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Research Article

Determination of Major, Minor and Trace Elements in Tef Using Portable Total X-Ray Fluorescence (TXRF) Spectrometer

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Abstract

Tef [*Eragrostis tef* (zucc.) Totter] is an indigenous cereal crop from Ethiopia. Its grains are milled and made to make foods and beverages. The levels of selected elements in tef flours sampled from Addis Ababa were analyzed using a portable total x-ray fluorescence spectrometer (PTXRF). The following concentrations (mean ± SD, mg/100g dry weight basis) were recorded in brown and white tef, respectively: K (486.80 ± 8.48) and (383.70 ± 3.25); Mg (209.15 ± 2.55) and (183.38 ± 11.73); Ca (116.15 ± 0.35) and (83.85 ± 0.78); Na (2.85 ± 0.20) and (2.32 ± 0.35); Fe (22.60 ± 0.02) and (16.05 ± 1.63), Zn (3.35 ± 0.12) and (2.70 ± 0.00); Cu(0.39 ± 0.01) and (0.43 ± 0.03); Pb (0.05 ± 0.00) and (0.05 ± 0.00); Se (0.009 ± 0.00) and (0.009 ± 0.00) and As (0.0035 ± 0.00) and (0.0035 ± 0.00). PTXRF is a useful technique for quantitative elemental analysis of tef flour samples. Tef is a high potassium and low sodium food source. Statistical analysis of variance (ANOVA) using Excel revealed that brown tef flour sample has higher mineral contents than white tef flour. Tef is a good source of dietary minerals and micronutrients for human-animal consumption.

Keywords: *Tef; Major; Minor; Trace; Essential Minerals; Toxic Minerals; Portable X-Ray Fluorescence*

Introduction

Tef (*Eragrostis tef* (Zucc.) Totter) 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 [1-4]. Tef is a member of the millet/Poaceae/ family that is tolerant to a wide range of climatic conditions. It can grow in altitudes ranging from sea level to 2800m above sea level under different moisture, soil, temperature and rainfall regimes [2]. 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. Its grains are milled and made to make foods and beverages for human consumption. Although tef 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.

Tef (*Eragrostis tef* (Zucc.) Totter) is an important food security crop in Ethiopia and the East African Highlands. In Ethiopia, the crop occupies over 2.8 million hectares equivalent to 25 - 30% of the total area covered by cereals [5]. Tef is a daily food staple for about 60 million inhabitants in the country. The principal use of tef grain for human food in Ethiopia is in the form of Enjerra, a soft porous thin pancake with a sour taste [4,6]. Enjerra is made from flour of Tef, water and starter fluid saved from previously fermented dough [7]. Enjerra is a major food staple, and provides approximately two thirds of the diet in Ethiopia.

Recently, the nutritional benefits of this cereal have also being acknowledged, appreciated and accepted by many other people around the world [3,8-10]. As tef grain flour was characterized by its excellent nutritional value as compared to other cereal flours the effects of the substitution of wheat flour with different levels of tef grain flour on the physico-chemical, bread making properties of the flour were investigated and have proved that breads supplemented with tef flour, up to a 5% level, are organoleptically and nutritionally acceptable

[9]. The amount of tef produced worldwide is increasing rapidly due to the plant's popularity as an especially nutritious grain and as high quality, horse hay.

The grain has high nutritional values: a very high protein, carbohydrate, fat, vitamin A and C, fiber, Thiamin, Riboflavin, Niacin, and essential minerals like Calcium, Chloride, Chromium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Sodium and Zinc [11-13]. Tef grains contain the eight essential amino acids (isoleucine, leucine, methionine, lysine, phenylalanine, threonine, tryptophan and valine). The protein digestibility is high because the main protein fractions are the most digestible types. The main protein fractions (albumin) are rich in lysine. Tef is essentially free of gluten. Interest in Tef has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain, making it a suitable substitute for wheat, maize and other cereals in food applications especially for people with celiac disease [9,14].

The study by the Ethiopian Institute Biodiversity Conservation laboratory form 114 tef variants collected from Ethiopia indicated that Tef grains contain average of 9 - 15% protein, 2% - 4% fat, 2% - 4% fiber, 68% - 74% carbohydrate, 10% - 13% of moisture and ash content between 2% and 3% [15]. According Mengesha., *et al*. [16], Tef contains higher amounts of a number of minerals than wheat, barley or sorghum. Previous published and unpublished studies [12,13,16,17] on the levels of some essential metals in Tef flours using UV-Visible and FAAS reported higher contents of calcium, iron and zinc for Tef than other common cereals. However, information on the contents of major, minor and trace elements in the Tef flours are variable and controversial in the literature. The applications of PTXRF for soil and agricultural samples have been demonstrated [18], but no work has been reported the use of PTXRF for quantitative elemental analysis of Tef. There was also none or very little data on the status of all relevant major, minor and trace elements for human and animal health contained in tef grains. Therefore, the purpose of this study is to evaluate the application of portable x-ray fluorescence spectrometer and to determine the major, minor and trace elements in Tef flours using the technique. The study also compares the results with previous works on tef flour and other grains. The levels of 10 major, minor and trace elements, usually relevant to nutritional biochemistry and health in human beings and animals, (K, Mg, Ca, Na, Fe, Zn, Cu, Pb, Se and As) in two different types (White and Brown) of tef flours collected from Addis Ababa, Ethiopia were determined. Findings of this study will provide adequate information on the distribution of elements and will ensure the dietary safety of the food from Tef in terms of essential and non-essential elements.

Materials and Methods

Equipments and Reagents

A Bruker Tracer III-SD portable x-ray fluorescence (PXRF) spectrometer (#T3S2731; Bruker AXS Microanalysis GmbH, Germany) using radioisotope excitation method were used for the investigation at the Soil-Plant Spectral Diagnosis Laboratory in the International Centre for Research in Agroforestry (ICRAF), Nairobi, Kenya. Portable XRF data were obtained using 2048 channels. Spectra were acquired using a voltage of 15 keV, an anode current of 25 μA, and no filter for 180 seconds per assay (170 seconds live time) for light (Na-Ca) elements and 40 keV, an anode current of 15 μA, and a yellow filter for 60 seconds per assay for heavy (Sc-Pb) elements. Two different forms of sample presentation were used: (I) direct measurement of 100-200 mg of plant sample on the nose of the instrument with a vacuum < 5 torr for light elements and (II) placement of 2 g of plant sample in a sample cup with 4 μm prolene as a film at the base of the cup with no vacuum (e.g. dry air conditions). The Bruker S1PXRF v.3.8.30 software was used for qualitative data with the net photon counts for all elements present calculated. The data were the processed using the Bruker S1CalProcess v.2.2.35 with empirical calibrations to give the ppm elemental concentrations.

Sample collection and preparation

About 50 mg of each White and Brown tef flours, purchased and milled in a commercial mill house, were randomly collected from tef bags in a household from Addis Ababa. The Tef flour samples were kept in polyethylene bags and transported to the Laboratory for total elemental concentrations of some essential metals and potentially hazardous metals using a Bruker Tracer III-SD portable x-ray fluorescence (PXRF) spectrometer. The milled Tef flours were analysed as loose powders without any further sample preparation and digestion.

Results and Discussion

Determination of elements in Tef flours

Tef is composed of complex carbohydrates with slow digestible starch and free of gluten, essential amino acids and fatty acids, fibers, phyto-chemicals and minerals [4,19]. Tef is a more nutrient-dense, gluten free cereal option with lower glycemic index for many people with celiac disease and diabetics [8,14].

The consumption of tef integrates a diet enriched with mineral elements that carryout beneficial functions and health effects in the human and animal body as components of structural proteins, cofactors and activators of enzymes, regulators of nerve transmissions, muscle contraction, osmotic pressure and salt water. Tef is also a potential link to transfer bio-active non-nutrient and anti-nutrient contaminants and heavy metals from the environment to the animals or human through the food chain. Exposures to these contaminants through the food chain are associated with multiple health effects with varying degree of severity and conditions. Thus, the analysis of the total concentration of some essential major, minor, trace elements and potential toxic heavy metals in tef and other food resources, depending on many complex biological and agronomic factors, has implications for both human and animal health upon consumption.

Metals contained in samples can be determined by classical methods (Gravimetric or electrochemistry), neutron activation analysis (NAA), spectroscopic methods (FAAS, GFAAS, HGAAS, ICP-OES, ICP-MS and TXRF) and chromatographic method (IC) [20]. A portable total x-ray Fluorescence spectrometer technique is used for this work. This technique unlike the other techniques has advantages in that the analysis is non-destructive, there is less error associated within the preparation of material for metal determination and consequently very rapid. Another advantage is that the spectral line interferences are unlikely. The instrument is also simple to operate. The main disadvantage of the technique is the lack of standards made of a matrix the same or similar to the plant tissue. However, portable total x-ray fluorescence spectrometer is still a useful technique for quantitative elemental analysis of tef flour samples and gives similar results with other techniques.

The concentration of K in both tef varieties was found to be the highest followed by Ca and Mg. Tef, however, has relatively low level of sodium in this work. To the contrary, the content of sodium reported in tef flours in an unpublished thesis work using FAAS by Zelalem [13] is about ten times greater than what is found in here by TXRF. This needs confirmation in the future work. Kocho and Bulla flours have been reported to have about 20 times more sodium than tef flours [21]. Tef is therefore a high potassium and low sodium food source. Eating more potassium-rich foods can lower your risk of high blood pressure, stroke, and heart disease. One of the biggest problems with the traditional western diet is too much sodium, and too little potassium. Studies suggest boosting your potassium intake and curbing salt and sodium can slash your stroke risk and may also lower your odds of developing heart disease. Potassium, a mineral, works by protecting blood vessels from oxidative damage and keeps vessel walls from thickening. Adults should aim to get 4,700 mg of potassium a day. Tef then can help to contribute this goal to a large extent if consumed daily.

Iron contents in the tef grain flours ranged within the limits of 16.05 mg/100g to 22.60 mg/100g DM. Zinc, Copper and Selenium contents in the tef grain flours ranged within the limits of 33.5 to 27; 3.9 to 4.3 and to 0.09 μg/g DM respectively. Zinc and Copper contents in the tef grain flours are significantly greater than wheat but lower than other cereals such as millet, maize and barley grown in healthy soils. Selenium contents in the tef flours are very small compared to wheat and other cereals for adequate contribution from tef. The concentration of Pb (0.5 μg/g) in these tef flours is above the permissible limit (0.3 μg/g) for plant based foods grown in uncontaminated soils [22]. Arsenic contents in tef flours on the other hand are below the maximum permissible limit. The concentration of minerals in plant-based foods is usually related to its level in the soil and its bioavailability.

The difference in mineral content between and within tef varieties is wide ranging. Red/Brown tef has higher potassium, magnesium, calcium, iron and zinc content than White tef. Similar results were found by Abebe., *et al* [12]. On the other hand, white tef has a higher copper content than Brown tef (Table 1, 2 and 3). Ketema [6] analyzed 12 genotypes of tef grown in different agro-ecologic settings and

5 varieties grown in a greenhouse in Great Britain and reported that genetic and environmental factors affect the mineral content of tef. This may partly explain the high variability in the mineral content reported in different studies.

Elements	Brown Tef (mean ±SD)	White Tef (mean ±SD)
Potassium, K	486.80 ± 8.48	383.70 ± 3.25
Magnesium, Mg	209.15 ± 2.55	183.38 ± 11.73
Calcium, Ca	116.15 ± 0.35	83.85 ± 0.78
Iron, Fe	22.60 ± 0.02	16.05 ± 1.63
Zinc, Zn	3.35 ± 0.12	2.70 ± 0.00
Sodium, Na	2.85 ± 0.20	2.32 ± 0.35
Copper, Cu	0.39 ± 0.01	0.43 ± 0.03
Lead, Pb	0.05 ± 0.00	0.05 ± 0.00
Selenium, Se	0.009 ± 0.00	0.009 ± 0.00
Arsenic, As	0.0035 ± 0.00	0.0035 ± 0.00

Table 1: *The observed average metal concentrations (mg/100g dry weight) in Brown and White Tef flour samples using portable XRF spectrometry.*

Tef Variety	K	Ca	Mg	Na	Fe	Method	References
White Tef	205.59 ± 7.17	180.7 ± 16.65	153.16 ± 9.45	22.89 ± 1.42	15.95 ± 1.75	FAAS	Zeleke, 2009
Red Tef	215.81 ± 8.13	178.54 ± 9.50	161.91 ± 7.76	22.06 ± 1.46	24.62 ± 1.10	FAAS	Zeleke, 2009
White Tef	383.70 ± 3.25	83.85 ± 0.78	183.38 ± 11.73	2.32 ± 0.35	16.05 ± 1.63	PXRF	This study, 2016
Brown Tef	486.80 ± 8.48	116.15 ± 0.35	209.15 ± 2.55	2.85 ± 0.20	22.60 ± 0.02	PXRT	This study, 2016
White Tef	NR	124	NR	NR	37.7	FAAS	Abebe., *et al.* (2007)
Brown Tef	NR	155	NR	NR	> 150	FAAS	Abebe., *et al.* (2007)

Table 2: *Comparison of the Levels of essential major and minor elements (Mean ± SD mg/100 g, dry mass) in Tef flour from Ethiopia by different methods.*

NR = Not Reported; ND = Not Detected

Tef Variety	Zn	Cu	Se	As	Pb	Method	References
White Tef	2.98 ± 0.12	1.08 ± 0.08	NR	NR	NR	FAAS	Zeleke, 2009
Red Tef	4.79 ± 0.32	2.51 ± 0.30	NR	NR	NR	FAAS	Zeleke, 2009
White Tef	2.70 ± 0.00	0.43 ± 0.03	0.009 ± 0.00	0.0035 ± 0.00	0.05 ± 0.00	PXRF	This study, 2016
Brown Tef	3.35 ± 0.12	0.39 ± 0.01	0.009 ± 0.00	0.0035 ± 0.00	0.05 ± 0.00	PXRF	This study, 2016
White Tef	2.86	NR	NR	NR	NR	FAAS	Abebe., *et al.* (2007)
Brown Tef	4.02	NR	NR	NR	NR	FAAS	Abebe., *et al.* (2007)

Table 3: *Comparison of the Levels of essential and non-essential trace elements (Mean ± SD mg/100 g, dry mass) in Tef flour from Ethiopia by different methods.*

NR = Not Reported; ND = Not Detected

Tef has been claimed to be superior to most common cereals in its mineral content, particularly in calcium and iron. Comparing uncontaminated Tef to Barley, Wheat, Maize and Sorghum, Mengesha [11] and Mohammed., *et al*. [9] reported that tef is superior in its mineral content, particularly in calcium and iron. However, the very high mineral (i.e. iron) content of tef has been contested and in many instances attributed to soil contamination [6,12,23]. The variability in the results could be due to error associated to sampling, sample preparation and treatment before measurement and method selection. For example, Hallberg and Bjorn-Rasmussen [24] reported that Tef's iron content is not different from other cereals by showing that iron content drops from 39.7 mg/100g to 3.5 mg/ 100g DM when grains are washed with dilute hydrochloric acid.

On the other hand, washing with acid is likely to lead to loss of acid-labile intrinsic iron and thus may underestimate the iron content. For instance, Areda., *et al*. [23] reported that acid washing of Tef grains led to a 50 percent greater loss of iron than washings with de-ionized water. More recently, Baye., *et al*. [25] examined the content of iron, zinc and calcium in Tef, Barley, Wheat, and Sorghum before and after washing with de-ionized water. He found that washing the tef grain significantly decreased in the iron content as well as the variability between replicates is found to be relatively high; suggesting that soil contamination in Tef is relatively high compared to other cereals. The mineral contamination of tef is probably due to its small size, and suggests increased contact with soil over a larger area [25].

The contamination of cereal grains by iron from the soil in Ethiopia, particularly in tef, has often been associated with traditional methods of threshing grain under the hooves of cattle [26]. More recently, Ambaw [27] compared the iron content of the same tef variety between laboratory (manual threshing) and traditional threshing. Traditional threshing led to 30 to 38 percent increase in iron content mainly due to soil contamination. The iron content of the laboratory threshed Tef was 16 mg/100g DM, which was still higher than what is found in many cereals. This suggests that although the intrinsic iron content of tef may not be as high as previously thought, tef is still a better source of iron than other cereals like wheat, barley, sorghum, and maize. In contrast to iron, Baye., *et al*. [25] showed that under the same conditions, the values reported for calcium and zinc are consistent and are less affected by washing. Zinc and Copper contents are significantly greater than wheat but lower than other cereals such as millet, maize and barley grown in healthy soils. Selenium contents in the tef grains are very small compared to wheat and other cereals [20]. Lead contents in the tef grains in this study which is 0.5 μg/g were higher than the maximum permissible limit set by FAO/WHO guidelines [22]. Similarly, high levels of lead contamination in some vegetables from Ethiopia have been reported by Deribachew., *et al*. [28]. While the controversies about the content of iron in tef continue to be a question to find the truth; the significant contamination of tef and other plant based foods from Ethiopia by the toxic element lead probably from the environment (and particularly from the soil), dietary contribution and its effects rather needs further investigation. If Tef flours for analysis are found from commercial milling houses, the possibility of contamination of some trace elements from the milling machine is also another possibility that should be considered and investigated.

Comparison of minerals in Tef with other cereals

Despite the differences and high variability in the mineral contents of Tef in different studies as described above, tef has a higher iron, calcium and copper content than other common cereals (Table 4 and 5). The zinc content of tef is also higher than that of sorghum and wheat. The zinc content of tef, however, is less than the contents in maize, barely and millets [29]. Tef grain iron is apparently high intrinsically or due to agronomic practice in Ethiopia and/or availability boosting through fermentation process in bread making [7,10,25]. Because of these, the prevalence of iron deficiency anemia is low and the prevalence of zinc deficiency is high among tef injera consumers in Ethiopia [30-34].

Food Type	K	Ca	Mg	Na	Fe	Metd.	References
Barley, Flour	NR	45.0	NR	NR	8.4	FAAS	Abebe., *et al.* (2007)
Maize, Flour	NR	16	NR	NR	3.6 - 4.8	FAAS	Abebe., *et al.* (2007)
Wheat, Flour	NR	15.2 - 39.5	NR	NR	3.7	ICP-MS	Elis 2008
Sorghum	NR	5.0 - 5.8	NR	NR	3.5 - 4.1	FAAS	Baye K (2014)
Rice	NR	23	NR	NR	1.5	FAAS	Baye K (2014)
Kocho	275.3- 438.0	49.8 -58.4	18 -29	46.2 - 68.8	9.25 - 13.5	FAAS	Atlabachew., *et al.* (2008)
Bulla	70.8 - 87.5	38.5 - 44.6	5.84 - 8.95	40.2 - 44.2	3.65 -5.98	FAAS	Atlabachew., *et al.* (2008)
White Tef	383.70 ± 3.25	83.85 ± 0.78	183.38 ± 11.73	2.32 ± 0.35	16.05 ± 1.63	PXRF	This Study, 2016
Brown Tef	486.80 ± 8.48	116.15 ± 0.35	209.15 ± 2.55	2.85 ± 0.20	22.60 ± 0.02	PXRT	This study, 2016

Table 4: *Comparison of selected major and minor metal concentrations (mg/100 g, dry mass) in White Tef and Brown Tef with some reported values in barley, wheat, maize, sorghum, rice, Kocho and Bulla.*

NR = Not Reported; ND = Not Detected

Food Type	Zn	Cu	Se	As	Pb	Metd	References
Barley, Flour	3.57	0.28 - 0.50	NR	NR	NR	FAAS	Abebe., *et al.* (2007)
Maize, Flour	2.6 - 4.6	1.30	NR	NR	NR	FAAS	Abebe., *et al.* (2007)
Wheat, Flour	1.7	0.23	NR	NR	0.0013	ICP-MS	Elis 2008
Sorghum	1.4 - 1.7	0.41	NR	NR	0.0060	FAAS	Baye K (2014)
Rice	2.2	0.16	NR	NR	NR	FAAS	Baye K (2014)
Kocho	0.71 -3.1	0.29 - 0.38	NR	NR	ND	FAAS	Atlabachew., *et al.* (2008)
Bulla	1.63 - 2.2	0.20 - 0.35	NR	NR	ND	FAAS	Atlabachew., *et al.* (2008)
White Tef	2.70 ± 0.00	0.43 ± 0.03	0.009 ± 0.00	0.0035 ± 0.00	0.05 ± 0.00	PXRF	This study, 2016
Brown Tef	3.35 ± 0.12	0.39 ± 0.01	0.009 ± 0.00	0.0035 ± 0.00	0.05 ± 0.00	PXRF	This study, 2016

Table 5: *Comparison of selected trace metal concentrations (mg/100 g, dry mass) in White Tef and Brown Tef with some reported values in barley, wheat, maize, Kocho and Bulla.*

NR = Not Reported; ND = Not Detected (Below the Detection Limit 0.3 mg/100 g for lead)

Statistical Analysis

A statistical analysis of variance (ANOVA) using Excel and SAS software revealed that there is a significant difference between the mineral levels of the two types of tef flour samples at 95% Cl ($p < 0.005$). In general, red/brown tef flour sample has higher mineral contents than the white tef flour. Furthermore, Tef is rich in its mineral contents compared to Maize, Wheat, Bulla or Kocho which are alternatively used in Ethiopia.

Conclusion

The concentration of K in both tef varieties was found to be the highest followed by Ca and Mg. Tef, however, has relatively low level of sodium. It is therefore a high potassium and low sodium food source. Among minor and trace elements analyzed the concentration of Fe was the highest followed Zn, Cu and Se. Zinc and Copper contents in the tef grain flours are significantly greater than wheat but lower than other cereals such as millet, maize and barley grown in healthy soils. Selenium contents in the tef flours are very small compared to wheat and other cereals for adequate contribution from tef. Relatively significant amounts of toxic lead and non-significant amounts Arsenic were detected in the samples. Statistical analysis of variance (ANOVA) using Excel and SAS software revealed that the red/brown

Tef flour sample has higher mineral contents than the white Tef flour. Furthermore, Tef is rich in its mineral contents compared to Wheat, Bulla or Kocho which are alternatively used in Ethiopia. Tef is a good source of dietary minerals and micronutrients for human-animal consumption.

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Bibliography

1. Vavilove NI. "The origin, variation, immunity and breeding of cultivated plants". Ronald Press. New York (1951): 37-38.
2. Ketema S. "Tef (Eragrostis tef), breeding, agronomy, genetic resources, utilization and role of Ethiopian Agriculture". Institute of Agricultural Research (1993): 102.
3. Stallknecht GF., *et al*. "Tef: Food Crop for Humans and Animals". In: J Jamick and JE Simon (eds.), New Crops. Wiley, New York. USA (1993): 231-234.
4. Baye K. "Tef: nutrient composition and health benefits". The Ethiopia Strategy Support Program, International Food Policy Research Institute and Ethiopian Development Research Institute, Addis Ababa, Working Paper 67 (2014): 1-18.
5. Berhe T and Gebretsadik Z. "Increasing the productivity of tef: New approaches with dramatic results". In: Girma A., Sintayehu M. (Eds), Tef: the story of Ethiopian Biodiversity, Addis Ababa, Forum for Environment, (2010): 1-52.
6. Ketema S. "Tef, Eragrostis Tef (Zucc.) Trotter". Promoting the Conservation and Use of Underutilized and Neglected Crops series no. 12. Gatersleben: Institute of Plant Genetics and Crop Plant Research & Rome: International Plant Genetic Resources Institute (1997).
7. Mezemir S. "Probiotic potential and nutritional importance of Tef (Eragrostis tef (Zucc.) Trotter) Enjerra-A review". *African Journal of Food, Agriculture, Nutrition and Development* 15.2 (2015): 9964-9968.
8. Hopman E., *et al*. "Tef in the diet of celiac patients in The Netherlands". *Scandinavian Journal of Gastroenterology* 43.3 (2008): 277-282.
9. Mohammed MIO., *et al*. "Evaluation of wheat breads supplemented with Tef (Eragrostis tef (Zucc.) Trotter) grain flour". *Australian Journal of Crop Science* 3.4 (2009): 207-212.
10. Alaunyte I., *et al*. "Improving the quality of nutrient-rich Tef (Eragrostis tef) breads by combination of enzymes in straight dough and sourdough bread making". *Journal of Cereal Science* 55.1 (2012): 22-30.
11. Mengesha H. "Chemical Composition of Tef (Eragrostis tef) Compared with that of Wheat, Barley and Grain Sorghum". *Economic Botany* 20.3 (1966): 268-273.
12. Abebe Y., *et al*. "Phytate, Zinc, Iron and Calcium content of selected raw and prepared foods consumed in rural Sidama, Southern Ethiopia, and implications for bioavailability". *Journal of Food Composition and Analysis* 20 (2007): 161-168.
13. Zelalem T. "Levels of trace Cadmium and essential Zinc in wheat flour commercially available in Addis Ababa, Ethiopia". M.Sc. Thesis, Addis Ababa University, Addis Ababa, Ethiopia (2006): 1-40.

14. Gebremariam MM., *et al*. "Tef (Eragrostis tef) as a Raw Material for Malting, Brewing and Manufacturing of Gluten-Free Foods and Beverages: A Review". *Journal of Food Science and Technology* 51.11 (2012): 2881-2895.

15. Tesema A. "Variability of panicle characters of Tef (Eragrostis tef (Zucc.) Trotter) from south, north, east, west and central highlands of Ethiopia". In: Girma A., Sintayehu M. (Eds.), Tef: the story of Ethiopian Biodiversity, Addis Ababa, Forum for Environment (2010): 1-52.

16. Mengesha H., *et al*. "Genetic variability and interrelationship of characters in tef, Eragrostis tef (Zucc.) Trotter". *Crop Science* 5.1 (1965): 155-157.

17. Zeleke K. "Levels of essential elements in three Tef (Eragrostis tef (Zucc.) Trotter) varieties". M.Sc. Thesis, Addis Ababa University, Addis Ababa, Ethiopia, (2009): 1-76.

18. Mages M., *et al*. "The use of a portable total reflection x-ray fluorescence spectrometer for field investigation". *Spectrochimica Acta Part B* 58 (2003): 2129-2138.

19. Kebede EM and Yimer AM. "Determination of the levels of iron from red, mixed and white Tef (Eragrostis) flour by using Uv/Visible spectrometry". *Journal of Natural Sciences Research* 5.9 (2015): 34-41.

20. Elis. "The optimisation of a method for total selenium analysis and application to cereal grain foods". M. App. Sc. Thesis, Royal Melbourne Institute of Technology (RMIT) University, Melbourne, Australia (2008): 1-92.

21. Atlabachew A and Chandravanshi BS. "Levels of major, minor and trace elements in commercially available enset (Ensete ventricosum (Welw) Cheesman) food products (Kocho and Bulla) in Ethiopia". *Journal of Food Composition and Analysis* 21 (2008): 545-552.

22. WHO/FAO. "Food additives and contaminants". Joint WHO/FAO Food standards programs, Codex Alimenterius Commission, ALINORM 01/12A:1-289, Switzerland (2001).

23. Areda A., *et al*. "The Iron Content of Tef [Eragrostis tef (Zucc.) Trotter] and its Controversy". *SINET: An Ethiopian Journal of Science* 16 (1993): 5-13.

24. Hallberg L and Bjorn-Rasmussen E. "Measurement of iron absorption from meals contaminated with iron". The *American Journal of Clinical Nutrition* 34.12 (1981): 2808-2815.

25. Baye K., *et al*. "Changes in Mineral Absorption Inhibitors Consequent to Fermentation of Ethiopian injera: Implications for Predicted Iron Bioavailability and Bioaccessibility". *International Journal of Food Science and Technology* 49.1 (2014): 174-180.

26. Bezwoda WR., *et al*. "The Relationship between Marrow Iron Stores, Plasma Ferritin Concentrations and Iron Absorption". *Scandinavian Journal of Haematology* 22.2 (1979): 113-120.

27. Ambaw A. "Determination of Iron Fractions of Laboratory Threshed and Field Threshed Tef Injera". Addis Ababa University, Addis Ababa, Ethiopia (2013).

28. Deribachew B., *et al*. "Selected heavy metals in some vegetables produced through waste water irrigation and their toxicological implication in eastern Ethiopia". *African Journal of Food, Agriculture, Nutrition and Development* 15.3 (2015): 10013-10032.

29. Kibatu G., *et al*. "Level of zinc in maize seeds and maize growing soils of central Mecha, Amhara National Regional State of Ethiopia". *Ethiopian Journal of Science and Technology* 9.1 (2016): 1-14.

30. Bultosa G. "Physiochemical characteristics of grain and flour in 13 tef [Eragrostis tef (Zucc.) Trotter] grain varieties". *Journal of Applied Science Research* 3.12 (2007): 2042-2051.

31. Umeta M., *et al*. "Content of Zinc, Iron, Calcium and their absorption inhibitors in foods commonly consumed in Ethiopia". *Journal of Food Composition and Analysis* 18.8 (2005): 803-817.

32. Asfaw KG and Danno FI. "Effects of salinity on yield and yield components of Tef [Eragrostis tef (Zucc.) Trotter] Accessions and Varieties". *Current Research Journal of Biological Sciences* 3.4 (2011): 289-299.

33. Eba T. "Tef (Eragrostis tef) cultivars, morphology and classification. Part II". Exp. Sta. Bull. No. 66, Addis Ababa University, College of Agriculture, Dire Dawa, Ethiopia (1975).

34. Kefyalew T., *et al*. "Phenotypic diversity for qualitative and phonologic characters in germplasm collections of Tef Eragrostis tef". *Genetics resources and crop evolutions* 47 (2000): 73-80.

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