**Comparison of the Concentrations of Metals Found in three types of Teff Samples**

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**Abstract:** Teff (*Eragrostis tef* (Zuccagni)) is a self-pollinated, annual, warm season cereal crop, believed to have originated in Ethiopia and have been domesticated and used throughout the world due to its excellent nutritional value as grains for human consumption and as forage for livestock. The aim of this study was to determine the concentration levels of metals in teff samples across different locations and to compare with other reported values due to different agricultural applications. The mean concentration levels of the metals in the three types of teff samples collected from the three sampling areas were determined by using MP-AES and were an efficient method. The overall mean concentrations determined (mg/kg, air dry weight) for the teff samples collected from the Bure, Debre Markos and Bahir Dar sites were in the ranges of Fe (719) > Ca (289) > Zn (86) > Mn (33) > Cu (14), Fe (728) > Ca (256) > Zn (87) > Mn (29) > Cu (13) and Fe (668) > Ca (270) > Zn (73) > Mn (36) > Cu (13), respectively. The level of Fe in the white teff was higher than that of the red and mixed teff samples collected from the Bure site and it was higher in the red teff sample collected from the Debre Markos site. The amount of Fe determined in the three types of teff samples collected from the Bahir Dar site and the concentrations of Ca, Zn, Mn and Cu determined in the three types of teff samples were almost similar within each sampling sites. The one way ANOVA indicated the mean concentrations of the studied metals found in the three types of teff samples within and between the sampling sites was not significant at 95% confidence level.

**Keywords:** Comparison, Concentration, Digestion, Metals, Teff Types

**Introduction**

Teff is a staple food in Ethiopia, consisting of two-thirds of their cereal diet and is primarily used to make injera. "Injera" is an Amharic term for Ethiopian bread similar to pan cake, made usually from teff. Injera is thin, prepared from teff flour, water and starter (a fluid collected from previously fermented mix) after successive fermentations (Neela and Fanta, 2020). Teff can also be combined with other baking flours to produce baked products, such as muffins and cookies. Teff has also been linked to other health benefits, such as anemia due to its exceptionally high Fe content (Coleman, 2012). Teff is a self-pollinated, annual, warm season cereal crop, believed to have originated in Ethiopia and have been domesticated and used throughout the world due to its excellent nutritional value as grains for human consumption and as forage for livestock (FAO, 2010). Suitable conditions of temperature, soil and water are the main growth conditions for teff. Although teff is grown for its grain, the straw is also used as forage for livestock as well as to reinforce mud or plasters in construction of houses both in rural and urban areas (Kibatu et al., 2017).

Teff is a hugely important crop to Ethiopia both in terms of production and consumption (FAO/WHO, 2011). In terms of production, teff is the dominant cereal by area coverage and second only to maize in production and consumption. However, it has been historically neglected compared to other staple grain crops, yields are relatively low (around 1.26 tons/hectare) and some farmers under certain conditions sustain high losses which result in reduced quantity of grain available to consumers (Merga, 2018).
Many people do not like injera made from other cereals such as wheat and barley as they lack the required organoleptic properties of injera. In a number of cases, families sell other cereals for cheaper prices and buy teff for food when they have enough cereals. Many Ethiopian people are very comfortable with the taste of teff injera than any other food. Nutritionally teff is the most valuable grain in Ethiopia, which is considered an excellent source of fiber, Fe and Ca than other cereal grains (Umeta et al., 2005). Recently there is a growing interest in teff grain utilization because of nutritional merits (whole grain) and free of the protein gluten that make teff an increasingly important dietary component for individuals who suffer from gluten intolerance or celiac disease (Boka et al., 2013). The color of teff can vary from white (ivory) to dark brown (black) depending on the variety. As shown in Fig. 1 in Ethiopia three major categories can be identified, white (nech), red (quey) and mixed (sergegna).

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bio-available form. There is a significant body of evidence that minerals by themselves and in proper balance to one another have important biochemical and nutritional functions. The difference in mineral content between and within teff varieties is wide ranging (Kebede, 2009).

Materials and Methods

All measurements were performed using an Agilent 4200 Micro-wave Plasma Atomic Emission Spectroscopy (MP-AES), with nitrogen supplied from an Agilent 4107 nitrogen generator. The sample introduction system consisted of a micro mist nebulizer and double-pass glass cyclonic spray chamber. An External Gas Control Module (EGCM) accessory and auto sampler were used. The MP-AES was controlled using the intuitive MP Expert software, which recommends wavelengths for the selected elements and automatically sets the nebulizer flow rate and EGCM settings. Auto background correction was used to resolve the element emission line from the organic matrix (Agilent Technologies, Inc., 2016).
MP-AES consists of microwave induced plasma interfaced to an Atomic Emission Spectrophotometer (AES). It is used for simultaneous multi-analyte determination of major and minor elements. MP-AES employs microwave energy to produce a plasma discharge using nitrogen supplied from a gas cylinder or extracted from ambient air, which eliminates the need for sourcing gases in remote locations or foreign countries. Samples are typically nebulized prior to interaction with the plasma in MP-AES measurements. The atomized sample passes through the plasma and electrons are promoted to the excited state. The light emitted electrons return to the ground state light is separated into a spectrum and the intensity of each emission line measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent.

All chemicals used in this study were of analytical grade reagents. Perchloric acid (70%) and nitric acid (69-72%) were used (Sigma Aldrich Steinleim, Germany). The stock standard solutions of 1000 mg L\(^{-1}\) were prepared from the nitrate salts of the metals. The working standard solutions of the selected metals were prepared freshly from the intermediated standard solutions (100 mg L\(^{-1}\)) which was obtained by diluting stock standard solutions.

Sample preparation utilized PVC flasks, polyethylene conical flasks, filter paper, 50 mL volumetric beakers, round bottom flask, ceramic mortar and pestle (USA), digital analytical balance (four digits) and Kjeldahl technique (England) were the materials used. All the glassware used were first kept overnight in a 10% HCl solution and then repeatedly washed with distilled water and dried in an oven for 24 h before use.

**Sample Collection and Preparation**

Representative White, red and mixed (brown) teff samples were collected from different teff bags/containers from the north-western areas of Ethiopia (Bahir Dar, Bure and Debre Markos), which are one of the most teff productive regional areas in Ethiopia. From each teff sample types around 0.1 kg of sub-samples were collected from different teff containers. For each teff sample types a total of around 0.5 kg were collected through compositing. The samples were sampled by using auger sampler from the containers. Some unwanted materials in the teff samples were removed. In the laboratory the collected teff samples were washed with tap water and then with distilled water to eliminate adsorbed dust and other particulate matters. The samples were then air-dried for seven days to remove the moisture. The dried samples were ground by using a machine grinder and sieved to mesh size of 0.5 mm. Then the samples were stored in plastic bags (polyethylene) under airtight conditions until the time of digestion.

**Digestion of Samples**

Applying the optimized conditions, 0.5 g of powdered each types of teff samples were transferred into a 100 mL round bottom flask. Then 6 mL of 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 parameters temperature and time. The digest was allowed to cool to room temperature for 10 min without dismantling the condenser and for 10 min 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 digest 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 was carried out in triplicate for each sample. Digestion of the blank was also performed in parallel with the teff samples keeping all digestion parameters the same. Then the metal concentrations in the digested sample solutions were determined by using MP-AES (Agilent technologies, Inc., 2016).

**Results**

As shown in Table 1 the overall mean concentrations determined (mg/kg, dry weight) for the teff samples collected from the Bure site were, Fe (719) > Ca (289) > Zn (86) > Mn (33) > Cu (14). The overall mean concentrations determined (mg/kg, dry weight) for the teff samples collected from the Debre Markos site, Fe (728) > Ca (256) > Zn (87) > Mn (29) > Cu (13). Similarly the overall mean concentrations determined (mg/kg, dry weight) for the teff samples collected from the Bahir Dar site were, Fe (668) > Ca (270) > Zn (73) > Mn (36) > Cu (13).

**Discussion**

As shown in Table 1 the concentrations of the metals were carried out by using MP-AES and mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample. The results were expressed in terms of mean values ± SD. All the results obtained from the MP-AES that were expressed in terms of (mg/L) were converted into (mg/kg). Commonly, concentration units are presented using units in the form of mass per volume (e.g. mg/L) for water samples or mass per mass (e.g., mg/kg). However, units expressed as parts per a number (e.g., parts per million, ppm) may still be encountered.
The concentrations of Fe and Ca were relatively higher than the concentrations of the other studied metals in all the sampling sites. The level of Fe in the white teff was higher than that of the red and mixed teff samples collected from the Bure site and the level of Fe in the red teff was higher than that of the white and mixed teff samples collected from the Debre Markos site. The amount of Fe determined in the three types of teff samples collected from the Bahir Dar site were almost similar. The concentrations of Ca, Zn, Mn and Cu determined in the three types of teff samples were also similar within each sampling sites. The differences in the concentration of the studied metals across different locations are due to the difference of agricultural practices and usage of different fertilizers like urea and others.

As shown in Table 2, the Fe contents determined in teff samples in this study are more than the other reported values and it is within the range of Baye et al. (2014) study. The amounts of Ca determined in this study were relatively lower than the other reported values. The amount of Cu determined in this method was almost similar with the other reported values. The concentrations of Mn determined in this study were more than the reported values of the other studies. The amounts of Zn determined in this study were almost similar with the other reported values.

| Teff type | Fe | Ca | Cu | Mn | Zn | Method | Reference |
|-----------|----|----|----|----|----|--------|-----------|
| White     | 95-377 | 170-1240 | 25-53 | - | 24-68 | FAAS | Baye et al. (2014) |
| White     | 160±2 | 1807±15 | 11±0.1 | 48.4±0.04 | 30±0.12 | FAAS | Kebede (2009) |
| White     | 189 | 1560 | - | - | - | - | do Nascimento et al. (2018) |
| White     | 161±2 | 839±1 | 4±0.03 | 27±0.0 | PTXRF | Kibatu et al. (2017) |
| White     | 89.5-146 | - | - | - | - | ICP-OES | Girma and Meareg (2017) |
| White     | 485-1195 | 247-348 | 13-15 | 20-28 | 73-85 | MP-AES | This study |
| Red       | 116-1500 | 180-1780 | 11-36 | - | 23-67 | FAAS | Baye et al. (2014) |
| Red       | 246±1 | 1785±10 | 25±0.3 | 224±0.2 | 48±1 | FAAS | Kebede (2009) |
| Red       | 664±1110 | 233-297 | 13-14 | 41-45 | 77-102 | MP-AES | This study |
| Mixed     | 115-1500 | 788-1470 | 16 | - | 38-39 | - | Baye et al. (2014) |
| Mixed     | 201±1 | 1686±11 | 38±0.1 | 133±0.0 | 38±0.1 | FAAS | Kebede (2009) |
| Mixed     | 589 | 1570 | - | - | - | - | do Nascimento et al. (2018) |
| Mixed     | 226±0.02 | 1162±0.3 | 4±0.01 | - | 34±0.1 | PTXRF | Kibatu et al. (2017) |
| Mixed     | 76 | 1800 | - | - | 36 | - | Yilmaz and Arslan (2018) |
| Mixed     | 443 | - | 8 | - | - | - | Kibatu et al., (2017) |
| Mixed     | 252-694 | 253-286 | 12.8-13 | 24-36 | 69-87 | MP-AES | This study |

**Table 1:** Mean concentrations (mean ± SD, n = 3, mg kg⁻¹ (air dry weight)) in the ranges metals in each samples

**Table 2:** Comparison of the concentration of teff samples with other reported values

The concentrations of Fe and Ca were relatively higher than the concentrations of the other studied metals in all the sampling sites. The level of Fe in the white teff was higher than that of the red and mixed teff samples collected from the Bure site and the level of Fe in the red teff was higher than that of the white and mixed teff samples collected from the Debre Markos site. The amount of Fe determined in the three types of teff samples collected from the Bahir Dar site were almost similar. The concentrations of Ca, Zn, Mn and Cu determined in the three types of teff samples were also similar within each sampling sites. The differences in the concentration of the studied metals across different locations are due to the difference of agricultural practices and usage of different fertilizers like urea and others.

As shown in Table 2, the Fe contents determined in teff samples in this study are more than the other reported values and it is within the range of (Baye et al., 2014) study. The amounts of Ca determined in this study were relatively lower than the other reported values. The amount of Cu determined in this method was almost similar with the other reported values. The concentrations of Mn determined in this study were more than the reported values of the other studies. The amounts of Zn determined in this study were more than the other reported values.

**Conclusion**

Teff is a hugely important crop to Ethiopia both in terms of production and consumption (FAO/WHO, 2011). In terms of production, teff is the dominant cereal by area coverage and second only to maize in production and consumption. Teff is a self-pollinated, annual, warm season cereal crop, believed to have originated in Ethiopia and have been domesticated and used throughout the world due to its excellent nutritional value as grains for human consumption and as forage for livestock (FAO, 2010). Suitable conditions of temperature, soil and water are the main growth conditions for teff.

The mean concentration levels of the metals in the three types of teff samples collected from the three sampling areas were determined by using MP-AES and were an efficient method. The overall mean concentrations determined...
(mg/kg, air dry weight) for the teff samples collected from the Bure site were Fe (719) > Ca (289) > Zn (86) > Mn (33) > Cu (14). The overall mean concentrations determined (mg/kg, dry weight) for the teff samples collected from the Debre Markos site were Fe (728) > Ca (256) > Zn (87) > Mn (29) > Cu (13). Similarly the overall mean concentrations determined (mg/kg, dry weight) for the teff samples collected from the Bahir Dar site were Fe (668) > Ca (270) > Zn (73) > Mn (36) > Cu (13).

The statistical analysis of one way ANOVA indicated that there is no significant difference between the mean concentrations of the studied metals (Fe, Ca, Cu, Zn and Mn) found in the three types of teff samples within the sampling sites. Similarly one way ANOVA indicated that there is no significant difference between the mean concentrations of the metals between the sampling sites at 95% confidence level.

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Ethics

This article is my original research paper and not done elsewhere and it is an important issue, because it is food related study across agro-ecological locations. For further investigations similar but not the same researches can be done worldwide.

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