HAWASSA UNIVERSITY

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCES

CHEMISTRY DEPARTMENT

PHYSICOCHEMICAL CHARACTERIZATION OF THREE WHOLE GRAIN TEFF CULTIVARS GROWN IN ENOR ENER MEGER WEREDA, GURAGHE ZONE, CENTRAL ETHIOPIA REGIONAL STATE, ETHIOPIA

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HAWASSA, ETHIOPIA

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DECLARATION

I hereby declare that this MSc thesis is my original work and has not been presented for a degree in any other university, and all sources of material used for this thesis/dissertation have been duly acknowledged.

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This MSc thesis has been submitted for examination with my approval as thesis advisor.

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LIST OF ABBREVIATION

ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists
db	Dry basis
DNA	Deoxyribonucleic acid
ESA	Ethiopian Standards Agency
FAAS	Flame Atomic Absorption Spectrometry
IDL	Instrumental detection limit
LSD	Least significant difference
MDL	Method detection limit
SD	Standard Deviation
SNNPR	Southern Nations Nationalities and Peoples Region
SPSS	Statistical Package for the Social Sciences
%RSD	Percent relative standard deviation

ABSTRACT

Enor Ener Meger wereda is one of teff [Eragrostis teff (Zucc.) Trotter] growing and producing wereda's in Guraghe zone, Central Ethiopia Regional State, Ethiopia. However, there is no information regarding the chemical composition of 'teff' grown in this wereda. Thus, this study was conducted to generate information on the chemical composition (proximate and mineral content) of 'teff' grown in Enor Ener Meger wereda and compare the result among the selected whole grain 'teff' samples and with the national standards for 'teff' flour specifications. A total of 5.0 Kg of 'teff' grain composite samples for each type of 'teff' were collected from ten farmers selected randomly from two kebeles in Enor ener meger woreda and were analysed for their physicochemical composition and mineral content. The proximate composition of whole grain 'teff' flours was determined using Standard methods of analysis and Flame Atomic Spectrometer was used to measure minerals content of samples. The mean ranges of values for pH, moisture, total solids, total ash, crude protein, crude fiber, crude fat, total carbohydrate, and energy were 6.36–6.77, 10.78–14.19%, 85.80–89.22%, 1.73–2.53%, 9.64–10.94%, 2.08–2.60%, 2.39–2.57%, 67.92–72.58%, and 336.56–350.36 kcal, respectively for the three whole grain 'teff' samples. Similarly, the mean ranges of values for calcium, magnesium, copper, and manganese were 153.29–248.54 mg/Kg, 123.93–180.20 mg/Kg, 11.66–27.20 mg/Kg, and 45.47–170.27 mg/Kg, respectively for the three whole grain 'teff' samples. Results indicated that there were significant differences in most of the evaluated physicochemical composition and minerals content among the three whole grains 'teff' samples. The results showed that key whole grain 'teff' samples contains relatively high amount of protein, fat and fiber than white whole grain 'teff' flour samples. Furthermore, minerals such as Ca, Mg, and Mn were found to be the lowest in white whole grain 'teff' samples. This might suggest that key whole grain 'teff' varieties are nutritious than the white whole grain 'teff' varieties. However, key whole grain 'teff' varieties have lower market price and social values in the community to the contrary.

Key words: 'Teff', proximate composition, Minerals, Enor ener meger Woreda,

1. INTRODUCTION

1.1. Background of the study

'Teff' [*Eragrostistef* (Zucc.) Trotter] is one of the major and indigenous cereal crops in Ethiopia, where it was originated [1]. It is considered a low-risk crop from the perspectives that it can be cultivated in a broad range of ecological surroundings and under tough environmental conditions. 'Teff' can grow in altitudes ranging from sea level to 2800 meters above sea level under different moisture, soil, temperature, and rainfall regimes. It grows in dry as well as water-logged soils, can tolerate anoxic situations better than maize, wheat, and sorghum and is resistant to many pests and diseases [2]. 'Teff' is best suited for cultivation in a warm climate with temperatures ranging from 10 to 27 °C and altitudes of (1,000-2,100 m) [3].

Food products of 'teff' are rich in crude fiber as the whole grain flour of the crop incorporates the bran of the grain. The grain of the crop provides relatively higher protein content with an excellent balance and a complete set of essential amino acids [4]. The proteins found in 'teff' are essentially free of gluten the type found in wheat (alternative food for consumers allergenic to wheat glutens) [5]. The grain proteins are also presumed easily digestible because polyamines are very small [6]. 'Teff' grain micronutrient is also apparently high. Particularly in iron, a result of agronomic practices used in Ethiopia and fermentation on 'injera' making [7]. It was also recently proven to be a significant source of bioactive compounds including polyphenols, especially very rich in flavonoid derivatives [8, 9] which are rare in the other common grains. Ethiopia is the only country in the world where 'teff' is intensely grown and produced for human consumption. In Ethiopia, 'Teff' occupies over 2.8 million hectares equivalent to 25-30% of the total area covered by cereals. In terms of production, teff is the dominant cereal by area coverage and second only to maize in production and consumption [3].

'Teff' is the most popular grain for making 'injera', although other grains such as sorghum, maize, barley, wheat and finger millet are sometimes used [10]. 'Teff' grain flour is widely used in Ethiopia for making 'injera' (staples for the majority of Ethiopians, a fermented, pancake-like, soft, sour, circular flatbread), sweet unleavened bread, local spirit, porridges and soups [11, 12]. 'Teff' can also be combined with other baking flours to produce baked products, such as muffins and cookies.

In Ethiopia, three major categories of 'teff' can be identified, white (nech), red (quey) and mixed (sergegna) [13]. White 'teff' generally grows only in the Ethiopian highlands and requires relatively good growing conditions. This, along with its higher consumer preference, may justify why white 'teff' is the most expensive type of 'teff'. However, in recent years, red 'teff', which is believed to be more nutritious, is also gaining popularity among health-conscious consumers in Ethiopia. Recently, there is a growing interest on 'teff' grain utilizations because of nutritional merits (whole grain), and gluten-free nature of the grain, making it a suitable substitute for wheat and other cereals for consumers allergenic to wheat glutens [5]. Many gluten-free products may not meet the recommended daily intake for fiber, minerals, and vitamins [14]. Therefore, the main objective of this study was to investigate the physicochemical composition of three 'teff' samples collected from two kebeles of Enor Ener Meger wereda, Guraghe zone, Central Ethiopia Regional State, Ethiopia by using recommended physicochemical analysis methods.

1.2. Statement of the Problem

'Teff' is major cereal crop used for human consumption in Ethiopia. It is highly valued for its 'injera' making quality. It has good mineral content and generally higher amount of essential amino acids [16, 23]. 'Teff' grain is a good contender for a function food in health promotion and disease prevention. So, the study of 'teff' grain to give pay attention in order to aware its significance in major cereal food crop human health benefits. These physicochemical compositions and elements are function for biological activities when at optimum levels. The body health problem occur when use either excessive levels or deficiency of physicochemical compositions and mineral elements in different 'teff' varieties. The contamination of body by these elements with different discharging mechanism such as wastewater, agricultural imputes and pharmaceutical sources in several part of Guraghe zone. Until this moment there is no scientific researchers studied on the physicochemical cooperation and mineral elements in three different 'teff' varieties samples of Guraghe zone. Therefore, this study was conducted to determine the physicochemical compositions (pH, moisture content, total ash, crude protein, crude fat, carbohydrates, total fiber, and mineral content) of the three kinds of 'teff' and tried to

gained an understanding of the reason behind the high cost of white (nech) teff as compared to red (key) teff or mixed (sergegna) teff.

1.3. Objectives of the study

1.3.1. General objective

The general objective of this study was to determine the physicochemical compositions of three kinds of whole grain 'teff' samples collected from two kebeles of Enor Ener Meger wereda, Guraghe zone, Central Ethiopia Regional State, Ethiopia.

1.3.2. Specific objectives

The specific objectives of the study were;

- To determine physicochemical composition such as pH, moisture content, total ash, crude protein, crude fat, carbohydrates, total fiber, of three whole grain 'teff' samples obtained from Enor Ener Meger wereda, Guraghe zone, Central Ethiopia Regional State, Ethiopia.
- To determine the mineral content such as calcium (Ca), magnesium (Mg), copper (Cu), manganese (Mn), iron (Fe) and zinc (Zn) in three whole grains 'teff' samples obtained from Enor Ener Meger wereda, Guraghe zone, Central Ethiopia Regional State, Ethiopia.

1.4 Significance of the study

The study might be significant for producers, consumers and distributors by providing information about the physicochemical composition and mineral contents of 'teff' cultivated in the kebeles or sold in local markets. It might also be served as a baseline for future studies regarding the cultivation of good quality 'teff' grains in the study area.

1.5. Limitation of the study

The limitation of this study was the lack of hallow cathode lamps for the determination of iron and zinc using flame atomic absorption spectrometry (FAAS), which are important constituents of 'teff'. Furthermore, the samples for this study were taken from two kebeles and the total numbers of composite samples considered were three due to time and budget constraints, which might affect the conclusion of this study.

2. LITERATURE REVIEW

2.1. Description of 'teff

Teff [*Eragrostis teff* (ZUCC.) Trotter] is one of the major and indigenous cereal crops in Ethiopia [15]. 'Teff' is a fine stemmed tufted annual grass like cereal crop characterized by a large crown, many shoots and a shallow, diverse root system. It is a unique durable crop grown over a wide range of environmental conditions in Ethiopia and has been utilized as food and supplements for majority of the human diet in Ethiopia [5]. In Ethiopia; it is a major food grain, mainly used to make injera, a traditional fermented Ethiopian pancake and it is a daily food staple for more than 60 million people in the country [15].

'Teff' is predominantly grown in Ethiopia as a cereal crop, and its cultivation as a cereal grown for food is little known elsewhere in the world, its primary processing is mainly limited to indigenous processing to make injera, pancake-like fluffy soft bread, which is a staple food for most Ethiopians [15, 16, 17, 18]. The grain is also used to make local alcoholic drinks, called 'tella' and 'katikala' [15, 16]. Recently, 'teff' grain has been begun to be utilized in mixtures with soybean, chickpea and other grains in the baby food industry due to its high mineral content [5, 19].

2.2. 'Teff' Production and its distribution

'Teff' has been cultivated in the Horn of Africa for at least 2,000 years, Ethiopia is known as the center of origin [20]. 'Teff' is mainly grown in Amhara and Oromia regions, which together accounted for 86% and 88.85% of the total cultivated area and production, respectively [21]. 'Teff' is an important cereal crop in Ethiopia. The area under 'teff' cultivation is over one million hectares of land each year. During the 1994/95 cropping season 'teff' occupied 32% of the cultivated land under cereals, while maize occupied 19%, sorghum 16%, barley 15%, wheat 13%, millet 4% and oats 1% [22]; this is similar to previous production years and clearly shows the importance of 'teff' in Ethiopia. 'Teff' can serve as a temporary ground cover. It's very fast germination and fibrous root system development makes it an excellent choice for erosion control [23]. A smaller quantity of 'teff' is also produced in Tigray and Southern Nations Nationalities and Peoples (SNNPR) [24]. Depending on the varieties, the color of 'teff' grains can be ivory, light tan to deep brown or dark reddish brown purple [25].

'Teff' is cultivated in Ethiopia and believed to be a reach source of nutrients, but the consumption and awareness of the 'teff' is limited as compared to the Quinoa [26]. In Ethiopia, a country of nearly 90 million people, approximately 6 million households grow 'teff'. The production and consumption of 'teff' grain are matters of national policy, since food insecurity remains a serious problem. 'Teff' is now considered a luxury cereal and its consumption is mostly an urban experience, as most rural people are unable to afford 'teff' and rely mostly on less expensive grains to make 'injera'. The Ethiopian government banned the export of 'teff' grain (but not of 'injera') in 2006 [27].

2.3. Health Benefits of 'Teff'

Food products of 'teff' are rich in crude fiber as the whole grain flour of the crop incorporates the bran of the grain. As a result, the nutrient content of 'teff' flour is also higher [28, 29]. 'Teff' is nutrient rich with a protein content ranging from 10 to 12%, and high in iron and calcium [30, 31]. It is known to have excellent amino acid composition with lysine levels higher than wheat or barley and totally gluten free [32, 28]. It has high levels of minerals and important source of water soluble vitamins [33]. In contrast to most cereals crops, 'teff' was reported to contain vitamin C and is typically good in its fiber and starch levels [28]. Amino acid analyses of some 'teff' varieties were done by Jansen et al. [34] but they used few samples of unidentified varieties of 'teff' bought from markets, hence the data has little value for plant breeding and other studies [36]. 'Teff' varieties have different market and socio-cultural values. The higher consumer preference of white 'teff' may justify why it is the most expensive type. Recently, some health conscious Ethiopians prefer red 'teff' [35].

2.4. 'Teff': Physicochemical Composition

The proximate composition on dry basis of 'teff' is reported to be 9.4% – 13.3% protein, 73.0% carbohydrate 1.98% – 3.5% crude fiber, 2.0% – 3.1% fat and 2.7% – 3.0% ash [37]. Other study reported that 'teff' grains' have high nutritive value with carbohydrates (57.27%), protein (20.9%), essential amino acids (8.15%) with major leucine and lysine (1.71 and 1.35%, respectively), vitamin B1 (1.56%) and potassium and calcium (32.4% and 9.63%, respectively) [38].

'Teff' is also reported to have a higher content of iron, calcium, phosphorus, copper, and thiamine compared to other grains like wheat, barley, and sorghum [39]. It is also reported to be

free of gliadin [22] and could be suitable for use in the diet of patients suffering with celiac disease [40]. They also act as natural antioxidants for the food industry. At the same time, they might inhibit digestive enzymes and reduce food digestibility [41].

2.5. Selected Mineral Composition of 'Teff'

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 4% of the body's weight [42]. Minerals unlike carbohydrates, lipids and proteins, are inorganic and cannot be produced by living beings with very important functions in the body.

2.6. Calcium and Magnesium

Calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism [43]. 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 [44]. Magnesium is an essential nutrient considered beneficial to health. It turns on over one hundred enzymes and enables nerves and muscle functions [43]. A study reported that 'teff' grain contains 159 mg calcium/100g and 170 mg/100g for magnesium, while wheat flour contains (15 mg calcium/100g) and (25 mg Magnesium/100g) [28].

2.6.1. Iron

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 premenopausal 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 [45]. Iron absorption can be blocked by calcium, magnesium, manganese, zinc, antacids and tetracycline (a common antibiotic) [46]. Deficiency of iron results in anemia which is recognized by its

symptom such as low blood iron level, small and red blood cells and low blood hemoglobin values [43]. Iron toxicity usually results from a generic disorder called hemochromatosis. This disease causes over absorption and accumulation of iron, which can result in severe liver and heart damage [47].

2.6.2. Zinc

Zinc is an essential trace element that functions as a cofactor for certain enzymes involved in metabolism and cell growth, it is found in nearly 300 specific enzymes. As a component of many enzymes, Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy. Zn is vital for the healthy working of many of the body's systems; it plays an essential role in numerous biochemical pathways. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection. Zn plays a crucial role in growth and cell division where it is required for protein and DNA synthesis, in insulin activity, in the metabolism of the ovaries and testes, and in liver function [48]. Zn deficiency may occur due to insufficient dietary intake. It was reported that nearly two billion people in the developing world are deficient in Zn [49]. Early Zn deficiency also leads to impaired cognitive function, impaired immune function, behavioral problems, memory impairment, and problems with spatial learning and neuronal atrophy. Public health programs involving Zn supplementation and food fortification could help overcome these problems [50].

2.6.3. Copper

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. Copper is required with iron for synthesis of hemoglobin. It works with many enzymes such as those involved in protein metabolism and hormone synthesis [43]. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases [47].

2.6.4. Manganese

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function [46]. It is an important cofactor in the key enzyme of glucose metabolism. It also helps to maintain normal nerve, brain and thyroid function [51]. 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. Toxicity from manganese is uncommon [52]. Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent [53].

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The samples were taken from two kebeles in Enor Ener Meger Woreda. These kebeles were named Ener kola and Shmoro, Guraghe zone, central Ethiopia Region state as shown in Figure 3.1. Enor Ener meger is bounded by Enamor woreda in the north, Hadiya in the south, Yem leyu woreda in the west, and Endegagn in the east. The woreda capital, Mike is located 211 km from Addis Ababa, 245 km from Hawassa city and 56 km away from wolkite city. It contains twenty-four rural and three urban kebeles. Its mean annual temperature ranges from 12.6–25 °C. Mean annual rain fall ranges from 801 mm to 1400 mm.

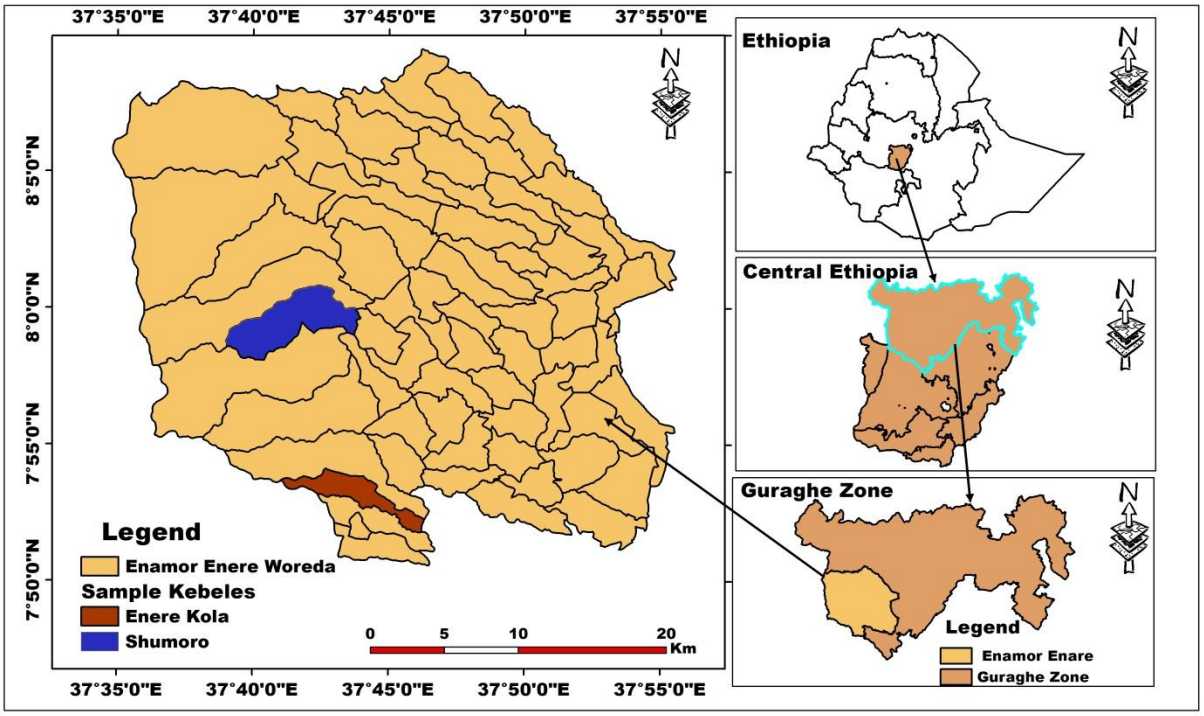

Figure 3. 1: Map of the study area

3.2. Sampling site, Sample Collection and Preparation

3.2.1. Sampling site and Sample collection

Three cultivars of 'teff' grain namely White (nech), Red (Key) and Mixed (Sergegna) were collected from two kebeles namely Ener Kola and Shemoro in Enor Ener meger woreda. These kebeles are a highly productive kebeles in the production of 'teff' in Enor Ener meger woreda. According to the report from Enor ener meger woreda office of agriculture, the selected kebeles have similar soil type (black soil) and climate condition and the climate zone is midland (woyna dega). About 0.5 Kg of each 'teff' samples (White, Red and Mixed 'teff') were collected from five farmers selected randomly from each kebeles. The 'teff' samples collected from the five farmers from Ener Kola kebele were mixed to give a total of 2.5 Kg of each 'teff' sample. Similarly, a total of 2.5 Kg of each 'teff' sample were collected from Shemoro kebele. Then, each 'teff' samples collected from the two kebelles were mixed and a total of 5 Kg of 'teff' sample were obtained for each type of 'teff' sample. Then, the 5 Kg 'teff' sample for each type of 'teff' was thoroughly mixed and reduced by half and kept in clean polyethylene bags and transported to the laboratory.

3.2.2. Sample preparation for mineral analysis

Once the soil and stone particles in the 'teff' samples were removed, 0.5 Kg of each 'teff' cultivars were washed with tap water and then with distilled water to remove adsorbed dust and particulate matters. Then, the samples were air-dried for five days to remove moisture. The dried samples were grounded and sieved to mesh size of 0.5 mm. Then, each powdered/flour samples were stored in clean and dried plastic bags (polyethylene) under airtight conditions before digestion.

3.2.3. Optimization of digestion procedure

Wet acid digestion is one of the methods widely used to get free metal ions in dissolved from complex organic matrix based on changing different digestion parameters, like volume ratio of reagents added, digestion temperature, and time. In this study he digestion procedures was optimized by varying the amount of HNO_3 (69-72%) and $HClO_4$ (70%) mixtures, digestion time and temperature for the digestion of 0.5 g of Red 'teff' flours. The digestion is assumed to be complete if the solution is clear and colorless [49–54].

3.2.4. Acid digestion of 'teff' samples

Applying the optimized conditions (i.e., 5 mL HNO_3 : 1 mL $HClO_4$ volume ratio of reagents, 240 °C digestion temperature and 2:30 hours digestion time), 0.5 g of powdered 'teff' sample was transferred into a 100 mL round bottom flask, and a mixture of HNO_3 (69-72%) and $HClO_4$ (70%) with a volume ratio of 5:1 (v/v) was added and the mixture was digested on a Kjeldahl digestion apparatus fitted with a reflux condenser by setting the temperature at 240 °C and digested for 2:30 hours. The digest was allowed to cool to room temperature for 10 minutes without dismantling the condenser and for 10 minutes after removing the condenser. To the cooled solution, 10 mL of distilled water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digested residue while filtering with filter paper (Whatman 125 mm diameter, Germany) into 50 mL volumetric flask.

The round bottom flask was rinsed subsequently with around 5 mL distilled water until the total volume reached around 40 mL. Then finally, the solution was filled to the mark (50 mL) using distilled water. The digestion sample was carried out in triplicate for each sample. Digestion of the blank was performed in parallel with the 'teff' samples keeping all digestion parameters the same. The metal concentrations in the digested sample solutions were determined by using AAS.

3.3. Apparatus/Instruments and Chemicals

3.3.1. Apparatus/Instruments

Portable pH Meter (pH-013M) was used to measure the pH of the 'teff' sample solutions; Round bottom flasks (250 mL) for digestion of 'teff' flour samples; Borosilicate volumetric flasks (50 and 100 mL sizes) were used for dilution of samples and preparation of metal standard solutions. Measuring cylinders (DURAN, Germany), pipettes (PYREX, USA), micro-liter pipettes (20-50 µL and 100-1000 µL size, DRAGON MED, Shangai, China) were employed for measuring different quantities of volumes of sample solution, acid reagents, and metal standard solutions.

Concentrations of metals were determined by flame atomic absorption spectrophotometer (BUCK SCIENTIFIC, Model 210VGP AAS, USA) equipped with a deuterium background corrector and hollow cathode lamps with a flame produced from air-acetylene as oxidant-fuel or flame atomizer.

3.3.2. Chemicals and Reagents

All reagents and chemicals used in this study were analytical grades. HNO_3 (69-72%) and $HClO_4$

(70%) (Riedel-de Haen) was used in the sample digestion steps. Stock standard solutions of concentration 1000 mg/L of the metals Ca, Mg, Cu and Mn (BUCK SCIENTIFIC, PURO-GRAPHIC) as nitrate salt standard solutions were used to prepare intermediate standard solutions. Distilled water was used for all dilutions. Distilled water was used for the dilution of the sample and intermediate metal standard solutions and for rinsing glassware and sample bottles.

3.4. Physicochemical Determinations

The physicochemical composition viz., pH, moisture, total solids, ash, crude protein, crude fat, carbohydrate, crude fiber and minerals contents of the flour of the 'teff' grains were determined according to standard methods of analysis [55]. Total solids were calculated as 100% minus the percent moisture content. Carbohydrates were calculated by difference. Energy values were obtained using the Atwater factors 3.47, 8.37 and 4.00 for protein, fat and carbohydrates, respectively [56]. For each physicochemical parameter, three replicates of each composite sample were analyzed.

3.4.1. Determination of pH

The pH of the 'teff' flour samples were measured using Portable pH Meter (PH-013M). 10% slurry of the sample was prepared by mixing 10 g sample in 100 mL of distilled water. The meter was calibrated and used to obtain pH readings of the slurry. The determination was done in triplicate.

3.4.2. Determination of Moisture

The percentage of moisture content was determined by AOAC (2000) Standard methods of analysis [55]. Accurately weighed 3.0 g well mixed 'teff' flour samples was placed in previously weighed petri-dishes and put it inside an oven adjusted at a temperature of 100 °C for 24 hours. The sample was reweighed until it attained practically constant weight. The percentage moisture content was calculated by the following formula:

$$\text{Moisture content (\%)} = \frac{W2-W3}{W2-W1} \times 100$$

Where, W1 = weight of crucible with lid; W2 = weight of crucible with lid and sample before drying; and W3 = weight of crucible with lid and sample after drying.

The moisture content determination was done in triplicate.

3.4.3. Determination of Total Ash

The ash content of samples was determined using the method as outlined in the Standard methods of analysis [55]. Briefly, 5.0 g of 'teff' samples was transferred into the pre-weighed crucibles and ignited in a muffle furnace at approximately 550 °C for 8 hours. Furnace was allowed to drop to below 250 °C and the crucibles transferred to desiccators to cool. The crucibles with the contents were weighed and the total ash content calculated and expressed as a percentage as follows:

$$\%\text{Ash} = \frac{[(Weight of\ crucible + Ash) - (Weight\ of\ empty)]}{(Weight\ of\ sample)} \times 100$$

The ash content determination was done in triplicate.

3.4.4. Determination of Crude Protein

The crude proteins were determined according to the AOAC method by the macro Kjeldahl method [55]. Crude protein determinations were done in triplicates. A weight of 1.5 g of the homogenous 'teff' flour sample was introduced into a Kjeldahl digestion flask together with 10.0 g of copper sulphate and sodium sulphate in the ratio of 5:1. 25 mL of concentrated sulphuric acid was added to the digestion flask and the digestion was carried at about 450 $^{\circ}$C in the fume cupboard until frothing ceased. A clear and light blue coloration was observed. The digest was cooled and diluted up to the mark with distilled water in 100 mL volumetric flask. 10 mL of the diluted mixture was poured into the distillation apparatus and 18 mL of 40% of sodium hydroxide was added.

25 mL of 2% boric acid was added into the receiving conical flask and two drops of bromocresol green and methyl red mixed indicator was added. The distillation was continued until boric acid solution turned from pink to yellowish green. After the distillation, the solution in the conical flask was titrated against a 0.1 N hydrochloric acid until the end point was reached. A blank was carried out using the same procedure as the sample. The nitrogen and protein content of the sample was calculated as follows:

$$\%\ \text{Nitrogen}\ (\ ^{w}/_{w}) = \frac{[(Sample\ titre - Blank\ titre) \times Normality\ of\ acid \times 14.007\ ]}{(\ 10 \times Weight\ of\ sample)}$$

$$\%\ Crude\ protein\ (\ ^{w}/_{w}\ ) = \%\ Nitrogen\ x\ 6.25$$

3.4.5Determination of Crude Fat

The fat content was determined according to the AOAC method [55]. Crude fat determinations were done in triplicates. Briefly, 5.0 g of sample was mixed with 0.88 mL of ammonia solution and 10 mL of 95% ethanol and mixed well. 25 mL of diethyl ether was added to the mixture and shaken vigorously for 1 minute. This was then followed by addition of 25 mL of petroleum ether and shaken vigorously to mix well. The mixture was then left to stand for an hour to allow aqueous and organic phase to separate. The fat extract (organic phase) was collected and the solvent was removed by distillation. The fat in the flask was dried in the oven at 100 ℃ for 30 minutes and the solvent was removed completely. The flasks were then cooled in a desiccator and were weighed for their mass of fat. The crude fat content was calculated as follows:

$$\% \text{ Crude fat} = \frac{[(Wt\ of\ flask+fat)-(Wt\ of\ empty\ flask-blank)]}{Wt.of\ sample} \times 100$$

3.4.6. Determination of Crude Fiber

The crude fiber content of the samples was determined according to AOAC methods [55]. Crude fiber determinations were done in triplicates. About 2.0 g of defatted sample was weighed in each of 600 mL beaker.

A 200 mL of 1.25% sulfuric acid solution was added to each beaker and allowed to boil on hot plate for 30 minutes by rotating and stirring periodically. During boiling the level was kept constant by addition of hot distilled water. After 30 minutes, 20 mL of 28% sodium hydroxide solution was added in to each beaker and again allowed to boil for another 30 minutes. The level was still kept constant by addition of hot distilled water.

The solution in each beaker was then filtered through crucibles containing sand by placing each of them on Buchner funnel fitted with rubber stopper. During filtration the sample was washed with hot distilled water. The final residue was washed with 1% sulfuric acid solution, hot distilled water, 1% sodium hydroxide solution and finally with acetone. Each of the crucibles with their contents was dried for 2 hours at 130 °C and cooled in desiccators and weighed (W1). Then, again they were ashes for 30 minutes at 550 °C in furnace and were cooled in desiccators. Finally the mass of each crucible was weighed (W2). The crude fiber was calculated from the equation:

$$\% \text{ Crude fiber} = \frac{[(Weight\ of\ paper + residue)-(Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

3.4.7. Determination of Total Carbohydrate

Total Carbohydrates was determined by difference method:

Total carbohydrates = 100 - [%Moisture + %Fat + %Protein + %Ash + %Crude fiber]

3.4.8. Total Energy Calculation (kcal/100gm)

Energy content was obtained by multiplying the mean values of crude protein, crude fat and total carbohydrate by the Atwater factors of 4, 9, and 4, respectively and taking the sum and expressing the result in kilocalories per 100 g sample as reported by [56].

The formula for calculating energy is shown below:

$$\text{Energy (kcal/100g)} = [(4\times \%\, protein) + (9 \times\ \%fat) + (4 \times \%\, total\ carbohydrate)]$$

3.4.9 Determination of Mineral Content

The determination of the concentration of selected major elements (Ca, and Mg), and trace elements (Cu and Mn) were carried out using Flame Atomic Absorption Spectrometer (Buck Scientific Model 210 AA) [15, 50]. Four working standard solutions of each metal were prepared from certified stock standard solution (1000 mg/L) in distilled water. Then, the working standard solutions for each metal were aspirated and absorbance will be recorded. Then, the absorbance values were plotted as a function of the concentration of working standards to generate a calibration curve. Once the calibration curve was linear and satisfied, the sample solutions were aspirated and their concentrations were read directly from the instrument.

3.5. AAS calibration and operating conditions

3.5.1. AAS Operating Condition

In this study, a total of four metals were analyzed using FAAS. For each metal, parameters such as lamp alignment, slit width, and wavelength was adjusted according to the requirement of the instrument after the respective hollow cathode lamp was inserted into the turret of the atomic absorption spectrophotometer. Table 3.1 showed the instrumental conditions for each metal analyzed.

Table 3. 1: FAAS instrumental operating conditions for the determination of minerals

Elements	Ca	Mg	Cu	Mn
Wavelength, nm	422.7	285.2	324.7	279.5
Slit width, nm	0.7	0.7	0.7	0.7

3.5.2. Calibration of AAS

Determinations of heavy metals concentration were done by using a flame atomic absorption spectra-photometer (BUCK SCIENTIFIC MODEL 210VGP**)**. Calibration of the atomic absorption spectrophotometer for each metal analyzed was done using the standard solution of the respective metals. The concentration of the prepared standard solutions of each metal is shown in Table 3.2.

Table 3. 2: Concentrations of working standards for each metal analyzed using FAAS

Metals	The concentration of the standards (mg/L)
Ca	0.15; 1.0; 2.0; 4.0
Mg	1.0; 2.0; 4.0; 6.0
Cu	0.01; 0.5; 1.5; 2.0
Mn	0.05; 0.1; 0.3; 0.5

3.6. The method detection limit (MDL)

Method detection limit is the minimum concentration of analyte that can be identified, measured and reported with 99% confidence that the analyte concentration if greater than zero [57, 58]. Method detection limit was calculated by multiplying the standard deviation of the blank by three times as follows [59]:

The method detection limits for the selected major and trace elements were estimated using blank digestate. Ten blank solutions were run with FAAS for the level of the metal in a similar manner as the sample and triplicate readings were recorded. Then, the standard deviations of the blank concentration was calculated and used to calculate the method detection limit of each

element. The instrumental detection limit for each element was read from the FAAS instrument manual.

3.7. Method of validation

Method validation is the process of providing that the analytical method is acceptable for its intended purpose appropriate quality assurance procedures and precautions were carried out to ensure the reliability of the results. Deionized water and analytical grade reagents were used throughout the study. Reagent blank determinations were used to correct the instrument readings. For validation of the analytical procedure, a recovery study was carried out by spiking red 'teff' flour sample, which was already analyzed for its mineral contents, with varying amounts of standard solutions of the metals. The precision and accuracy of the method employed in the determination of the selected major and trace elements in 'teff' flour samples were evaluated by the matrix spikes recovery analysis method. Thus, spiking experiment was done to evaluate the suitability of the method for its intended purpose.

3.7.1. The Accuracy of the Method

Recovery is one of the most commonly used techniques utilized for validation of the analytical results and evaluating how far the method is acceptable for its intended purpose. The accuracy of the digestion procedures were assured by spiking the samples with a standard solution of known concentration of the target analyses. Red 'teff' flour samples were analyzed with and without the spike. Digestion was carried out in triplicates. About 50.0 µL of a 1000 ppm stock standard solution of Ca, Mg, Cu and Mn were mixed at once with a 0.5 g of Red 'teff' flour sample taken in a 100 mL round bottom flask, and were digested in the same digestion procedure that was used for unspiked Red 'teff' flour sample. The digested spiked Red 'teff' flour sample was analyzed for tits respective metal content using FAAS. The percent recovery for each metal was calculated using the following formula [60]:

$$\%R = \frac{(\text{con.in spiking sample} - \text{conc.in unspiked sample})}{(\text{actual spike conc.})} \text{x}\,100$$

3.7.2. The Precision of the Method

The precision of the method expresses the variations within the same laboratory, when the same sample is analyzed by the same procedure and instrument, and/or by different analysts in a different time interval, was evaluated by calculating the standard deviation of the same samples digested and analyzed by the same procedure and by the same instrument.

3.8. Statistical Analysis

The data analysis for all parameters was done by using SPSS version 16.0, and the Microsoft excel program. The Mean, Standard deviation, and one-way analysis of variance (ANOVA) were done using the above statistical analysis tools and the mean values of nine replicates per 'teff' sample type were compared for their mineral content using the least significant difference (LSD) test at $P < 0.05$ probability levels [61].

4. RESULT AND DISCUSSION

4.1. Results of the physicochemical composition of 'teff' samples

The results of the physicochemical composition of whole grain 'teff' flour samples were presented in Tables 4.1. Except for moisture content, all parameters were stated on dry basis (%(w/w) db) while moisture was expressed on wet basis.

Table 4. 1: Physicochemical composition of the three 'teff' flour samples (mean ± SD, n= 3, %) except for pH

Parameters	**Sample type**		
	Key 'teff'	White 'teff'	Mixed 'teff'
pH	6.77 ± 0.01	6.36 ± 0.01	6.54 ± 0.01
Moisture	13.28 ± 0. 25	10.78 ± 0. 51	14.19 ± 0. 17
Total solids	86.72 ± 0. 25	89.22 ± 0. 51	85.80 ± 0. 17
Total ash	1.73 ± 0. 12	2.53 ± 0. 12	2.33 ± 0. 12
Crude protein	10.94 ± 0. 05	9.64 ± 0. 04	10.79 ± 0. 05
Crude fibre	2.60 ± 0. 05	2.08 ± 0. 08	2.35 ± 0. 05
Crude fat	2.53 ± 0. 12	2.39 ± 0. 02	2.41 ± 0. 02
Carbohydrates	68.92 ± 0. 17	72.58 ± 0. 57	67.92 ± 0. 16
Energy (kcal/100 g)	342.22 ± 1.37	350.36 ± 2.54	336.56± 0.39

4.1.1. PH

pH is a physicochemical property that indicate the bitterness or sourness of flours and their products. In the present study, the pH value of the whole grain 'teff' flour samples ranged from 6.36 to 6.77. The whole grain 'teff' flour samples had nearly a neutral pH as these might be due to the absence of fermentation as the samples were analyzed immediately. One-Way ANOVA analysis showed that the pH content was significantly different, $F(2, 6) = 3684.333$, $P < 0.001$, among the three 'teff' samples.

4.1.2.Moisture content

The mean moisture content of the whole grain 'teff' flour samples were ranged from 10.58 to 14.19%. Significant differences in the moisture content, $F(2, 6) = 79.471$, $P < 0.001$, were observed among the three whole grain 'teff' flour samples. The highest mean value (14.19%) of moisture content was recorded in mixed whole grain 'teff' flour samples, while the least mean value (10.58 %) of moisture was recorded in white whole grain 'teff' flour samples (Table 4.1). These results indicate that the moisture content of white whole grain 'teff' samples were below the maximum level of Ethiopian Standards [62]. Furthermore, key and mixed whole grain 'teff' flour samples were shown to have higher moisture content (13.28%, and 14.19%. respectively). However, the result of the moisture content of white whole grain 'teff' flour samples were similar with previous reports [63,64] and [65], who have found that the moisture content of Ethiopian whole grain 'teff' was within the range of 9.3–11.2% and 11% to 12.6% respectively. However, the results of the moisture content of key and mixed whole grain 'teff' flour samples are higher than the reported values [63, 64, 65] the moisture content of 'teff'.

4.1.3. Total solids content

The total solids content is a measure of the amount of material remaining after all the water has been evaporated. There was a significant differences in the total solids content $F(2, 6) = 79.471$, $P < 0.001$, among the three whole grain 'teff' flour samples. The highest mean value (89.22 %) of total solids content was recorded in white whole grain 'teff' flour samples, while the least mean value (85.80 %) of total solids was recorded in mixed whole grain 'teff' flour samples (Table 4.1).

Total solids content, and hence moisture content of food is of great importance to every food processor as a number of biochemical reactions and physiological changes in food depend very

much on the moisture content or total solids content. Furthermore, total solid or moisture content has an effect on the stability and quality of foods.

4.1.4. Total ash content

The mean values for total ash content of the whole grain 'teff' flour samples ranged from 1.73% to 2.53%. Significant differences, $F(2, 6) = 39.000$, $P < 0.001$, were observed among the three whole grain 'teff' flour samples. However, Post hoc testing revealed that the total ash obtained for white whole grain 'teff' flour samples (2.53 ± 0. 12%) and mixed whole grain 'teff' flour samples were statistically similar. In this study the total ash content recorded for the three type of whole grain 'teff' flour samples falls below the maximum value of total ash reported in Ethiopian Standards [62]. However, the total ash content of both white and mixed whole grain 'teff' flour samples falls within the reported ranges (2.12 to 5.03 g/100 g) for Ethiopian 'teff' grain [66], except for key whole grain 'teff' flour samples.

4.1.5. Crude protein content

The crude protein content of the whole grain 'teff' flour samples ranged from 9.64% to 10.94% (Table 4.1). Significant differences, $F(2, 6) = 753.129$, $P < 0.001$, were observed among the three whole grain 'teff' flour samples in the level of crude protein. The highest mean value of crude protein (10.94 %) was found in key whole grain 'teff' flour samples while the lowest mean value (9.64%) of crude protein was recorded in white whole grain 'teff' flour samples. These values were below the protein content reported in Ethiopian Standards [62].

The mean values of protein content recorded for all the three type of whole grain 'teff' flour samples in the present study fall within the reported ranges of (8.7–11.1%) and (8-11%) respectively [15, 67]. However, the mean values of protein content recorded for all the three type of whole grain 'teff' flour samples were below the reported mean values (13.3% and 12.8-20%,) of protein content of 'teff' grains [39, 68, 69]. This variation between the current findings and literature data could be mainly attributed to the difference in the varieties of the teff grains and their growing environmental conditions.

4.1.6. Crude fiber content

The crude fiber content of the whole grain 'teff' flour samples ranged from 2.08 % to 2.60% (Table 4.1). There were significant differences, $F(2, 6) = 55.462$, $P < 0.001$, in crude fiber content among the three whole grain 'teff' samples considered in this study. The highest crude fiber content (2.60%) was found in key whole grain 'teff' flour samples while the lowest crude fiber content was found in white whole grain 'teff' flour samples. The crude fiber content of all the three whole grain 'teff' flour samples were below the maximum allowed total crude fiber reported in Ethiopian Standards [62] and they were within the range of values (2.6 - 3.8%) reported [15] and within the range of values (1.9 - 3.5%) reported [37]. However, the crude fiber content of the three type of whole grain 'teff' flour samples obtained were below values (3%) reported [64, 70] and it was also lower than the report [21, 71] who have reported that the fiber content of whole grain teff is 8.57% and 9.8%, respectively. These inconsistencies in fiber content with literature data might be associated with 'teff' varietal differences used.

4.1.7. Crude fat content

The crude fat content of the whole grain 'teff' flour samples ranged from 2.39 % to 2.53 % (Table 4.1). There was no significant differences, $F(2, 6) = 3.815$, $P = 0.085$, in crude fat content among the three whole grain 'teff' samples considered in this study. Although it is statistically similar, numerically, the highest mean value (2.53 %) of crude fat content was recorded in the key whole grain 'teff' flour samples, while the least mean value (2.39 %) of crude fiber was recorded in the white whole grain 'teff' samples. The crude fat content of all the three whole grain 'teff' flour samples were within the reported ranges in Ethiopian Standards [62].

The fat content in the present study is in line with the findings [37, 38, 71] of the researchers who have reported that Ethiopian teff crude fat contents ranged from 2- 6%, 2-3.1%, and 2-3% respectively. However, the crude fat content of all the three whole grain 'teff' samples were below the values (3.2% and 4.4%) as it was reported [69, 72].

4.1.8. Total carbohydrate content

The total carbohydrate content of the whole grain 'teff' flour samples ranged from 67.92 % to 72.58% (Table 4.1). Significant differences, $F(3, 32) = 141.517$, $P < 0.001$, ($p < 0.05$), in total carbohydrate content were observed among the three whole grain 'teff' flour samples. The highest mean values (72.58%) of carbohydrate content was recorded in the white whole grain

'teff' flour samples while the lowest mean value (67.92 %) of total carbohydrate was recorded for the mixed whole grain 'teff' flour samples. These values of total carbohydrate content of all the three whole grain 'teff' flour samples were above the reported value of carbohydrate in Ethiopian Standards [62].

The findings reported by different researchers [63, 64, 66] are in close agreement with the mean values of carbohydrates recorded for all the three type of whole grain 'teff' flour samples considered in this study. The highest mean of carbohydrate content recorded for the white whole grain 'teff' flour samples might be due to low values obtained for moisture and crude fiber content. Since carbohydrate contents of plant food are calculated by difference, a decrease in moisture, protein, fat and crude fiber contents of the sample will ultimately affect the carbohydrate contents' and contribute to observed increases in total carbohydrate content.

4.1.9. Total Energy content

The total energy content of the whole grain teff flour samples ranged from 336.56 Kcal to 350.36 Kcal (Table 4.1). Significant differences, $F(3, 32) = 50.961$, $P < 0.001$, ($p < 0.05$) in total energy content were observed among the three whole grain 'teff' flour samples. The highest mean energy content (350.36 Kcal) was recorded in the white whole grain 'teff'. On the other hand, the lowest mean value of total energy was recorded in mixed whole grain 'teff' flour samples.

In this study, the calorie value recorded for all the three whole grain 'teff' flour samples were in close agreement with the finding of [21, 71], who have reported a mean energy value of 362.65 Kcal and (357 Kcal) for teff grain respectively. The lower mean energy values obtained for mixed 'teff' flour samples could be related to reduced proteins, carbohydrates, and fats. Consequently, a low levels in these major energy-yielding nutrients leads to a reduction in calorie values.

4.2. Results of optimization of digestion procedure

The result of the optimization experiment was summarized in Table 4. 2. Optimized procedures were selected based on the usage of lesser reagent volume, shorter digestion time and reasonable mild temperature for obtaining clear and colorless solutions of the resulting digests. Based on this fact the optimized digestion conditions for the whole grain 'teff' samples in this study were (5 mL HNO_3: 1 mL $HClO_4$) volume ratio of reagents, 240 °C digestion temperature and 2:30

hours digestion time. The experimental variables that were attempted during optimization of the digestion of 0.5 g of key whole grain 'teff' flour samples were shown in appendix I.

4.3. Calibration curve for each element analyzed

The correlation coefficients for the calibration curves for all the selected metals analyzed using FAAS were greater than or equal to 0.999 which assured the linearity of instrumental response for individual metals or these correlation coefficients showed that there was a very good correlation (linear relationship) between concentration and absorbance. The equation of the graph and its R^2 value for each metal are shown in Table 4.2. The calibration graphs for the selected metal analyzed were shown in appendix II.

Table 4. 2: Calibration graph equation and R^2 value

Metal	**Calibration Equation**	**R^2 Value**
Ca	Y= 0.0506x + 0.0026	0.9999
Mg	Y = 0.0016x + 0.0001	0.9995
Cu	Y = 0.0518x + 0.0012	0.9996
Mn	Y = 0.1139x + 0.0011	0.9994

4.4. Method Detection Limits Values

The method detection limit values for each element analyzed in whole grain 'teff' flour samples were shown in Table 4.3 and they were above the detection limit of the instrument.

Table 4. 3: Method detection limit for the elements determined in whole grain 'teff' flour samples

Element	**Ca**	**Mg**	**Cu**	**Mn**
IDL mg/Kg	0.05	0.005	0.005	0.03
MDL mg/Kg	0.5	0.05	0.01	0.05

The method detection limits estimated indicated that if the concentration of the respective element is at least equal to the detection limit, it can be detected but not quantified.

4.5. Results of the mineral contents of 'the whole grain 'teff' flour samples

The results of the concentration of the elements analyzed for each type of whole grain 'teff' flour samples were presented in Tables 4.4.

Table 4. 4: Results of the mineral contents (mean + SD, n = 9, mg/Kg) of the three type of whole grain 'teff' flour samples

Element	Sample type		
	Key whole grain 'teff' flour samples	White whole grain 'teff' flour samples	Mixed whole grain 'teff' flour samples
Calcium	207.29 ± 0. 42	153.29 ± 0. 44	248.54 ± 0. 34
Magnesium	151.72 ± 1.38	123.93 ± 0. 22	180.20 ± 0. 28
Copper	27.20± 0. 30	21.17 ± 0. 32	11.66 ± 0. 14
Manganese	170.27 ± 0. 47	45.47 ± 0. 37	151.33± 0. 15

4.5.1. Calcium and Magnesium

The calcium and magnesium content in the analyzed whole grain 'teff' flour samples ranged from 153.3 to 248.5 mg/Kg and 123.9 to 180.2 mg/Kg respectively (Table 4.4). The highest calcium and magnesium content were found in mixed whole grain 'teff' flour sample followed by key 'teff' flour samples and white 'teff' flour samples. One-Way ANOVA analysis showed that there was a significant difference in the calcium content, $F(2, 24) = 124469.470$, $P < 0.001$, and magnesium content $F(2, 24) = 10440.512$, $P < 0.001$, among the three whole grain 'teff' flour samples. The calcium content of all the three whole grain 'teff' flour samples were above the reported value of calcium in Ethiopian Standards [62].

The observed level of calcium in key whole grain 'teff' sample were within the range of values (180-1780 mg/Kg) reported by [35], but it was lower than the reported value (1785 ± 10 mg/Kg) of calcium in key 'teff' [73]. Similarly, the observed level of calcium in mixed 'teff' flour samples were lower than the calcium concentration (788-1470 mg/Kg; 1686 ± 11 mg/Kg; 1570 mg/Kg; 1800 mg/Kg) reported [35, 73, 74, 75] in mixed 'teff' flour samples. The calcium content of white 'teff' flour in this study was lower than the reported value (170-1240 mg/Kg) of

calcium in white ‘teff’ flour [35], (1807 ± 15 mg/Kg) of calcium in white ‘teff’ [73], and (1560 mg/Kg) of calcium in white ‘teff’ flour [74].

The observed level of magnesium in all the three type of whole grain ‘teff’ flour samples were below the values (3800 mg/Kg; 1580 mg/Kg,;1810 mg/Kg; 1840 mg/Kg) reported [33, 37, 68, 69, 76, 77] by these studies respectively.

4.5.2. Copper

The copper concentration of the whole grain ‘teff’ flour samples ranged from 11.7 to 27.2 mg/Kg (Table 4.4). The lowest mean value (11.7 mg/Kg) was recorded in mixed whole grain ‘teff’ flour samples and the highest mean value (27.2 mg/Kg) was recorded in key whole grain ‘teff’ flour samples. There was a significant differences in the copper content, $F(2, 24) = 7885.828$, < 0.001, among the three whole grain ‘teff’ flour samples.

The observed copper concentration in all the three type of whole grain ‘teff’ flour samples were below the values previous reported (1.01 mg/100 g or 10.1 mg/Kg) [69], and (2.6 mg/100 g or 26.0 mg/Kg) [37, 64, 78, 79, 80] respectively. Moreover, the observed content of copper in key whole grain ‘teff’ flour samples was lower than the reported value (25 ± 0.3 mg/Kg) of copper in key ‘teff’ flour sample [73], but it was within the range of copper level (11-36 mg/Kg) reported in key ‘teff’ flour sample [35]. Similarly, the observed content of copper in mixed ‘teff’ samples was lower than reported values (4 ± 0.01 mg/Kg; 8 mg/Kg) of copper in mixed ‘teff’ [2, 17] and it was higher than reported values (16 mg/Kg; 38± 0.1 mg/Kg) of copper in mixed teff [4, 21]. The observed content of copper in white ‘teff’ samples was higher than reported values (4 ± 0.03 mg/Kg; 11 ± 0.1 mg/Kg) of copper in white ‘teff’ [2, 73] and it was found to be within the range of reported copper level (25-53 mg/Kg) in white ‘teff’ [35].

4.5.3. Manganese

The manganese content of the whole grain ‘teff’ flour samples varied from 45.5 to 170.3 mg/Kg. The lowest value (45.5 mg/Kg) was obtained for white whole grain ‘teff’ flour samples and the highest value was obtained for key whole grain ‘teff’ flour samples. Manganese content was significantly different, $F(2, 24) = 323538.225$, < 0.001, among the three whole grain ‘teff’ flour samples.

The observed content of manganese in key whole grain ‘teff’ flour samples was lower than the reported value ((224 ± 0.2 mg/Kg) of manganese in key ‘teff’ [73]. Similarly, the observed content of manganese in white whole grain ‘teff’ flour samples was lower than the reported value (48 ± 0.04 mg/Kg) of manganese in white ‘teff’ [73]. Similarly, the observed content of manganese in mixed whole grain ‘teff’ flour samples was higher than the reported value 133 ± 0.2 mg/Kg) of manganese in mixed ‘teff’ [73].

4.6. Results of the Recovery test of Elements

Recovery experiment was carried out using key whole grain ‘teff’ flour samples. The results of the %recovery experiment for the analyzed elements were shown in Table 4.5.

Table 4. 5;- Recovery test results (mean ± SD, n=3, mg/Kg) of key whole grain ‘teff’ flour samples

Metal	**Conc. of unspiked sample (mg/Kg)**	**Amount added (50 µL of 1000 mg/L = 0.5 mg/Kg)**	**Conc. Spiked sample (mg/Kg)**	**Recovery (%)**
Ca	207.29 ± 0. 42	0.5	208.24 ± 0.20	98.0
Mg	151.72 ± 1.38	0.5	152.26 ± 0.23	108.0
Cu	27.20± 0. 30	0.5	27.75 ± 0.49	110.0
Mn	170.27 ± 0. 47	0.5	170.75 ± 1. 20	96.0

The %recovery values for all the analysed elements lie within the range 96.0% to 110.0%, which is in the acceptable range (80–120 %), which suggested that the experimental procedures and the method of analysis were accurate and valid.

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

In this study, the physicochemical characterization of three whole grains ‘teff’ grown in Enor Ener Meger wereda, Guraghe zone, Central Ethiopia Regional State, Ethiopia, was investigated. Physicochemical quality parameters such pH, moisture, total solids, total ash, crude protein, crude fiber, crude fat, total carbohydrate, total energy, and minerals such as calcium, magnesium, copper and manganese were analyzed to evaluate the nutritional value of three whole grains ‘teff’ samples. Overall, the physicochemical characterization revealed the high nutritive value of all the three types of whole grains ‘teff’ samples. However, the nutritional composition of key whole grain ‘teff’ is better, especially in minerals and crude protein, fat, and fiber, than mixed and white whole grain teff. In addition, highest level of calcium and magnesium were recorded in mixed whole grain ‘teff’. Thus, the study's findings highlight the need for increased awareness regarding the nutritional qualities of red whole grain ‘teff’ and its acceptance among the majority of the community. Furthermore, the high market prices of white whole grain ‘teff’ should be reevaluated as it has low nutritional quality as compared to red and mixed whole grain ‘teff’. However, further studies are required on the nutritional quality analysis of the various varieties of ‘teff’ grown in the country to conclude conclusively.

5.2. RECOMMENDATION

Based on the finding of the current result the following ideas were recommended.

- The producers, consumers and distributors to set the ‘teff’ price based on nutrition.
- Further study should be undergone on all trance metals by using more sensitive instruments, taking time and increasing budget in the future.
- The farmers are advised to use natural fertilizers instead of artificial fertilizers.

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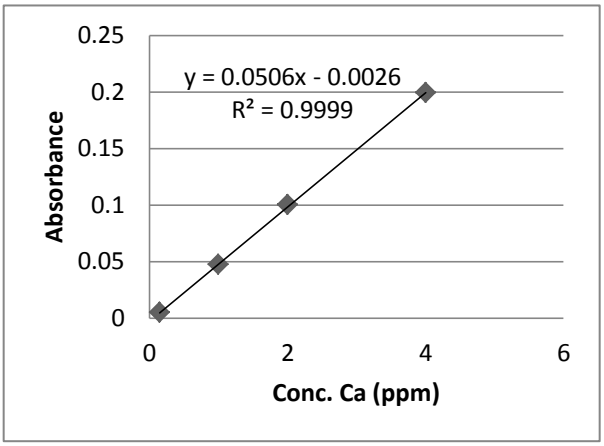
APPENDICES

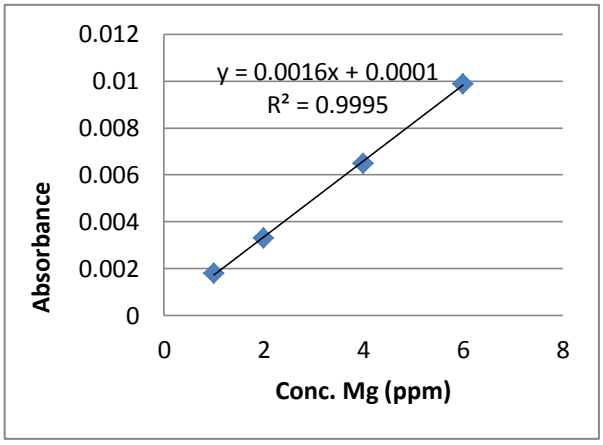
Appendix I: Reagent ratios and volumes, temperature and time attempted during optimization of digestion of 0.5 g of Red 'teff' flour samples.

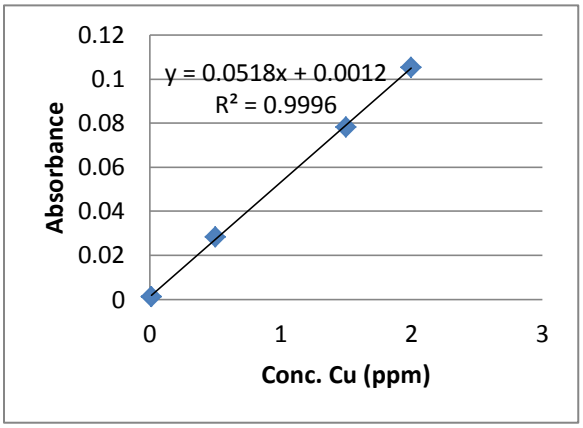
Trials	Reagent volume (mL)			Temperature (°C)	Time (hour)	Results
	HNO3	HClO4	Total			
1	1	1	2	240	3:00	Yellow with suspension
2	2	1	3	270	2:00	Cloudy yellow
3	3	1	4	300	2:30	Nearly colorless
4	4	1	5	240	2:30	Slightly colorless
5	6	1	7	240	2:30	Clear colorless
6	3	2	5	240	2:30	Slightly colorless
7	4	2	6	240	2:30	Nearly colorless
8	4	1	5	270	2:30	Nearly colorless
9	5	2	7	240	2:30	Clear colorless
10	5	1	6	270	1:30	Yellow with suspension
11	5	1	6	150	1:00	Yellow with suspension
15	5	1	6	240	2:30	Clear colorless*

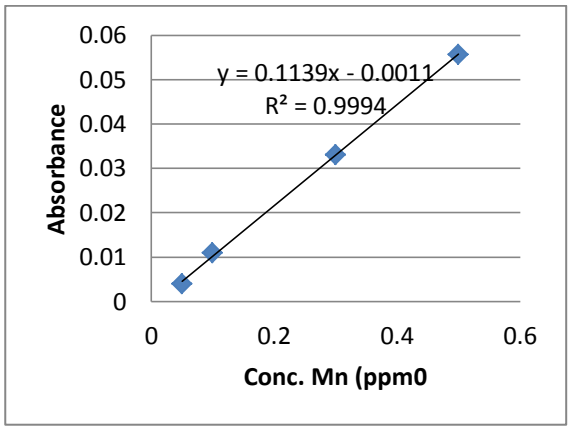
*The optimized conditions for the three parameters (reagents volume ratio, time and temperature).

Appendix II: Calibration graphs









Appendix III: ANOVA analysis results

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
pH	Between Groups	.246	2	.123	3684.333	.000
	Within Groups	.000	6	.000		
	Total	.246	8			
Moisture (%)	Between Groups	18.764	2	9.382	79.471	.000
	Within Groups	.708	6	.118		
	Total	19.472	8			
Total solids (%) db	Between Groups	18.764	2	9.382	79.471	.000
	Within Groups	.708	6	.118		
	Total	19.472	8			
Total ash (%) db	Between Groups	1.040	2	.520	39.000	.000
	Within Groups	.080	6	.013		
	Total	1.120	8			
Crude proteine (%) db	Between Groups	3.024	2	1.512	753.129	.000
	Within Groups	.012	6	.002		
	Total	3.036	8			
Crude fiber (%) db	Between Groups	.401	2	.200	55.462	.000
	Within Groups	.022	6	.004		
	Total	.422	8			
Crude fat (%) db	Between Groups	.037	2	.018	3.815	.085
	Within Groups	.029	6	.005		
	Total	.065	8			
Carbohydrates (%) db	Between Groups	36.116	2	18.058	141.517	.000
	Within Groups	.766	6	.128		
	Total	36.882	8			
Energy (kcal/100 g)	Between Groups	288.702	2	144.351	50.961	.000
	Within Groups	16.996	6	2.833		
	Total	305.698	8			

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Calcium (mg/L)	Between Groups	41074.925	2	20537.463	124469.470	.000
	Within Groups	3.960	24	.165		
	Total	41078.885	26			
Magnesium (mg/L))	Between Groups	14247.432	2	7123.716	10440.512	.000
	Within Groups	16.376	24	.682		
	Total	14263.807	26			
Copper (mg/L)	Between Groups	1105.476	2	552.738	7885.828	.000
	Within Groups	1.682	24	.070		
	Total	1107.159	26			
Manganese (mg/L)	Between Groups	81423.787	2	40711.893	323538.225	.000
	Within Groups	3.020	24	.126		
	Total	81426.807	26			