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Levels of Trace and other Selected Metals in Mixed Teff Grain Samples

Abraha Gebregewergis Weldehawaria

Department of Chemistry, Food Science and Nutrition Research, Ethiopian Institute of Agricultural Research, Ethiopia

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Email: abrahag1981@gmail.com

Abstract: Teff *(Eragrostis tef* (Zuccagni)) is a hugely important crop to Ethiopia both in terms of production and consumption. In terms of production, teff is the dominant cereal by area coverage and second only to maize in production and consumption. The principal use of teff grain for human food in Ethiopia is in the form of Injera, a soft porous thin pancake with a sour taste. In this study, the levels of some selected essential and trace metals in the mixed teff grain samples were determined in the samples collected from the three selected sampling areas (Bure, Debre Markos, and Bahir Dar) of Ethiopia. After proper sample pretreatment, 5 mL HNO_3: 1 mL $HClO_4$, 240°C, and 2:30 h were the optimized digestion conditions. Then using the optimized conditions sample preparation was made and the levels of metals were determined by the Micro-Plasma Atomic Absorption Spectroscopy (MP-AES). The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Al (713-832) >Fe (252-694) >Ca (253-286) >Zn (69-87) >Mn (24-36) >Cu (13) >Pb (1.8-2.8) >Cd (1.8). The results indicated that mixed teff grain is a good source of essential metals but not free from the toxic metals Cd and Pb, the concentrations of Cd and Pb were higher than the WHO guidelines (0.1 and 0.2 mg/kg, respectively). The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with a standard solution and the percentage recoveries varied from 92 to 104%, which is good and is in the allowed range of 90% ±10. ANOVA indicated that there is no significant difference between the mean concentrations of Cd and Pb among the mixed teff samples, but a significant difference for the other studied metals at a 95% confidence level.

Keywords: Essential Metals, Micro-Plasma Atomic Absorption Spectroscopy, Mixed Teff Grain (*Eragrostis Tef* (Zuccagni)), Trace Metals

Introduction

Teff [*Eragrostis tef* (Zuccagni) Trotter] is one of the major and indigenous cereal crops in Ethiopia, where it is believed to have originated (Grant and Greg, 2006). It is considered a low-risk crop from the perspective that it can be cultivated in a broad range of ecological surroundings and under tough environmental conditions where most other cereals fail. The crop is preferred by both farmers and consumers due to its beneficial traits associated with its agronomy and utilization (Ketema, 1997). Teff is most known for its minute seed head, with nearly two million seeds per pod and a diameter of only 0.7 to 1.0 mm. Teff in Amharic means the lost seed because if dropped, it is so easily lost. In Ethiopia, teff is harvested by hand when the vegetative part of the plant turns yellow. Oxen are used to trample the grass to separate the seed from the rest of the plant (Ketema, 1997). With a population exceeding 110 million people, Ethiopia is the only country in the world where teff is intensely grown and produced for human consumption. Teff is a staple food in Ethiopia, consisting of two-thirds of their cereal diet, and is primarily used to make Injera. 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 has been domesticated and used throughout the world due to its excellent nutritional value as grains for human consumption and as forage for livestock. It can grow in altitudes ranging from sea level to 2800 m 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. 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 the construction of houses both in rural and urban areas (Kibatu *et al*., 2017). Teff 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. The principal use of teff grain for human food in Ethiopia is in the form of injera, a soft porous thin pancake with a sour taste.

Teff is a hugely important crop to Ethiopia both in terms of production and consumption. 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 a reduced quantity of grain available to consumers (Merga, 2018). Nevertheless, the high Fe content of teff has been a source of controversy as some authors have estimated it to be high (Melaku *et al*., 2005; Saturni *et al*., 2010), whereas others reflect this high level to soil contamination during the traditional method of threshing and are thus of limited biological utility (Melaku *et al*., 2005).

Literature Review

The study by the Ethiopian Institute Biodiversity Conservation Laboratory from 114 teff variants collected from Ethiopia indicated that teff grains contain an 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%. Teff contains higher amounts of several minerals than wheat, barley, or sorghum. Previously published studies on the levels of some essential metals in teff flours using UV-Visible and FAAS reported higher contents of Fe, Ca, and Zn in the teff than other common cereals. However, information on the contents of major, minor, and trace elements in the teff flours is variable and controversial in the literature (Kibatu *et al*., 2017). Therefore, the purpose of this study is to determine the metal contents in teff using the MP-AES.

Teff was estimated to account for 16% of cereals grain production and was cultivated on 2.7 million hectares based on Ethiopian Central Statistics Authority. In the central, northern, and western parts of the country, it is the staple food (Melaku *et al*., 2005). Teff is a high-status cereal crop and a family who do not depend on teff as daily food is considered poor in these parts of the country. The main reason behind such attitude is the long historical, socio-economic, and cultural values of teff developed by the society. Injera from whatever crop is the most common form of food in the central and north part of Ethiopia. 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 several 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 (Melaku *et al*., 2005). Recently there is a growing interest in teff grain utilization because of its nutritional merits (whole grain) and free of the protein gluten that makes teff an increasingly important dietary component for individuals who suffer from gluten intolerance or celiac disease (Boka *et al*., 2013).

Chemical Composition of Teff

The genetic and phenotypic diversity of teff in Ethiopia is a national treasure of potentially global importance. Teff is tolerant to many extreme environmental conditions including water-logging and storage pests. Although teff performs well on various soil types the average grain yield in Ethiopia is about 0.7 tons/ha. The yield of teff is even lower in the drier part of the country. This low yield is attributed to nutrient deficiencies mainly of N and P and the susceptibility of the crop to lodging at higher N. The grain proteins offer an excellent balance among the essential amino acids. Teff is rich in carbohydrates, and fiber and has a complete set of essential amino acids. Teff is also particularly high in iron and has more calcium, copper, and zinc than other cereal grains. They also contain polyphenols (produced as secondary plant metabolites) which affect the nutritional properties. The demand for gluten-free foods is certainly increasing. Interest in teff has increased noticeably due to its very attractive nutritional profile and gluten-free nature of the grain making it a suitable substitute for wheat and other cereals in their food applications as well as foods for people with celiac disease. Many gluten-free products may not meet the recommended daily intake of fiber, minerals, and vitamins (do Nascimento *et al*., 2018). The amount of teff produced worldwide is increasing rapidly due to the plant's popularity as an especially nutritious grain. The grain has high nutritional values, a very high protein, carbohydrate, fat, vitamin A and C, fiber, thiamin, riboflavin, niacin, and essential minerals like Ca, Cl^-, Cr, Cu, Fe, Mg, Mn, P, K, Na, and Zn.

Minerals

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. In cases where concentration and bio-availabilities in the food supply are low, fortification has been popular (Miller, 1996). There is a significant body of evidence that minerals by themselves and in proper balance with one another have important biochemical and nutritional functions. The difference in mineral content between and within teff varieties is wide-ranging. Red teff has a higher Fe and Ca content than mixed or white teff (Abebe *et al*., 2007). On the other hand, white teff has a higher Cu content than red and mixed teff (Table 1). (Ketema, 1997) analyzed 12 genotypes of teff 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 Fe content of teff.

The zinc content of teff is higher than that of sorghum and wheat. However, the very high mineral (Fe) content of teff has been contested and in many instances attributed to soil contamination (Ketema, 1997; Abebe *et al*., 2007). For example, Hallberg and Björn-Rasmussen (1981) reported that teff's Fe content is not different from other cereals by showing that Fe content drops from 39.7 to 3.5 mg/100 g when grains are washed with dilute hydrochloric acid. However, washing with acid is likely to lead to loss of acid-labile intrinsic Fe and thus may underestimate the Fe content. For instance, reported that acid washing of teff grains leads to a 50% greater loss of Fe than washings with de-ionized water. Comparing uncontaminated teff to barley, wheat, maize, and sorghum, Mengesha (1966) reported that teff is superior in its mineral content, particularly in Ca and Fe. More recently Baye *et al*. (2014) examined the content of Fe, Zn, and Ca in teff, barley, wheat, and sorghum before and after washing with de-ionized water. Mengesha (1966) found by washing the grain significantly decreased the iron content as well as the variability between replicates. Despite this decrease, the variability between replicates for teff remained relatively high suggesting that soil contamination in teff is relatively high compared to other cereals.

The mineral contamination of teff is probably due to its small size and suggests increased contact with soil over a larger area (Baye *et al*., 2014). The contamination of cereal grains in Ethiopia particularly in teff has often been associated with traditional methods of threshing grain under the hooves of cattle. More recently compared the Fe content of the same teff variety after laboratory (manually) and traditional threshing. Traditional threshing led to a 30-38% increase in Fe content mainly due to soil contamination. The Fe content of the laboratory threshed teff was 160 mg/kg DM, which was still higher than what is found in many cereals. This suggests that although the intrinsic Fe content of teff may not be as high as previously thought, teff is still a better source of Fe than other cereals like wheat, barley, sorghum, and maize. Iron is necessary for red blood cell formation and is required for oxygen transport throughout the body. According to Besrat *et al*. (1980), the Fe content of 35 samples of acid-washed white and red teff grain was 36 to 78 mg/kg on Dry Matter bases (DM). Lovis (2003) reported that teff grain contains 58 mg/kg Fe while wheat flour contains 44.1 mg/kg Fe. Mengesha (1966) reported values of 196 and 115 mg/kg Fe for two teff cultivars, while values of 40 mg/kg Fe and 78.5 mg/kg Fe for two wheat cultivars. According to values of Fe content of teff seed ranged between 209 and 755 mg/kg, sorghum 50 to 156 mg/kg, and millet 390 mg/kg.

Calcium is essential for developing and maintaining healthy bones and teeth, and assists in blood clotting, muscle contraction, nerve transmission, and oxygen transport. Lovis (2003) reported that teff grain contains 1590 mg/kg Ca while wheat flour contains 1500 mg/kg Ca.

Zinc is an essential part of more than 200 enzymes included in digestion, metabolism, reproduction, and wound healing. It plays a critical role in immune response and is an important antioxidant. Mengesha (1966) reported that two teff cultivars contain 67 and 68 mg/kg Zn while wheat contains 39.5-60 mg/kg Zn. Lovis (2003) reported that teff grain contains 20 mg/kg Zn but wheat flour contains 8.5 mg/kg Zn.

Copper is both an essential nutrient and a drinking-water contaminant. It has many commercial uses. It is used to make pipes, valves, and fittings and is present in alloys and coatings. Sensitivity to the toxic effects of excess dietary copper is influenced by its chemical form, species, and interaction with other dietary minerals. Cu does not appear to be a cumulative toxic hazard for man, except for individuals suffering from Wilson's disease. Cu is not considered to be mutagenic, carcinogenic, or affect reproduction. Teratogenicity/embryotoxicity is observed in some animal studies. Cu is essential to normal red blood cell formation and connective tissue formation. It acts as a catalyst to store and release Fe to help hemoglobin formation. Lovis (2003) reported that teff grain contains 7 mg/kg Cu while wheat flour contains 1.82 mg/kg Cu. Mengesha (1966) reported values of 520 mg/kg Cu and 640 mg/kg Cu for two teff cultivars while 550 mg/kg Cu for two wheat cultivars.

Manganese is a key component of enzyme systems, supports brain function, and is required for blood sugar regulation. It is one of the most abundant metals in the Earth's crust, usually occurring with iron. It is used principally in the manufacture of Fe and steel alloys, as an oxidant for cleaning, bleaching, and disinfection as potassium permanganate, and as an ingredient in various products. Mn is an essential element for humans and other animals and occurs naturally in many food sources. The most important oxidative states for Mn are Mn^{2+}, Mn^{4+}, and Mn^{7+}. Mn is naturally occurring in many surface water and groundwater sources, particularly in anaerobic or low oxidation conditions and this is the most important source of drinking water. The greatest exposure to Mn is usually

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from food (WHO, 2003). Mengesha (1966) reported values of 21.2 mg/kg Mn and 30 mg/kg Mn for two teff cultivars while 12 mg/kg Mn and 36 mg/kg Mn for two wheat cultivars. Lovis (2003) reported that the Mn content of teff grain was 64 mg/kg and wheat flour was 7.92 mg/kg.

Aluminum is a major component of the earth's crust. It occurs in the environment in the form of silicates, oxides, and hydroxides. It is released to the environment both by natural processes and from anthropogenic sources, whereby natural processes far outweigh the contribution of anthropogenic sources. Mobilization of Al through human actions is mostly indirect and occurs as a result of the emission of acidifying substances into the atmosphere. Al is highly concentrated in soil-derived dust from natural processes, coal combustion, and activities such as mining and agriculture. Al and its compounds appear to be poorly absorbed in humans; the mechanism of gastrointestinal absorption has not yet been fully elucidated. Variability results from the chemical properties of the element and the formation of various chemical species occur, which are dependent upon the pH, ionic strength, presence of competing elements, and complexion agents within the gastrointestinal tract.

Cadmium is a relatively rare element, released into the air, land, and water by human activities. In general, the two major sources of contamination are the production and utilization of Cd and the disposal of wastes containing Cd. The pathway of human exposure to crops is susceptible to increases in soil Cd. The Cd uptake by plants from soil is greater at low soil pH. Tobacco is an important source of Cd uptake in smokers.

Lead is a classical chronic or cumulative poison. In humans, Pb can result in a wide range of biological effects depending upon the level and duration of exposure. Health effects are generally not observed after a single exposure. Many of the effects that have been observed in laboratory animals have also been observed in humans, including hematological effects, neurological and behavioral effects, renal effects, cardiovascular effects, and effects on the reproductive system. In addition, Pb has been shown to have effects on bone and the immune system in laboratory animals. Children are more vulnerable to the effects of Pb than adults. Pb is associated with impaired neurobehavioral functioning in children.

Importance of Teff

Teff has its own agronomic and food qualities that make it the most important crop to the farm household compared to other crop enterprises (Ketema, 1997). Teff is a better choice and provides some harvest when it grows in moisture stress agro-ecology where other crops face potential starvation that leads to total production loss (Tesemma, 2001). The other quality of teff is its storability in various locations without being infected by storage pests compared to other staples and making it an alternative cereal cash crop for most Farmers. It is also the most desirable crop because of its straw quality for livestock feed, best injera quality, and the ability to provide more satisfaction from a small weight of the grain, (Mengistu and Mekonnen, 2010).

Consumption of Teff in Ethiopia

In the past, a major determinant of grain consumption was its production. However, with improvement in market linkages, this picture is gradually changing. In the Oromia region for instance, where regional teff production is the second highest next to the Amhara region in Ethiopia, its consumption expenditure is only 8%. In contrast, the Afar region, little known for its teff production has comparatively higher teff consumption expenditure (10%) (Berhane *et al*., 2011). Today several factors including agro-ecology (Baye *et al*., 2013), livelihoods, and income (Berhane *et al*., 2011) determine cereal consumption in Ethiopia.

Statement of the Problem

Teff is generally accepted that the grain is highly nutritious although there is some debate about its precise nutritional value of it. Regarding the Fe content, some authors have estimated it to be high and others reflect this high level is attributed to the dust and dirt that cling to the grain (Grant and Greg, 2006). Whereas other studies revealed that the Fe content of teff varies between geographical regions possibly as a result of soil contamination (Saturni *et al*., 2010; Bokhari *et al*., 2012). A literature review indicated that the mineral contents of teff flour were determined by using different techniques at different times. But the results reported by the different kinds of literature are controversial and have differences; this may be due to sampling error. Most of the research was done by collecting teff flour samples from commercially available mill houses; this may increase the contamination levels of the samples. Therefore collecting teff grain samples and determining the mineral contents of it by using the MP-AES is very important and accurate results can be obtained.

According to (Berhane *et al*., 2011) Amhara region is the highest teff producer in Ethiopia. But no reported literature is present about the levels of metals in teff samples collected from the described sampling areas. Additionally, no research is done on the analysis of minerals of teff grain using microwave plasma atomic emission spectrometer. Hence, determining the mineral contents of mixed teff samples by using microwave plasma atomic emission spectrometer is a new method in Ethiopia and since the method is a multi-element system and cost-effective, analyzing mineral contents in mixed teff samples using this instrument is important.

Table 1: Mineral content of teff grain compared to other cereals (mg/kg) (Baye *et al*., 2014)

Metal	White teff	Red teff	Mixed teff	Maize	Sourghum	Wheat	Rice
Fe	95-377	116-1500	115-1500	36-48	35-41	37	15.0
Zn	24-68	23-67	38-39	26-46	14-17	17	22.0
Ca	170-1240	180-1780	788-1470	160	50-58	152-395	230.0
Cu	25-53	11-36	16	13	4.1	2.3	1.6

Significance of the Study

Since teff is one of the major indigenous cereal crops in Ethiopia, identifying its metal contents of it is very important. Additionally, MP-AES is a multi-element analysis method, therefore many elements can be determined within a short period compared to other spectroscopic methods. The generated analytical data and information on the chemistry of the teff can be used as baseline information for future research in various fields related to Ethiopian teff productive areas.

Experimental

Instrumentation

MP-AES consists of a microwave-induced plasma interfaced with 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 before 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 is measured at the detector. Most commonly determined elements can be measured with a working range of low part per million (ppm) to weight percent. MP-AES is a technique comparable to traditional AA and AES but with several potential advantages including lower cost of operation and elimination of the requirement for flammable gasses. MP-AES instruments are benchtop instruments. While the technique is mature, there are a limited number of manufacturers supplying commercial MP-AES instruments.

All measurements were performed using an Agilent 4200 MP-AES (USA), 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 autosampler 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. Instrument operating conditions are given in Table 2. The sample introduction system consisted of PVC peristaltic pump tubing, a single-pass glass cyclonic spray chamber, and the One Neb nebulizer. The Agilent MP Expert software was used to automatically subtract the background signal from the analytical signal.

Chemical Reagents and Materials

All chemicals used in this study were analytical-grade reagents. Perchloric acid (70%) and nitric acid (69-72%) were used (Sigma Aldrich Steinleim, Germany). The reference standards of the metals under study were the products of Perkin Elmer (Boston, USA). The stock standard solutions 1000 mgL^{-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 mgL^{-1}) which were obtained by diluting stock standard solutions.

The materials used in the laboratory were sample preparation utilized PVC flasks, polyethylene conical flasks, filter paper, 50 mL volumetric beakers for sample and solution preparation, round bottom flask, ceramic mortar and pestle (USA) for grinding and homogenizing the samples, digital analytical balance (four-digit) and Kjeldahl technique (England) for digestion. All the glassware used was 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.

Description of the Study Area

The mixed teff samples were collected from the most teff production areas of three different localities of the Amhara regional state of Ethiopia. Particularly from Debre Markos, Bure, and Bahir Dar which are located in the northwestern part of the Amhara regional state. The geographical locations (latitude, longitude, and elevation) of sampling sites are described as follows. Bahir Dar is located at a latitude of 11°35'37.1" N and a longitude of 37°23'26.8" E in the northern hemisphere. Bahir Dar is located at the exit of the Abbay from Lake Tana at an altitude of 1,820 meters above sea level. The city is located approximately 578 km northwest of Addis Ababa. Debre Markos is a city in northwest Ethiopia. It is located in the Misrak Gojjam Zone of the Amhara administrative region, it is located at a latitude and longitude of 10°20′N 37°43′E coordinates and an elevation of 2,446 meters above sea level.

Debre Markos is located approximately 306 km far apart from Addis Ababa. Bure is a town in western Ethiopia located in the Mirab Gojjam Zone of the Amhara region, this town is located at a latitude and longitude of 10°42′N 37°4′E with an elevation of 2091 meters above sea level. Bure is located approximately 414 km far apart from Addis Ababa. The reason for the selection of these places was based on the availability of the teff and its popularity in consumption.

Sample Collection and Preparation

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

Optimization of Digestion Procedure

Wet acid digestion is one of the methods that are involved to get free metal ions in dissolved form from complex organic matrix based on changing different digestion parameters like volume ratio of reagents added, digestion temperature, and duration of time. One of the wet acid digestions can be carried out by the Kjeldahl apparatus in which organic components are assumed to decompose in the form of different gaseous forms and other metallic elements are left in the solution except for those easily volatile metals like Hg. Moreover, it is assumed that digestion is assumed to be complete if the solution is clear and colorless.

Different digestion procedures were carried out for the teff samples using HNO_3 and $HClO_4$ acid mixtures by varying the volume of the acid mixture, digestion time, and digestion temperature (Boke *et al*., 2015). 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 for the resulting digests. Based on this fact the optimized digestion conditions for the mixed 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 h digestion time. Compared to (Zeleke, 2009), the optimum conditions used in this digestion process were better both in digestion time and temperature. According to (Zeleke, 2009) 3:00 h and 300°C were the optimum conditions. The optimized condition in this study is also better than other different optimized conditions in terms of the reagents used. In this experiment, only two acids (HNO_3 and $HClO_4$) were used for the digestion of the samples, but others were used more. Therefore, the optimized conditions in this study were economical in terms of time and reagent volumes used. The optimum conditions for digestion of the mixed teff samples in this study are shown in Table 3.

Digestion of Samples

Applying the optimized conditions, 0.5 g of powdered 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 the dissolution of filter paper by the digested residue while filtering with filter paper (Whatman 125 mm diameter, Germany) into a 50 mL volumetric flask.

Table 2: Agilent 4200 MP-AES operating conditions.

Parameters	Fe	Ca	Cu	Zn	Mn	Al	Cd	Pb
Wavelength (nm)	372.0	422.7	324.8	213.9	403.1	396.1	228.8	405.8
Background correction	Auto	Auto	Auto	Auto	Auto	Auto	Auto	Auto
EGCM setting	High	High	High	High	High	High	High	High
Replicates	3	3	3	3	3	3	3	3
Pump speed (rpm)	15	15	15	15	15	15	15	15
Blank subtraction	On	On	On	On	On	On	On	On
Stabilization time (s)	20	15	15	15	15	15	16	20
Sample uptake time (s)	30	27	25	25	25	30	30	30
Sample uptake fast pump	On	On	On	On	On	On	On	On
Rinse time (s)	10	10	10	10	10	10	10	10
Read time (s)	3	3	3	3	3	3	3	3
Nebulizer flow (L/min)	0.65	0.6	0.7	0.95	0.9	0.95	0.5	0.75

Table 3: Reagent ratios and volumes, temperature and time attempted during optimization of digestion of 0.5 g of mixed teff sample.

Trials	Reagent volume (mL) HNO_3	$HClO_4$	Total	Temperature (ºC)	Time (h)	Results
1	1	1	2	240	2:30	Yellow with suspension
2	2	1	3	240	2:30	Cloudy yellow
3	3	1	4	240	2:30	Nearly colorless
4	4	1	5	240	2:30	Slightly colorless
5	5	1	6	240	2:30	Clear colorless*
6	6	1	7	240	2:30	Clear colorless
7	3	2	5	240	2:30	Slightly colorless
8	4	2	6	240	2:30	Nearly colorless
9	4	1	5	240	2:30	Nearly colorless
10	5	2	7	240	2:30	Clear colorless
11	5	1	6	240	0:30	Yellow with suspension
12	5	1	6	240	1:00	Yellow with suspension
13	5	1	6	240	1:30	Cloudy light yellow
14	5	1	6	240	2:00	Light yellow
15	5	1	6	240	2:30	Clear colorless*
16	5	1	6	240	3:00	Clear colorless
17	5	1	6	150	2:30	Cloudy yellow with suspension
18	5	1	6	180	2:30	Cloudy yellow with suspension
19	5	1	6	210	2:30	Slightly yellow
20	5	1	6	240	2:30	Clear colorless*
21	5	1	6	270	2:30	Clear colorless
22	5	1	6	300	2:30	Clear colorless

The round bottom flask was rinsed subsequently with around 5 mL of 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 mixed teff samples keeping all digestion parameters the same. Then the metal concentrations in the digested sample solutions were determined by using MP-AES.

Instrument Calibration

Calibration standard solutions were prepared for each of the metals which were prepared from the MP-AES standard stock solutions that contained 1000 mgL^{-1}. These intermediate standards were diluted with distilled water to obtain five working standards for each metal of interest. Then Fe, Ca, Cu, Zn, Mn, Al, Cd, and Pb were analyzed with MP-AES. Three replicate determinations were carried out on each sample. All the above-listed metals were determined by emission/concentration mode and the instrument readout was recorded for each solution. The same analytical procedure was employed for the determination of elements in the digested blank solutions. The correlation coefficients for each metal show that the change in emission with concentration is in good correlation. Five points calibration curve was established by running a series of the prepared working standard solutions for each metal. Correlations coefficients obtained for the calibration curves were >0.999. After calibration, the sample solutions were aspirated into the MP-AES instrument and readings of the elemental concentrations were recorded. Three replicate determinations were carried out on each sample. Method detection and quantification limits

The Limit of Detection (LoD) is a measure of how sensitive the analytical method is and is the lowest concentration or weight of analyte that can be measured at a specific confidence level. For the determination of the Limit of Detection of the analytical method (LoD), triplicates of eight blanks were prepared in parallel and analyzed for their metal contents. The Standard Deviation (SD) of the eight blanks was calculated and multiplied by three ($LOD = 3SD_b$) to determine the method detection limit. The Limit of Quantification (LoQ) is the smallest quantity of analyte that can be measured with acceptable accuracy and precision and it is described as ten times the standard deviation. In this study, the limit of detections for all of the eight elements in the samples was smaller than all the results obtained and are given in Table 4.

The calibration curve for each element was constructed using an appropriate standard at a series of concentrations. A regression equation for each metal was constructed and the best fit of the equation was checked using the correlation coefficient (R^2). In all the cases, the regression coefficient (R^2) was found to be above the accepted linear range value of 0.999. As can be seen from Table 4, the method detection limits are low (≤0.56 mg/kg) enough to determine the metals in the samples at trace levels. The calibration curves were with good correlation coefficients.

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.

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Table 4: The wavelength, method detection and quantification limit, correlation coefficient, and calibration curve equations

Metals	Wavelength (nm)	IDL (mg/kg)	MDL[1] (mg/kg)	MQL[2] (mg/kg)	Correlation coefficient	Calibration curve equation*
Fe	372.0	0.10	0.40	1.30	0.9999	I = 5032C+81.40
Ca	422.7	0.10	0.56	1.90	0.9992	I = 68218C+8746.00
Cu	324.8	0.10	0.40	1.30	0.9998	I = 10654C+9283.00
Zn	213.9	0.10	0.22	0.70	0.9990	I = 2448C-198.70
Mn	403.1	0.10	0.15	0.50	0.9999	I = 27752C-255.50
Al	396.1	0.10	0.17	0.50	0.9990	I = 17002C-25.00
Cd	228.8	0.01	0.02	0.10	0.9996	I = 7863C+733.90
Pb	405.8	0.01	0.06	0.20	0.9997	I = 2889C+11.22

Due to the absence of Certified Reference Material (CRM) for the mixed teff samples in the laboratory, the validity of the digestion procedures was assured by spiking the samples with a standard solution of known concentration of the target analytes. From the stock solution of 1000 mg/L, 120 µL of Fe, 44 µL of Ca, and 15 µL of Zn were added to 0.5 g of mixed teff sample. For Cu intermediate standard solution of 100 mg/L was prepared and 30 µL of Cu was added to 0.5 g of mixed teff sample. For Cd and Pb intermediate standard solutions of 10 mg/L were prepared and 45 µL of Cd and 63 µL of Pb were added to 0.5 g of mixed teff sample. The spiked and non-spiked samples were digested and analyzed in similar conditions using the optimized procedure. Metals were analyzed in triplicate standard metal solutions to evaluate the efficiency of the procedure and the percentage recoveries lie within the range from 92 to 104% and were calculated using the following formula.

$$\%\mathrm{Recovery} = \frac{spiked\ sample - unspiked\ sample}{amount\ added} \times 10$$

Results and Discussion

Calibration Curves of Standards for Each Metal

The calibration curves were prepared from standards of known concentration covering the concentration range expected in the sample. Then, the curves were established at five concentration levels corresponding to 0, 5, 10, 15, and 20 mg/L for Fe, Ca, Cu, Zn, Mn, Cd, and Pb and 0, 1, 2, 3, and 4 mg/L for Al. All the working standards of metals solution used for the calibration curve exhibited very good linearity with squared regression coefficients (R^2) values ranging from 0.9990 to 0.9999.

The Concentrations of Metals in the Mixed Teff Samples

As shown in Table 5 the concentrations of the metals were carried out by using MP-AES and the precision of the results was determined by calculating the Relative Standard Deviation (RSD). The relative standard deviation of the results is in the range of mean ±10 which is good and in the recommended range. Mean values were determined from triplicate analysis of each sample and triplicate samples were used for each sample site. As such the mean values determined were triplicate of triplicate analysis for each metal and the results were in terms of mean values ± SD (where n = 3 for each of the metals in this study).

All the results obtained from the MP-AES that are expressed in terms of (mg/L) were converted into (mg/kg) using the following equation:

$$\frac{mg}{kg} = \frac{c \times v \times D}{w} \qquad (1)$$

where, C = Concentration in mg/L, V = Volume in liter, W = Weight of sample in kilogram and D = Dilution factor (considered as unity).

The overall mean concentration levels in the mixed teff samples for elements Al, Fe, Ca, Zn, Mn, Cu, Pb, and Cd were 766, 511, 268, 76, 32, 13, 2.5, and 1.8 mg/kg, respectively. As shown in Table 7 the results of concentrations of the metals showed that the mixed teff samples collected from the Bure site have higher amounts of Ca and Zn compared to the samples collected from Debre Markos and Bahir Dar. The concentrations of Fe and Al collected from Bahir Dar were higher compared to that of samples collected from the Bure and Debre Markos sites.

In figure 1A, higher concentrations of Al and Fe are observed in the mixed teff samples collected from the Bahir Dar and Debre Markos sampling sites, and a relatively lower amount of Zn is observed compared to that of Al, Fe, and Ca collected from the three sampling sites. Similarly, in Fig. 1B, relatively higher concentrations of Mn and Cu are observed compared to that of Pb and Cd in the mixed teff samples collected from the three sampling areas. In short, the mean concentrations of the metals (mg/kg) in the mixed teff samples collected from the three sampling areas can be ordered as Al>Fe>Ca>Zn>Mn>Cu>Pb>Cd.

Comparison of Levels of Metals in Tef with other Cereals

As can be seen from Table 6, diversified concentration ranges of the studied metals were noticed compared with other cereals like maize, sorghum, wheat, and rice. Between teff and wheat, teff and rice, comparable Ca concentrations are observed, and between teff and maize comparable Cu concentrations occur. For the other metals, higher concentrations between teff and the maize, sorghum, wheat, and rice

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with significant differences have been noticed. This variation may be due to species variability and variations in agricultural practices. The results obtained in this study indicated that Fe, Cu, and Zn are more in teff than cereals like sorghum, wheat, and rice, Ca is more in teff than maize, sorghum, and rice, Ca in teff is comparable with wheat, Cu in teff is comparable with maize.

Analysis of Variance (ANOVA)

In this study one-way ANOVA (one treatment factor with two or more treatment levels) was used. Microsoft excel 2008 was used for the preparation of calibration curves and the data analysis. One-way ANOVA was used to compare the mean values of the metals between different sampling sites. As shown in Table 7, the statistical analysis of ANOVA indicated that there is a significant difference among the mean concentrations of Al, Ca, Cu, Fe, Mn, and Zn found in the mixed teff samples collected from the three sampling areas at 95% confidence level. But there is no significant difference in the mean concentrations of Cd and Pb between the mixed teff samples. The presence of significant differences may be due to the presence of different geographical distribution, rainfall, soil composition, harvesting, and storing methods.

Table 5: Mean concentrations (mean ± SD, n = 3, mg kg-1, dry weight) of metals in each sampling site

	Concentrations (mg/kg) in (mean ± SD) of metals in the mixed teff samples from			
Metals	Bure	Debre markos	Bahir dar	Overall mean
Fe	252±2	588±3	694±4	511.0
Ca	286±5	253±9	265±1	268.0
Cu	13±1	13±1	13±0.2	13.0
Zn	87±3	73±3	69±3	76.0
Mn	36±3	24±2	36±2	32.0
Al	713±2	752±8	832±4	766.0
Cd	1.8±0.2	1.8±0.2	1.8±0.2	1.8
Pb	2.8±0.3	2.8±0.1	1.8±0.1	2.5

Table 6: Comparison of the mineral content of teff grain compared to other cereals (mg/kg) (Baye *et al*., 2014)

	Metal concentrations (mg/kg)			
Cereals	Fe	Ca	Cu	Zn
White teff	95-377	170-1240	25-53	24-68
Red teff	116-1500	180-1780	11-36	23-67
Mixed teff	115-1500	788-1470	16-00	38-39
Maize	36-4800	160-0000	13-00	26-46
Sourghum	35-4100	50-5800	4.10	14-17
Wheat	37-0000	152-3950	2.30	17-00
Rice	15-0000	230-0000	1.60	22-00
This study				
Mixed teff	252-6940	253-2860	13.00	69-87

Table 7: Analysis of Variance (ANOVA) between and within mixed teff grain samples at a 95% confidence level.

Metal	Comparison	SD (mg/kg)	Df	F_{cal}	F_{crit}	Remarks
Fe	Between samples	231	2	6001	5.14	Significant difference among the sample means
	Within samples	2.98	6			
Ca	Between samples	16.6	2	10.4	5.14	Significant difference among the sample means
	Within samples	5.15	6			
Cu	Between samples	0.28	2	20.1	5.14	Significant difference among the sample means
	Within samples	1.24	6			
Zn	Between samples	9.50	2	12.0	5.14	Significant difference among the sample means
	Within samples	2.74	6			
Mn	Between samples	6.62	2	7.13	5.14	Significant difference among the sample means
	Within samples	2.48	6			
Al	Between samples	61.0	2	171	5.14	Significant difference among the sample means
	Within samples	4.65	6			
Cd	Between samples	0.46	2	5.00	5.14	No significant difference among the sample means
	Within samples	0.20	6			
Pb	Between samples	0.28	2	1.57	5.14	No significant difference among the sample means
	Within samples	0.35	6			

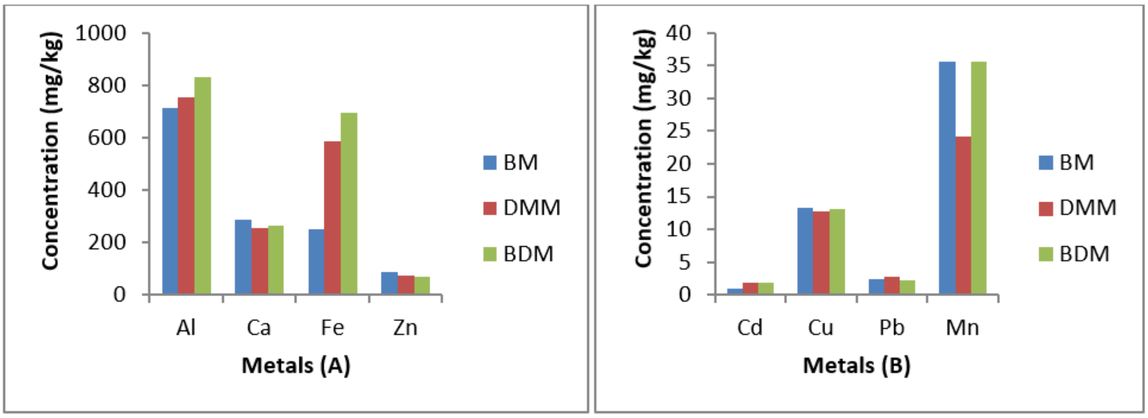

Fig. 1: The concentrations of metals (mg/kg) in mixed teff samples collected from the three sampling areas. (Where: BM = Bure mixed teff, DMM = Debre Markos Mixed teff, and BDM = Bahir Dar Mixed teff)

Conclusion

An efficient digestion procedure for the determination of metals in the mixed teff grain samples was optimized and validated through the spiking method and a good percentage recovery was obtained for the metals of interest. The mean concentration levels of the metals in the mixed teff samples collected from Debre Markos and Bahir Dar sampling areas were higher. The overall mean concentrations determined (mg/kg, dry weight) were in the ranges of Al (713-832) >Fe (252-694) >Ca (253-286) >Zn (69-87) >Mn (24-36) >Cu (13) >Pb (1.8-2.8) >Cd (1.8).

The results indicated that mixed teff grain is a good source of essential metals but not free from the toxic metals Cd and Pb, the concentrations of Cd and Pb are higher than the WHO guidelines (0.1 and 0.2 mg/kg, respectively). From the results of this study, it is possible to conclude that the samples collected from the three sites accumulated relatively larger amounts of Al and Fe among the determined metals and lower amounts of Cu, Cd, and Mn. The accuracy of the optimized procedure was evaluated by analyzing the digest of the spiked samples with a standard solution and the percentage recoveries varied from 92 to 104% which is good and is in the allowed range of 90% ±10.

By using ANOVA, there is no significant difference between the mean concentrations of Cd and Pb among the mixed teff samples, but there is a significant difference for the other studied metals of the mixed teff samples at a 95% confidence level. Since the levels of the toxic elements, Cd and Pb in the mixed teff samples are above the allowable limit, it is possible to conclude that a person who consumes mixed teff produced from this sampling areas is not free from the risks of Cd and Pb, this may be caused due to the contamination of the samples. In this study, the amount of the essential metal Cu found in the mixed teff samples is enough for human consumption but relatively lower than the levels of Fe, Co, Zn, and Mn. In soil, Cu is relatively immobile, since it binds strongly with organic matter and it seldom leaches and its availability to plants strongly depends on the soil type, mainly on the organic matter content and pH. The uptake of Cu by plants is affected by many factors including the soil pH, the prevailing chemical species, and the concentration of Cu present in the soil. Cu uptake in plants is among the lowest of all the essential elements (Burkhead *et al*., 2009).

References

Abebe, Y., Bogale, A., Hambidge, K. M., Stoecker, B. J., Bailey, K., & Gibson, R. S. (2007). 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(3-4), 161-168. doi.org/10.1016/j.jfca.2006.09.003

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Picq, C., & Guyot, J. P. (2014). Changes in mineral absorption inhibitors consequent to fermentation of Ethiopian injera: Implications for predicted iron bioavailability and bioaccessibility. International Journal of Food Science & Technology, 49(1), 174-180. doi.org/10.1111/ijfs.12295

Baye, K., Mouquet-Rivier, C., Icard-Vernière, C., Rochette, I., & Guyot, J. P. (2013). Influence of flour blend composition on fermentation kinetics and phytate hydrolysis of sourdough used to make injera. Food Chemistry, 138(1), 430-436. doi.org/10.1016/j.foodchem.2012.10.075

Berhane, G., Zelekawork, P., Kibrom, T., & Seneshaw, T. (2011). Food grain consumption and calorie intake patterns in Ethiopia. IFPRI Ethiopia strategy support program II (ESSP II) working paper 23. Addis Ababa. International Food Policy Research Institute. Available at: https://www.researchgate.net/.../257716460_Patterns_in_Foodgrain_Consumption.

Besrat, A., Admasu, A., & Ogbai, M. (1980). Critical study of the iron content of tef (Eragrostis tef). Ethiopian medical journal, 18(2), 45-52. doi.org/10.3389/fpls.2015.00177

Boka, B., Woldegiorgis, A. Z., & Haki, G. D. (2013). Antioxidant properties of Ethiopian traditional bread (Injera) as affected by processing techniques and tef grain (Eragrostis tef (Zucc.)) varieties. Canadian Chemical Transactions, 1(1), 7-24. https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.700.4851&rep=rep1&type=pdf

Boke, A., Megersa, N., & Teju, E. (2015). Quantitative determination of the heavy metal levels in the wild edible plant parts and their corresponding soils of the central and western regions of the Oromia state, Ethiopia. J. Environ. Anal. Toxicol, 5(5), 1. doi.org/10.4172/2161-0525.1000299

Bokhari, F., Derbyshire, E., Li, W., Brennan, C. S., & Stojceska, V. (2012). A study to establish whether food-based approaches can improve serum iron levels in child-bearing aged women. Journal of Human Nutrition and Dietetics, 25(1), 95-100. doi.org/10.1111/j.1365-277X.2011.01185.x

Burkhead, J. L., Gogolin Reynolds, K. A., Abdel-Ghany, S. E., Cohu, C. M., & Pilon, M. (2009). Copper homeostasis. New Phytologist, 182(4), 799-816. doi.org/10.1111/j.1469-8137.2009.02846.x

Coleman, J. M. (2012). Assessing the potential use of teff as an alternative grain crop in Virginia (Doctoral dissertation, Virginia Tech). https://vtechworks.lib.vt.edu/handle/10919/77012

do Nascimento, K. D. O., Paes, S. D. N. D., de Oliveira, I. R., Reis, I. P., & Augusta, I. M. (2018). Teff: suitability for different food applications and as a raw material of gluten-free, a literature review. Journal of Food and Nutrition Research, 6(2), 74-81. http://article.foodnutritionresearch.com/pdf/jfnr-6-2-2.pdf

Grant, V., & Greg, M. (2006). A demand and supply analysis of prospects for the Australian health grains industry. A report for the rural industries research and development corporation.

Hallberg, L., & Björn-Rasmussen, E. (1981). Measurement of iron absorption from meals contaminated with iron. The American journal of clinical nutrition, 34(12), 2808-2815. doi.org/10.1093/ajcn/34.12.2808

Ketema, S. (1997). Teff (Eragrostis tef (Zucc.) Trotter). Promoting the conservation and use of underutilized and neglected crops. Institute of plant genetics and crop plant research. International Plant Genetic Resources Institute, 12, 163-173.

Kibatu, G., Chacha, R., & Kiende, R. (2017). Determination of major, minor, and trace elements in tef using portable total X-ray fluorescence (TXRF) spectrometer. EC Nutrition, 9 (1), 51-59.

Lovis, L. J. (2003). Alternative to wheat flour in baked goods. Published by the America Association of Cereal Chemists, Inc. Journal of Cereal Food World, 48 (2), 62-63.

Melaku, U., West, C. E. & Habtamu, F. (2005). Content of zinc, iron, calcium, and their absorption inhibitors in foods commonly consumed in Ethiopia. Journal of Food Composition Analysis, 18, 803-817.

Mengesha, M. H. (1966). Chemical composition of teff, [Eragrostis tef.) compared with that of wheat, barley, and grain sorghum. Ecological Botany, 20, 268-273.

Mengistu, D. & Mekonnen, L. (2010). Integrated agronomic crop management to improve teff productivity under terminal drought. Department of dry-land crop and horticultural sciences, MekelleUniversity, Mekelle, Ethiopia.Available at:https://www.researchgate.net/.../221923107_Integrated_Agronomic_Crop_Management.

Merga, M. (2018). Progress, achievements, and challenges of tef breeding in Ethiopia. Ethiopian Institute of Agricultural Research, Asossa Agricultural Research Center. Journal of Agricultural Science and Food Research, 9 (1), 204. Available at: https://www.omicsonline.org/.../pprogress-achievements-and-challenges-of-tef-breeding-

Miller, E. (1996). Physico-chemical properties of teff. Minerals in food chemistry. Available at: https://www.scribd.com/document/328314097/Food-Chemistry-O-R-Fennema.

Saturni, L., Gianna Ferretti, G. & Bacchetti, T. (2010). The gluten-free diet. Safety and Nutritional Quality Nutrients, 2 (1), 16-34.

Tesemma, T. (2001). An overview of teff and durum wheat production in Ethiopia. Available athttps://evans.uw.edu/.../Evans_UW_Request_98_Productivity_of_Ethiopian_Ag_Octo.

WHO. (2003). Background document for preparation of WHO guidelines for drinking-water quality, Geneva. World Health Organization (WHO/SDE/WSH/03.04/103).

Zeleke, K. (2009). Levels of essential elements in three teff [Eragrostis tef (Zucc.) Trotter] varieties. Master Thesis, Addis Ababa University. Available at https://books.google.com.et/books?id=HYoZBQAAQBAJ.