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Determination of the Levels of Iron from Red, Mixed and White Teff (Eragrostis) Flour by using UV/Visible Spectrophotometery

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Abstract
A simple and sensitive Spectrophotometric method has been used for the determination of iron concentration in Teff flour and its concentration was compared between the different varieties. The method is based on the reaction of iron with *o*-phenathroline reagent at acidic condition. The absorbance-concentration plot was linear over the range 0.1-2 mg L^{-1} with correlation coefficient of 0.999. This method was applied successfully for determination of iron in teff flour samples. The result shows that the concentration of iron was the highest for the red teff with a value of 2472.7mg/kg, mixed teff have the next highest iron content with 1440.9 mg/kg, while white teff has the least iron concentration with 881mg/kg. *This project work revealed that Red/brown teff*, the least expensive form and the least preferred type, has the highest iron content.
Keywords: Teff (*Eragrostis*), o-phenanthroline, UV/Visible Spectrophotometery.

1. Introduction

Iron is a critical nutrient that the human body requires in order to function properly. An iron deficiency can lead to impaired immunity, increased fatigue and weakness, and it can cause maternal mortality in pregnant women (1). Iron is vitally important in biochemical processes including oxygen transport, oxygen storage, and oxidative energy productions (2). About two- thirds of the iron in the human body is found in hemoglobin, the protein responsible for the transportation of oxygen to tissues (1). Adult males and females require different amounts of iron per day. According to Institute of Medicine Food and Nutrition Board typically, women require about 15 milligrams per day while men only require about 10 milligrams. Children and pregnant women especially need iron.

People in the world use different kind of cereal crop as the main item of their food due to their diverse ecological, environmental and preparation custom difference. Teff (*Eragrestis*) is a fine stemmed, tufted annual grass and it is categorized in a cereal crop, preferred over other cereal crops in Ethiopia, the leading producer of Teff. Teff is estimated to feed millions of people and is extremely important for at least 60 million people comprising rural producers, processors and consumers in Ethiopia (17). Apart from serving as food, Teff has a lot of potential industrial uses but unfortunately has not been commercially processed to any significant extent.

Red Teff is one of the three Teff species in Ethiopia. It is less utilized for making Ethiopia bread (Injera) as a result of less soft, porous or sponge and traditional bias which fails to recognize the unique quality characteristics. The species has high yield, high multiplication ratio and better storability, than the preferred indigenous White and Sirgegna Teff (17).

Teff is well known by Ethiopians and Eritreans for its superior nutritional quality. It contains 11% protein, 80% complex carbohydrate and 3% fat. It is an excellent source of essential amino acids, especially lysine, the amino acid that is most often deficient in grain foods. Teff contains more lysine than barley, millet, and wheat and slightly less than rice or oats. Teff is also an excellent source of fiber and iron, and has many times the amount of calcium, potassium and other essential minerals found in an equal amount of other grains. When teff is used to make "enjera", a short fermentation process allows the yeast to generate more vitamins (3).

While the reported high iron content of teff seed has been refuted, the lack of anemia in Ethiopia is considered to be due to the available iron from enjera (4). Teff is the main staple in the northern, western and central parts of the country (5). Some scientists think that the high results about Teffs iron content are due to ferruginous soil ground into the outside surface of the grains. There can be concluding that teff contains a high level of iron. This means that the most people in Ethiopia eat their daily recommended nutrient intake of iron. Unfortunately this does not mean that the people in Ethiopia does not have anemia, this is probably caused by a lack of foods that are rich in ascorbic acid, which improve the absorption of iron (6).

The growing awareness of the importance of analysis of trace, minor and major elements of food for human health has generated interest in different food preparation practice and in consequence, the need for their quantitative and qualitative determination. As a result studying about the elemental and nutritional content of these cereal crops species is essential for both producers as well as for the consumers.

It is important to study the iron concentration in food because iron is an essential element in the metabolism of almost all organisms. Iron is a critical constituent of many important proteins and enzymes in the human body, for example iron can be found in the regulation of cell growth and differentiation, hemoglobin, and mycoglobin. Iron helps strengthen the immune system, as it is a major component of cell fight infections and provides the human body with energy by being involved in chemical reactions that converts food into energy. In addition, iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Iron deficiency is common only among children and pre-menopausal women. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health (7). According agency of Toxic Substances and Disease Registry deficiency of iron deprives body tissues of oxygen and results in anemia which is recognized by its symptom such as low blood iron level, small red blood cells and low blood hemoglobin values, fatigue, paleness, dizziness, sensitivity to cold, irritability, poor concentration and heart palpitation. The aim of this project was to study the iron contents of the three species of Teff of Ethiopia origin and relative comparison of the preferred variety of Teff to bring out the differences between them, to create an awareness about the importance of iron in our diet and increase their daily dietary intake of iron in a way that did not deviate from their traditional diet. To achieve these, three species of Teff of selected varieties were processed to flour and analyzed its iron content by UV/Visible spectrophotometric techniques (22).

The aim of this study is to determine the level of iron from different varieties of Teff flour by using UV/Visible Spectrophotometric Techniques. That means the study includes determining the concentration of iron in Teff flour and comparing the amount of iron content from the different varieties of Teff flour such as red, white and mixed Teff flour that obtained from Arba Minch town market by using UV/VIS spectrophotometric techniques. This investigation gives awareness to the people about the concentration of iron to be sure about micro chemical composition of Teff, because they are a lot of doubts about this, and secondly, it helps probably to think that which varieties of Teff is rich of iron.

2. Review of Literature

2.1. Spectrophotometric Determination of Iron

To become familiar with the principle of calorimetric analysis and to determine the iron content of known sample calorimetric analysis is based on the change in the intensity of a solution with variation in concentration. Colorimetric methods represent the simplest forms of absorption analysis. The human eye is used to compare the color of the sample solution with a set standard until a mach is found. An increase in sensitivity and accuracy result when a spectrophotometry is used measure the color intensity. Basically, it measures the fraction of an incident beam of light which is transmitted by a sample at a particular wave length.

In the determination of iron (II) in aqueous solutions, a tricyclic nitrogen heterocyclic compound, 1, 10-phenanthroline ($C_{12}H_8N_2$, ortho-phenanthroline or o-Phen) is used as the ligand that reacts with metals such as iron, nickel, ruthenium, and silver to form strongly coloured complexes. With ferrous ions (Fe^{2+}), it reacts in a ratio of 1:3 to form an orange red coloured complex $[(C_{12}H_8N_2)_3Fe]^{2+}$ in aqueous medium as per the following equation.

$$Fe^{2+} + 3\ Phen \longrightarrow Fe(Phen)_3{}^{2+}$$

The ligand is a weak base that reacts to form phenanthrolinium ion, $phenH^+$, in acidic medium. Accordingly, the complex formation may be represented as follows,

$$Fe^{2+} + 3PhenH^+ \longrightarrow Fe\ (Phen)\ _3{}^{2+} + 3H^+$$

The molar absorption coefficient (£) of the ferrous complex, $[(C_{12}H_8N_2)_3Fe]^{2+}$ so obtained is 11,100 $dm^3\ mol^{-1}\ cm^{-1}$ at the wavelength of maximum absorbance, $\lambda_{max\ =}$ 508 nm (18).

2.2. The History of Teff

The history of teff traces back to thousands of years ago. Teff is a type of tufted grasses originally grown predominantly in Ethiopia and Eritrea as a staple grain of their cuisines. Teff belongs to the plant genus of Eragrostis. Today, there are 34 different teff cultivars based on their characteristics of culms, panicle branches, leaves, flowers, and grains. The nutritional value of teff plant is suitable for both human and livestock. Grain teff comes in a small granule size and its color varies from white to dark brown. Plain teff grain has a slightly sweet taste. However, it is usually mixed with other flours to make injera, which is soft flat bread, in Ethiopia. Teff crop has a strong adaptive capacity to endure high heat and bright light so that it survives in difficult climates. It is also known as being relatively disease-free crop, having short growing period and long storage life (19).

2.3 Nutritional of value of Teff grain

Teff grain is the seed of an Ethiopian grass. The raw unprocessed grain goes in to baked to add texture flavor and neutrino in the same manner you might add nuts to your brownies. Mills also grinned Teff in to flour to thicker soups and gravies or to be cords as porridge one cup of raw teff contains high concentration of a few vitamins and several essential minerals. The grain is also rich in amino acid. Teff has Avery high calcium content and contains high levels of phosphorous, iron, copper, aluminum, barium and thiamine. The principle use of teff grain for human food is the Ethiopian bread (enjera). Enjera is a major food staple and provides approximately two-thirds on the dist in Ethiopia white the reported high iron content of teff seed has been refuted the lack of anaemia in Ethiopia is considered to due to the available iron from enjera. Enjera is described as soft porous thin pan cake, which has as our taste-teff is low in gluten and there for, the bread reaming quit flat when eaten. In Ethiopia Teff flour is often mixed with other cereals flours, but the flavor and quality of enjera made from mixture is considered less tasty.

2.3.1 Micro components

As these components are present in very small quantities in the foods, they don't determine their texture or their appearance, but their nutritional value, and its function to help human body to accomplish it different functions. Micro-components are enzyme cofactors and then, they play an important job in the development of metabolism reactions.

The grain of teff has a very big nutritive value, with a grain protein content (10-12%) similar to other cereals. Besides providing protein and calories, teff is a good source of minerals, particularly iron. It has very high calcium content and contains high levels of phosphorus, copper, aluminium, barium and thiamine (8). But the bigger nutritional importance that teff has, is the lack of gluten in the grain. This made it useful for patients with the celiac disease. The teff starches had ash contents in the range of 0,13 – 0,23%. This is a value comparable to typical cereal starch ash (0,1 – 0,2%) reported by Swinkels, 1985. Phosphorus content is similar to that of rice starch.

Chemical concentration rates between different macro-components of teff grain are essential to teff's texture, appearance and characteristics. Teff is low in hydrolyzed lipids, but high in protein, carbohydrates, and fiber. The excellent amino acid composition of teff is higher than that of barley, wheat and corn. Micro components in teff grain, on the other hand, promote human health. Teff whole grain has high calcium content (34 mg/100g), and contains high levels of iron (3.9 mg/100g), copper (04 mg/100g), and thiamin (0.51 mg/100g). Since teff grain is so small in size (1,000 weight 0.3 to 0.4 g), flour can consist of both the bran and the germ which are the most nutritious parts of any grain. The small granule size of teff starch is also responsible for the considerably low paste viscosity, high water absorption index and low water solubility index. (9)

2.3.2 Physiological function of iron

Iron is essential in oxygen supply as a component of haemoglobin and for oxygen storage as a component of myoglobin. Iron is also a component of enzymes with functions in the metabolism of energy and proteins and in the synthesis of proteins, tissues, hormones and neurotransmitters. Because iron easily reacts with oxygen, mechanisms have evolved that tightly limit the uptake of iron and control the reactivity of iron in the body (23).

2.3.3 Iron requirements in infancy

Full term, normal birth weight infants below 6 months do not generally need any iron in addition to the amounts provided by human milk. The newborn infant has a high blood concentration of hemoglobin, which declines during the first few weeks of life. The iron that was bound in hemoglobin is then transferred to iron storage. Up to around 6 months of age the iron need of the infant is covered by the release of endogenous iron. Iron concentrations in the blood of infants, but not in human milk, depend on maternal iron status. At around 6 months, additional intake beyond what is available in breast milk becomes necessary. Estimated daily iron requirements in 6-12 month old children are 0.9-1.3 mg/kg body weight (10).

2.3.4 Iron absorption

Iron absorption depends on total iron intake, dietary factors and the iron status of the individual. Bioavailability of iron differs between different types of foods, and is assumed to be about 10 % from a mixed diet. Bioavailability of iron is lower in cereal-based foods (non-haem iron) than iron in meat products (haem iron). Phytates inhibit uptake of iron from many vegetable sourcesof iron, including iron in soy-based formulas. Humans do not actively excrete iron and protection from iron overload occurs through down-regulation of absorption (10). However, research indicates that down-regulation of iron absorption may be less effective up to 9 months of age (10).

2.3.5 Normal Iron Metabolism

Well-balanced diet contains sufficient iron to meet body requirements. About 10% of the normal 10 to 20 mg of dietary iron is absorbed each day, and this is sufficient to balance the 1 to 2 mg daily losses from desquamation of epithelia. Greater iron utilization via growth in childhood, greater iron loss with minor hemorrhages, menstruation in women, and greater need for iron in pregnancy will increase the efficiency of dietary iron absorbtion to 20% iron absorbtion is regulated by:

- Dietary regulator: a short-term increase in dietary iron is not avidly absorbed, as the mucosal cells have accumulated iron and "block" additional uptake.
- Stores regulator: as iron stores increase in the liver, the hepatic peptide **hepcidin** is released that diminishes intestinal mucosal iron ferroportin release and the enterocytes retain any absorbed iron and are sloughed off in a few days; as body iron stores fall, hepcidin diminishes and the intestinal mucosa is signaled to release their absorbed iron into circulation.

The composition of the diet may also influence iron absorbtion. Citrate and ascorbate (in citrus fruits, for example) can form complexes with iron that increase absorbtion, while tannates in tea can decrease absorbtion.

The iron in heme found in meat is more readily absorbed than inorganic iron by an unknown mechanism. Non-heme dietary iron can be found in two forms: most is in the ferric form (Fe^{3+}) that must be reduced to the ferrous form (Fe^{2+}) before it is absorbed. Duodenal microvilli contain ferric reductase to promote absorbtion of ferrous iron. Only a small fraction of the body's iron is gained or lost each day. Most of the iron in the body is recycled when old red blood cells are taken out of circulation and destroyed, with their iron scavenged by macrophages in the mononuclear phagocyte system, mainly spleen, and returned to the storage pool for re-use. Iron homeostasis is closely regulated via intestinal absorption. Increased absorption is signaled via decreased hepcidin by decreasing iron stores, hypoxia, inflammation, and erythropoietic activity. The 'set point' for hepcidin synthesis may also be infuenced by the bone morphogenetic protein pathway (11)

2.3.6 Recommendations for iron intake in infants and young children

A recommend intake of iron has not been set for infants below the age of 6 months because of the assumption that breast milk provides the iron to fulfill the iron requirements at this age. From 6 months to 5 years of age, Swedish nutrition recommendations (SNR) state 8 mg/day as the recommend intake (RI). The RI is based on calculations of iron requirements in infancy and childhood in the Nordic nutrition recommendations (NNR) (20). The up-regulation of iron absorption when iron stores are low has not been accounted for in the factorial model from which the iron requirements of infants are obtained (10). This could lead to an overestimation of requirements. Consequently, the scientific background to determine iron requirements of infants would be strengthened by information from intervention studies. The concentration of iron in human milk is approximately 0.3 mg/L and the literature show gastrointestinal absorption to be about 50 %. In infants who are exclusively breastfed, the iron intake is approximately 0.2 mg/day if they consume 700 ml milk per day. Infants consuming infant formula as the sole source of nutrition may reach an intake of 6 mg/day iron from formulas complying with iron content regulations (12). The higher concentrations of iron in infant formula than in breast milk could, at least partly, be explained by previous assumptions of much lower bioavailability of iron from infant formula than from breast milk. However, lower iron levels in infant formula have been recommended in recent years (13).

Important iron sources in older children and adults are meat and meat products, pulses, cereals and green vegetables. Breast milk provides sufficient iron for most infants below 6 months. In the second half of infancy and in young children, iron- fortified products are important iron sources.

2.4 Comparison Concentration of Iron from Teff and Other Cereals

Different authors have demonstrated the high iron content and the relatively favorable phytate: iron molar ratio, teff enjera was the best source of bioavailable iron of all foods analysed by (16), between the Ethiopian foods.

Table 1: Comparison Concentration of Iron from Teff and Other Cereals

Food type (n)	Iron mg/100g
Teff injera, unfermented	30.3±3.0
Teff injera, fermented	34.7±4.1
Maize injera, unfermented	4.2±0.7
Sorghum injera, unfermented	9.2±2.1
Sorghum injera, fermented	8.1±1.7
Wheat injera, fermented	3.5±0.8
Maize bread	5.2±1.2
Sorghum bread	6.8±0.2
Wheat bread	5.4±1.2
Maize porridge	3.6±1.2
Sorghum porridge	6.3±1.3
Maize, boiled	3.5±0.7
Sorghum, boiled	3.6±0.8

Analyzing this table, it is evident that teff enjera has a bigger iron content that other Ethiopian foods. In the particular case of teff, this value decrease 4 times, after the fermentation. In the sorghum case, the value decrease 5 times. It is easy to understand why nutritionists in Ethiopia are promoting the practice to ferment teff.

3. Material and Methods

3.1 Sample Collection and Preparation

The Teff samples (White, Red and Mixed Teff grain) used for this study were collected from Arba Minch market. Depending on the varieties three type of teff flour were prepared in Arba Minch university chemistry department laboratory. Powder forming process was performed by grinding the teff grain with grinding machine.

3.1.1 Digestion Process

1g of each varieties of flour was transferred to deferent 250 ml beaker and 50 ml of 1 M HCL was added to each beaker and stirred with a stirring rod. Then the beakers were covered with a watch glass and placed it under a fume hood and heated on hot plate for 30 minutes at a temperature of 50^0C. After 30 minutes, the hot plate was turn off and cooled the beaker until it touches and solution was kept for overnight.

3.1.2 Filtration and Preparation of Sample with a Reagent to Form a Coloured Solution

Each solution was poured into three different separatory funnel which have a piece of filter pare and the filtration was continued by dropping a small amount of deionized water until all of the digested flour rinsed into the funnel. The filtrate was collected by using 50ml graduating cylinder and the solution was transfer to a clean 100 ml beaker. The pH of the solutions was measured in the beaker with universal pH indictor paper. To adjust the pH of the solutions between 3 and 4, 8 ml of acetic acid / sodium acetate buffer was added to each of the solution in the beakers followed by stirring and the pH of the solutions was measured in the beaker again. The solutions were transferred in to three different 100 ml volumetric flasks labeled W1, R1 and M1. Then 2ml of hydroxylamine hydrochloride and 5 ml of 1, 10-Phenanthroline solution was added to each flask. The solutions were allowed to stand for at least 15 minutes until the reagents for full colour development and diluted to the mark with deionized water and mixed well and ready for further analysis.

3.2 Standard Solution Preparation

3.2.1 Standard ferrous solution (Stock Solution)

Standard ferrous solution **(**10 ppm (10 mg/ dm^3)) were prepared by weighing 0.0702 g of analytical grade ferrous ammonium sulphate hexahydrate, $[Fe(NH_4)_2(SO_4)_2.6H_2O]$ and quantitatively transferred to a volumetric flask of 1L capacity. Sufficient amount of distilled water was added to dissolve it and 2.5 cm^3 of concentrated sulphuric acid finally diluted the solution to the mark.

3.2.2 Preparation of 1, 10-phenanthroline

1, 10-phenanthroline was prepared by dissolving 100 mg of the reagent in 100 cm^3 of distilled water. The reagent can be stored in a bottle.

3.2.3 Hydroxylamine hydrochloride

Hydroxylamine hydrochloride was prepared by dissolving 10 g of hydroxylamine hydrochloride in 100 cm^3 of distilled water**.**

3.2.4 Sodium acetate (0.1M)

Sodium acetate (0.1M) was prepared by dissolving 10 g of sodium acetate in100 cm^3 of water.

3.2.5 Acetic acid (0.1M)

Acetic acid (0.1M was prepared by diluting about 6 cm3 of glacial acetic acid to 100 cm^3

3.2.6 Acetic acid-sodium acetate buffer (pH = 4.5)

Acetic acid-sodium acetate buffer (pH = 4.5) was prepared by mixing 65 cm^3 of 0.1M acetic acid and 35 cm^3 of 0.1 M sodium acetate in a 100 cm^3 flask.

3.2.7 Preparation of Iron Standard Calibration Solutions

Six calibration standards iron-phenantroline complex solution were prepared with a concentration of 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 ppm, respectively by taking 1, 3, 5, 10, 15 and 20 ml of the standard ferrous ion solution into a series of 100 ml standard flasks labeled from 1 to 6. Then 2 ml of hydroxylamine hydrochloride, 5 ml of 1, 10-Phenanthroline and 8 ml of acetic acid /sodium acetate buffer solution was added to each flask. The solutions were allowed to stand for at least 15 minutes until the reagents for full colour development and diluted to the mark with deionized water and mixed well. Blank solution was prepared in exactly the same manner as above except omitting the standard iron solution.

3.3 Measuring the Absorbance Value

For the measurements of absorbance *SANYO SP65* Spectrophotometer was used. The absorption spectrum for the 2.0 ppm standard solution against the reagent blank in the range of 400 –700 nm was recorded and the wavelength which gives maximum absorbance (λ_{max}) was selected. The absorbance was measured for all the standard solutions at the maximum wavelength (λ_{max}) three times and the readings were recorded. Similarly, the absorbance of the unknown samples were measured and recorded.

3.4 Calculations

To get the standard calibration curve a graph was plotted absorbance v_s concentration of the standard solution and the equation of linear region of the curve obeys beer-lamberts law was calculated and used for the estimation of unknown sample. As a result, the concentrations of the ferrous ions present in the unknown sample solutions were calculated by using approximate linear region equation.

4. Results and Discussion

4.1 Calibration curve

The range of linearity of concentration vs. absorbance curve is of great importance in determining the elemental concentration of the Teff samples and ensures the accuracy of the UV/Visible Spectrophotometer and to establish that results of the determination were true and reliable. Data presented in Table (2) illustrated the absorbance values of the 1, 10-phenantroline and iron (II) complexes of standards. The calibration curve for Fe standards are shown in Figure 4.1. The calibration curves obtained were fairly linear.

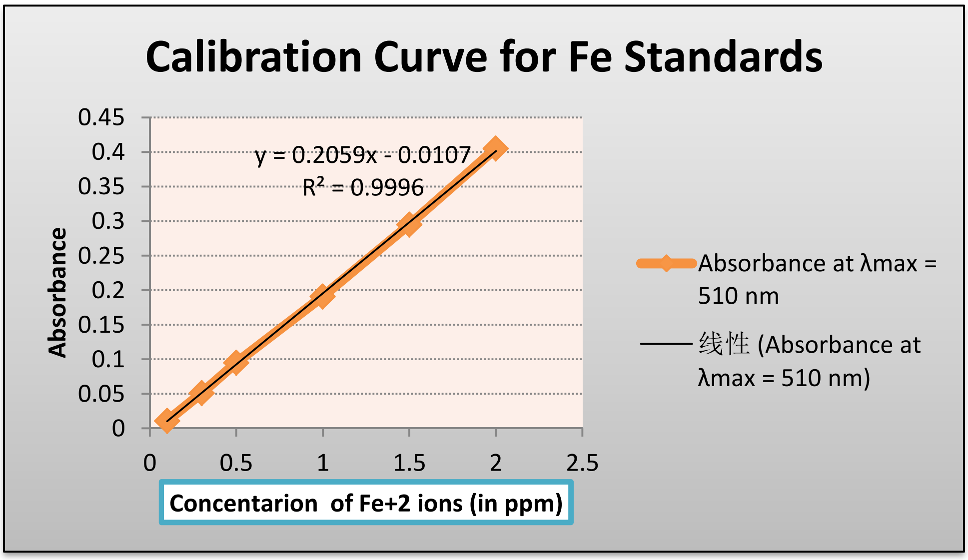

Fig. 4.1: Correlation of iron concentration (x) vs absorbance (y), determined by the UV/Visible spectrophotometric method at 510 nm

The spectroscophotometric methods of determination of iron in the form of Fe (II) complex with 1, 10-phenantroline was used to measure the contents of the metal in the Teff flour. The mean absorbance determined spectrophotometrically was plotted against standard concentrations of iron and linear regression was performed (Fig. 4.1). The linear regression equation was y = 0.205x-0.010 with a correlation coefficient (R^2) 0.999 (n = 6) indicating a good linearity. The mean iron content of flour samples were showed in Table 3. The simple method (spectrophotometric reading) produced accurate results.

Table 2: Absorbance values of the iron phen. complexes of standards

Std No	V. of standard/sample solution(ml)	Conc. Of F_e^{2+} ions (ppm)	Absorbance at λmax
1	1	0.1	0.011
2	3	0.3	0.0507
3	5	0.5	0.1146
4	10	1.0	0.1816
5	15	1.5	0.288
6	20	2.0	0.4196

Table 3: The mean iron content of teff flour samples Absorbance at λmax = 510 nm

Types of Samples	Conc. of Fe^{2+} ions (mg/L)	Absorbance at λmax = 510 nm
White teff flour	6.93 ± 0.142	0.132
red teff flour	19.55 ± 0.141	0.389
brown teff flour	11.34 ± 0.153	0.222

Values are means ± standard deviations (n = 3)

In recent years, awareness that trace elements play a very important role, either beneficial or harmful, in human health has increased. Because most essential **trace metals** especially iron are present in food products in very low concentration, precise and accurate analysis is most essential if meaningful results are to be obtained (14).

4.2. Mean Iron Concentration of Red, Mixed and White teff Flour and Comparison between them

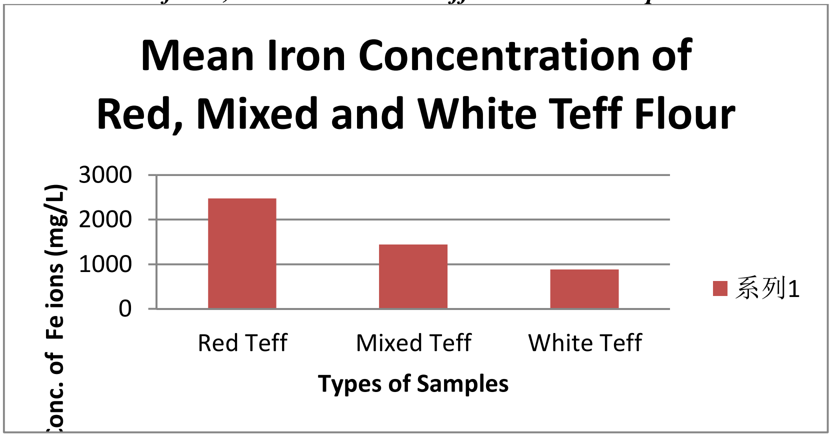

Fig 4.2 Graph show the iron concentration

The amount iron in 1kg of red teff was found to be 2472.0 mg, which was the highest of all varieties of teff that analyzed in this project study. This result is in agreement with the study by (15) *Red/brown teff*, the least expensive form and the least preferred type, has the highest iron content. From this study the amount of iron in *mixed* (red/brown and white) teff flour was 1440.9 mg this value makes the variety to be the second or moderate iron content. The possible explanations for this result may be, though to our knowledge no studies investigating the iron content of the soil and its possible effect on the iron contents of the grain have been conducted, it may be possible that the high iron content of the soil contributes to the iron content of the grain. The amount of iron in white teff was very small in one kg of the sample its amount contains were 881mg. However, *White* teff is the preferred type but only grows in certain regions of Ethiopia requires the most rigorous growing conditions, and is the most expensive type of teff (15). Generally, fig 4.2 shows that the iron concentration to be the highest for the red teff with a concentration of 2472.0 mg/ kg, mixed teff have the next highest iron content with 1440.9 mg/kg, while white teff has the least iron concentration with 881mg/kg.

5. Conclusions

A simple and sensitive spectrophotometric method has been used for the determination of iron concentration in Teff flour and its concentration was compared between the different varieties. The method is based on the reaction of iron with o-phenathroline reagent at acidic condition. The absorbance-concentration plot is linear over the range 0.1-2 mg L^{-1} with correlation coefficient of 0.999. This method was applied successfully for determination of iron in flour samples. The result showed that the iron concentration to be the highest for the red teff with a concentration of 2472.7mg/kg, mixed teff have the next highest iron content with 1440.9 mg/kg, while white teff has the least iron concentration with 881mg/kg. *Red/brown teff*, the least expensive form and the least preferred type, has the highest iron content. Even though, the amount of iron in *White* teff is very small and the most expensive type it is the most preferred variety and used as a status symbol in Ethiopia.

From this project we can suggest that; in persons living in areas of the country where consumption of red teff is most prevalent, hemoglobin levels were found to be higher with a decreased risk of anemia related to parasitic infection (15). As studies of the increased health benefits associated with high iron contents in red teff become elucidated, there should be more acceptance of this grain in society. And since the study was restricted on the concentrations of iron further study should be conducted on the contents and levels of other micro nutrients and minerals of the three varieties of teff.

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