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Studies on Commercially available Enset (*Ensete ventricosum*(Welw.), Cheesman) food Products (Kocho and Bulla) for Major, Minor and Trace Elements

BY

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Studies on Commercially available Enset (*Ensete ventricosum*(Welw.), Cheesman) food Products

(Kocho and Bulla) for Major, Minor and Trace Elements

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By

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Dedication

To my mother, Abaynesh and my Father, Atlabachew

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Abstract

Studies on Commercially available Enset (*Ensete ventricosum* (Welw.), Cheesman) food Products (Kocho and Bulla) for Major, Minor and Trace Elements

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Kocho and Bulla are starchy foods obtained from fermenting edible part of the leaf sheath and corm of enset plant (Ensete ventricosum (Welw.), Cheesman). In the present study the level of selected metals (Na, Mg, Mn, Ni, Pb, K, Ca, Fe, Zn, Cu, Co, Cr, Cd) in Kocho and Bulla sampled from different supermarkets of Woliso (Oromiya region) and Welkite (SNNPRG) of Ethiopia were analyzed. Known weight of oven dried Kocho and Bulla samples were digested by wet digestion using 2 mL of HNO_3 and 2 mL of $HCLO_4$ for 2 h at variable temperature (120 - 270 ^oC). The contents of the minerals in the digests were analysed using flame atomic absorption spectrometer. The following concentration ranges (μg/g) were recorded in Kocho and Bulla, respectively: K (2753 – 4380) and (708 – 875); Na (462 – 688) and (402 – 442); Ca (498 - 584) and (385 - 446) ; Mg (180 - 290) and (58.4 - 89.5); Fe (92.5 -135) and (36.5 - 59.8); Zn (31 - 32.08) and (22 -

44.3); Cu (3.4 – 4.3) and (2.01 - 3.53); Mn (8.58 -10.13) and (1.0 - 4.98); Ni (≤ 5.61) and (<4.0]; Cr (5.96 - 6.42) and (≤ 5.38); Co (5.5 - 6.1) and (5.0 - 5.01). Where as Cd and Pb were not detected in both types of food stuffs. The concentration of K was highest followed by Ca, Na and Mg in both food stuffs. From trace elements analysed, Zn was found to be highest next to Fe. Generally Kocho contained higher concentration compared to Bulla for the majority of the mineral nutrients identified. In general, Kocho and Bulla are rich in Ca and Zn compared to other similar food stuffs and contains comparable concentration of Cu, Fe and Mn. Beside Kocho and Bulla are free of heavy metal (Cd and Pb) contaminations compared to others.

Key Words: Kocho, Bulla, Amicho, SNNPRG, Oromia, Enset, FAAS, Wolkite, Woliso, Major, Minor, Trace, Element

1. Introduction

Agriculture in Ethiopia is characterized by diverse farming practices. Farmers with various ethnic back ground and cultural diversity living in the country's diverse agro-ecological zones have developed farming systems, characterized by a high degree of species diversity.

Root and tuber crops are widely cultivated in southern Ethiopia, which are supporting a considerable portion of the country's population as source of food. Prominent among these are: potato (Solanum tuberosum L.), sweet potato (Ipomoea batatas L.), enset (Ensete ventricosum (Welw.), Cheesman), godere (Colacasia esculanta L.), yams (Dioscorea spp.), Ethiopian dinch (Coleus parviflorus), koteharrie (Diaspora bulbiferous) and anchote (Coccinia abyssinica). Among these, enset, anchote and some yams are endemic to Ethiopia [1].

Enset is one of the seven species of the genus Ensete which is a monocarpic, perennial giant herbaceous plant reaching 4-8 meter or even up to 11 meter in height. It is the main crop of a sustainable indigenous Ethiopian system that help ensures food security in a country that is food deficient. Enset is related to and resemble the banana plant and is produced primarily for the large quantity of carbohydrate-rich food found in a false stem (pseudo stem) and an underground bulb (corm) [2, 3].

Enset (Ensete ventricosum (Welw.) Cheesman) belongs to the family Musaceae [4, 5]. Morphologically it resembles a banana plant but bananas belong to the related genus Musa. Both Enset and Musa have a large underground corm, a bundle of leaf sheaths (pseudo stem), and large paddle-shaped leaves. Both enset and banana are herbaceous perennial monocarpic crops; they produce flower only once at the end of their life cycle [4, 6, 7].

In spite of the extensive distribution of wild enset, it is only in Ethiopia that the plant has been domesticated. Wild enset propagates naturally by seed, and restricted in Ethiopia to elevation of approximately 1,200 to 1, 600 meters above sea level. However, almost always propagate domesticated enset vegetatively [8].

1.1. Major enset growing area in Ethiopia

Enset is one of the potential indigenous crops for food production [9-12] and can be grown everywhere in Ethiopia. According to several authors [13, 14], the enset cultivation system is economically viable and is one of the few successful indigenous and sustainable agricultural systems. It is sustainable because it has been providing food for humans for generation from the same plot and maintains the quality of life of the people. It grows in a wide range of environmental conditions. Even though it is grown in many administrative regions, the dwellers of the central and southwestern parts of Ethiopia are the only people that use enset as a staple and co-staple crop [15, 16]. At present, enset is important for about one-fifth of the total population of Ethiopia and cultivation is estimated to cover more than 224,400 hectares of land [14]. The majority of enset production is confined to Sidamo, Shoa, Keffa, Gamo Goffa and Illubabor administrative regions.

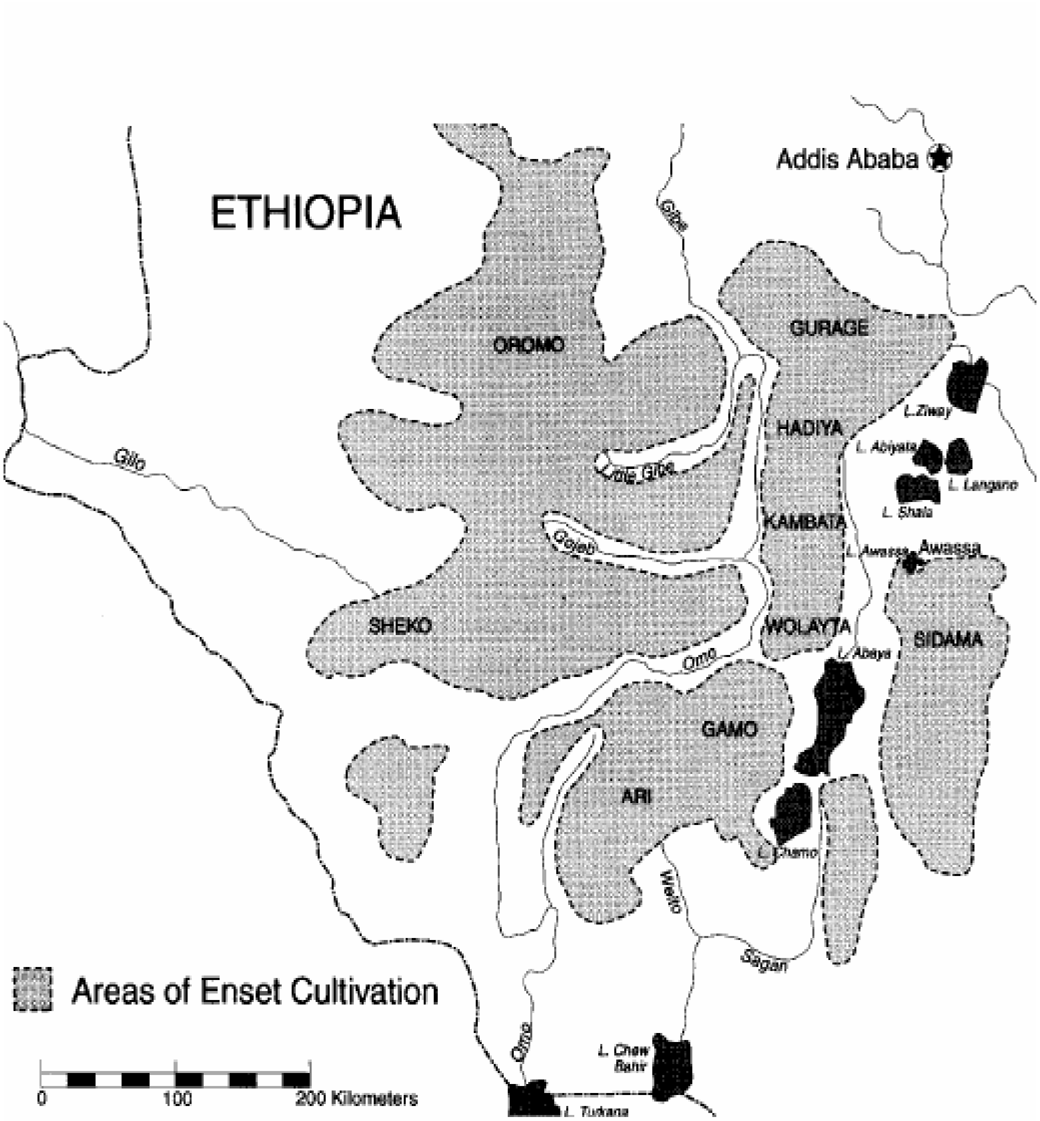


Figure 1 Major enset growing area of Ethiopia.

1.2. Food uses of enset

The plant is perhaps the biggest vegetable of all and looks like a banana "tree." The food, however, comes mainly from the lower trunk, filled with starchy pith, which on the largest specimens can be a meter in diameter and three meters tall. A second food comes from underground, where a corm may be almost a meter long and a meter in diameter, packed with starch like some giant potato. The edible parts are formed by the pseudo-stem and the underground corm rather than by the fruit [8,9, 21].

Nutritive value of starchy foods depends mainly on their nutrient content, physico-chemical properties of their starches and the existence of anti-nutritional activities and toxic substances.

Figure 2 Enset plant

The major foods obtained from enset are kocho, bulla and amicho.

Kocho is the bulk of the fermented starch obtained from the mixture of the decorticated (scarped) leaf sheaths and the grated corm (underground stem base). Kocho needs a lengthy period of processing and preparation, which is carried out by women. The first stage involves removing the leaf stalks and grading of the corm. Then the fibers are separated out and the pulp is crushed to extract the starch. This is put in a pit about 1.5 m deep and 1 m diameter, wrapped airtight with enset leaves before being packed down with stones. It is then allowed to ferment – a process, which may last anything from 4 months to three years. The pit is opened at intervals to allow aeration, and the enset leaves are replaced. This is repeated until the desired fermentation quality is reached or the food is needed. Finally, the fermented starch is dried and treated as flour. This can be used to prepare a pancake – like bread, which is eaten with milk and cabbage. *Kocho* is increasingly exported to urban markets [2, 8, 10, 18-22],
Kocho can be stored for a long period of time without spoiling. The quality of Kocho depends on the age of the harvested enset plant, the type of clone (variety), and harvesting season. Moreover, within one plant, the quality is influenced by the part of leaf sheath and corm processed [210, 21].

Bulla is the small amount of water-insoluble starchy product that may be separated from kocho during processing by squeezing and decanting the liquid. After decanting, the bulla is left to dry and fermented in a way similar to kocho or can be directly cooked without fermentation. It is considered the best quality enset food and is mainly from fully matured enset plant [8,21].

Amicho is the fleshy inner portion of the enset corm, which may be cooked and eaten separately, tasting similar to potato [2, 8, 22].

Kocho and bulla foods are extremely popular at restaurants that serve the Ethiopian delicacy of *Kitfo* (raw ground beef mixed with butter and spice) [8].

The fiber obtained from enset is used to make bags, ropes, cordage and mats. Enset leaves and dried leaf sheath are also used for wrapping materials. Furthermore, the leaves are also used as a plate for serving food. The dried midribs and petiole are used for making mats and rope (in place of nail) in house construction and as fuel. Enset leaves are used as cattle feed especially during the dry seasons when feed is scarce. Some enset clones are used as local medication for different illness and damage such as bone fracture, bone breakage and diarrhea for both human beings and animals [8, 10, 23.]

The mineral contents of enset products vary widely due to numerous factors, such as disease, age of harvested enset, sanitation, soil contamination and others. The general chemical composition of enset as reported by Cheesman, Agren and Gibson [4, 24] the products are high in carbohydrate and low in protein and fat.

The main feature of enset foods is their high energy value (1410–1950 kJ per 100 g dry matter of kocho, 1580–1850 kJ per 100 g dry matter of bulla), derived almost entirely from carbohydrate. Fresh kocho contains 47–62 g moisture per 100 g. Per 100 g dry matter the approximate composition of kocho is: protein 1.1–2.8 g, fat 0.2–0.5 g, carbohydrates 95–98 g, fibre 2.3–6.2 g, ash 1.7 g, Ca 60 mg, P 68 mg, Fe 7 mg, thiamine 0.06 mg, riboflavin 0.08 mg, niacin 0.6 mg. The moisture content of bulla ranges from 44–55 g per 100 g fresh material. Per 100 g dry matter the approximate composition of bulla is: protein 0.4–0.8 g, fat 0.2–0.4 g, carbohydrates 93–98 g, fiber 0.6–0.8 g, ash 0.2 g, Ca 91 mg, P 44 mg, Fe 5.8 mg, thiamine 0.02 mg, niacin 0.2 mg [24].

Wolde-Gebriel *et al* [21] reported that Bulla is more energy rich (8.5 MJ kg^{-1}) than kocho (6.5 $MJkg^{-1}$). All foods have a low protein content (4-22 gkg^{-1}) and no vitamin A.

Abebe *et al* [17] also reported the mineral concetration of some of the minerals (Fe, Zn and Ca) in Bulla and Kocho sampled from Sidama Zone, Southern part of Ethiopia.

The study of the life process shows that many vital functions are dependent on the presence of the major, minor and specific trace elements. Because of that, minerals are the most important nutrient factors for growth and maintenance of human life. The concentration of major and minor elements in living tissue can be expressed in grams per kilogram. On the other hand, the concentration of trace elements in living tissue varies between 0.01 and 100 mg kg^{-1}. The concentration of major, minor and trace elements in food gives important information about dietary habits of special group, health situation of individuals and origin of elements [25, 26].

Enset is a plant that grows in most part of Ethiopia. It is a draught resistant plant and its products can be kept for a long period of time without being spoiled. It is used as the main food for 7-10 million people in the highlands of south and south western Ethiopia. Therefore studies on major, minor and trace elements are necessary to assess its role as source of essential nutrients.

1.3. Mineral nutrition

Humans require a suite of mineral elements in varying amounts for proper growth, health maintenance and general well being [28,29]. Plant-derived foods have the potential to serve as dietary sources for all human-essential minerals, and with a well-balanced diet that includes mixed sources of grains, fruits, vegetables, roots and tuber crops, plant foods can make a significant contribution to daily mineral needs at all stages of the life cycle [30].

Plants can manufacture vitamins, essential amino acids, and fatty acids but they can not manufacture minerals. To get the minerals they need, plants rely on the soil in which they grow. Plants draw in minerals from the soil one tiny, some times microscopic, parcel at a time, through their vast, tentacle-like complex of roots and then put those minerals to work doing the job of sustaining life. In ideal word we would take in our daily requirement of minerals by eating plants that grow in mineral rich soils [28-30]

In plants, minerals follow the same path as water. Some mineral ions diffuse in-between the cells. In contrast to water, some minerals are actively taken up by plant cells. Mineral nutrient concentration in roots may be 10,000 times more than in surrounding soil. During transport throughout a plant, minerals can exit in xylem and enter the cells that require them. Mineral ions cross plasma membranes by a chemiosmotic mechanism. Plants absorb minerals in ionic form: for example, nitrate (NO_3^-), phosphates (HPO_4^-), potassium ion (K^+), calcium (Ca^{2+}), etc; all have difficulty crossing a charged plasma membrane [26-30].

There are marked differences between the elementary composition of the soil and that of plants or animals. Plants have the faculty of assimilating large amounts of certain elements out of proportion to their abundance in the soil and animals have a similar but less pronounced ability to screen out certain elements from the plants. The ability of plants to absorb minute amounts of needed elements, while keeping out larger amounts of unnecessary ones [36].

Many individuals both in developed and developing countries are failing to attain recommended mineral intakes [31]. Whereas an increased consumption of plant food products would be beneficial, it appears that behavioral and/or environmental factors will continue to limit their consumption [32]. Thus, as an alternative strategy, efforts are underway to increase the nutrient composition of those plant foods which people do eat, as an attempt to ensure adequate attainment of dietary nutrients in all individuals [32, 33].

Currently, the achievable densities of minerals in our existing agricultural crops means that few individual plant foods are able to supply the daily recommended intake for any given mineral in an average or reasonable serving size. This problem of low mineral density is particularly troublesome in staple foods, such as cereal grains tuber crops and root crops, which make up a large proportion of daily food intake in the developing world [28-33].

The amount of an element in a plant or animal must not be considered a criterion of its relative value. For some elements present in very small quantities are as essential to growth as those, which compose the greater portion of the plant or animal. The number of elements in a plant is determined by their presence in the soil and air. The amount or proportion depends on many factors: species, edge and root distribution of the plant, physical and chemical nature of the soil, proportion and distribution of the element, method of cultivation, and general climatic condition. In an animal, however, the most important single factor is the food, which the animal consumes [34-36].

1.4. Importance and classification of elements in food

Foods and beverages ingested by human represent a potentially proficient pathway of exposure to toxic and nutritionally important major, minor and trace elements. Many mineral elements occur in living tissues, foods and diets in such small amounts that they are frequently described as traces [36].

Vitamins and minerals play essential catalytic roles in metabolism and have other functional roles as well. Although needed in minute amounts, most vitamins are not synthesized in the human body in sufficient quantity, and therefore, together with essential minerals must be obtained from food or other exogenous sources.

Minerals are inorganic substances found in the body that function in conjunctions with enzymes, hormones, vitamins and other compounds. They play important roles in nerve transmission, muscle contraction, blood formation and metabolism of macronutrients and energy production. Some minerals can either block or enhance absorption of other nutrients, including other minerals and some vitamins. They are present in the skeletal system and other hard tissues and constitute approximately 4% of the body's weight [37-46].

The bulk of human body is composed of six major elements; oxygen, carbon, hydrogen, nitrogen, calcium and phosphorous and the six minor elements; sulfur, potassium, sodium, chlorine, magnesium, and silicon. If noble gases are excluded as unlikely to have a physiological function, seventy one elements of the periodic system remains and because of their low concentration in living mater, are termed the trace elements [37].

The concentration of major and minor elements in living tissues can be expressed in grams per kilograms. On the other hand, the concentration of trace elements in living tissues varies between 0.01 and 100 mg kg^{-1}. It may not be appropriate to classify them as essential or toxic elements. It is logically wrong to establish a category of "toxic" elements, because any element may be potentially toxic and this property is but a function of concentrations to which humans are exposed. Essentiality of the trace elements is established when a further reduction below the range of tolerable levels, better known as "range of safe and adequate intakes", results in a consistent and reproducible impairment of a physiological function [38, 39, 46].

1.5. Classification of trace elements

All major and minor elements are important; besides that, some of the trace elements, e.g. Cr, Fe, Co, Cu, Zn, Se, Mo and I are essential trace elements; and some of them; Mn, Si, Ni, B, V, and Sn are probably essential trace elements; and further some of them As, Cd, Pb, Al and Hg are considered potentially toxic, some possibly essential elements for animal and human life. Actually all essential elements may also be toxic in animals and humans if ingested at sufficiently high levels and for a long enough period [38-42, 46].

Essential trace elements are required by man in amounts ranging from 50 µg day^{-1} to 20 mg day^{-1}. The organism can neither grow nor complete its life cycle without the element in question. The element should have a direct influence on the organism and be involved in its metabolism. The effect of the essential elements cannot be wholly replaced by any other elements [45-47].

The bioavailability of essential elements depends on their chemical form, the composition of the diet and health situation of the individuals. Thus, establishment of the optimum daily requirements and determinations of actual daily intake of essential elements are important problems of trace elements in nutrition [36, 47]. The physiological role of K, Ca, Fe, Zn, Cu, Na, Pb, Mg, Co, Ni, Cd, Mn and Cr are briefly described below.

Potassium (K)

Potassium is the most abundant mineral found in the human body next to P and Ca. It is a major electrolyte of intracellular fluid. It is of great physiological importance, contributing to the transmission of nerve impulses, the control of skeletal muscle contractility, and the maintenance of normal blood pressure. It is obviously essential for plants and can not be entirely replaced by any other elements. Efficiency symptom includes irregular heart beat, loss of appetite and muscle cramps [49].

Calcium (Ca)

Calcium forms a vital part of bone and tooth structure, and is also important as a positive ion (Ca^{2+}) in blood clotting, muscle contraction, and nerve impulse transmission. It also participates in glycogen metabolism [37, 48]. Inadequate intake of calcium increases the risk of osteoporosis (bone loss with no apparent cause). Excess intake of calcium may cause kidney stones and reduces mineral absorption in general [49].

Magnesium (Mg)

Magnesium is essential to maintain both the acid-alkaline balance in the body and healthy functioning of nerves and muscles (including the heart), as well as to activate enzymes to metabolize blood sugars, proteins and carbohydrates [50]. A 2:1 ratio of calcium to magnesium is essential to maintain strong bones. Indications of a magnesium deficiency may be muscle twitches, nervousness, abnormal heart beat and disorientation. Excess intake of magnesium causes weakness in people with kidney failure [49].

Iron (Fe)

Iron carries oxygen to the cells and is necessary for the production of energy, the synthesis of collagen, and the functioning of the immune system. Iron deficiency is common only among children and pre-menopausal women. Great care must be taken not to take too much iron, as excess amounts are stored in the body's tissues and adversely affect the body's immune function, cell growth and heart health [51, 52]. Iron absorption can be blocked by calcium, magnesium, manganese, zinc, anti-acids and tetracycline (a common antibiotic) [50].

Deficiency of iron results in anemia which is recognized by its symptom such as low blood iron level, small and red blood cells and low blood hemoglobin values [48]. Iron toxicity usually results from a generic disorder called hemochromatosis. This disease causes over absorption and accumulation of iron, which can result in sever liver and heart damage [49].

Manganese (Mn)

Manganese is an essential element to both plants and animals. It is necessary for normal bone metabolism and important enzyme reactions. It also helps to maintain normal nerve, brain and thyroid function [50]. While a deficiency of this mineral is uncommon, it is often lost in processed foods [53, 54]. A deficiency of manganese may affect brain health, glucose tolerance, normal reproduction, and skeletal and cartilage formation. Grains and cereal products are the best food sources of manganese, while animal products are the poorest. Toxicity from manganese is uncommon [55]. Exposure to high level of Mn can cause both mental and emotional disturbance, along with increased slowness and clumsiness of the body movements. This disease is called manganism. Any brain injury due to the accumulation of Mn in the brain is permanent [56].

Copper (Cu)

The essential role of copper in maintaining normal health in both animals and humans has been recognized for many years. The average daily dietary requirement for copper has been reported by many scholars. Copper is required with iron for synthesis of hemoglobin. It works with many enzymes such as those involved in protein metabolism and hormone synthesis [48, 49, 56]. Deficiency of copper causes low white blood cell count and poor growth. Excess intake of copper can cause vomiting, nervous system disorder and Wilson's diseases [49].

Zinc (Zn)

Zinc is an essential element found in the tissue of animals and plants even at normal ambient concentrations. However, if plants and animals are exposed to large concentrations of bioavailable Zn, significant bioaccumulations can result, with possible toxic effects. [49].

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function. If zinc is removed from the catalytic site, activity is lost;

replacement of zinc restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these functions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc mal-absorption resulting in severe deficiency, have provided much insight into the functional outcomes of zinc deficiency. These include impairments of dermal, gastrointestinal, neurologic and immunologic systems [58].

Lead (Pb)

Lead serves no useful purpose in the human body, and its presence in the body can lead to toxic effects, regardless of exposure pathway. Lead toxicity can affect every organ system. On a molecular level, proposed mechanisms for toxicity involve fundamental biochemical processes. These include lead's ability to inhibit or mimic the actions of calcium (which can affect calcium-dependent or related processes) and to interact with proteins (including those with sulfhydryl, amine, phosphate, and carboxyl groups).

Acute high lead exposure can cause serious physiologic effects, including death or long-term damage to brain function and organ systems. Effects of lead exposure vary according to exposure timing and levels, and other factors, and some effects may be latent [59].

Cadmium (Cd)

Cadmium has no known nutritional value, and it is highly toxic to both plants and animals. The biochemical effects of Cd in humans include interference with enzymatic activity, the ability of interacting with nucleic acids and damaging kidney, hypertension and anosmia (absence of smell)

[60]. Cadmium is known to accumulate in the kidney. This kidney damage leads to calcium deficiency in the rest of the body, particularly in the skeleton [61].

Sodium (Na)

Sodium is an essential mineral or micronutrient which along with potassium helps to regulate the body's fluid balance. Sodium deficiency is not common, and according to some experts the average Western diet provides more than 5 times the recommended daily allowance of sodium. Excess sodium intake is linked with high blood pressure and heart disease. The current recommendation is to consume less than 2,400 milligrams (mg) of sodium a day. This is about 1 teaspoon of table salt per day. It includes all salt and sodium consumed, including sodium used in cooking and at the table [62].

Chromium (Cr)

Chromium exists in 3 main forms: metallic state, trivalent and hexavalent forms. While hexavalent chromium is recognised as an industrial toxin linked to lung cancer, trivalent chromium is acknowledged as an essential nutrient. The latter is known to improve insulin sensitivity and, therefore, to influence carbohydrate, fat and protein metabolism. Diabetes and coronary heart disease are associated with low chromium concentration in human tissue [63].

Nickel (Ni)

No sign of nickel deficiency has been described for humans. Nonetheless, the presence of nickel enzymes in lower forms of life and the response of experimental animals to low dietary nickel are persuasive pieces of circumstantial evidence that nickel is essential for humans. Of course, the potential importance of nickel in human nutrition is not limited to deficiency. Like other mineral elements, nickel ingested in high amounts can have adverse effects. However, because of excellent homeostatic regulation, life-threatening toxicity of nickel through oral intake is unlikely. Generally, greater than 250 µg/g of diet are required to produce signs of nickel toxicity (such as depressed growth and anemia) in animals; by weight extrapolation, this indicates that ingestion of over 250 mg of soluble nickel daily could produce toxic symptoms in humans. But the generality "nickel is relatively nontoxic" cannot be made for humans [64].

Cobalt (Co)

The only known animal requirement for cobalt is as a constituent of vitamin B_{12}, which has 4% cobalt in its chemical structure. This means that a cobalt deficiency is really a vitamin B_{12} deficiency. Cobalt deficiency symptoms include a loss of appetite, emaciation, weakness, anemia, and decreased production. Excessive amounts of cobalt produce cardiomyopathy with a high mortality risk. No RDA (Relative Dietary Allowance) or Estimated Safe and Adequate Daily Dietary Intake has been set for cobalt [64].

1.6. Analysis of metals in plant materials

Metals may be determined satisfactorily by a variety of methods, with the choice often depending on the precision and sensitivity required. Several spectrometry techniques have been used for macro and trace elements determination in plants or biological materials.

Atomic absorption spectrometry with flame (FAAS), hydride (HGAAS) or electrotermal atomization (ETAAS) [65, 66 70], graphite furnace atomic absorption (GFAA) [70], inductively coupled argon plasma optical emission spectrometry (ICP-OES) [67, 70], direct current argon plasma optical emission spectroscopy (DCP-OES) [66], and inductively coupled plasma mass spectrometry (ICP-MS) [40,69, 70] are most commonly used for the determination of metals in plant materials because of their inherent selectivity, sensitivity, precision and accuracy.

1.7. Sample decomposition

The various techniques for elemental determination do not all requires the same degree of sample matrix break down. The choice of a digestion technique should take into account the objective of the final determination and factors such as the matrix composition, the element contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [65, 66, 75].

The purposes of sample decomposition are [65]:

- Converting all the species in which a given element is present in such a way that it becomes present in one defined form.
- Eliminating interfering substances from the matrix.
- Obtaining the element in a homogeneous and easily accessible matrix.

The different decomposition methods could be classified in to three categories.

I. Wet digestion

The majority of wet digestion methods involve different combinations of five acids (HNO_3, H_2SO_4, $HClO_4$, HCl, and HF) and H_2O_2. The choice of an individual acid or combinations of acids depends upon the nature of the matrix to be composed. The procedure could take place either in open system or closed system (bomb decomposing with conventional or microwave heating) [40, 60,65, 71-72].

II. Dry ashing

Dry ashing is usually performed by placing the sample in an open inert vessel and destroying the combustible (organic) portion of the sample by thermal decomposition using a muffle furnace. After decomposition the residue is dissolved in acid and transferred to a volumetric flask prior to analysis. Typical ashing temperatures are 450 to 550 °C. Magnesium nitrate is commonly used as an ashing aid. Dry ashing is also conducted at 50-100°C under reduced pressure in an oxygen plasma discharge. Keeping its advantage and simplicity, it has some limitation. These are [40,65-76]:

- Losses due to retention to the ashing container.
- Losses due to volatilization.
- Contamination from the ashing container.
- Contamination from the muffle furnace.
- Physical loss of 'low density' ashes when the muffle door is opened (air currents).
- Difficulty in dissolving certain metal oxides.
- Formation of toxic gases in poorly ventilated area.

III. Fusion

Fusion is a powerful technique especially both for organic matrices and those with a high silica and alumina content. Since solid and aggressive fusion reagents are difficult to purify, fusion cannot be recommended as ultra trace analysis. A second disadvantage is that the method is carried out in contact with ambient air. Risks of volatilizations are large [73, 75].

1.8. Purpose and scope

Since enset (*Essete ventricosum*) products such as Bulla and Kocho are one of the main energy sources and serve as the staple and co-staple food for many peoples of Ethiopia, the knowledge of their mineral concentrations are of particular interest. However, information on the contents of major, minor and trace elements in enset products are scares in the literature.

Recently the mineral contents of unprocessed edible part of enset (fresh sample taken from the plant) was determined and reported by Debebe [27]. The purpose of this study is to determine the major, minor and trace elements in commercially available enset food products (Bulla and Kocho) and to compare the result with unprocessed edible part of enset. Furthermore, the findings of this study will provide adequate information on the distribution of major, minor and trace metals and it will ensure the dietary safety of Kocho and Bulla consumed in the region in terms of essential and/or non essential elements.

1.9. Specific objectives

The specific objectives of this study are:

- To develop suitable digestion method for Bulla and Kocho samples.
- To determine the major, minor and trace elements (K, Ca, Fe, Zn, Mn, Pb, Mg, Co, Ni, Cd, Cu, Na and Cr) content of Bulla and Kocho.

- To compare the levels of minerals in Bulla and Kocho with that of unprocessed edible part of enset.
- To compare the levels of minerals in Bulla and Kocho with that of other similar food products.

2. Experimental

2.1 Equipment and reagents

2.1.1. Equipments

A drying oven (DIGITHEAT, J.P.SELECTA,S.a, Spain) was used to dry kocho and bulla samples. A blending device (Moulinex, France) was used to grind and homogenize the dried kocho sample. A digital analytical balance (Mettler Toledo, Model AG204, Switzerland) with ± 0.0001 g precision was used to weigh kocho and bulla samples. A 250 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered kocho and bulla samples. A refrigerator (Hitachi, Tokyo, Japan) was used to keep the digested sample till analysis. **BUCK SCIENTIFIC MODEL 210 VGP** (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium ark back ground correctors was used for analysis of the analyte metals (Na, Mg, Mn, Ni, Pb, K, Ca, Fe, Zn, Cu, Co, Cr, Cd) using air-C_2H_2 flame. Na and K were determined in the emission mode of the spectrometer.

2.1.2. Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. (69-72 %) HNO_3 (Spectrosol, BDH, England) and 70% $HClO_4$ (Aldrich, A.C.S. Reagent

Germany) were used for digestion of bulla and kocho samples. Lanthanum nitrate hydrate (98%, Aldrich, Muwaukee, USA) was used to avoid refractory interference (for realizing calcium and magnesium from their phosphates). Stock standard solutions containing 1000 mg/L, in 2% HNO_3, of the metals Na, Mg, Mn, Ni, Pb, K, Ca, Fe, Zn, Cu, Co, Cr, Cd (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for preparation of calibration standards and in the spiking experiments. Deionized water was used throughout the experiment for sample preparation, dilution and rinsing apparatus prior to analysis.

2.2. Procedures

2.2.1. Cleaning apparatus

Apparatus such as volumetric flasks, measuring cylinder and digestion flasks were washed with detergents and tap water, rinsed with deionised water, soaked in 50 % nitric acid for two days, rinsed with deionised water five times, dried in oven and kept in dust free place until analysis begins.

2.2.2. Description of sampling sites

For the collection of Kocho and Bulla, Woliso (Oromiya region) and Wolkite (SNNPRG) were chosen. The reason for selection of these places was based on two things. First, the mineral contents of unprocessed edible part of enset (*Ensete ventricosum*) from Woliso and Wolkite was determined and reported by Debebe [27]. Therefore, in order to relate and compare the mineral contents of unprocessed edible part with the processed one, these places were selected. Secondly, commercially available bulla in urban markets particularly Addis Ababa (Ethiopia) super markets may not be pure, meaning that it may be mixed with wheat flour. So, in order to have precise and accurate information, the samples were collected from the source (production area) (Woliso and Wolkite) where the kocho and bulla samples are sold without mixing with wheat flour.

Woliso and Wolkite are found in the South Western part of Ethiopia. They are found under comparable climatic condition. Their geographical location and distance from Addis Ababa (capital city of Ethiopia) are given in Table 1.

Table 1 Geographical location and distance from Addis Ababa of sampling sites.

Site	Latitude	Longitude	Distance (km)
Wolliso	8^0 31'N	37^0 58'E	120
Wolkite	8^0 15'N	37^0 47'E	155

2.2.3 Collection and preparation of Kocho and Bulla samples

Kocho sampling

The processed Kocho looks like semisolid dough containing a lot of fiber.
The fermented material after extraction from the pit was squeezed, drained and chopped finally to make a starchy paste. The paste can be cooked for human consumption it is either rolled and baked to make bread or mixed with other products to make porridge. Commercially available Kocho is both

the squeezed and the semisolid one. But the purpose of this study is to analyze the mineral contents of readily consumable commercially available Kocho (squeezed out Kocho or starchy paste). Squeezed out and ready to be cooked Kocho samples were collected from Woliso and Welkite supermarkets. To do this, ten supermarkets from each sampling sites were randomly selected. Half kilogram (500 g) of kocho from each supermarket was collected. From each 500 g, 200 g was taken oven dried at 70 ^{o}C for 64 h and grinded in a blender to homogenize and reduce the size of some caked Kocho particles during drying. Finally two bulk samples one from each stated areas were prepared for analysis. Six with 0.5 g aliquot (three from each bulk sample) were taken for final digestion.

Bulla sampling

Like Kocho, Bulla is available in different supermarkets but it is in the form of flour, meaning that it is the dried one. The dried one is the purest form of it. The dried one looks like a flour and packed with different packing materials and ready for sell and directly processed for meal like any other flour types. Sampling was done similar to kocho except that the bulk bulla sample was oven dried at 60 ^{o}C for 24 h and no grinding step was used. Because the bulla sample was dried and it was in the form of flour when collected from the supermarkets but to be sure for complete removal of moisture it was allowed to dry in the oven.

2.2.4. Digestion of Kocho and Bulla Samples

Debebe [27] has optimized different conditions by varying digestion time, reagent volume, volume ratio of reagents and digestion temperature for unprocessed edible part of esnet (*Ensete ventricosum*). Finally he selected the procedure involving 2 mL of HNO_3 (69-70%) and 2 mL of $HClO_4$ (70%) for digestion time of 2 and ½ hours. The efficiency of his procedure and other parameters for the processed plant materials (Kocho and Bulla) have been checked. However a procedure which fulfilled stated criteria was selected.

Applying the optimized procedure, 0.5 g of dried and homogenized Kocho and Bulla samples were transferred into a 250 mL round bottomed flask. To this was added 4 mL of a mixture of HNO_3 (69- 72%) and $HClO_4$ (70%) with a volume ratio of 1:1 and the mixture was digested on a micro Kjeldahl digestion apparatus by setting the temperature first to dial at 4 (120 ^oC) for 30 min and then increased to dial 6 (180 ^oC) for the next 30 min and then increased to 9 (approximately 270 ^oC for the remaining 1 h then after the digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 10 min after removing the condenser. To the cooled solution 15 mL of deionized water was added to dissolve the precipitate formed on cooling and to minimize dissolution of filter paper by the digest residue while filtering with Whatman®, (110 mm, diam), filter paper. The round bottom flask was rinsed subsequently with 5 mL deionized water until the total volume reached around 45 mL. To this final solution, 1% lanthanum nitrate solution was added and the solution was filled to the mark (50 mL) with deionized water. Triplicate digestions were carried out for each bulk sample. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS. Ten blank solutions were prepared following the same digestion procedure as the sample.

2.2.5. Determinations of major minor and trace metals

Secondary standard solutions containing 10 mg/L were prepared from the atomic absorption spectroscopy standard stock solutions that contained

1000 mg/L. These secondary standards were diluted with deionized water to obtain four working standards for each metal of interest. Na, K, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu were analyzed with FAAS (BUCK SCIENTIFIC MODEL 210GP) equipped with deuterium arc background corrector and standard air-acetylene flame system using external calibration curve after the parameters (burner and lamp alignment, slit width and wavelength adjustment) were optimized for maximum signal intensity of the instrument. Three replicate determinations were carried out on each sample. Hallow cathode lamp for each metal operated at the manufacturer's recommended conditions were used at its respective primary source line. The acetylene and air flow rates were managed to ensure suitable flame conditions. The first two elements (Na and K) were determined by emission mode while the remaining eleven were determined by absorption/concentration mode. The reason for using emission mode is to avoid ionization interference. The same analytical procedure was employed for the determination of elements in ten-digested blank solutions. The operating conditions for FAAS employed for each analyte are given in Table 2.

Table 2 Instrumental operating conditions for determination of metals in Kocho and Bulla samples using flame atomic absorption spectrophotometer.

Element	Wavelength (nm)	Detection limit (mg/L)	Slit width (nm)	Lampcurrent (mA)	Energy
K	766.5	0.010	0.7	2.0	-
Na	589.0	0.002	0.2	2.0	-
Ca	422.7	0.010	0.7	2.0	3.012
Mg	285.2	0.001	0.7	1.0	4.091
Cu	324.7	0.020	0.7	1.5	3.970
Zn	213.9	0.005	0.7	2.0	3.238
Mn	279.5	0.001	0.7	3.0	3.961
Ni	341.5	0.040	0.2	3.0	3.059
Fe	248.3	0.030	0.2	7.0	3.003
Co	240.7	0.050	0.2	4.5	2.900
Cr	357.9	0.050	0.7	2.0	3.750
Cd	228.9	0.005	0.7	2.0	3.207
Pb	283.2	0.100	0.7	2.0	3.62

2.2.6. Recovery test

The efficiency of the optimized procedure was checked by adding known concentration of each metal in 0.5 g sample. The procedure was as follow:
50 μg of 1000 mg/L Ca, K, and Na were spiked at once in to 0.5 g of Bulla sample and the remaining metals (10 μg of Mg and Zn; 0.5 μg of Cu; 15 μg of Fe and 0.25 μg of Mn were spiked at once in to another round bottomed flask containing 0.5 g of bulla. similarly 0.5 μg of Co and Cr were spiked at

one in a flask containing 0.5 g of Bulla sample. For spiking Kocho sample, 250 μg of K, 0.4 μg of Cr and 1 μg of Co and 0.5μg of Cu, were spiked from 1000 mg/L of stock solution of each metals in to a flask containing 0.5 g of Kocho sample and the remaining metals (50 μg of Na, Ca and Mg, 10 μg of Zn, 2.5 μg of Mn, and 25 μg of Fe, were spiked at once in 0.5 g of Kocho and 0.5 μg of Ni was spiked in an other flask containing the same gram of Kocho sample and the same digestion process was followed. Each sample was determined for their respective spiked metals by atomic absorption spectrophotometer. Each recovery test for both samples was performed in triplicates.

2.2.7. Method detection limit

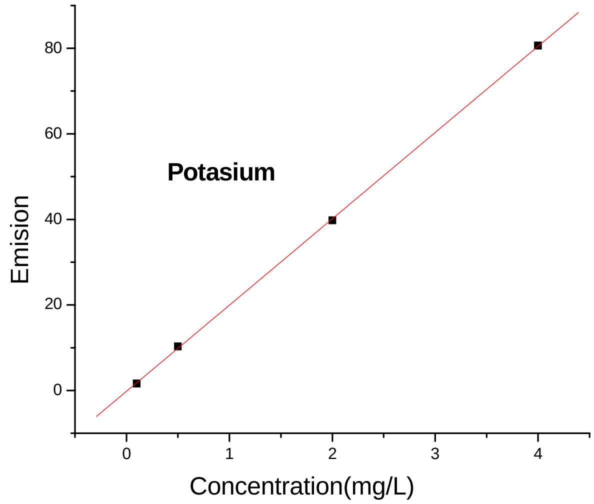
Ten blank samples were digested following the same procedure as the samples and each of the samples were determined for the elements of interest (Na, K, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu) by atomic absorption spectrophotometer. The standard deviation for each element was calculated from the ten blank measurements to determine method detection limit.

Chapter Three

3. Results and Discussion

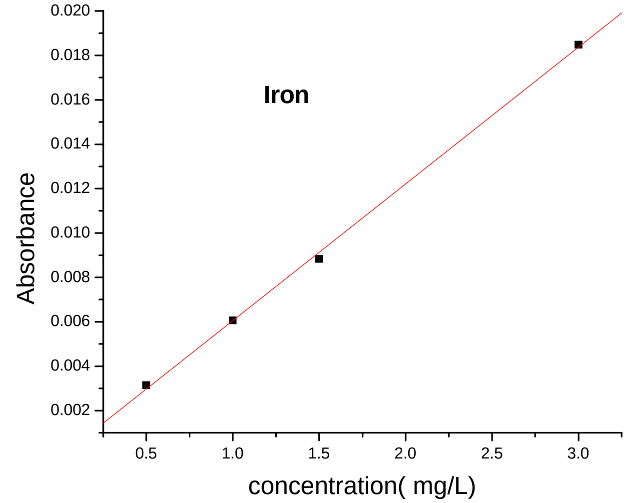
3.1. Instrument Calibration

The qualities of results obtained for major, minor and trace metals analysis using AAS are seriously affected by the calibration and standard solution preparations procedures. The instrument was calibrated using four series of working standards. The working standard solutions of each metal were prepared freshly by diluting the intermediated standard solutions mentioned under section 2.2.5. Concentrations of the intermediate standards, working standards and value of correlation coefficient of the calibration graph for each of the metals are listed in Table 3. The calibration graph of each of metals of interest is shown in Figure 3.



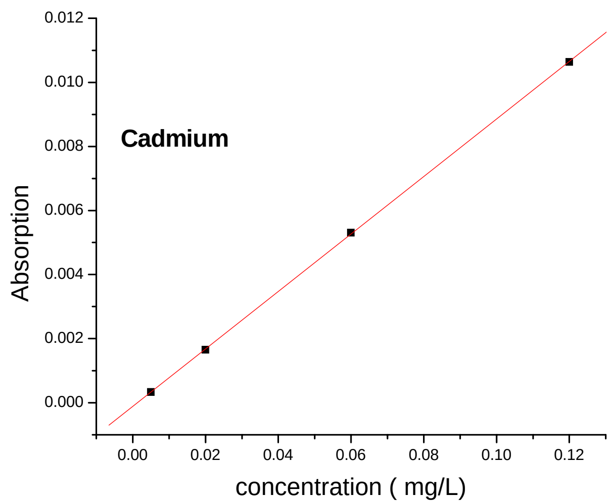
$Y = -0.2189 + 20.1761X$
$R = 0.9999$

Figure 3a. Calibration graph of K
Standard solution.



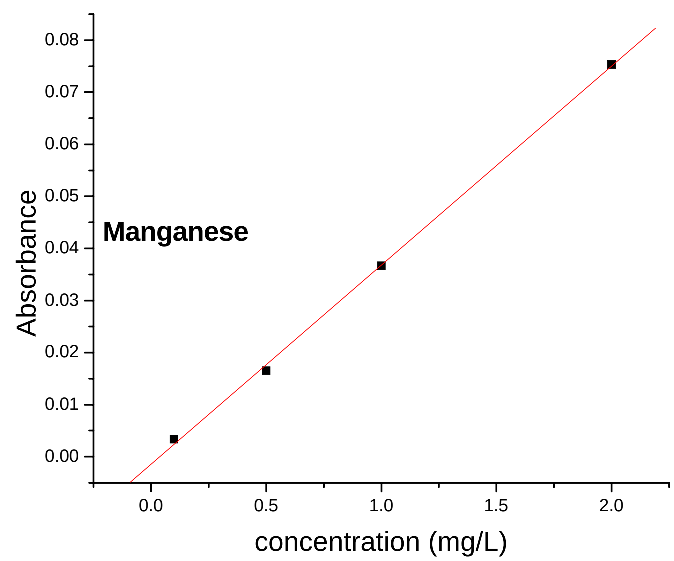
$Y= -1.06286\ E^{-4}+0.00616X$
$R= 0.9995$

Figure 3b. Calibration graph of Fe
Standard solution



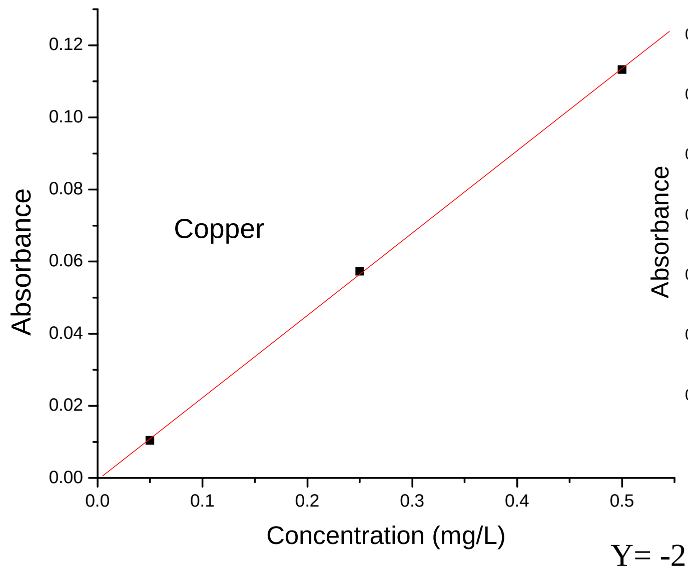
$Y= -1.1688E^{-4} + 0.09974X$
$R = 0.9999$

Figure 3c. Calibration graph of Cd
Standard solution



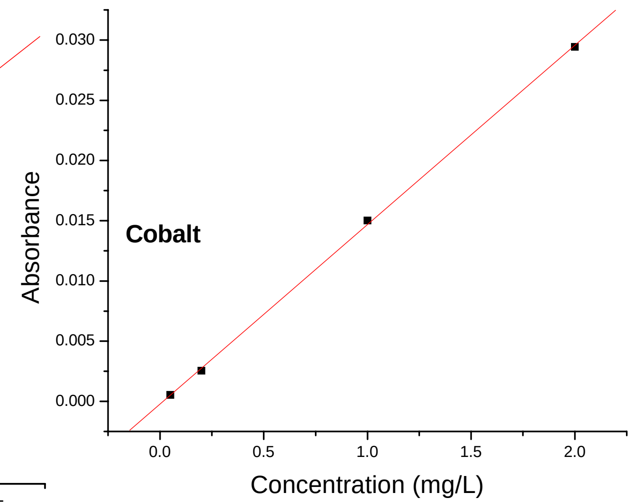
$Y= -0.00144 + 0.03823X$
$R= 0.9996$

Figure 3d. Calibration graph of Mn
Standard solution



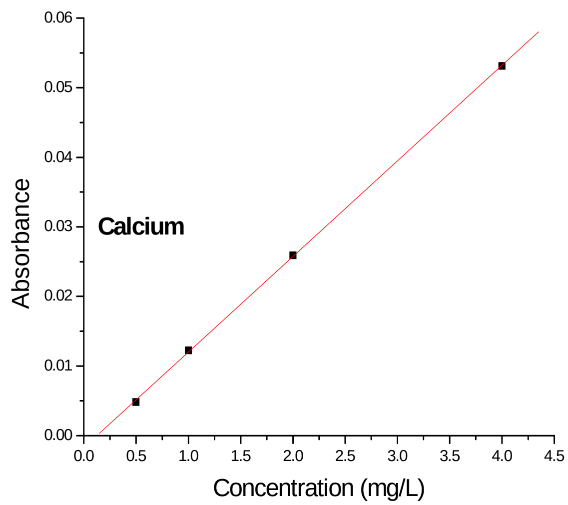
Y= -5.4941 E^{-4} + 0.22826X
R= 0.9999

Figure 3e. Calibration graph of Cu Standard solution

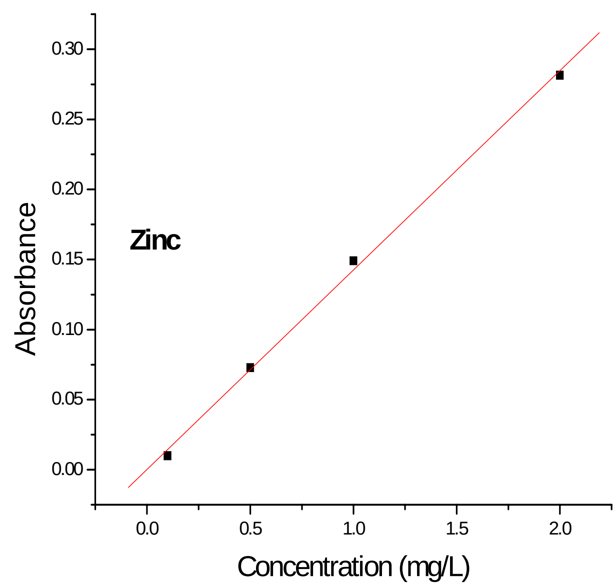


Y= -2.2371 E^{-4} + 0.0149X
R = 0.9998

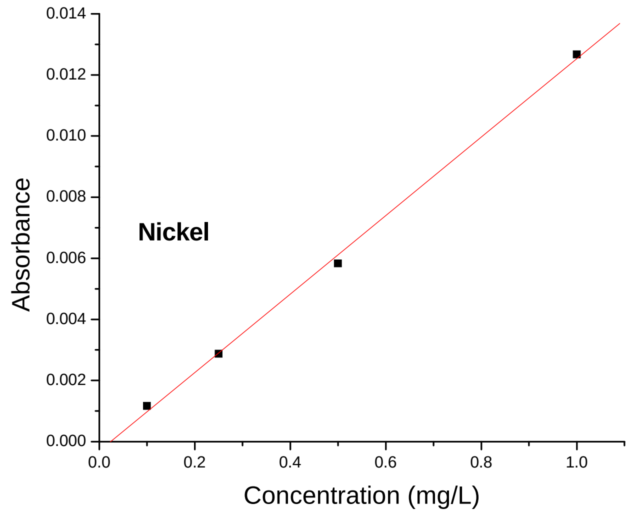
Figure 3f. Calibration graph of Co Standard solution

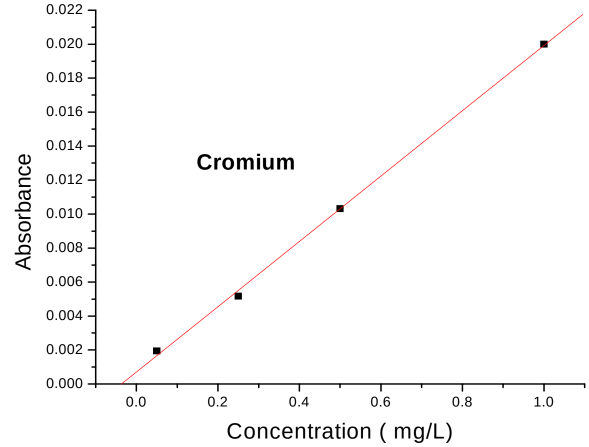


Y= -0.00175 + 0.01374X
R= 0.9999
Figure 3g. Calibration graph of Ca Standard solution



Y= 2.23656 E^{-4} + 0.14229X
R= 0.9991
Figure 3h. Calibration graph of Zn Standard solution

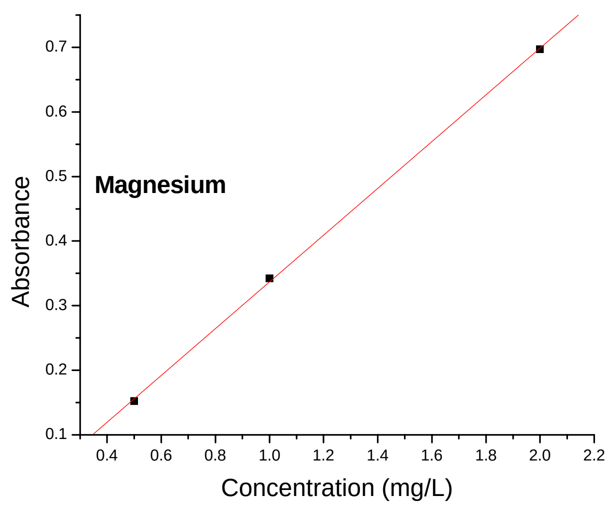




Y= -3.0479 E^{-4} + 0.0284
R= 0.9991

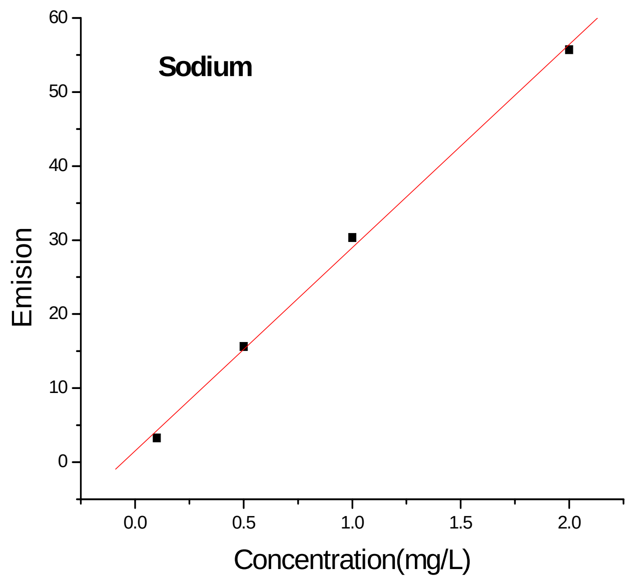
Figure 3i. Calibration graph of Ni Standard solution

Y= 7.1038 E^{-4} + 0.01921
R= 0.9999
Figure 3j. Calibration graph of Cr Standard solution



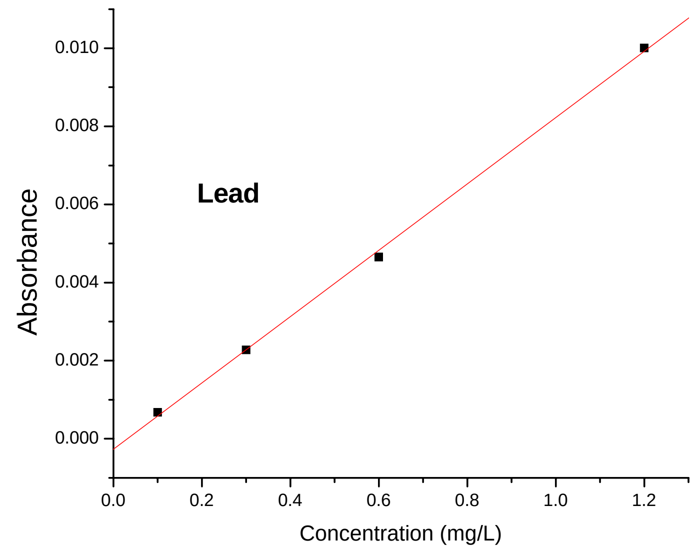
Y= -0.02514 + 0.36201X
R= 0.9998

Figure 3k. Calibration graph of Mg Standard solution



Y= 1.52515 + 27.4447X
R= 9988

Figure 3l. Calibration graph of Na
Standard solution



$Y = -2.6822E^{-4} + 0.00849X$
R= 0.9995

Figure 3m. Calibration graph of Pb
Standard solution

Figure 3 (a-m). Calibration graph of metals' standard solution

Table 3 Working standards and correlation coefficients of the calibration curves for determinations of metals using flame atomic absorption spectrophotometer

No	Metal	Concentration of intermediate standard (mg/L)	Concentration of standards, in mg/L	Correlation coefficient of calibration curves
1	K	10	0.1, 0.5, 2, 4	0.9999
2	Na	10	0.1, 0.5, 1, 2	0.9998
3	Ca	10	0.5, 1, 2,4	0.9999
4	Mg	10	0.5, 1, 2, 3	0.9998
5	Cu	10	0.05, 0.1, 0.2, 1	0.9999
6	Zn	10	0.1, 0.5, 1,2	0.9991
7	Mn	10	0.1, 0.5, 1, 2	0.9997
8	Ni	10	0.05, 0.25, 0.5	0.9989
9	Fe	10	0.5, 1, 1.5,3	0.9995
10	Co	10	0.05, 0.2, 1,2	0.9998
11	Cr	10	0. 1, 0.5, 1, 2	0.9993
12	Cd	1	0.005, 0.02, 0.06, 0.12	0.9999
13	Pb	10	0.1, 0.3, 0.6, 1.2	0.9995

3.2. Moisture Content of Kocho and Bulla.

As mentioned in the previous sections Kocho contains a lot of water (moisture) before and after it has been squeezed out. Similarly Bulla also contains appreciable amount of water in it. However commercially available Bulla in supermarkets is the dried and packed one like other flour types. But the wet and the flour type (the dried one) can be cooked with similar procedure. Agren and Gibbson[24], Abebe *et al* [17] and Wu Leung *et al* [80] determined the moisture content of Kocho and Bulla. According to their report, the range of the moisture content of squeezed out Kocho and Bulla respectively was 36-62 and 44-57 respectively. Since the moisture content of Kocho and Bulla varies with temperature and other factors, it is difficult to express the mineral contents in them as a wet weight basis. Therefore to have constant result, Kocho sample was oven dried prior to digestion so as to express the result in terms of dry mass basis. Whereas Bulla sample was initially dried when bought from the supermarkets but to be quit sure it was allowed to dry in the oven for 24h.

3.3. Optimization of Digestion of Kocho and Bulla Samples

One of the basic requirements for sample preparation for analysis is to get an optimum condition for digestion. The optimum condition is the one which leads:

- Minimum reagent volume consumption
- Minimum digestion time
- Minimum residue (clear solution)
- Ease of simplicity.

Debebe [27] has optimized different conditions by varying digestion time, reagent volume, volume ratio of reagents and digestion temperature for unprocessed edible part of esnet (*Ensete ventricosum*). Finally he selected the procedure involving 2 mL of HNO_3 (69-70%) and 2 mL of $HCLO_4$ (70%) for digestion time of 2 and ½ hours. Having this as a base, his procedure was checked whether it works for the processed enset sample or not. All his procedures were evaluated and it was effective for Kocho and Bulla. However, slight modification was made on digestion time and temperature. In his case, he used 2 and ½ hours for a temperature adjusted at dial 8 (approximately 240 ^{o}C) for the whole duration. But for the present experiment, a digestion time of 2 hours with variable temperature gave clear solution and selected for both types of samples.

Therefore, based on the listed criteria on his paper and presently confirmed one, the optimum digestion procedure chosen was the one that fulfilled the stated criteria for complete digestion of 0.5 g of the dry sample, with 2 mL HNO_3 (69-72%) and 2 mL $HClO_4$ (70%) for a total of 2 hrs.

3.4. Evaluation of Analytical Figures of Merit

3.4.1. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at two levels: repeatability and reproducibility. For these guidelines, a simple assessment of repeatability will be acceptable. The precision of an analytical procedure is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements.

In this study the precision of the results were evaluated by the pooled standard deviation, and relative standard deviation of the results of three samples (n = 3) and triplicate readings for each sample meaning that a total of 9 measurements for a given bulk sample. These parameters are useful in estimating and reporting the probable size of indeterminate error. The results of the present analysis are reported with corresponding pooled standard deviation of nine measurements for a bulk sample and triplicate reading per sample and relative standard deviation. Table 7and 8 shows % RSD of each metal in each food types.

3.4.2. Method Detection Limit

Method detection limit is defined as the minimum concentration of analyte that can be measured and reported with 99% confidence that the analyte concentration is greater than zero [73]. In other word, method detection limit is the lowest analyte concentration that produces a response detectable above the noise level of the system, typically three times the noise level but not necessarily quantitated as an exact value. For the present study, replicate analyses for ten blank samples for some of the elements and six blank samples for the others were performed and the pooled standard deviation of the ten blank reagents was calculated. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. As can be seen from Table 4, the method detection limit of each element is above the instrument detection limit.

Table 4 Method detection limit for Kocho and Bulla samples (n =10 for all metals except for Pb, Cd, Cr, Ni, and Co, for which, n =6).

Metal	MDL (mg/g)	Instrument detection limit mg/L
K	0.006	0.01
Na	0.001	0.002
Ca	0.006	0.01
Mg	0.003	0.001
Cu	0.002	0.02
Zn	0.003	0.005
Mn	0.001	0.01
Ni	0.004	0.04
Fe	0.005	0.03
Co	0.005	0.05
Cr	0.005	0.05
Cd	0.002	0.005
Pb	0.013	0.1

MDL = method detection limit.

3.4.3. Evaluation of Analytical Method

The ability to provide timely, accurate and reliable data is central to the role of analytical chemistry. Method validation is the process of providing that analytical method is acceptable for its intended purpose. Therefore analysts are increasingly impelled to validate analytical procedures and to estimate uncertainty associated to the results. Since there is no certified reference material (Kocho and Bulla) in our laboratory, the validity of the optimized digestion procedure for Kocho and Bulla were checked by carrying out with a lower level of traceability, such as spiked samples. As shown in Table 5 and 6, the percentage recovery for Kocho and Bulla samples are between 90 to 110% (100 ± 10), which are within the acceptable range for all metals except for Mg, K, and Cr in Kocho sample, for which a recovery of 89%, 89% and 85%, respectively was obtained. Similarly, for Cr and Co in Bulla a recovery obtained was 85% and 87%, respectively. The lower recovery for above elements may be attributed to the matrix analyte interaction may be high and that is why their recover value decreased.

Table 5 Recovery test for Bulla samples.

Metal	[a] Conc. in sample (μg/g)	Amount added (μg/g)	[b] Conc. in spiked sample (μg/g)	[c] % Recovery
K	875	100	966 ± 5.1	91 ± 5.1
Na	402	100	494 ±2	91.3±6
Ca	446	100	552.3 ± 0.6	106.3 ± 0.6
Mg	89.5	20	118.3 ± 0.2	94.2 ± 1
Cu	3.53	1.0	4.49 ± 0.09	96.7 ± 8.7
Zn	44.3	20	62.75 ± 1.74	93.7± 9.1
Mn	1.0	0.5	1.48 ± 0.05	96 ± 11
Cr	5.38	1.0	6.21± 0.19	85 ± 10
Fe	59.82	30	90.9 ± 2.5	110 ± 8
Co	5.01	1.0	5.89 ± 0.11	87±5

[a] average value of 9 measurements (μg/g).

[b] values are mean ± SD of triplicate readings of triplicate analyses.

[c] values are mean ± SD of triplicate readings of triplicate analyses.

Table 6. Recovery test for Kocho samples.

Metal	[a] Conc. in sample (μg/g)	Amount added (μg/g)	[b] Conc. in spiked sample	[c] % Recovery
K	4380	500	4823 ± 30	89 ± 6.1
Na	462.	100	552.9 ± 4	90.3±5.2
Ca	583.9	100	676.2 ± 2	92.3 ± 2
Mg	290.4	100	379.4 ± 4	89 ± 4
Cu	3.77	1.0	4.7± 0.2	93.7 ± 10
Zn	32.08	20	50.42 ± 0.27	91.77± 1.38
Mn	8.58	5.0	13.38 ± 0.4	96 ± 7.2
Ni	5.61	1.0	6.49 ± 0.29	89 ± 6.2
Fe	134.92	50	183± 3	96.8 ± 5
Cr	6.42	0.8	7.1 ± 0.3	85±10
Co	6.1	2.0	7.89 ± 0.04	90.2±5.7

[a] Average value of 9 measurements (μg/g).

[b] Values are mean ± SD of triplicate readings of triplicate analyses.

[c] Values are mean ± SD of triplicate percentage recovery values of triplicate analyses.

3.4.4. Determination of Major Minor and Trace Metals

The concentration of thirteen elements (major, minor and trace) (K, Ca, Fe, Zn, Mn, Pb, Mg, Co, Ni, Cd, Cu, Na and Cr) in the digested and diluted solutions of Kocho and Bulla were identified with flame AAS. Among the identified elements except Cadmium (Cd) and lead (Pb) which are below the method detection limit, all have been identified and shown in Table 7 and 8 with their respective % RSD. There is a wide range of the mineral content of the two starchy fermented foods prepared from enset. The most abundant metal among the macro element is K, followed by Ca, and Mg where as Fe and Zn contents of Kocho and Bulla are the predominant among the tested minor and trace nutrient heavy metals. The remaining metals vary from place to place and among food types. The content of Na, major nutrient for human but non-essential alkali metal in plant nutrition, was found to be higher than all heavy metals.

Table 7 Mean concentration (X ± SD, n = 9, μg/g dry weight) and relative standard deviation (% RSD) of major, minor and trace elements in Kocho and Bulla samples from Woliso.

Metal	Woliso bulla	% RSD	Woliso kocho	% RSD
K	875 ± 5	0.60	4380 ± 20	0.46
Na	402 ± 6	1.5	462 ± 3	0.65
Ca	446 ± 3	0.67	584 ± 6	1.02
Mg	89.5 ± 1.6	1.8	290 ± 14	4.83
Cu	3.53 ± 0.3	8.5	4.3 ± 0.36	8.37
Zn	16.3 ± 1.1	6.7	27.08 ± 0.9	3.3
Mn	1.0 ± 0.11	11	8.58 ± 0.35	4.1
Co	5.01 ± 0.0.3	5.98	6.1 ± 0.25	4.09
Fe	59.8 ± 2.1	3.6	135 ± 9	6.4
Cr	ND[a]	-	6.42 ± 0.34	5.3
Ni	ND[a]		ND[a]	-
Cd	ND[a]	-	ND[a]	-
Pb	ND[a]	-	ND[a]	-

[a] Concentration of the tested heavy metal below the method detection limit.

Table 8 Mean concentration (X ± SD, n = 9, μg/g dry weight) and relative standard deviation (% RSD) of major, minor and trace elements in Kocho and Bulla samples from Welkitie.

Metal	Welkite bulla	% RSD	Welkite kocho	% RSD
K	708 ± 5	1	2753 ± 22	1
Na	442 ± 5	1	688 ± 5	1
Ca	385 ± 8	4	498 ± 11	2
Mg	58.4 ±1.2	2.1	180 ± 11	8
Cu	2.01 ± 0.15	7.46	3.4 ± 0.26	7.6
Zn	22 ± 0.7	3.1	31 ± 0.8	2.5
Mn	4.98 ± 0.25	5.0	10.13 ± 0.43	4.3
Co	5.89 ± 0.67	11.4	5.5 ± 0.08	2.2
Fe	36.5 ± 1.33	3.6	92.5 ± 4.1	4.4
Cr	5.38 ± 0.28	5.2	5.96 ± 0.3	5.03
Ni	ND[a]	-	5.61 ± 0.38	6.7
Cd	ND[a]	-	ND[a]	-
Pb	ND[a]	-	ND[a]	-

[a] Concentration of the tested heavy metal below the method detection limit.

3.4.6. Distribution Pattern of Metals in Different Processed Enset Foods

Plants accumulate minerals essential for their growth from the environment and can also accumulate metals such as Cd, Co and Ag, Na, which have no known direct benefit to the plant [74]. Plants have developed several biochemical processes for the mobilization and uptake of minerals. To chelate and solubilize soil minerals, the roots secrete metal-chelating molecules such as phytosiderophores. Metal-chelating proteins such as metallothioneins also function in plants to bind metals and enhance transport and metabolism. This biochemical complexation maintains the metal in a soluble form that is available for metabolism, and may explain the relatively high bio-availability of minerals found in plants [74, 76]. Enset plant is one of those plants which have long root. As reported by Debebe 27], enset plant is known to accumulate essential and non-essential plant nutrients and hence its processed foods.

The corm and the false stem of enset after they have been grated or decorticated and mixed up, the watery part are allowed to squeezed out to get Bulla and the solid material gives kocho after fermentation. Then these enset products allowed fermenting for a set period of time, which depends upon the weather condition (i.e. the temperature and amount of fermented kocho added as a yeast), harvesting season meaning that age of the plant and variety or clone of the species [8,10, 18].

Therefore the major, minor and trace metals in Kocho and Bulla and their quality will be affected by different factors. Physical and chemical properties of the soil, application of natural (manure) and artificial fertilizers, increasing industrialization and associated pollution of the biosphere, storage and processing of Kocho and Bulla, age of harvested (processed) enset plant, climatic condition of the region and other factors are the main contributors for the mineral contents of Kocho and Bulla.

3.4.6.1. Concentration of Metals in Kocho

As it can be seen from Table 7 and 8 and Figure 4 and 5, there is a variation in concentration of macro and micro nutrients within the processed food types and there is slight variation in some metals along with geographical location. Except for Cd , Ni and Pb, which are below detection limit in all food stuffs, and Cr in Woliso Bulla, the selected elements are successfully determined except for nickel and Mn whose precision is low (% RSD greater than ten) (Table 7, 8). The pattern of concentration of elements in Kocho collected from Woliso was decreased as K > Ca > Na > Mg > Fe > Zn > Mn > Cr > Co > Cu. Like wise the pattern of concentration of elements in Kocho collected from Welkite was decreased as K > Na > Ca > Mg > Fe > Zn > Mn > Cr > Ni > Co > Cu.

As can be seen from the trend, the table and the figure mentioned above Kocho can be good source of major, minor and trace metals that are essential to human.

Kocho contains higher amount of K, followed by Ca, Na and Mg. The higher levels of K and Mg in the kocho according to Mirschner [78] was due to the fact that nutrient elements such as N, P, K, S, and Mg are highly mobile in the plant tissue and trans-located from old plant tissue to new plant tissue.

Generally, since the soils, which have been used for cultivating the plant, are highly fertilized with manure and organic residues, they were high in available potassium, calcium and magnesium. Hence, the plant has high amount of these metals [10].

From observed result, Kocho is known to contain higher Na concentration next to K and Ca. As Grusak [35] reported, Na and Cr are required by humans, but not by plants. Fortunately for humans, however, plants can acquire these elements through non-specific influx processes using existing transporters localized to their roots. In fact, a wide range of plant's non-essential elements (both benign and detrimental) has been measured in plant tissues, with concentrations sometimes reaching dramatic levels if soil availability is high. Therefore enset plant is also known to accumulate these nutrients extensively and that is why Na concentration is relatively higher next to K and Ca in both Kocho and Bulla.

The concentration of Fe and Zn were higher than the entire trace metals in the samples from both sites where as Cd, Ni and Pb were not detected in the sample. Of course Cd and Pb have no nutritional value for our body; there low concentration is appreciated.

It has been reported that Fe, Cu, and Zn, are the main elements that plant could accumulate and pass up the food chain. Therefore, the detection of Cu and the high concentration of Zn from trace metals next to iron in Kocho may be because of the fact that these ions are readily transferred from the soil to plants, and accumulate in the root and tuber of the false stem of enset plant and hence in Kocho.

Since the soil types of enset growing areas of Ethiopia are moderately acidic to slightly basic with the pH ranges from 5.6 to 7.3 , the plant expected to have a better accumulation of micronutrients like iron and zinc [10,17,82,].

As mentioned before the nutritional value of Kocho has not yet been analyzed except for few metals. The mineral content of Ca, Zn and Fe were analyzed and reported by different scholars (Table 9). Presently analyzed concentrations of Ca and Fe are almost comparable with the one reported in the literature.

Table 9 Data from other sources (literature).

Food type	Elements (μg/g)			
	Zn	Fe	Ca	Ref
Bulla	0.7-0.9	36-65	400-470	17
	-	58	910	24
		11-77	440-650	80
Kocho	3.2-7.2	36-101	1400-2260	17
	-	70	600	24
	6	37	320	77
	-	53	1200	80

As can be seen from Table 9, the reported concentration range (in μg/g) of Ca, Fe and Zn respectively were 320-2260, 36-110 and 0.7-6. Where as the concentration range of these metals in presently studied Kocho were found to be 493-583.9, 92-134.9 and 27.8-31 respectively of Ca, Fe and Zn. Except for Zn the concentration of the other two metals lie within the range. The variation may be attributed from different reason. The previous results were conducted on Kocho from Sidama (southern part of Ethiopia) and Areka, which is found in Wolayta zone (southern part of Ethiopia). As mentioned before the mineral concentration of Kocho is affected by different reasons, of these, age of harvested enset plant, precaution taken during processing, physical and chemical nature of the soil and its mineral nutrients, ...etc. therefore either of the reasons may lead such variations. Secondly some of the authors did not mentioned their method of analysis and sample decomposition technique and others used dry ashing technique as sample decomposition method, which is known to have a problem in analyte loss. Therefore such discrepancy may lead variation in Zn concentration.

Looking at the tables and figures mentioned above, Kocho food contains appropriate amount of trace elements in it. And toxic metals are below their maximum recommended range.

Except for few metals, the trends for metal accumulation in Kocho from the two stated areas were almost the same and for some metals their concentrations were almost comparable. This is because these areas are located within the same geographical location. As a result of this, they will have comparable climatic conditions.

Except for few metals, the concentration of analyzed minerals in Kocho from Woliso is higher than Kocho from Welikite. This result is also confirmed by the mineral content of unprocessed edible part of enset plant analyzed and reported by Debebe [27]. The detail comparison is given in the next section. From this we can deduce the possible reason for the higher concentration of metals in Kocho sampled from Woliso. As mentioned before there are different factors affecting the quality and hence mineral contents of Kocho. Of these, physicochemical property of the soil, age of harvested enset and variety or clone of enset processed may be some of the possible reasons. As information gathered from the farmers, farmers living around Woliso area have more cattles and apply manure (cow dung) to the enset plant and that is why Kocho from Woliso and its surrounding is more whitish than Kocho from Welkite and its surrounding. It is believed that Kocho and Bulla from fully matured enset contains less fiber, more flour and white in color. Therefore if the mineral content of the soil is rich enough, the plant will readily absorb it and get mature within a few years.

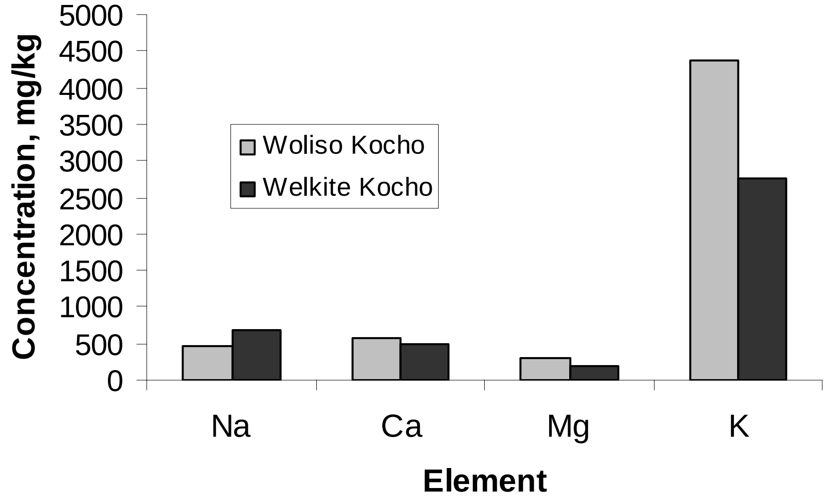


Figure 4 Average metal concentrations (for major and minor metals) in Kocho collected from Woliso and Welkite

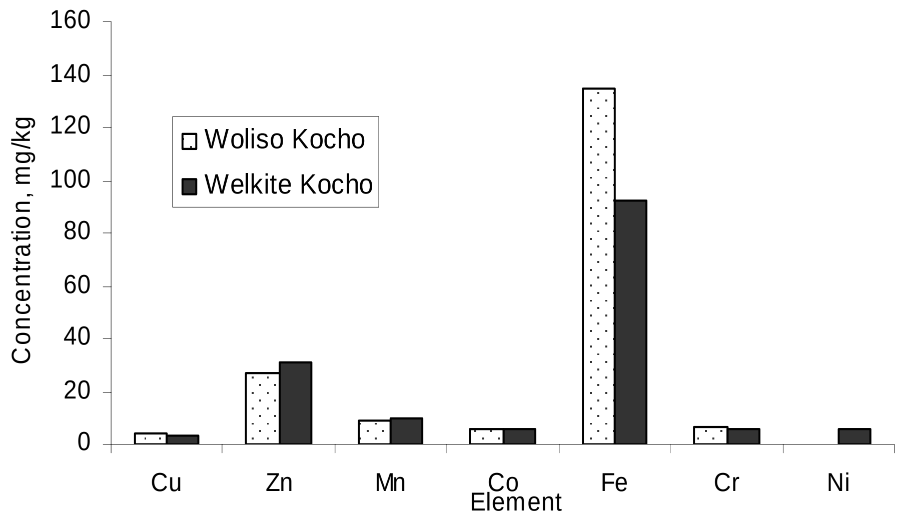


Figure 5 Average metal concentrations for trace metals in Kocho collected from Woliso and Welkite.

3.4.6.2. Concentration of Metals in Bulla

As shown in Fgure 6, 7, Table 7and 8, the trend in average concentration of metals in Bulla sampled from Woliso is K > Ca > Na > Mg > Fe > Zn > Co > Ni > Cu > Cr > Mn. Similarly, the pattern of the average concentration of metals in Bulla sampled from Welkite is K > Na > Ca > Mg > Fe > Zn > Cr > Co> Mn > Cu. The average concentration of metals in Bulla sampled from Woliso and Welkite respectively are K (875, 708), Na (402, 688), Ca (446, 385), Mg (89.5, 58.4), Zn (16.3, 22), Co (5.01, 5.0), Cu (3.53, 2.01), Mn (1.0, 4.98), Fe (59.8, 36.5) Cr (nil, 5.38) µg/g of dry mass. Like Kocho, Cd, Ni and Pb were not detected because they were below method detection limit. Like wise Cr was below detection limit in Woliso Bulla.

The concentration of K is the highest in Bulla from both places followed by Ca, Na and Mg. Since these nutrients are essential to the plant except for Na, the plants takes relatively large amount of them and accumulate in different part of the plant tissue and their natural abundance is also higher in the soil [82]. That is why these minerals are in larger quantity in both Kocho and Bulla. Where as the concentration of Cd, Ni and Pb were not detected. This may be due to low natural abundance in the soil, low absorption constant and liable complexetion.

In addition to the major nutrients, the concentration of iron and zinc are higher than the rest of the micronutrients.

As mentioned before the mineral contents of Bulla have not yet been analysed and reported except for Ca, Fe and Zn [17, 80, 24] The concentration ranges these metals reported in the literature (Table 9) are Zn (0.7-0.9 mg/kg), Ca (400-910 mg/kg) and Fe (11-77 mg/kg).

Except for Zn, the concentrations of Fe and Ca in the present study are within the range and follow the same pattern with the reported values. The possible reasons for deviation in Zn concentration are described under section 3.4.6.1.

In this finding, the concentration patterns of the metals in Bulla from both places follow the same pattern except for few trace metals.

Generally the concentration of metals in Bulla from Woliso is higher than Bulla from Welkite except for the elements Na, Zn, Mn and Cr. But the variation is not that much significant for the majority of the elements identified. The reason is already mentioned under section 3.4.6.1.

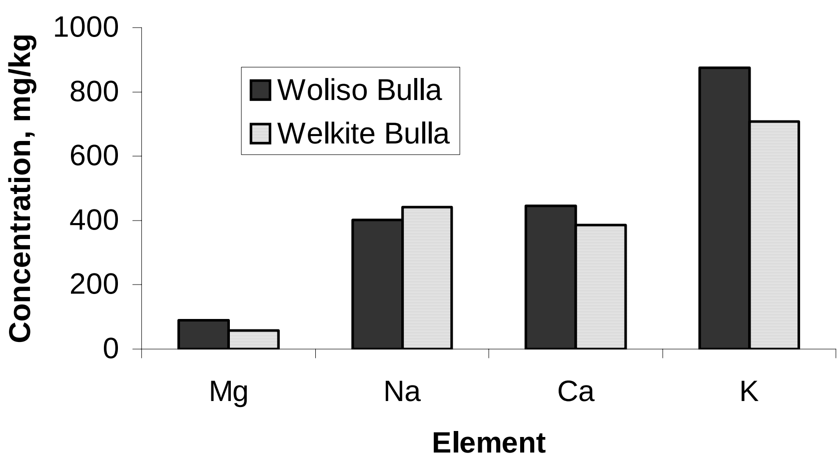


Figure 6 Average metal concentrations for major and minor metals in Bulla collected from Woliso and Welkite.

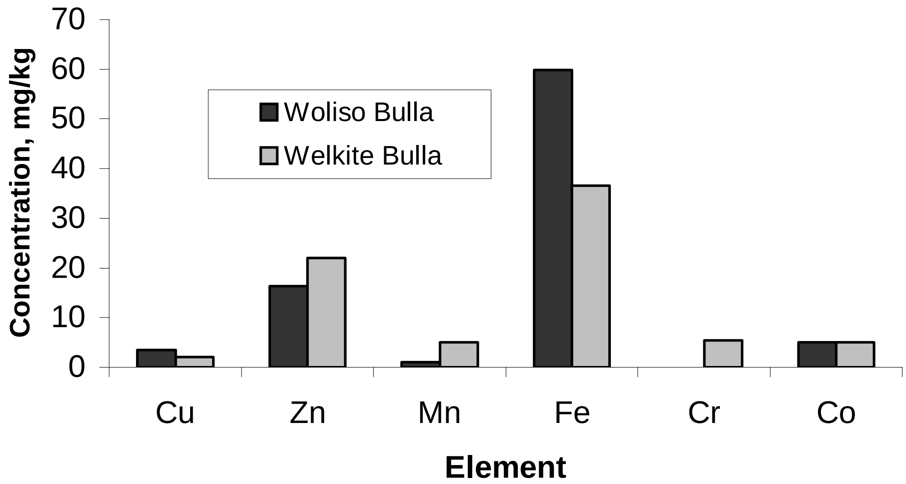


Figure 7 Average metal concentrations for trace metals in Bulla collected from Woliso and Welkite.

3.4.6.3. Correlations among Levels of Metals in Kocho and Bulla

As with all crops, the mineral composition of Kocho and Bulla varies from place to place depending on the climate, the soil mineral content and its pH, the Enset clone variety, age of the plant being processed and other factors

Knowing the distribution of metals in a given food stuff is essential to

- Recommend it as a source of certain nutrient
- Fortify or supplement deficient nutrient or
- Enhance or stop the use of the food as a staple or co-staple food.

Bulla is the purest form of all Enset foods. Meaning that, its purity is with respect to its physical characteristics (odor, and test), its pH, calories content [18], lower contents of fiber and ash as compared to Kocho. In the following section, the comparative study of the mineral contents of Kocho and Bulla is explained in detail.

Ca, Mg, Na, and K

The concentrations of Ca, Mg, K and Na in Kocho and Bulla collected from Woliso and Welkite are given in Table 7 and 8. The comparative results of these nutrients in Kocho and Bulla within their respective site are also shown in Figure 8 (a and b).

From Figure 8 (a and b), the concentration of the four metals in Woliso Kocho is higher than Woliso Bulla. The same trend has been observed for Welkite Kocho and Bulla]. From this it is possible to conclude that the concentration of the major metals in Kocho is higher than in Bulla collected from both places. Whether the difference is significant or not, it has been discussed in the latter section under the section statistical analysis.

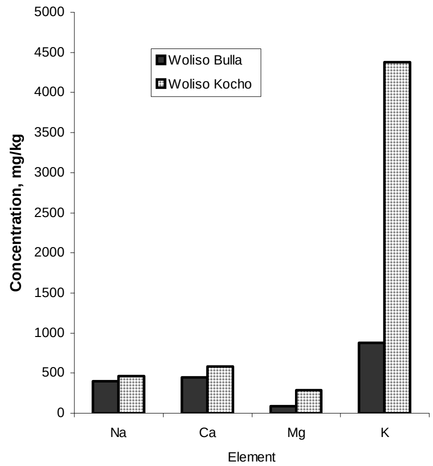
The overall comparative study of these metals in Kocho and Bulla along with their sampling site is shown in Figure 9 and the range of each metal in Kocho and Bulla excluding the sample site is shown in Table 10

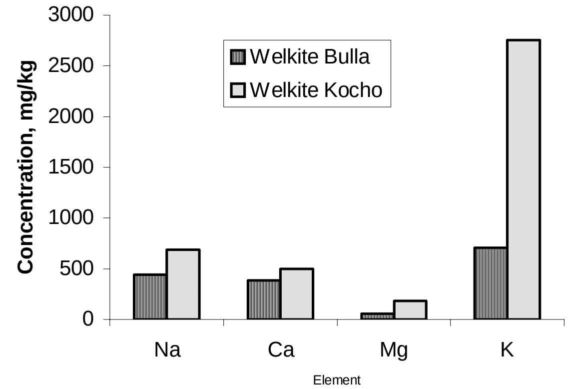
As can be seen from the Figures 9 and Table11, the concentrations of the four nutrients are higher in Kocho compared to Bulla irrespective of the sampling site. Concentration of K in the four samples is higher with the range of (708 – 875 μg/g) in Bulla and (2753 – 4380 μg/g) in Kocho, where as the range of Ca, Mg and Na in Kocho and Bulla respectively is Ca (498 - 584) and (385-446 μg/g), Mg (180 - 290) and (58.4 - 89.5) and Na (462 – 688) and (402- 442).

Histogram in Figure 9 clearly shows that, among the four samples Woliso Kocho contains the highest level of K. The concentration pattern of this metal in the four samples could be arranged in decreasing order: Woliso Kocho > Welkite Kocho > Woliso Bulla > Welkite Bulla.

Except for Na, the same trend was followed for the Ca and Mg. But for Na the trend follows: Welkite Kocho > Woliso Kocho > Welkite Bulla > Woliso Bulla. However Na is still higher in Kocho. As reported by Wu Leung and Ababe *et al* [17, 80] the concentration of Ca is higher in Kocho than compared to Bulla.

From this trend it is possible to conclude that Kocho contains higher concentration of K, Na, Mg, and Ca as compared to Bulla. As stated before, Bulla is obtained from decantation of the watery part during Kocho processing. Therefore only small amount of water-soluble species of these metals will pass in to Bulla and the majority of the metal ions will remain in the bulk of the decorticated and grated false stem and corm. Therefore the pattern of metals in Kocho and Bulla follows the same trend irrespective of the sampling site.





(b)

(a)

Figure 8 A comparative study of Average concentrations of major and minor metals in Bulla and Kocho from (a) Woliso (b) Welkite.

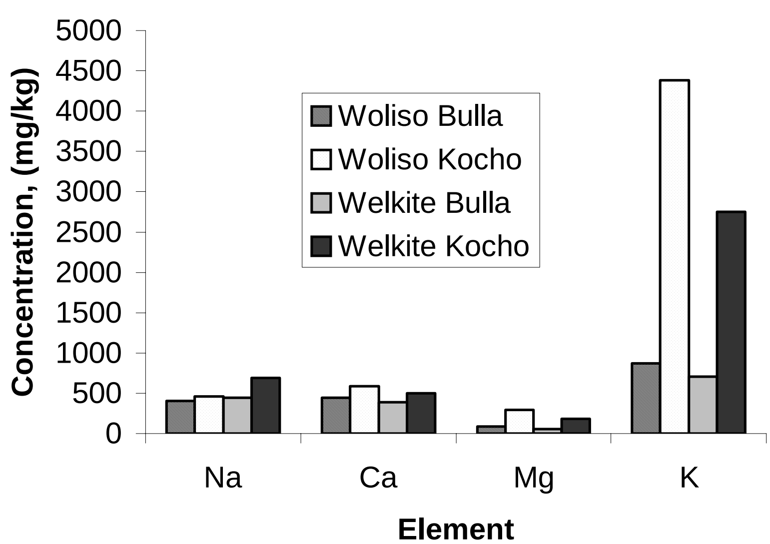


Figure 9 Over all site wise comparative study of average concentrations of major metals in Bulla and Kocho from Woliso and Welkitie.

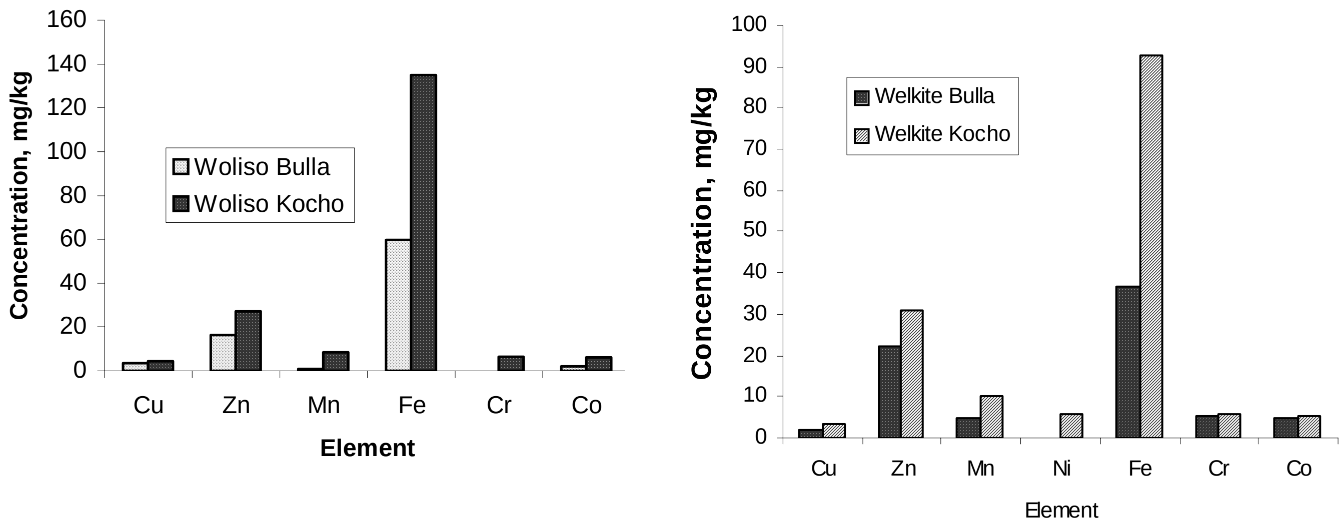
Fe, Zn, Cu, Mn, Ni, Cr, Cd, Co, and Pb

When the concentration of trace metals in Kocho and Bulla from their corresponding sampling site were compared, Kocho has relatively higher metal concentrations. The comparative results are presented in Figure 10a for Woliso samples and 10b for Welkite samples. And the overall comparative result between Kocho and Bulla are presented in Figure 11.

For most of the metals Woliso Kocho has relatively more metal concentration and followed by Welkite Kocho and Woliso Bulla. While Welkite Bulla has the least metal concentration for the majority of the major, minor and trace metals.

In both Kocho and Bulla the trend for most metals is the same. The concentration of Fe is higher in all the four samples followed by Zn. From Figure 11, the trend for Fe is: Woliso Kocho > Welkite Kocho > Woliso Bulla > Welkite Bulla. For zinc the trend is reversed meaning that Welkite Kocho has higher amount of Zn followed by Woliso Kocho and Welkite Bulla followed By Woliso Bulla. For Mn the trend is similar to that of Zn. Where as Co, Cu and Cr followed the Fe trend except the concentration of Co and Cr in Welkite Kocho and Bulla is comparable. Similarly, Pb and Cd were not detected in all the samples. For Ni, it was bellow detection limit except in Welkite Kocho.

The concentration of Fe and Zn was analysed and reported by Abebe *et al.* and Amede [17, 77] and the trend of this metals are similar to the present study. Meaning that Kocho contains higher amount of these metals compared to Bulla. The overall concentration range of these metals is given in Table 10. The reason for the higher concentration of these metals in Kocho is already mentioned in the preceding sections.



(a) **(b)**

Figure 10 A comparative study of Average concentrations of trace metals in Bulla and Kocho from (a) Woliso (b) Welkite.

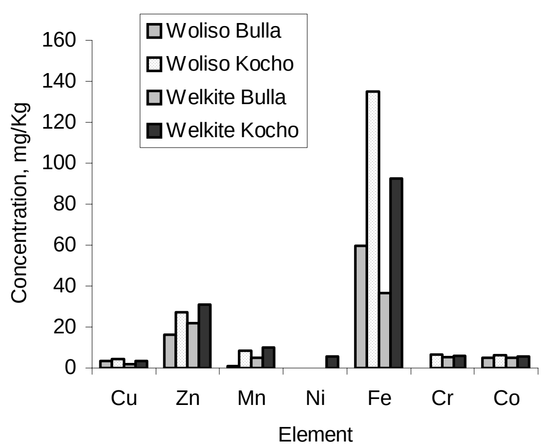


Figure 11 Over all site wise comparative study of average concentrations of trace metals in Bulla and Kocho from Woliso and Welkitie.

Table 10 Range of metal concentration in Kocho and Bulla from both studied areas.

Metal	Range of metal concentrations (μg/g)	
	Bulla	Kocho
K	708 - 875	2753 - 4380
Na	402 - 442	462 - 688
Ca	385 - 446	498 - 584
Mg	58.4 - 89.5	180 - 290
Cu	2.01 - 3.53	3.4 – 4.3
Zn	22 - 44.3	31 - 32.08
Mn	1.0 - 4.98	8.58 -10.13
Ni	ND[a]	≤ 5.61
Fe	36.5 - 59.8	92.5 -135
Cr	≤5.38	5.96-6.42
Co	5.0 - 5.01	5.5 - 6.1
Cd	ND[a]	ND[a]
Pb	ND[a]	ND[a]

[a] Concentration of the tested heavy metal below the method detection limit

3.4.6.4. Correlations among Levels of Metals in Unprocessed Edible Part of Enset and Processed Enset Foods (Kocho and Bulla)

The term 'food processing' covers an enormous field – from simple boiling to the use of irradiation. But for the present study food processing infers to converting unedible food material to the edible ones. As mentioned in the previous sections, Kocho and Bulla are nothing but they are obtained by fermenting the scraped and decorticated edible part of enset leaf sheath and the corm. During this processes a number of physicochemical processes will be taking place. As reported by many authors [17, 81], food processing will enhance the nutritional quality of food, and some anti-nutritional elements will also be minimized during fermentation. For example as reported by Abebe *et al.* [17], the phytate concentration of fermented enset dramatically decreased as compared to unfermented one. Therefore the bioavailabilities of mineral nutrients from processed enset foods are greatly enhanced.

Food processing has inevitable consequences on the nutritional value of foods. Beside this enhancement in nutritional quality, there may be loss of some food nutrients and enhancements of certain nutrients through contamination.

Knowledge of the various food processes and the nutrient losses that occur during each process will allow improvements to be made and losses as well as contamination to be minimized. The macro- and micro-nutrients (carbohydrate, protein, fats, vitamins and minerals) contained within foods all show varying degrees of stability when foods are stored or processed.

The mineral contents of unprocessed edible part of enset (Ensete ventricosum) were analyzed and reported by Debebe [27]. He analyzed the plant by decorticating and grating the corm and the false stem. Then he dried it, grind, digest with optimized acid mixtures and finally analyzed the selected metals by AAS. Table 11 shows the concentration range of each element found from his identification in unprocessed edible part of enset sampled from Woliso and Welkite.

Ca, Mg, and K

As can be seen from Table 11 and 12, there is a dramatical decrease in the concentration of these metals in processed edible part of enset (Kocho and Bulla). As mentioned in the previous sections, a number of processes followed during enset food processing from decortications to fermentation and preparing for cooking. During these processes some metals are definitely lost from Kocho and Bulla. Differences between species and varieties or clones of enset are not the only sources of variation of mineral contents of Kocho and Bulla and unprocessed edible parts of enset. Cultural practices (such as location and age of processed enset plant, plant density, fertilizer application, and duration of processing), storage conditions, and traditional processes can strongly affect different characteristics of Kocho and Bulla, which determine their mineral content and nutritional value. Consecutive changes in Kocho and Bulla nutritive value may be consequence variation of kinds of risks, such as toxicity or decrease in processing aptitude or nutrient contents.

Broadly speaking, minerals can be lost during and after enset fermentation by one of three different means:

- Intentional losses such as that occur when the edible part of false stems and corm are gratted, decortecated and peeled. i.e during removal of the inedible fractions of plant, (the fiber from foods during processing and unedible upper part of the false stem). However, these processes can incur losses of minerals; with careful control of the processes, nutrient losses can be minimized.

- Inevitable processing losses that result from squeezing out of the watery part from Kocho before cooked. This is one of the processes, which lead much amount of nutrient to be lost. Before Kocho is ready to be cooking, the watery part has to be removed by squeezing. During this time, much of the soluble mineral nutrients leached out from the flour with the liquid. From the present study it is possible to conclude that alkaline and alkaline earth metals do not form a complex species in the plant material and due to this the majority of the nutrients will be leached out together with the water during squeezing process.

- Accidental losses or avoidable losses due to inefficient processing or storage systems. This process is also leads contamination as well as loss of mineral nutrients. Selecting well aged plant, duration for fermentation as well as mechanism of allowing the decorticated and grated enset food to be fermented and mechanism of storage after fermentation are other processes which lead loss of mineral nutrients or contamination and hence increment in some mineral nutrients.

Therefore the concentrations of Ca, Mg and K in processed enset foods are greatly reduced as compared to unprocessed one. Since the mineral content of unprocessed edible part is analyzed carefully and in a controlled manner as Debebe [27] mentioned, it is difficult to clearly point out the exact reason for reduction of these nutrients in processed foods. But in general the above-mentioned reasons hope fully contribute reasonable effect.

Table 11 [a]Metal content of unprocessed edible part of enset and processed foods of enset (Kocho and Bulla) (dry mass basis) from Woliso

Sample type	K (μg/g)	Ca (μg/g)	Mg (μg/g)	Na (μg/g)	Fe (μg/g)
[b] Upr from woliso	26,200 -32,200	39,000-39,100	24,900- 26,900	NR	18.2-54.4
[d]Ws Bulla	874.5	446	89.5	402	59.8
[d]Ws Kocho	4380	584	290	462	135

	Cu	Zn	Mn	Ni	Cr	Cd	Co	Pb
	-----	-----	-----	-----μg/g-----	-----	-----	-----	
[b]UPr from Ws	5.1-5.2	11.8-42.3	2-4	ND[c]	6.3-7.6	0.6-1.8	7.9-10.5	ND[c]
[d]Ws Bulla	3.53	27.1	1.0	ND[c]	ND[c]	ND[c]	5.01	ND[c]
[d]Ws Kocho	4.3	22	8.58	ND[c]	6.42	ND[c]	6.1	ND[c]

[a] Results reported in terms of (i) range of average concentration of metals calculated for triplicate measurements for unprocessed edible part and (ii) average concentration of metals calculated for triplicate sample each with triplicate analysis.
[b] Results taken from ref. [27].
[c] Concentration of the tested metal below method detection limit.
[d] Results of the present study.
Upr = unprocessed enset.
Ws= Woliso.
NR = Not reported
ND = not detected

Table 12 [a]Metal content of unprocessed edible part of enset and processed foods of enset (Kocho and Bulla) (dry mass basis) from Welkite

Sample type	K (μg/g)	Ca (μg/g)	Mg (μg/g)	Na (μg/g)	Fe (μg/g)
[b] Upr from Wk	14,100 -17,700	36, 100-36,700	25,500- 25,600	NR	21.8-29.7
[d] Wk Bulla	708	385	58.4	442	36.5
[d] Wk Kocho	2753	498	180	688	92.5

	Cu	Zn	Mn	Ni	Cr	Cd	Co	Pb
	----	----	----	μg/g	----	----	----	----
[b]UPr from Wk	1.5-2.6	18.2-21.8	4-5	ND [c]	5.8-6.9	0.6-1.8	2.8-5.7	ND [c]
[d]Wk Bulla	2.01	31	4.98	ND [c]	5.38	ND [c]	5.0	ND [c]
[d]Wk Kocho	3.4	16.3	10.1	5.61	5.96	ND [c]	5.5	ND [c]

[a] Results reported in terms of (i) range of average concentration of metals calculated for triplicate measurements for unprocessed edible part and (ii) average concentration of metals calculated for triplicate sample each with triplicate analysis.
[b] Results taken from ref. [27].
[c] Concentration of the tested metal below method detection limit.
[d] Results of the present study.
Upr = unprocessed enset.
Wk = Welkite.
NR = not reported
ND not detected

Fe, Zn and Mn

Unlike the above mineral nutrients, these metals show a slight increment in processed foods as compared to the unprocessed ones. From Table 11 and 12, the concentration range of Fe, Zn and Mn in unprocessed edible part and in processed enset foods (including Kocho and Bulla) respectively from Woliso and Welkite are: Fe (18.2-54.4, 59.8-135) and (21.8-29.7, 36.5-92.5), Zn (11.8 - 42.3, 22-27) and (18.2-21.8, 16.3 – 31), and Mn (2-4, 1.0 - 8.58) and (4-5, 4.98- 10.13). In case of Fe, its concentration range in processed foods from both Woliso and Welkite increased compared to unprocessed one. Similarly, Mn also showed a slight increment in processed foods. But the concentration range of Zn in processed foods from Woliso is lower (below the range) than unprocessed edible plant material. Whereas its concentration range in processed foods from Woliso is slightly higher than the range in the unprocessed edible part of the plant sampled from Welkite.

As tried to point out in the above section, as there is nutrient loss during processing, there may be contamination starting from the harvesting area and time to the storage area and time.

Particularly the increment in Fe concentration in processed food is already expected because every processing utilities are made of iron and wood. Beside this there may be contamination with the dust particles during fermentation and storage. Beside this some minerals may me released out from the matrix during fermentation process.

The pulp produced from both the corm and the pseudostem must be sealed. The enset leaves lining the pit walls are folded over to cover as much of the top of the pulp as possible during fermentation. More leaves are placed over the top, tucked in down the sides and fastened with dried leafsheaths tied around them. Unless serious protection is taken, dust particles and rain may be mixed with the product of interest. More than three pits will be used for complete fermentation. Finally after fermentation is completed, before use, the fermented Kocho have to be refined. This is done immediately before cooking. First, as much liquid as possible is removed and wringing it out, like wet clothes are wrung. Then the wrung out product forms a crumbly substance which can be pulled loose from the fiber. At this point there are still many fibers which need to be reduced to a manageable size. The obtained flour is pounded into a mass and chopped rapidly with iron knife. The resulting crumbs are kneaded and the mass reformed to be chopped again. This is done repeatedly until the flour like substance is considered fine enough [2, 8, 23]. Therefore through all these processes there is iron contamination in the final product and that is why iron showed an increment in the processed food materials.

Cu, Cd, Pb, Cr, Co, and Ni

The concentration range of Cu, Cr and Co in the processed food types are below the concentration range compared when with their respective unprocessed edible plant material. However, the decrease in concentration range is not that much significant as compared to the major metals. This may be beside loss in concentration; there may be substantial contamination during processing. In addition to this, these metals may form water insoluble complex in the plant material so that they will not be easily leached out during squeezing.

Pb and was not detected in processed foods and unprocessed edible plant materials except for some plant unprocessed edible plant material collected from Welkite. Whereas Cd was not detected in processed foods but it was detected in unprocessed edible plant material. The reason is already mentioned in the previous sections why minerals lost during enset processing. Like wise Ni was below detection limit in some unprocessed edible plant materials and processed ones. But generally speaking enset plant growing in Woliso and Welkite as well as their respective processed foods did not contain Pb and Cd which are below our method detection limit (Table 11).

3.4.6.5. Comparison of minerals in enset foods and cereal flours

In those areas where enset is a staple and co-staple food, people prepare meal from Kocho or Bulla only. But some times they use mixture of either of the following: Kocho with flour (maize or wheat flour), Bulla with wheat flour and Bulla with Kocho [8]. In urban area where enset food is not a staple food, most of the time they use mixture of Bulla and wheat flour and prepare delicious porridge. Depending upon the individual interest, up to 30% of wheat flour can be mixed with the starchy enset food, Bulla. Mixture of Kocho with flour is not that much common in urban areas but some individuals mix around 10% flour in Kocho and prepare different types of meals. Therefore comparison of the mineral contents of enset food with cereal flours is crucial so as to know the dietary habits of those individuals who rely on enset as a staple and co-staple food.

Wheat, barley, Tef and maize are basic food for human. Their contents in macro, micro and trace elements depend on plant needs as well as amounts available from the soil.

Many authors reported the concentration of metals in cereal grain and cereal flours, which are grown in different part of the world including Ethiopia. Abebe *et al.* [17], Wojciechowska-Mazurek *et al.* [75], Chaven *et al.* [57] and Sager *et al.* [84] are some of them who reported certain selected mineral nutrients in the four major food types (maize, barley, Tef, and wheat). Table 12 summarizes the results. From the table, it is possible to see that the concentration range of some metals is higher in Kocho and Bulla as compare to some cereal flours. For example, the concentration ranges of Ca (μg/g) in Kocho, Bulla, wheat flour, barley flour, Tef flour, and maize are 498 - 584, 385 – 446, and 414-444, 395-450, < 1500, and 13-120, respectively. The concentration range of Ca in Kocho and Bulla is as good as with Barley flour and wheat flour. As compared to “Keyi Tef” flour from Ethiopia, Kocho and Bulla contains lesser amount of this mineral whereas maize flour contains least amount of this mineral.

The concentration ranges of Mn in Kocho, Bulla, wheat flour, barley flour, Tef flour, and maize are : 8.58 -10.13, 1.0 - 4.98, 24.1-46, 9.0-15, NR, 0.6-2.6, respectively. Therefore the trend for Mn becomes, wheat flour > barley > Kocho > Bulla > Maize. “Keyi Tef” contains the highest amount of Fe followed by Kocho. While Bulla, whole wheat, maize and barley white flour contains comparable Fe concentration range. White wheat contains the least Fe concentration range.

Pb and Cd have been analyzed in some places in cereal flours but their concentration was very small and not detected in Kocho and Bulla. The concentration range of Zn and Cu is almost comparable in all food types mentioned before except for white wheat flour, which contains smaller concentration of Zn.

Even though the mineral contents of plant foods are affected by different factors, it is possible to conclude something from results shown in Table 13. Kocho and Bulla are rich in Ca and Zn and contains comparable concentration of Cu, Fe and Mn. Beside this Kocho and Bulla are free of heavy metal contaminations like others do.

Table 13 Comparison of observed metal concentration (mg/kg, dry mass) in Kocho and Bulla with some reported values in barley, wheat, and maize and Tef.

Food types	Ca (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Ref.
Barley, white flour	450	NR	NR	84	17
	395-421	9.0-15	2.8-5	31-38	84
Red Tef: whole grain	1550	NR	NR	>1500	17
Maize, white four	120	NR	NR	49	17
	13-76	3.6-7.0	0.6-2.6	14.7-27	84
Whole wheat	NR	46	5	43	57
	414-444	24.1-46	3.2-6.2	19-41	84
White wheat flour	NR	7	2	11	57
Whole cereal flour	NR	NR	<2	NR	75
[a]Kocho	498 - 584	8.58 -10.13	2.87- 3.77	92.5-34.9	Pr
[b]Bulla	385 - 446	1.0 - 4.98	2.01 - 3.53	36.5-59.8	Pr

	Zn (mg/kg)	Pb (µg/kg)	Cd (µg/kg)	Ref.
Barley, white flour	35.7	NR	NR	17
	14.7-21.7	NR	NR	16
Red Tef: whole grain	40	NR	NR	17
Maize, white four	21.5	NR	NR	17
	12.8-14.1	NR	NR	84
Whole wheat	35	NR	NR	57
	18.1-34	NR	NR	84
White wheat flour	8	NR	NR	57
Whole cereal flour	19-41	13-60	5-80	75
[a]Kocho	31 - 32.1	ND	ND	Pr
[b]Bulla	22 - 44.3	ND	ND	Pr

[a,b] The range includes sample from Woliso and Welkite.

NR: not reported; ND: not detected; Pr, present study.

Note: [Pr], WoLiso and Welkite (Ethiopia); [3] Ethiopian cereals; [14], Poland cereals; [15], different countries; [16], Australia.

The Food and Nutrition Board [83] declared the RDA (Relative Dietary Allowance) of each mineral nutrient for all age levels of an individual. The source of the majority of the mineral nutrients is food of plant origin. As it was discussed in the previous sections, about 20 % of the Ethiopian population are relay on enset foods. Therefore it is better to compare the mineral contents of enset foods with that of RDA value so as to know individual's dietary habit. Ca, Fe, Mg, Zn and K are nearly comparable with the RDA values recommended by the Food and Nutrition Board by assuming that an average of 1 kg of Kocho and Bulla are sufficient to an individual adult per day. Similarly Na, Mn, Cu and Cr may not fulfill the individuals recommended value. However almost all people can use other vegetables such as cabbage together with Bulla and Kocho foods and possibly they will get some of deficient nutrients from these vegetables.

In general, enset foods will contribute the major portion of the mineral nutrients to the individual. Beside Kocho and Bulla are free of heavy metal contamination.

3.5. Statistical analysis

In this study samples were collected from two-studied areas from different randomly selected supermarkets. Each samples where mixed thoroughly and one representative bulk sample was taken for each food types. From each bulk samples three aliquots were taken, digested and analyzed. During this processes a number of random errors may be introduced in each aliquots and in each replicate measurements. And again there may be differences in results of analysis between food samples within inter-sites and intra-sites. Therefore depending upon the type and nature of results at hand, there are different statistical methods used to check whether there is a difference in results of analysis or not; and if there is a difference, statistical analysis will tell us the difference is significant or not. Of these, analysis of variance (ANOVA) is the best method [85].

Analysis of variance (ANOVA) is used to test hypothesis about differences between two or more means. For the present study, the significance of variation within sample and between samples has been studied using one-way ANOVA. Such calculation can be made using detail calculations following the formula. It can also be made on a computer using excel and Minitab [83]. For the present study Minitab was used to calculate the presence or absence of significant difference in mean concentration of each metal between Kocho and Bulla from Woliso and Welkite. The following results were obtained.

No significant difference ($P \geq 0.05$) at 95% confidence interval was observed in Na concentration between Woliso Bulla and Woliso Kocho, Woliso Bulla and Welkite Bulla and Welkite Bulla and Welkite Kocho. However a significant difference ($P < 0.05$) in mean concentration of Na at 95% confidence level was observed between Woliso Bulla and Welkite Kocho, Woliso Kocho and Welkite Kocho and Welkite Kocho and Woliso Bulla. This result reveals that there is inter-site variability in mean concentration of Na, in Kocho Food and there is absence of inter-site variability of Na in Bulla food.

Similarly, there is a significant difference ($p < 0.05$) in mean concentrations of K, Ca and Ni at 95% confidence interval between food types, i.e. between Bulla samples, Kocho samples and between Kocho and Bulla samples except for Ca, and Ni for which there were lack of a significant difference between Woliso Bulla and Woliso Kocho and Woliso Bulla and Welkite Bulla. Like wises absence of significant difference ($p \geq 0.05$) in mean concentration of Cu and Co at 95% confidence interval in all food types sampled from Woliso and Welikite was observed.

For Mn and Zn, the difference in their mean concentration between Woliso Bulla and Woliso Kocho, Woliso Bulla and Welkite Bulla and Woliso Bulla and Welkite Kocho, were significant ($P < 0.05$) at 95% confidence interval; whereas absence of significant difference ($P \geq 0.05$) were observed for these metals for the remaining pair of analysis.

Woliso Bulla and Welkite Kocho and Welkite Kocho and Woliso Bulla showed a significant difference in the mean concentration of Fe and Mg and insignificant difference for the remaining pairwise analysis for Fe concentration. Similarly, mean concentration of Mg showed a significant difference when compared with Woliso Bulla and Woliso Kocho and Woliso Bulla and Welkite Bulla.

Absence of insignificant difference in some mineral nutrient in both samples from Welkite and Woliso may indicates that since these area are under the same geographical location and share common climatic conditions. Similarly presence of significant difference in concentration for some minerals indicates that either of the studied area contains higher concentration of mineral nutrient in the soil or well aged plant have been harvested and well managed precautions have been followed during processing.

4. Conclusion and Recommendation

Processed enset (*Ensete ventricosum) (*Welw.) Cheesman food products Kocho and Bulla are carbohydrate rich foods and mainly used as a staple and co-staple foods in Southern Nation Nationality Peoples of Ethiopia and are underutilized foods for nutrition and income in other parts of the country and out of the country.

In this study commercially available Kocho and Bulla were analyzed for their contents of Na, K, Ca, Mg, Mn, Cd, Co, Cr, Zn, Ni, Pb, Fe, and Cu.

The optimized wet digestion method for Kocho and Bulla analysis was found efficient for the majority of the minerals and it was evaluated through the recovery experiment and a good percentage recovery was obtained (100 ± 10) for the majority of the minerals identified.

In this study the levels of thirteen elements in the two starchy food stuffs collected from two of the major Kocho and Bulla producer areas (Woliso and Welkite) were analyzed. The results showed that both Kocho and Bulla contains appropriate concentration of major, minor and trace metals. For the majority of the mineral nutrients, their concentration was higher in Kocho compared to Bulla and for the majority of the elements Woliso Kocho and Bulla contained the higher concentration. The ANOVA results suggest that there were significant variation in the level of some elements between the foodstuffs within the sampling site and between the sampling sites, which could be attributed to different factors such as age and clone (varity) of enset species processed [10], different precaution taken during processing, fermentation and storage conditions and length of fermentation. For some elements the variations were insignificant, which could be attributed to having similar climatic conditions of the sampling sites because they are located within the same geographical location.

Pb and Cd were not detected in both food staffs from both places. Likewise Ni was not detected in Woliso Kocho and chromium in Woliso Bulla. The results revealed that Kocho and Bulla accumulate Ca and Na next to K. From trace elements analyzed, Fe concentration was found highest followed by Zn. Except for Zn, these results were also comparable with reported literature values analyzed from other parts of the country.

The results of the present study were compared with the mineral contents of unprocessed edible plant material reported by Debebe [27] and there is a significant difference in major minerals concentration between processed enset foods and unprocessed ones. Meaning that the major portion of their concentration has been lost during processing through intentional losses such as that occur when the edible part of false stems and corm are gratted, decortecated and peeled, inevitable processing losses that result from squeezing out of the watery part from Kocho before cooked or accidental losses or avoidable losses due to inefficient processing or storage systems. For some trace metals, their concentration in processed enset foods showed a higher concentration compared to unprocessed one, which may be attributed to contamination during processing. For the majority of trace elements, their concentration range was lower compared to unprocessed ones but the decrease was not that much significant compared to major and minor metals. This may be due the above-mentioned reasons and they may form complex species in the plant material and may not easily leached out during processing and squeezing steps.

For some metals, their concentration was compared with cereal flours. For some of the elements, extracted cereal flours contains lower concentration range while whole grain contains higher concentration range compared to Kocho and Bulla. For some metals, their concentration range was comparable with Kocho and Bulla while others are lower in their concentration in Kocho and Bulla. Generally speaking, mineral contents of Kocho and Bulla satisfy the individuals' need equally with cereal flours.

Since Kocho and Bulla are staple and co-staple foods for about 20% of the Ethiopian population, a complete food composition table should be crucial. The present study will give brief information about the mineral contents of Kocho and Bulla. But to have complete and general information further research should focus on these foods from other part of the region. Secondly, the mineral status of the soil in which enset is growing abundantly in the region should be studied so as to supplement fertilizer for the deficient nutrients.

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