of wheat and barley flour were Mn (5.7), Fe (43.8), Zn (28), Mg (243.4) and Mn (5.6), Fe (44.2), Zn (10.4) and Mg (284.4) respectively. The wheat and barley samples collected from the study areas showed the presence of Mn, Fe, Zn, and Mg. Mg was the highest content metal in the studied samples. The decreasing order of metal concentrations in wheat and barley flour were Mg > Fe > Zn > Mn.

4.6. Distribution of Levels of Metals in wheat bread and barley bread samples.

The average concentration of metals in studied sites expressed in mg/kg of dry matter of wheat bread and barley bead samples were Mn (5.7), Fe (46.6), Zn (29.5), Mg (316.4) and Mn (6.7), Fe (45.4), Zn (16.3) and Mg (329.86) respectively. The wheat and barley bread samples collected and prepared from the study areas showed the presence of Mn, Fe, Zn, and Mg. Levels of metal Mg in barley bread higher than wheat bread. Levels of Mn, Fe and Zn metals in wheat bread higher than in barley bread. Mg was the highest content metal in both wheat and barley bread samples. The decreasing order of metal concentration was Mg > Fe > Zn > Mn. The concentration of wheat and barley bread was often greater than that of regular barley and wheat flour due to its higher protein content. Bread flour typically contains around 12-14% protein, compared to 8-11% in all-purpregular wheat flour (52). This higher protein content is crucial for developing gluten, which gives bread its structure and chewiness. Additionally, bread flour can absorb more water than regular wheat flour, leading to higher hydration levels in dough's made with bread flour(53). This is important for achieving the desired texture and crumb structure in bread.

4.7. Compare the level of selected metals in cereal samples wheat, barley flour and their processed food in the study area.

The concentrations of metals (Fe, Zn, Mn and Mg) were determined by AAS varied in the wheat, barley and its processed food samples. The concentrations Fe, Zn, Mn and Mg in the wheat and barley grain samples lower than its processed food samples. The concentration of metals in each samples varied in the decreasing order of Mg > Fe > Zn > Mn. The order of metals contents in the raw grain and processed foods were different from each other. This is due to the differences in food processing practices. The comparison Show below in the graph of concentration versus metals. The concentration of metals (Mg, Zn, Fe and Mn) in wheat bread samples higher than wheat flour.

The order of Mn < Zn < Fe < Mg.

Similarly concentration metals in barley four less than barley bread. The order of Mn < Zn < Fe < Mg.
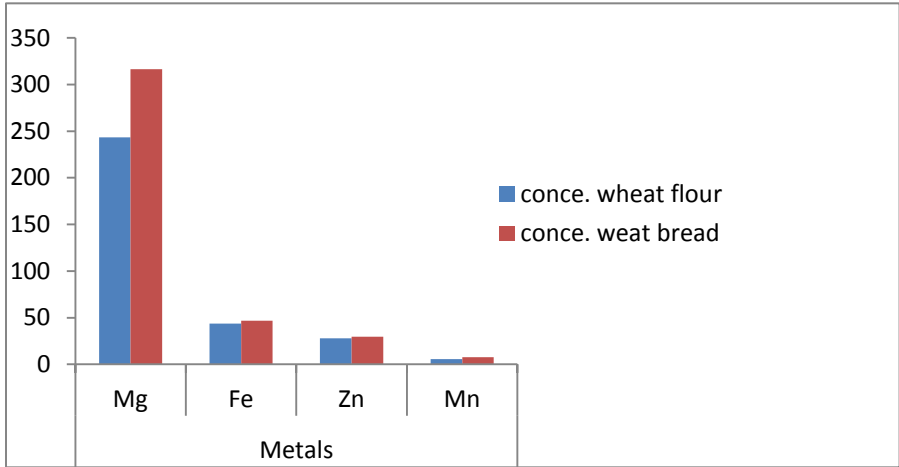
Figure 6: Comparison of the Concentration of metals in wheat flour and wheat bread samples.
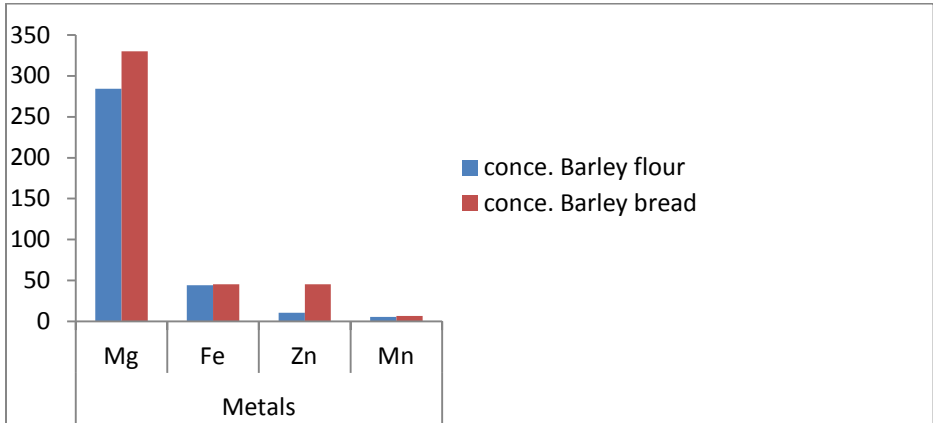
Figure 7: Comparison of the Concentration of metals in barley flour and barley bread samples.

4.8. Statistical Analysis.

The mean significant difference between the values of samples obtained in this study was evaluated by indepenance t-test at 95% confidence level. There are 4 degrees of freedom for metal measurement and the critical value at $t_4 =$ from the table is 2.78 (P= 0.05). The t- calculated value of for manganese and iron determination was less than the t-critical value. That means there is no significant difference in concentration of Mn and Fe between wheat and barley flour. The calculated t-values of zinc and magnesium were greater than the critical t-value. There is a significant difference between the mean concentrations. The t- calculate value of Mn, Fe, Zn and Mg in wheat flour with wheat bread and barley flour with barley bread are greater than t- critical value. There is mean significant difference in concentration between them respectively.

4.9. Comparison of the levels of metals in barley, wheat with literature values

Several studies have been done on barley and wheat grain by different investigators in different countries. However, there is no detailed study conducted on the levels of metal (Mg, Fe, Zn and Mn) contents in barley wheat grains cultivated in Guna Begemidr woreda. Therefore, the results of present study have been compared with the results reported from other countries in the literature as summarized in Table 12 and 13. There are variations in the levels of individual metal in the barley and wheat grain from different countries. The variations are expected due to differences in the soil properties, geographical locations, climatic conditions and agricultural practices in different countries.Table12: Comparison of essential metals (Mg, Fe, Zn and Mn) concentration (mg/kg, dry weight basis) in barley grain with reported values.

	Selected metals				Country	Reference
Concentration in Barley cereal sample in (mg/kg)	Mg	Fe	Zn	Mn		
	1650	87	175	13	Egypt	(53)
	2086	937	33.7	23.9	Pakistan	(54)
	NR	1546	53	38.7	Pakistan	(55)
	940	56.9	24.6	14.6	Poland	(56)
	1810	82.9	69	12.3	Saudi Arabia	(57)
	650	30	21	NR	UK	(68)
	NR	31.85	3.85	1.67	Ethiopia	(99)
	NR	NR	18.8	NR	Iran	(60)
	284.4	44.2	10.4	5.6	Ethiopia	This study

NR = not reported

The table 12 shows that the levels of metals found in the present study are lower than the literature values. However, the concentration Zn in this study higher than reported value in Ethiopia and the concentration Fe and Mn in this study higher than reported value in UK and Ethiopia.

Table13: Comparison of essential metals (Fe, Zn and Mn) concentration (mg/kg, dry weight basis) in wheat grain with reported values.

Metals concentration ±SD			References
Fe	Zn	Mn	
422.5	99.5	500	(59)
8.168± 1.717	6.154±0.313	4.309 ±6.187	(61)
8.168 +0.662	6.314 ± 0.211	2.866 ± 0.981	(61)
43,8 ± 0.001	28 ± 0.001	5.7 ±0.022	This study
10.64	8.54	7.67	(59)
37.9	38.9	17.6-24.2	(62)

NR= not reported

In this (table 13), the mean value of Mn from the present study in wheat samples were lower than mean values reported by others. The mean value of Fe from the present study in wheat grain samples higher than the values reported by others. The mean value of Zn in the current study was higher than the mean value reported by Tegegne and Shart but lower than Abrar. The mean concentration of Fe, Mn and Zn in wheat samples collected from three Kebeles in Guna Begemidr Woreda was found to be less than the limits approved by FAO/WHO.

5. CONCLUSION

In this study, the levels of metals (Mg, Fe, Mn and Zn), in cereal grains and its processed foods were determined by AAS. An efficient digestion procedure for determination of metals in wheat, barley and its processed food samples collected from the selected areas were validated through spiking method and a good percentage recovery was obtained for the metals of interest. The concentration of metals in wheat flour and wheat bread samples were recorded as the order Mg (243.4 mg/kg)> Fe (43.8 mg/kg)> Zn (28 mg/kg) >Mn (5.7 mg/kg) and Mg (316.4 mg/kg)> Fe (46.6 mg/kg) >Zn (29.5 mg/kg) >Mn (7.2 mg/kg) respectively. And the concentration of metals in barley flour and barley bread samples were recorded as the order Mg (284.4 mg/kg)> Fe (44.2 mg/kg)> Zn (10.4 mg/kg) > Mn (5.6 mg/kg) and Mg (329.86 mg/kg)> Fe (45.4 mg/kg)> Zn (16.3 mg/kg) >Mn (6.7mg/kg) respectively. Among the metals, Mg was the highest and Mn was the lowest concentration in all samples. The t-independent test result at 95% confidence level suggests that there were significant difference in the mean concentration of metals in wheat flour with wheat bread and barley flour with barley bread. However, in wheat flour and barley flours were no significance differences among mean concentration of Mn and Fe metals. Generally, the concentration of metals in the wheat, barley and its processed foods were different from each other. The concentrations Fe, Zn, Mn and Mg in the wheat and barley grain samples lower than its processed food samples. This is due to the differences in food processing practices. Barley, wheat and its processed foods are sources of essential metals.

6. Recommendation

Representative sample of wheat and barley from different geographical conditions like soil type, Soil P^H, and climate has not yet been studied. Future studies should focus on the determination of other important minerals and none essential metals in barley and wheat grains and its processed food samples in selected area. I recommend applying other instrument like inductive coupled plasma optical emission spectroscopy (ICP-OES)

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7. APPENDEX: T-test for sample analysis

T-test for Wheat flour and Wheat bread

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
| | | F | Sig. | T | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
									Lower	Upper
Mn	Equal variances assumed	.208	.672	-25.900	4	.000	-.340000	.013128	-.376448	-.303552
	Equal variances not assumed			-25.900	3.899	.000	-.340000	.013128	-.376821	-.303179
Fe	Equal variances assumed	4.000	.116	-8.573	4	.001	-.014000	.001633	-.018534	-.009466
	Equal variances not assumed			-8.573	2.560	.006	-.014000	.001633	-.019741	-.008259
Zn	Equal variances assumed	5.565	.078	-4.287	4	.013	-.008333	.001944	-.013730	-.002937
	Equal variances not assumed			-4.287	2.384	.037	-.008333	.001944	-.015530	-.001136
Mg	Equal variances assumed	.064	.813	-91.974	4	.000	-.365333	.003972	-.376362	-.354305
	Equal variances not assumed			-91.974	3.950	.000	-.365333	.003972	-.376417	-.354250

T-test for Barley flour and Barley bread

Independent Samples Test

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
| | | F | Sig. | T | Df | Sig(2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
									Lower	Upper
Mn	Equal variances assumed	.000	1.000	-5.345	4	.006	-.006667	.001247	-.010130	-.003204
	Equal variances not assumed			-5.345	4.000	.006	-.006667	.001247	-.010130	-.003204

		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Fe	Equal variances assumed	.182	.692	-4.250	4	.013	-.005667	.001333	-.009369	-.001965
	Equal variances not assumed			-4.250	3.938	.014	-.005667	.001333	-.009392	-.001942
Zn	Equal variances assumed	3.935	.118	-5.227	4	.006	-.025667	.004910	-.039300	-.012033
	Equal variances not assumed		.	-5.227	2.316	.025	-.025667	.004910	-.044256	-.007078
Mg	Equal variances assumed	2.295	.204	-45.782	4	.000	-.174667	.003815	-.185259	-.164074
	Equal variances not assumed			-45.782	2.660	.000	-.174667	.003815	-.187737	-.161597

T-Test for Wheat and Barley flour

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means					95% Confidence Interval of the Difference	
		F	Sig.	T	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Mn	Equal variances assumed	12.633	.024	-.780	4	.479	-.006667	.008551	-.030407	.017073
	Equal variances not assumed			-.780	2.043	.516	-.006667	.008551	-.042724	.029391
Fe	Equal variances assumed	2.000	.230	-1.732	4	.158	-.002000	.001155	-.005206	.001206
	Equal variances not assumed			-1.732	3.200	.176	-.002000	.001155	-.005548	.001548
Zn	Equal variances assumed	4.000	.116	61.025	4	.000	.088667	.001453	.084633	.092701
	Equal variances not assumed			61.025	2.725	.000	.088667	.001453	.083767	.093567
Mg	Equal variances assumed	.106	.762	-44.500	4	.000	-.205000	.004607	-.217790	-.192210
	Equal variances not assumed			-44.500	3.884	.000	-.205000	.004607	-.217942	-.192058

8. Appendix Figure

Magnesium (Mg) calibration curve: $y = 0.0019x + 0.0012$, $R^2 = 0.9989$

Iron (Fe) calibration curve: $y = 0.0202x + 0.0004$, $R^2 = 0.9999$

Zinc (Zn) calibration curve: $y = 0.0999x + 0.0563$, $R^2 = 0.9993$

Manganese (Mn) calibration curve: $y = 0.0265x + 0.0186$, $R^2 = 0.9997$