ADDIS ABABA UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CHEMISTRY

DETERMINATION OF THE LEVELS OF TRACE METALS IN ETHIOPIAN LANDRACE LENTILS (*Lens Culinaris Medik.*)

By: Sintayehu Leshe Kitaw

Advisor: Merid Tessema (PhD)

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A Graduate project submitted to the Department of Chemistry in partial fulfillment for the Requirements of the Degree of Masters of Science in Chemistry

June 2010

Acknowledgments

I am greatly indebted to my advisor Dr Merid Tessema for his guidance and encouragement while I carried on this project work. Once again, thank you, doctor.

I also thank all my instructors and members of the chemistry department of Addis Ababa University.

I like to express my appreciation to Ato Erdaw Tachibele (instructor at the Nursing School of Addis Ababa University) for his genuine encouragement, and material support.

I like to greatly thank Ato Kuyyu Ketema (Head of North Shewa Education Office), Ato Solomon Abebe (Kuyyu Woreda Administrator) and Ato Girma Worku (Chief of Kuyyu Woreda Education Department) without whom my study in the program could not have been achieved.

I am very delighted to express my appreciation to Ephrem Lemma and Tesfaw Ashagre for their true and brotherly companionship in helping me get a laptop service.

I interestingly need to extend my appreciation to Rediet Ayalew for his excellent conduct and characteristics except his limitations in reading hard working.

Finally, I would like to express my deepest gratitude and affection to my wife, Alemtsehay Dhabi, for the endurance and patience she showed me when I was away from home leaving the entire burden of caring for our lovely children, Hanna and Al'azar. I deeply regret for not accompanying you while you were pregnant and delivered our little Robel recently. Thank you Very much Alem.

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List of abbreviations and their full expressions

CACC	Credit acceptance corporation committee
IBS	Irritable bowel syndrome
FAAS	Flame atomic spectrometry
PMT	Photo multiplier tube
RSD	Relative standard deviation
SD	Standard deviation
MDL	Method detection limit
MQL	Method quantization limit
WL	Wavelength
SW	Slit width
DL	Detection limit
LC	Lamp current

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DETERMINATION OF THE LEVELS OF TRACE METALS IN ETHIOPIAN LANDRACE LENTILS (*Lens Culinaris Medik*)

Abstract

The concentrations of trace essential metals (Co, Cu, Fe, Mn, Ni, and Zn) and toxic heavy metals (Cd, Pb) in the Ethiopian landrace lentil samples were determined by using flame atomic absorption spectrometry. A wet digestion procedure, using mixtures of HNO_3, $HClO_4$ and H_2O_2, was developed for the decomposition of powdered lentil samples. The accuracy of the method was checked by the standard addition method. The contents of the analyzed heavy metals ranged between 0.009 and 0.013 for Cd, 0.285 and 0.360 for Co, 0.226 and 0.282 for Cu, 9.17 and 11.91 for Fe, 6.7 and 8.2 for Mn, 0.120 and 0.244 for Ni, 0.142 and 0.176 for lead and 8.62 and 10.03 for Zn, all in mg/100 g. The results were compared with values reported in the literature.

Keywords: lentil, atomic absorption spectrometry, wet digestion, mineral nutrition, toxic metals, legumes.

1 INTRODUCTION

1.1 History, origin and geographical distribution

Lentils are an ancient world legume and were probably one of the first plant species to be domesticated [1]. They are considered to be one of the oldest food crops of humankind that researchers have traced back to 7000-8000 BC [2, 3]. They originated in the fertile crescent of the Mediterranean region and dated back to the beginning of agriculture itself. They are mentioned in Egyptian texts dating back to 1085 BC, and were a popular crop during the Romans and Greeks. Lentils are also mentioned in the first book of the Old Testament of the Bible (Genesis 27) that Esau sold his birthright to Jacob for a dish of lentils. They are also mentioned in Holy Quran (Second Surah, Al-Baqarah) as "Manna-o-Salva" which the Jews asked Mosses to request from God. Presently, the cultivated lentil (Lens culinaris Medik) is thought to have originated in western Asia from where it spread to Egypt and southern Europe. From there, the distribution widened northward to Europe, eastward to India, Pakistan and China and southward to Ethiopia and later to the new world including Latin America, Mexico, Chile, Argentina, Colombia and more recently Canada [4]. In tropical Africa, it is grown in Sudan, Eritrea, Ethiopia (mainly in the northern, central and eastern highlands), Kenya, Tanzania, Malawi, Zimbabwe, Madagascar and Mauritius. It is also cultivated in Morocco, Tunisia, Algeria, Libya, Egypt and South Africa. Lens culinaris belongs to the genus Lens, which is classified taxonomically in the order Rosales, sub-order Rosineae, family Leguminoseae and sub-family papilionacea. Until the work of Ladizinsky in [5], the genus Lens was considered to comprise five annual species, of which only one is cultivated and is botanically known as Lens culinaris Medik. Ladizinsky [6], on the basis of cytological evidence and morphological distinction, has suggested only two species: Lens culinaris and Lens nigricans, in the genus Lens. All other types are regarded as sub-species either of Lens culinaris or Lens nigricans. Lens culinaris comprises 3 sub-species: culinaris, orientalis and odemensis. The former is cultivated and the later two are wild. Lens nigricans contains two sub-species: nigricans and ervoides, both of which are wild. Within the cultivated species of lentil, there are two races: microsperma and macrosperma [3]. The microsperma with small and

rounded seeds mostly with red or orange cotyledons are preferred in south Asia, Afghanistan, Ethiopia and Egypt.

Macrosperma with larger and more flattened seeds, normally yellow cotyledons are predominant in southern Europe, North Africa and North and Latin America. However, the two races overlap in their distribution in western Asia and southeastern Europe [1]. Evidence suggests that the cultivated lentil was domesticated from orientalis ancestors. This progenitor derivative relationship has been suggested by morphology [2], ecology/archeology, numerical taxonomy and pollen morphology [7], and cytology and hybrid fertility [8].

Nutritionists rank lentil as an excellent source of human diet which is high in protein; is a major source of complex carbohydrates; is high in fibers; rich in vitamins A and B, potassium and iron; and low in sodium and fat. Lentils are often used as meat extenders or substitutes because of the high protein content and quality and are also used in gluten-free, diabetic, low salt, low calorie, low cholesterol and high fiber diets. Lentils are becoming increasingly popular as important sources of vegetable protein. High protein content, i.e., 22 to 34.6% [8] and 55% starch [9], low level of antinutrients and an ability to grow in low water stress conditions are the main attributes that make lentils important legume crops.

1.2 Production and international trade

According to FAO statistics, the annual world lentil production in 1999–2003 amounted to 3.1 million ton per year from 3.8 million hectare. The main producers were India (948,000 ton per year from 1.43 million hectare), Canada (616,000 ton per year from 554,000 hectare) and Turkey (473,000 ton per year from 490,000 hectare). In tropical Africa, the main producer is Ethiopia (47,000 ton per year from 78,000 hectare). About 60% of the lentil production in Africa (including North Africa) comes from Ethiopia, where the area under lentil cultivation has been decreased since the mid 1980s, but in the late 1990s, this trend has been reversed due to the release of cultivars with resistance to rust and fusarium wilt. World lentil exports in 1998–2002 amounted to about 1 million ton per year. The main exporters were Canada (430,000 ton per year), Turkey (127,000 ton per year), Australia (124,000 ton per year) and India (120,000

ton per year). The main importers were Egypt (90,000 ton per year), Sri Lanka (86,000 ton per year) and Turkey (81,000 ton per year) [10-12].

Table 1 Lentil production (tones) in major growing countries during 2001-05

Country	2001	2002	2003	2004	2005	Average
India	915,200	974,400	880,000	1,100,000	1,000,000	973,920
Canada	566,300	353,800	519,900	962,000	1,277,900	735,980
Turkey	520,000	565,000	540,000	540,000	555,000	544,000
Australia	266,000	67,000	103,811	52,300	210,000	139,822
Nepal	143,084	148,384	149,963	158,671	160,716	152,164
USA	131,450	113,760	110,770	189,690	234,190	155,972
China	125,000	125,000	132,000	145,000	135,000	132,400
Syria	177,467	132,805	168,437	125,300	153,665	151,535
Bangladesh	126,000	117,000	116,000	122,000	121,000	120,000
Iran	104,399	38,430	120,000	125,000	125,000	118,279
Ethiopia	65,250	41,670	35,275	35,275	63,357	47,517

Source: FAOSTAT 2007

1.3 Ecology and climatic conditions

Lentil is often grown on nutrient poor soils without or with little fertilizer applications. Superior morphological (root length, root hairs) and physiological (exudation of proteins and enzymes) root traits facilitate efficient use of soil nutrients. Identification of lentil germplasm with superior root traits, therefore, may help to sustain lentil production in nutrient poor soils of many developing countries including the Indian subcontinent, Bangladesh, west Asia, North

Africa, Sudan, Yemen, Ethiopia, Eritrea and South America. The identified germplasm may be directly introduced or used for targeted breeding of nutrient efficient and drought tolerant varieties.

Lentil is grown as a summer annual in temperate regions and as a winter annual in subtropical regions. In the tropics, it is cultivated at higher elevations (1800–2500m; 1800–2700m in Ethiopia) or as a cool season crop. It grows at mean temperatures of 6–27°C, but lentil is not suited to the hot and humid tropics. Intense or prolonged frost and temperatures much higher than 27ºC seriously affect growth. Lentil requires an annual rainfall of about 750 mm, with dry conditions around harvest time, but an annual rainfall of 300–2400 mm is tolerated. It is moderately tolerant to drought, but differences exist between cultivars. Lentil normally requires long day lengths for flowering, but the response varies among genotypes, and some cultivars are day length insensitive. In Ethiopia lentil is grown in the short rainy season ('belg', February–May) and during the main rainy season ('kiremt', June–December), the latter being predominant. To avoid water logging the 'kiremt' crop is sown on Vertisols at the end of the rainy season (September) and grown on residual soil moisture. Lentil can be grown on a wide range of soil types, from sandy to heavy clay soils, but does not tolerate flooded or waterlogged soils. A pH near 7.0 is best for lentil production, but it tolerates a pH of 4.5–9.0. Lentil is generally very sensitive to salinity [11-13].

1.4 Uses of lentils as food

Lentil is primarily grown for its mature seeds, which are consumed mainly in sauces and soups. In Ethiopia, it is used in 'kik wot' (sauce of split seeds), soup (from whole seeds or flour), 'nufro' (boiled and salted), 'azifa' (cooked and mashed) and 'elbet' (paste from flour). Many other dishes are prepared from lentil in different countries. Some of these include spicy lentil salad, lentil burgers with coriander-yoghurt sauce, lentil and mushroom cottage pie and lentil potatoes. In India, split seeds (dhal) are used in soups and the whole seed is eaten salted and fried. The seeds are ground into flour used for cakes and bread and for the preparation of special foods, e.g. for infants and invalids. Young pods, sprouted seeds and leaves are eaten as vegetable. Lentil seeds are occasionally fed to animals as a source of protein, particularly to

poultry. The seeds are believed to remedy constipation and other intestinal problems. In India they are applied as a poultice to slow-healing sores. In Ethiopia, the seeds are credited with aphrodisiac properties.

In Ethiopia, lentil is grown for human consumption as a rich source of protein (23 – 24%) [14], and therefore, may correct important amino acid deficiencies of cereals when used mixed with tef, wheat and barley. It is a cash crop fetching the highest price in domestic market compared to all other food legumes and cereals. It is also an important export crop for Ethiopia. The crop is generally grown in rotation with cereals to break cereal disease cycles and to fix atmospheric nitrogen, thus reducing the demand of other cereal crops for nitrogen fertilizers. Ethiopia is one of the major lentils producing countries in the world and the first in Africa (FAOSTAT, 2006). However, its average seed yield has remained very low, 0.64 ton per hectare (CACC, 2002). Among the major reasons are: susceptibility of the landraces to various diseases and their inherently low yield potential. These problems necessitated the introduction of lentil germplasms to improve production and productivity.

Fig 1 Common legume crops

Accordingly, ten improved varieties were released for production in Ethiopia. However, in the long run, these varieties may replace indigenous landraces ultimately resulting in genetic erosion. In addition, farmers are eager to replace lentils with remunerative crops, which could

exacerbate the threat of genetic erosion. These facts suggest the importance of exhaustive germplasm collections before possible loss of genetic diversity. Prior knowledge of genetic variability within the germplasm available at genebank is important to identify the areas of major priority for conservation and for improvement programs. Lentils are a crossover ingredient; a mainstay of humble peasant dishes; they figure as well in haute fare. These petite legumes add a hearty note to soups and stews; they are a tasty addition to salads. In Europe, lentils are enjoyed as a side dish simply dressed with butter or olive oil. Cooked lentils also serve as a bed for meats or fish, sopping up the rich juices.

1.5 Lentil varieties, taxonomy and common names

There are many types of lentils, but most are permutations of the brown, green, and red varieties. Brown lentils are the most common type used in the U.S. This category is bold and earthy tasting. Sold with the seed coat on, brown lentils are small in size and grayish brown with a yellow interior. The smaller Chinese brown lentils are also common. Brown masoor lentils are actually red lentils in their seed coats. Green lentils are a dull olive color. They are also known as Chilean or Egyptian lentils. Green lentils are generally smaller and rounder than brown. Red lentils are bright orange, pink and shiny. They are peeled off their seed coat and split horizontally. Used extensively in Indian cuisine, red lentils are quick cooking and delicate in flavor. Taxonomically, lentil belongs to the kingdom-plantae, family-fabacea, subfamily-faboidea, genus-lens, and species-Lens culinaris Medik (also Lens esculenta Moench). Some vernacular names include Adas (Arabic), Mercimek (Turkey), Misser (Ethiopia), Masser (India), Lentille, Lentille Cultivée, Lentilles (French), Linse, Linsen (German), Lente, Lenticchia, Lenticchie (Italia).

1.6 **Health benefits of lentils**

Dry lentil seeds are good sources of high quality protein, vitamins, and a balanced range of minerals. They are also excellent sources of complex carbohydrates and dietary fibers. Lentils, like most other legumes, are loaded with fiber. One serving of lentils (about one cup) has nearly eight grams of fiber, both soluble and insoluble, both of which are needed for a healthy

digestive system. Because the fiber content of lentils is so high, they can help preventing many digestive illnesses such as constipation and irritable bowel syndrome (IBS). Lentil seeds, which are highly accepted in most parts of the world, provide one of the best means of fighting malnutrition among people in developing countries. In several countries, reduction in the consumption of legumes in general and lentils in particular, has been related to the presence of antinutritional factors in these legumes. Cheap methods are required to improve nutritional quality by either removing antinutritional compounds or by raising the levels of nutritional enhancers. Traditional methods such as germination and fermentation tend to improve the nutrient quality of foods. Fermentation is generally achieved by native microflora. Fermented food is a widely exploited source of valuable protein. Among the legumes, soybeans have been extensively used for this purpose, although other legumes such as beans and chickpeas are also occasionally used [15].

Fig 2 Tomato lentil soup

Fermentation is associated with many chemical changes that enhance organoleptic response, contents of free sugars and vitamins as well as bioavailability of minerals [16]. and results in the breakdown of some of the antinutritional endogenous compounds.

1.7 The chemical composition of lentils

1.7.1 Crude protein content

Seed protein content varies considerably among varieties and plant breeders could utilize this information to produce cultivars yielding high protein content. Krober *et al* [17] in their survey of the protein content of lentils grown in widely differing locations in India showed that there were significant differences in the crude protein of lentils grown in different locations. Protein content of lentils was reported in the range of 22 to 34.6% [18]. One hundred grams of decorticated lentil seeds contain 25.8 g protein [7]. Sulieman [19] in his survey found the protein content of lentils grown in Sudan ranged between 32.4 and 35.6%.

1.7.2 Lipids

Glycerol esters of fatty acids which make up to 99% of the lipids of plant and animal origin have been traditionally called oils and fats. Nawar [20] reported that in addition to their role as vitamins carriers, dietary lipids frequently provide a significant proportion of calories. Dietary lipids supply essential fatty acids. Hundred grams of dried lentil seeds contain 0.6 g fat [17]18. Decorticated lentil seeds contain 1.83g fat/100g decorticated seeds [21]. Sulieman [19] found the fat content of lentil ranged between 1.95 and 2.4%.

1.7.3 Fiber

The term crude fiber is defined as the washed, dried organic residue remaining after acid and alkaline hydrolysis of a defatted material. Fibers become of considerable interest in human nutrition because of some beneficial attributes, along their adverse effect [21]22. One hundred grams of decorticated lentil seed contain 0.9g fibers [7]. Sulieman [19] found the fiber content of lentil ranged between 1.17 and 2.43%.

1.7.4 Ash

Ash, a residue that remains after ignition of organic matter, is used as starting point for determination of elemental composition of food material. It is required by the human body for good health and growth [21]. Sulieman [19] found the ash content of lentil ranged between 2.7 and 3.8%.

1.7.5 Carbohydrates

Carbohydrates are the chief sources of energy by providing 70 – 80% of the calories in the human diet [23]. One hundred grams of dried lentil seeds contain 65.0 g total carbohydrate [18]. Hulse [21] reported that one hundred gram of decorticated lentil seed contain 58.9 gram of total carbohydrate.

1.7.6 Minerals

Minerals are present in foods at low but variable concentrations and in multiple chemical forms. The role of minerals in food is to provide a reliable source of essential nutrients in a balanced and bioavailability form. Essentiality of inorganic elements is not always easy to prove. The minerals considered to be essential for normal body functions include the major elements (Sodium, Potassium, Calcium, Magnesium, Phosphorus, and Chloride) and trace elements (Cobalt, Chromium, Copper, Iron, Manganese, Molybdenum, Selenium and Zinc).

In cases where concentration and/or bioavailability in food supply are low, fortification has been popular. Bioavailability of minerals is defined as the fraction of the ingested nutrients that is absorbed and subsequently utilized for normal physiological functions. Physiological requirements for different inorganic nutrients vary widely depending upon age, sex, stage of growth, pregnancy and lactation. Owing to the presence of anti-nutritional factors like phytic acid and polyphenols, which complex with divalent cations, the bioavailability of minerals for human consumption is poor. Nutritional quality of the grain is expected to improve once the

anti-nutritional factors are reduced or eliminated. Bioavailability of minerals can be improved by suitable processing methods such as cooking, germination or fermentation [24].

1.8 Nutrient values of lentils

Among the cool season legume crops, lentils are the richest in their important amino acid contents (lysine, arginine, and leucine) and other sulphur containing amino acids. The composition of mature raw lentil seeds per 100 g edible portion is: 11.2 g water, 1413 kJ (338 kcal) energy, 28.1 g protein, 1.0 g fat, 57.1 g carbohydrate, 30.5 g dietary fiber, 51 mg Ca, 107 mg Mg, 454 mg P, 9.0 mg Fe, 3.6 mg Zn, 0.48 mg thiamin, 0.25 mg riboflavin, 2.6 mg niacin, 0.54 mg vitamin B_6, 433 μg foliate and 6.2 mg ascorbic acid. The essential amino-acid composition per 100 g edible portion is: 251 mg tryptophan, 1957 mg lysine, 238 mg methionine, 1383 mg phenylalanine, 1006 mg threonine, 1392 mg valine, 2034 mg leucine and 1212 mg isoleucine [25]. The main limiting amino acids are methionine and cystine. Antinutritional factors include trypsin inhibitors, haemagglutinins, tannins, phytate and oligosaccharides, but the levels are considerably lower than those in pea and faba bean, and lentils are considered more easily digested.

1.9 Trace metals analysis in legumes

The use of fertilizers, herbicides, pesticides, selected hybrid seeds, wide spread irrigation and modern farm equipments has in recent years increased to boost the yield of crops and vegetable production to support the increasing population and rural to urban migration. Such progress, nevertheless, is often associated with various forms of environmental hazards. Regular soil spraying with pesticides increase abundance of harmful chemicals that is subsequently stored in ground minerals and water. Fertilizers frequently contain trace amounts of arsenic and heavy metals; so their repeated use may cause toxicity of soils.

Crops can accumulate these trace heavy metals in or on their tissue, thus they are intermediate reservoirs, through which trace elements from soils, and partly from water and air, transfer to man and animals. Growth media including soil, nutrient solution, water and air are main sources of heavy metals to crops which enter by roots or foilages through adsorption or absorption.

Groundwater, the main source of water supply for agriculture and other purposes, is susceptible to pollution from sources such as farm animals, man, sewages, polluted streams and refuse disposals. Besides, salt intrusion from coast that can extend several kilometers inland is another factor determining suitability of groundwater for irrigation as excess salt interferes with osmotic process and metabolism.

Different crop species accumulate different metals depending on environmental conditions, metal species and available forms of the heavy metals. Many plants are found to be in a position to take up large quantities of certain elements from the environment and said hyper accumulators of heavy metals [26]. Trace elements play an important role in chemical, biological, biochemical, metabolic, catabolic and enzymatic reactions in the living cells of plants, animals and human beings. More than sixty elements are found in human body in various forms among which twenty five are considered essential to human health out of which fourteen existed usually less than 1 $\mu g g^{-1}$ of tissue and so termed trace. Cobalt is essential component of vitamin B_{12}; zinc is found in several enzymes and genetic material transcription; copper is a key component of redox enzymes and chromium has a role in glucose metabolism, iron in oxygen transport and so enables metabolism [27].

Though required in very small amounts, deficiency of trace elements cause diseases, whereas their presence in excess may result in toxicity to human life disturbing normal functioning of organs and central nervous system. Anemia from iron deficiency affects more than half of pregnant women and at least one third of children under five years [28]. In Ethiopia, the Food and Agricultural Organization estimate indicated that the prevalence of iron deficiency is 85 % among children and 58 % among pregnant women while in Kenya similar survey estimated 60 % and 70% respectively [29]. On the other hand, trace metals like lead, cadmium and mercury are known for their detrimental health effect. Cadmium, for example, has been considered as an

extremely significant pollutant, even in small amounts, affecting all forms of life because of its high toxicity and great solubility in soil and water. No level of lead in blood as well should be considered safe for children due to its neurotoxicity [30, 31].

As food is the major intake source of toxic trace metals by human beings, contamination of food has become a burning issue in recent years particularly in most metropolitan cities [32]. This is attributed mainly to the problem of environmental pollution due to rapid urbanization and industrialization with improper environmental planning leading to discharge of industrial, agricultural and sewage effluents into water bodies, lands and air. Added to the heavy release of trace metals, their geo-accumulation, bioaccumulation, bio-magnification in bio-system and non-biodegradability exponentially enhances their concentration across the food chain and the effect on human beings [25]. Once they enter the body, they may alter their oxidation state, may form complexes with other biological molecules, but their essential integrity remains constant [33].

Determination of inorganic elements in agricultural products is also attracting considerable attention for a new application, which is to identify the geographic origin of the agricultural products to provide necessary information for consumers, agricultural farmers, retailers, and administrative authority. The technique was based on the observation that differences in the composition of inorganic elements are based on the differences in cultivation conditions: features of the soil, such as its composition, pH, soil type, moisture, organic constituents, temperature and humidity.

Another reason for analysis of metals in crops is for diagnosis of deficiency of essential metal nutrients so that appropriate minerals can be supplied for the proper growth of crops. At High concentrations of heavy metals, metabolic processes of the plants can be interfered, resulting in poor growth and sometimes even death [34]. A study conducted in China estimated that the output of rice grain was reduced by more than ten million tons annually because of heavy metal pollution of soil, particularly from application of sewage sludge and industrial byproducts.

The study of inorganic elements in biological samples is now well expanding into a field called bioinorganic chemistry concerned with the study of elemental uptake, physiological action, storage, excretion and introduction as probes or drugs when necessary. Therefore, the determination of the metal contents of lentils and other crops across different parts of the globe were conducted from the following viewpoints: health risk assessment, nutrient content analysis for consumers, to trace geographic origin of food products, nutritional status assessment of growing plants and assay of suitability of soil and water for farming.

Although investigation of plants for their metal concentrations is indispensable, a survey of literature indicates that such a study is scarce in Ethiopia. Among the previous studies, metal concentrations in fruits [35] and leafy vegetables in Addis Ababa [36, 37] are some to be mentioned. Trace metal assessment, particularly in lentil is currently not available at least to my knowledge although exposure of the crop to the metals is inevitable. Therefore, this study aims to fill the gap at least partially in the area and initiate others on locally improved lentil varieties like Teshale, Ada'a, Alem Tena, Alemaya and other crops widely used throughout the country.

1.10 Mineral Nutrition

Minerals are nutrients that exist in the body and are as essential as our need for oxygen to sustain life. In the body, only 5% of the human body weight is mineral matter, vital to all mental and physical processes and for total well being. They are most important factors in maintaining all physiological processes; are constituents of the teeth, bones, tissues, blood, muscle and nerve cells [38].

Acting as catalysts for many biological reactions within the human body, they are necessary for transmission of messages through the nervous systems, for digestion, metabolism and utilization of all nutrients in foods. Vitamins cannot be properly assimilated without the correct balance of minerals. For example, calcium is needed for vitamin "C" utilization, zinc for vitamin "A", and magnesium for "B" complex vitamins [38, 39]. Minerals are very important in keeping the blood and tissue fluids from either becoming too acid or too alkaline and they allow other nutrients to pass into the blood stream, and aid in transporting nutrients to the cells. They also draw chemicals in and out of the cells. A slight change in the blood concentration of important

minerals can rapidly endanger life [38]. No single mineral can function without the others since the functions of the mineral nutrients are synergistically related [37, 38]. Acquiring the proper balance of minerals in the body can make the difference between disease and health.

Plant derived foods have the potential to serve as dietary sources of minerals for all humans [39, 40]. Minor and serious health conditions such as energy loss, premature aging, diminished senses and degenerative diseases like heart disease and cancer are evidences of mineral malnutrition. Many individuals, both in developed and developing countries, are failing to attain recommended mineral intakes [41]. In most cases, these could be prevented with proper mineral supplementation. In this line, an increased consumption of plant and plant related food would be beneficial. However, only 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 [42-44]. Thus, alternative strategies and 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 [43-44].

The nutritional value of a diet in terms of macronutrients and trace nutrients is dependent on the bioavailability of the mineral for physiological processes in the organism much more than its contents in the soil and air. The proportion depends on many factors such as species, root distribution of the plant, physical and chemical nature of the soil, proportion and distribution of the element, method of cultivation and general climatic conditions.

1.11 Importance of Minerals in Humans

Minerals are inorganic substances found in the body that function in conjunction 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 3% of the body's weight [45-52].

Minerals in food become part of tissue structure like in bone and teeth. They help maintaining acid-base balance, keep the body pH neutral and regulate body processes such as in enzyme systems; function in nerve impulse transmission and muscle contraction and help releasing energy from food [53].

The increasing use of highly refined foods which are low in minerals, vitamins, etc., contributes to health problems. Nutritional deficiency may lead to disease and the disease may lead to nutritional deficiencies. Many times, minerals are discussed separately but it is important to note that their actions within the body are interrelated; no single mineral can function without the others since they are synergistically related.

1.12 Analysis of metals in lentils

Some transition metals at trace level in our metabolism play effective roles for our healthy life. Heavy metals normally occurring in nature are not harmful, because they are only present in very small amounts. However, if the levels of these metals are elevated, then they can show negative effects. The essential metals can also produced toxic effects when the metal intake is excessively elevated. Due to the importance of trace elements on human metabolism, their analysis is an important part of public health studies [54]. Therefore, in order to be aware about the levels of metals in food, especially in staple foods, their analysis is mandatory.

Metals may be determined satisfactorily by a variety of methods; with the choice often depending on the precision and sensitivity required. Several spectrometric techniques have been used for macro and trace element determinations in plants or biological materials. The different techniques so far reported for the determination of metals in plant products are: Direct current argon plasma optical emission spectroscopy (DCP-OES) [55], Flame atomic absorption spectrometry (FAAS) [55-59], graphite furnace atomic absorption (GFAA) [59], inductively coupled argon plasma optical emission spectrometry (ICP-OES) [59, 60] and inductively

coupled plasma mass spectrometry (ICP-MS) [59-61]. These methods are most commonly used for the determination of materials because of their inherent selectivity, sensitivity, precision and accuracy.

In this project, flame atomic absorption spectroscopy, FAAS, is used for the analysis of metals in lentil samples. Most determination methods require pretreatment of samples before analysis. One of these sample pretreatments involve sample matrix break down or sample decomposition.

1.13 Sample Decomposition

Sample dissolution is an integral part of sample treatment due to the intrinsic requirement of most quantitative analytical techniques for samples to be in liquid form. The majority of methods for mineral constituents require the organic matrix of the foods to be removed by extraction and/or concentration before they can be applied. The various techniques for elemental determination do not all require the same degree of sample matrix breakdown. Sample decomposition is useful for 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 and obtaining the element in a homogeneous and easily accessible matrix. The choice of decomposition techniques should take into account the objective of the final determination and factors such as the matrix composition, the elemental contents, the possible interferences, the risk of loses and contaminations, the practicality and possible safety hazards in the laboratory [56]. The different decomposition methods could be classified in to dry ashing, wet digestion and microwave digestion [62].

1.13.1 Dry ashing techniques

Dry ashing is a sample preparation method generally convenient to be applied for subsequent trace metal determination in food materials. Dry ashing or oxidation is usually performed by placing 0.1 to 1 g of the sample in an open vessel and removing the organic matter from the samples by thermal decomposition normally in the presence of an ashing agent using a muffle furnace. Typical ashing temperatures are 450 to 550 ^{o}C at atmospheric pressure and the ash

residues are dissolved in an appropriate acid. The degree of volatilization loss is a limiting factor and depends on: (i) the applied temperature (ii) the form in which the analyte is present in the sample and (iii) the chemical environment in the ashing stage. Oxidizing reagents may be used as ashing aids in order to prevent the volatilization of analytes and also to speed up the ashing process. High-purity magnesium nitrate and magnesium oxide are commonly used for that purpose [63]. The application of dry ashing methods is simple and large quantities of food samples may be treated at the same time. This procedure permits the preconcentration of trace elements in the final solution which is useful when very low concentrations are to be determined. The ash is also completely free of organic matter which is a prerequisite for some analytical addition of an ashing aid; on the other hand, it increases the content of inorganic salts significantly which might be a problem for the subsequent techniques in the determination of trace elements. It might also contribute to contamination necessitating careful blank control.

1.13.2 Wet ashing techniques

Wet digestion methods include sample decomposition by an acid or mixtures of acids, carried out in open vessels, in tubes, on a hot plate or in an aluminum heating block or in closed vessels at elevated pressure (digestion bombs) with thermal or microwave heating. The applicability of this technique is strictly dependent on the type of food; carbohydrates are easily mineralized with nitric acid at 180 ^{o}C, while fats, proteins, and amino acids cause incomplete digestion due to the relatively low oxidation potential of nitric acid at 200 ^{o}C; these materials require the addition of sulfuric and/or perchloric acid with all the problems related to their use at high temperature and pressure. The type of acid used in the preparation procedure can have important consequences in the measurement step. It is commonly known that in all atomic spectrometric techniques nitric acid is the most desirable reagent. In spite of the occasionally observed signal suppression in its presence (e.g., in ICP-OES), no severe analytical problems are encountered in practice with nitric acid at concentrations up to 10%, sometimes higher, in all atomic spectrometric techniques as long as its concentration is similar in calibration and sample solutions. Hydrogen peroxide, added in most mineralization procedures, is also rarely responsible for analytical problems [64]. The presence of hydrochloric acid is not troublesome in ICP OES analysis; however, its exclusive use is prohibited in GFAAS analysis because of the

possible formation of volatile and difficult-to-dissociate analyte chlorides that could cause vapor phase and/or spectral interference [65]. Because of its high viscosity, utilization of sulfuric acid is usually avoided in spite of its efficiency in digestion of organic matrices. Its presence is particularly undesirable in analytical techniques where the sample introduction is by nebulization (FAAS, ICP-OES and ICP-MS).

1.13.3 Microwave Assisted Digestion

Microwave assisted digestion with nitric acid, mixtures of nitric and hydrochloric acids without or with the addition of hydrogen peroxide is a widely used technique for the dissolution of food samples. Microwave heating has several advantages over conventional

heating on a hot plate as the energy is generated in the digestion mixture and not transferred by conduction. Among the key advantages of microwave assisted digestion are the much shorter digestion times and the reduced need for aggressive reagents to obtain complete digestion. There are two different systems available for microwave assisted digestion: pressurized closed vessel systems and open focused micro wave systems that work under atmospheric pressure. Microwave assisted digestion in closed vessels under pressure has gained popularity as a simple and fast dissolution technique that minimizes acid consumption, the risk of sample contamination, and loss of volatile elements. One of the limitations is the time required for cooling before the vessels can be opened, which may take hours, depending on the type of equipment used. The main advantages of open focused micro wave radiation are safety, versatility, control of microwave energy released to the sample, and the possibility for programmed addition of solutions during the digestion. However, loss of volatile elements cannot be excluded in open vessel digestion, and the results of low level elements might be affected by the high amount of reagents used and hence the increased risk of sample contamination. This risk can be minimized by using vapor phase acid digestion which has proven to be very effective in minimizing the residual carbon content [66].

In this study, the levels of the essential trace metals(Co, Cu, Fe, Mn, Ni, Zn) and toxic heavy metals (Pb and Cd) in lentil samples freshly collected from different farms in Ethiopia will be determined using flame atomic absorption spectroscopic technique.

2 SIGNIFICANCE and OBJECTIVES OF THE STUDY

2.1 Significance of the study

Ethiopia is a major lentil producing country in Africa but with low productivity yet. Lentil produced in the country is still used as a supplemental food despite its excellent protein, carbohydrate, fiber and minerals contents. The fact that no analysis was made on the levels of essential and toxic minerals in lentils produced in Ethiopia makes this project work significant and initiative for further detailed research.

2.2 Objectives of the Study

Since Lentils serve as the most important supplemental food for many peoples of Ethiopia, the knowledge of its mineral nutrition is of particular interest. Although little information is available on the levels of all essential elements in the Ethiopian lentil in the literature, several investigations have been carried out on the levels of many metals in other samples using different methods such as ICP-OES [62]67 and FAAS [62,67] employing dry ashing or wet digestion techniques. Since no single mineral can function independently, the presence or absence of other essential elements can, in one way or another, affect the activity of the other element in the living body. Therefore, the determination of the levels of mineral elements, which are potentially related, is necessary.

2.2.1 General objective

The purpose of the study is to determine the levels of eight selected metallic elements in lentil samples collected from three different locations of the country.

2.2.2 Specific objectives

The specific objectives of this study are to:

- develop a suitable method for lentil sample digestion
- determine the levels of Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn in lentil samples
- compare the levels of these metals in lentil samples
- compare the levels of metals determined in lentil with other similar legume crops and

3 EXPERIMENTAL

3.1 Equipment and Reagents

3.1.1 Equipments

A drying oven (DIGITHEAT, J.P.SELECTA, S.a, Spain) was used to dry the washed lentil seed samples. Mortar and pestle was used to grind and powder the dried lentil samples. A digital analytical balance (Mettler Toleado, Model AG204, Switzerland) with $\pm$0.0001 g precision was used to weigh the lentil samples. 100 mL round bottomed flasks fitted with reflux condensers were used in Kjeldahl apparatus hot plate to digest the dried and powdered lentil samples. A refrigerator (Hitachi) was used to keep the digested samples cool till analysis. BUCK SCIENTIFIC MODEL 210 VGP (East Norwalk, USA) atomic absorption spectrophotometer equipped with deuterium arc background correctors were used for analysis of the analyte metals using air-acetylene flame.

3.1.2 Reagents and chemicals

Reagents that were used in the analysis were all analytical grade. HNO_3 (69-72%) and $HClO_4$ (70%) [RESEARCH-LAB FINE CHEM INDUSTRIES MUMBAI 400 002 (INDIA)] were used for the digestion of lentil samples. Stock standard solutions containing 1000 mg/L, in 2 % HNO_3, of the metals Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn (BUCK SCIENTIFIC PURO-GRAPHIC^{tm}) were used for the preparation of calibration standards and spiking experiments.

De-ionized water was used throughout the experiments for sample preparation, dilution and rinsing apparatus prior to analysis and during analysis.

3.2 Procedures

3.2.1 Cleaning Apparatus

Apparatus such as volumetric flasks, measuring cylinders and digestion flasks, and all the necessary materials used for the experiment were washed with detergents and tap water, rinsed with deionised water, soaked in chromic acid solution for 24 hrs, rinsed with de-ionized water many times and kept in dust free place until digestion.

3.2.2 Collection and Preparation of lentil samples

3.2.2.1 Collection of samples

Recently harvested lentil samples were collected from three locations of the country: Dejen area (Gojjam), Welenchitti area (East Shewa) and Menz-mama Midir (North Shewa, Amhara). As the reliability and accuracy of analytical information largely depends on the history of the sample [68], efforts were made to record necessary information about the sample for later consideration. The samples were packed into Polyethylene plastic container bags, labeled and transported to the laboratory for further treatment.

3.2.2.2 Sample Preparation

Each of the lentil samples are thoroughly washed with tap water and there after rinsed in distilled water so as to remove surface contaminants like soil, dust and spray residues. The samples are then placed in acid washed clean porcelain crucibles labeled according to the sample and oven dried at 85 ^{0}C for 48 hrs in drying oven (DIGITHEAT, J.P. SELECTA, S.A. SPAIN). At this stage, care was taken to avoid any source of contamination, especially

micronutrients. The dried lentil samples were ground and homogenized into fine powder with a grinding device (MOULINEX, FRANCE) and stored in polyethylene bags for digestion.

3.2.3 Digestion of lentil Samples

For lentil samples, in most cases, dry ashing [69] and wet digestion [62] are commonly used for analysis by FAAS. Different combinations of mineral acids have been employed for the decomposition of lentil flour by wet digestion. Mustafa S. *et al* [62] used a procedure involving mixtures of 12 mL HNO_3 and H_2O_2 (2:1 volume ratio) to digest lentil for four hours until they found a colorless solution at a temperature of 350^0C.

In this study, for the digestion of lentil flour sample, different conditions such as digestion time, volume ratio of reagents and digestion temperature were tried to achieve optimum conditions, as indicated in Table 3 a-c. The optimum conditions for lentil sample digestion are Nitric acid, Perchloric acid and Hydrogenperoxide mixture with (4:1:1v/v) ratio, digestion temperature of 300^0C and digestion duration of 3 hrs. Applying the optimized procedure, 0.5 g of dried and homogenized lentil sample was transferred into a round bottomed flask. Then 6 mL mixture of HNO_3 (69-72%), $HCIO_4$ (70%) and H_2O_2 (4:1:1v/v) was added and the mixture was digested in the Kjeldahi digestion apparatus by setting the temperature first at 120^0C for 30 minutes and them increasing to 300^0C for the remaining 2 1/2 hrs. Then after, the digested solution was allowed to cool for 10 min without dismantling the condenser from the flask and for 5 min after removing the condenser. To the cooled solution, deionized water was added to dissolve the precipitate formed on cooling. Then the solution was poured into 50 mL volumetric flask. The volumetric flask was filled to the 50 mL mark with deionised water. Blank solutions were prepared following the same digestion procedure as for the sample. Triplicate samples and sextet blanks were digested. The digested samples were kept in the refrigerator, until the level of all the metals in the sample solutions were determined by FAAS.

3.2.4 Determinations of the essential and toxic metals

Secondary standard solutions containing 10 mg/L were prepared in 1000 mL volumetric flask from the atomic absoroption spectroscopy standard stock solutions that contained 1000 mg/L. Three working standards for each metal of interest were prepared from these secondary standards. These working standards were prepared freshly for each element from the secondary standards by appropriately diluting with deionized water for calibration purpose as shown in Table 4. Then, Mn, Co, Cu, Zn, Ni, Cd, Pb and Fe were analysed by FAAS 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) were optimized for maximum signal intensity of the instrument. For each element, respective hollow cathode lamp was inserted in to the atomic absorption spectrophotometer, and the solution was successively aspirated into the flame. The acetylene and air flow rates were managed to ensure suitable flame conditions. Three replicate determinations were carried out for each sample. The elements were determined by absorption/concentration mode and then the instrument readout was recorded for each solution manually. The same analytical procedure was employed for the determination of elements in the six digested blank solutions. The instrument operating conditions for FAAS employed for each analyte are given in Table 2.

Table 2 Instrument parameters for Mn, Co, Cu, Zn, Ni, Cd, Pb and Fe determination in lentils by FAAS

Element	WL (nm)	SW (nm)	LC (mA)	LR (mg/l)	Energy (erg)
Cd	229	0.7	2.0	1.12	3.07
Co	241	0.2	4.5	5.25	2.75
Cu	325	0.7	1.5	3.00	3.33
Fe	248	0.2	7.0	3.75	3.23
Mn	280	0.7	3.0	1.87	3.97
Ni	232	0.2	7.0	5.25	2.93
Pb	217	0.7	3.0	15.00	3.13
Zn	214	0.2	2.0	0.75	3.10

4 RESULTS AND DISCUSSION

4.1 Optimization of digestion of lentil samples

A series of procedures involving some changes in reagent volume, reagent composition, digestion temperature and time were tested. Accordingly, sixteen procedures were tested for digestion of the lentil samples. This procedure was developed with some modification of the procedure used to determine the levels of trace metal contents in commercial powdered soup samples by atomic absorption spectroscopy [62]. The optimized procedures and conditions indicated in (Tables 3a -c) were used throughout the analysis.

Table 3a Optimized reagent volumes for digestion of 0.5 g of lentil samples

Trials	Reagents	volume Ratio (mL)	Observations
1	HNO_3	6	Clear yellow
2	HNO_3:$HClO_4$	5:1	Light yellow
3	HNO_3:$HClO_4$	4:2	Light yellow
4	HNO_3:$HClO_4$	3:3	Clear/suspension
5	HNO_3:$HClO_4$:H_2O_2	4:1:1	Clear (optimized)
6	HNO_3:H_2O_2	4:2	Yellowish

Table 3b Optimized temperature for digestion of 0.5 g lentil samples in HNO_3/$HClO_4$/H_2O_2 mixture

Trials	Temperature (^oC)	Observations
1	180	Yellow
2	210	Light yellow
3	240	Clear suspension
4	270	Clear suspension
5	300	Clear (optimized)
6	330	Clear

Table 3c Optimized Time for digestion of 0.5 g lentil samples in $HNO_3/HClO_4/H_2O_2$ mixture

Trials	Time (hr)	Observations
1	2:00	Yellow
2	2:30	Clear yellow
3	3:00	Clear (optimized)
4	3:30	Clear

The optimized procedure was selected depending upon: clarity of digests, minimal reflux time/digestion time, minimal reagent volume consumption, absence of undigested lentil samples, simplicity and acceptable use of masses of lentil samples. Based upon these criteria, the optimal digestion procedure chosen was the one that requires 3 h for complete digestion of 0.50 g of lentils powders with 4 mL HNO_3 (69-72%), 1 mL $HClO_4$ (70%) and
1 mL H_2O_2 as indicated in Table 3a. However, the other tested procedures have some limitations. They require higher reagent volume, longer digestion time and higher temperature. In addition, they result in the formation of turbid digests and coloured digested solutions. As wet digestion is used, it is necessary to prepare reagent blanks for each digestion employed. Reagent blanks were also prepared and digested usig the same procedure as for the sample and were used to correct for impurities present in the acid and water.

4.2 Instrument calibration

The qualities of results obtained for essential metals analysis using FAAS are seriously affected by calibration and standard solution preparation procedures. The instrument was calibrated using three series of working standards. The working standard solutions of each metal were prepared fresh by diluting the intermediate standard solutions. Concentrations of the working standards and value of correlation coefficient for each metal is shown in Table 4.

Table 4 Series of working standards and correlation coefficients of the calibration curves for the determination of metals in the lentil varieties using FAAS

Metal	Concentration of Standards (mg/L)	Regression Equation (A* = mC + b)	Correlation coefficient (R)
Cd	0.25, 0.50, 1.00	$A = 0.10C - 0.006$	0.9993
Cu	0.50, 1.00, 1.50	$A = 0.05C - 0.0013$	0.9968
Co	0.25, 0.50, 1.00	$A = 4.28C + 9.1 \times 10^{-5}$	0.9995
Pb	1.20, 2.40, 4.80	$A = 5.63C \times 10^{-4} - 7.73 \times 10^{-6}$	0.9991
Mn	0.25, 0.50, 1.00	$A = 0.02C + 1.13 \times 10^{-4}$	0.9999
Ni	0.25, 0.50, 1.00	$A = 8.8 \times 10^{-3}C + 8.45 \times 10^{-5}$	0.9996
Zn	0.20, 0.40, 0.80	$A = 0.10C + 4.5 \times 10^{-6}$	0.9997
Fe	0.50, 1.00, 1.50	$A = 2.30 \times 10^{-3} C + 1.33 \times 10^{-5}$	0.9992

* A = Absorbance, C = Concentration in mg/L

4.3 Evaluation of analytical results

4.3.1 Precision

The precision of a method is the degree of closeness of the results and is usually reported as percentage relative standard deviation. It is often subdivided into repeatability and reproducibility. Repeatability expresses the closeness or agreement between a series of measurements obtained from multiple sampling and sample analysis. It is often expressed as the standard deviation or relative standard deviation of replicate measurements.

For the percentage RSD to be ideal in terms of statistical significance, it would be necessary to make quite a large number (in the order of 20) of measurements. However, as a compromise to save time, it is quite usual to make 6-10 repeated measurements. Similarly, there are pragmatic compromises that may be made in assessing reproducibility. What ideally required is to carry out replicates of the complete assay in different laboratories or on different days of experiment and find the percentage RSD of the results.

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, nine 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 the corresponding pooled standard deviation of nine measurements for a bulk sample and triplicate reading per sample and relative standard deviation as given in Table 6.

4.3.2 Method detection limit

Two important parameters in method validation are limit of detection and limit of quantification. 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. It is the concentration that gives a signal three times the standard deviation of the blank or background signal. Six blank samples were digested following the same procedure as for the samples and each of the samples were determined for the elements of interest (Mn, Co, Ni, Cd, Pb, Zn, Fe, and Cu) by atomic absorption spectrophotometery. The standard deviation for each element was calculated from the six blank triplicate measurements to determine method detection limit. In this study the detection limit of each element was calculated as three times the standard deviation of the blank ($3S_{blank}$, n=9), and is summarized in Table 5. As can be seen from the table, the method detection limit for each element is above the instrument detection limit.

4.3.3 Method limits of quantization

Limit of quantification is the lowest concentration of the analyte that can be measured in the sample matrix at an acceptable level of precision and accuracy. It is the same as the concentration which gives a signal ten times the standard deviation of the blank. Limit of quantification is the lowest limit for precise quantitative measurements. The quantification limit of each element was calculated as ten times the standard deviation of the blank ($10S_{blank}$, n=9). The results are summarized in Table 5.

Table 5 Method detection and quantization limit (n=9, DLM=3S_{blank} and MQL=10S_{blank} in mg/kg) for all metals determined in lentil samples.

Metal	MDL (mg/100g)	MQL (mg/100g)	DL (mg/l)
Cd	0.014	0.047	0.05
Co	0.11	0.37	0.05
Cu	0.045	0.15	0.02
Fe	0.09	0.30	0.03
Mn	0.10	0.34	0.01
Ni	0.054	0.18	0.04
Pb	0.019	0.063	0.01
Zn	0.032	0.11	0.005

4.3.4 Recovery test of the optimized procedure

Method validation is a way of testing a particular analytical method to see if it is suitable for its intended purpose. The validation process begins in method development in that the documentation must include a record of the method development process giving details of the conditions explored by the rationale in the progress of the process. The efficiency of the optimized digestion procedure was checked by adding known concentrations of each metal in 0.5 g sample. For the recovery analysis, 100, 50, 50, 50, 50, 50, 50 and 100 μg of Mn, Co, Ni, Cd, Pb, Cu, Zn and Fe respectively were spiked to the samples all at once. Each recovery test for the sample was performed in triplicates. Standard metal solutions were used to fortify the sample to the specified metal given in Table 6 and the percentage recovery was calculated using equation (1).

R = [(Amount after spike – amount before spike)/ Amount added] x100% ---------- (1)

Recoveries of the metals in the spiked lentil sample are between 89 % and 101.5 %. The results of this recovery test are indicated in Table 6. The mean percentage recoveries for all analytes were within an acceptable range (75-125%), indicating the laboratory performance for each analyte is in control. Recovery values in the above range are acceptable for both bulk and trace analysis and the digestion procedure is believed to remove metal fractions associated with organic matter. The lower recovery of cobalt may be due to incomplete digestion of the standard samples while the high recovery value of iron could be attributed to either contamination or incomplete digestion of the lentil samples.

Table 6 Analytical results for Recovery test of the optimized procedure for lentil samples

	Concentration (mg/100g)		
Metal	Added	Found	Recovery (%)
Cd	0.0	0.012 ± 0.0009	
	5.0	4.63 ± 0.32	92.4
Co	0.0	0.30 ± 0.014	
	5.0	4.75 ± 0.48	89.0
Cu	0.0	0.24 ± 0.004	
	5.0	5.23 ± 0.053	97.6
Fe	0.0	9.70 ± 0.32	
	10.0	19.85 ± 1.3	101.5
Mn	0.0	7.83 ± 0 .11	
	10.0	17.38 ± 1.7	95.5
Ni	0.0	0.23 ± 0.013	
	5.0	4.93 ± 0.30	94.0
Pb	0.0	0.16 ± 0.008	
	5.0	4.93 ± 0.39	95.4
Zn	0.0	9.97± 0.13	
	5.0	14.90 ± 1.1	98.6

4.4 Levels of metal contents in the analyzed lentil samples

Table 7 and figures 3(a-c) show the concentrations of various heavy metals such as Cadmium, Cobalt, Copper, Iron, Nickel, Lead and Zinc in the different lentil samples.

Cadmium

In the present investigation, the values of Cadmium range from 0.009 to 0.013 mg/100 g in various lentil samples. The maximum concentration (0.013 mg/100 g) of cadmium was recorded in samples collected from Dejen and Boset, while minimum concentration (0.009 mg/100g) was registered in lentil samples of Molale. Acute doses (10-30 mg/kg/day) of cadmium can cause severe gastrointestinal irritation, vomiting, diarrhea and excessive salivation, and doses of 25 mg of cadmium per kg body weight can cause death. Low level chronic exposure to cadmium can cause adverse health effects including gastrointestinal, hematological, musculoskeletal, renal, neurological and reproductive effects. The main target organ for cadmium following chronic oral exposure is the kidney [70]. Intake of cadmium can double if one smokes cigarettes because each cigarette contains about 0.2 mg/100 g cadmium, on the average [71].

Cobalt

Cobalt is one of the most important trace elements in the world of animals and humans. In the form of vitamin B_{12} (cobalamin), this metal plays a number of crucial roles in many biological functions. Cobalamin is necessary for DNA synthesis, formation of red blood cells, and maintenance of the nervous system, growth and development of children. The cobalt content in this study varies from 0.285 to 0.360 mg/100g. The lowest concentration (0.285 mg/100g) of cobalt was observed in lentils from Molale and Boset. On the other hand, lentil from Dejen showed highest concentration of cobalt (0.360 mg/100 g). Deficiency of cobalt in diet results in pernicious anemia, severe fatigue, shortness of breath and hypothyroidism, while overdose may lead to angina, asthma, cardiomyopathy, polycythemia and dermatitis. The safety limit for human consumption of cobalt is 0.05 to 1 mg/day in humans [72]. Thus the analyzed range of cobalt concentrations in lentils during the present investigation falls within the safety limit.

Copper

The acceptable limit for human consumption of copper is 1 mg/100 g [73]. When copper concentration exceeds its safe level, it causes hypertension, sporadic fever, uremias, coma etc. The present investigation reveals that the concentration of copper varies from 0.226 to 0.282 mg/100 g, which falls below the safe limits for human health and hygiene. The highest concentration of copper was found in Dejen lentil (0.282 mg/100 g), while the lowest concentration 0.226 mg/100 g was recorded in lentil from Boset. As it falls below the safety limit, lentils which contain copper can be consumed without any risk.

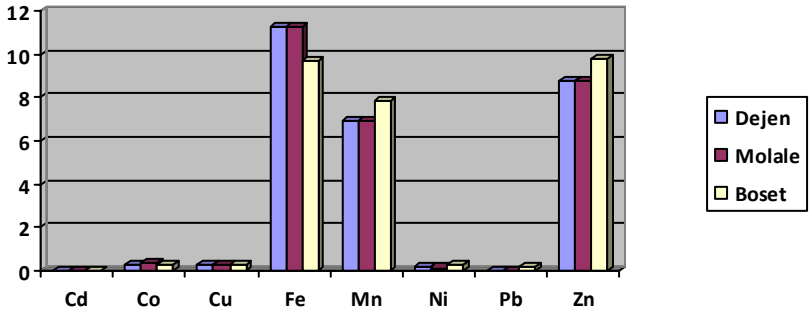

Fig 3 levels of metals contents(mg/100g) in various lentil samples

Iron

Iron is an essential element in the production of red blood cells. In the results, iron content was highest in Molale lentil (11.91), while it was found lowest in lentil from Boset (9.17 mg/100 g). The iron content ranges from 9.17 to 11.91mg/100 g. Low intake of iron may cause anemia, tiredness and pallid physique, while high intake may result into hepatic megaly, cardiac infraction and nephric malfunction. The acceptable limit for human consumption of iron is 8 to 11 mg/day for infants as well as adults [72]. In the present investigation, the value of iron was found slightly higher in lentil from Dejen.

Nickel

Nickel is found in soyabeans, lentils, nuts, grains and vegetables. Lentil from Boset showed high content of nickel (0.244 mg/100 g), while the lentil from Molale contain low value of nickel (0.120 mg/100 g). The content of nickel ranges from 0.120 to 0.244 mg/100 g in the different lentil samples. Deficiency of nickel has been linked with hyperglycemia, depression, sinus congestion, fatigue, reproductive failures and growth problems in humans, while excess intake leads to hypoglycemia, asthma, nausea, headache, and epidemiological symptoms like cancer of nasal cavity and lungs. The prescribed safety limit of nickel is 3 to 7 mg/day in humans [74]. In this study, the contents of nickel lie below the safety limit and the lentils can be consumed without any risk.

Lead

In the present study, the lead content varies from 0.142 to 0.176 mg/100 g, which slightly exceeds the safety limit (0.15 mg/100 g) for human consumption [74]. Lead was not detected in Dejen and Molale lentils while Boset lentil showed 0.155 mg/100 g of lead. Todd [75] emphasized that most of the accumulated lead is sequestered in the bones and teeth. This causes brittle bones and weakness in the wrists and fingers. Lead stored in bones can re-enter the blood stream during periods of increased bone mineral recycling (i.e., pregnancy, lactation, menopause, advancing age, etc.). The mobilized lead can be redeposited in the soft tissues of the body and can cause musculoskeletal, renal, ocular, immunological, neurological, reproductive, and developmental effects.

Zinc

Among all metals, zinc is the least toxic and essential element in the human diet as it is required to maintain the proper functions of the immune system. It is also important for normal brain activity and is fundamental in the growth and development of the foetus. Zinc deficiency in the diet may be more detrimental to human health than too much zinc in the diet. Although the average daily intake of zinc is 7-16.3 mg per day, the recommended dietary allowance is 15 mg

per day for men and 12 mg per day for women [72]. On the contrary, the high concentration of zinc in foods may cause vomiting, renal damage, cramps etc. The acceptable limit for human consumption of zinc is 15 mg/100 g [73]. In the present study, the concentration of zinc was found to be high in lentils from Boset (10.03 mg/100 g), while low concentration of zinc was observed in lentils from Molale (8.62 mg/100 g). The level of zinc ranges from 8.62 to 10.03 mg/100 g, which falls within the range of the recommended daily intake. The levels of minor and trace metals in Dejen and Boset lentils are shown in figures 3a and 3b respectively.

Manganese

Manganese is important for the synthesis of organic substances in plants and for the metabolism of a number of nutrient elements in plants. Plants need small amounts of manganese, from 0.0001% to 0.02%, and it is indispensable for their normal growth and development. For most cultivated plants, the critical concentration of manganese in leaves is 25 mg kg^{-1} dry matter [76]. In this study, the concentration of manganese ranged between 6.95 and 7.83 mg/100 g. The trend of concentration of various heavy metals in lentil samples studied in this work is as follows: Fe > Zn > Mn > Cu > Co > Ni > Pb > Cd.

Table 7 Average Levels of essential and toxic Metals (mg/100 g) in powdered lentil samples

	Dejen		Molale		Boset	
Metal	Mean*	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
Cd	0.0122 ± 0.0006	4.75	0.0103 ± 0.0008	7.26	0.0115 ± 0.0009	8.21
Co	0.31 ± 0.013	4.19	0.32 ± 0.012	3.75	0.30 ± 0.014	4.67
Cu	0.27 ± 0.003	1.1	0.25 ± 0.0012	0.47	0.24 ± 0.004	1.7
Fe	11.26 ± 0.32	2.84	11.25 ± 0.53	4.71	9.7 ± 0.32	3.3
Mn	6.95 ± 0.09	1.27	6.96 ± 0.20	2.88	7.83 ± 0.11	1.4
Ni	0.208 ± 0.012	5.76	0.132 ± 0.008	5.82	0.232 ± 0.013	5.62
Pb	BDL	-	BDL	-	0.155 ± 0.008	5.43
Zn	8.79 ± 0.11	1.25	8.73 ± 0.08	0.95	9.79 ± 0.13	1.33

4.5 Comparison of metal contents of lentil with other legumes

Like lentil, other legumes such as haricot bean, kidney bean, chick pea, and broad bean are basic food supplements that are rich in protein, carbohydrate, fiber and mineral nutrients. People sometimes use mixtures of two or more of these legumes with other cereal crops to enhance the nutritional values of their diets. Comparison of the values for lentil with other legumes is therefore very essential to know the dietary mineral intake of individuals who use legumes in their diets.

Many researchers have reported the levels of mineral nutrients in legumes as shown in table 12. From the table, it is possible to see that the concentration range of some metals is higher in lentil than other legumes. The concentration of iron in lentils (64.6-74.7) is the highest exceeded only by broad beans (77.8-82.4). Lentils are also the richest sources of the essential element, zinc, when compared to other legumes. The levels of cadmium, copper, nickel and lead in lentil and the other legumes are closely comparable [77].

Table 8 Comparison of metal contents of lentils with other legumes (mg/kg)

	Legumes				
Element	Lentil	Haricot Bean	Kidney Bean	Chick Pea	Broad Bean
Cd	0.008-0.01	nd-0.009	nd-0.011	0.004-0.015	0.007-0.016
Co	8.26 ± 2	-	-	-	-
Cu	2-3.3	2.4-3.7	2.6-3.5	3.2-4.9	3.9-5
Fe	64.6- 74.7	60.3-66.7	62-66.6	65.0-70.2	77.8-82.4
Mn	7.41-19.0	-	-	-	-
Ni	0.10-0.33	0.12-0.29	0.10-0.20	0.20-0.35	0.12-0.19
Pb	0.40-0.61	0.40-0.61	0.50-0.70	0.50-0.70	0.36-0.48
Zn	45.1-70.20	35.4-45	38.8-50.20	37.4-42.8	42.8-50

4.6 Comparison of the analyzed concentration of metals in lentil samples with reported (literature) values

There are numerous reports on the levels of cadmium [62, 77], cobalt [80], copper [62, 77, 81] iron [62, 77, 78, 81] manganese [62, 79] nickel [77], lead [62, 77] and zinc [62, 82] in lentil. The comparison of the values determined in this study with the reported values is presented in Table 9. From the table, one can see that the concentrations of the metals analyzed in this study are, in most cases, in the range of different reported values although results of this project are close to the lower margin of the range probably due to differences in instrumentation, lentil variety and soil type.

The level of cobalt reported in [80] (8.26 ± 0.2) is much higher than the value obtained in this study (0.285-0.360). The literature value may be higher due to contamination during the manufacturing process of the food items. The cadmium contents reported in [77] (0.013-0.024) are relatively in good agreement with the values in this study (0.009-0.013). The iron content determined in this work is also comparable with those values reported in [81]except that the maximum value in the literature is the highest.

Table 9 Comparison of the analyzed concentration of metals in lentil samples with literature values

	Concentration (mg/100 g)		
Metal	Analyzed	Reported	References
Cd	0.009-0.013	0.009-0.50	62, 77, 83
Co	0 .285-0.36	8.26 ± 0.2	80
Cu	0.226-0.282	0.250 - 0.770	62, 77, 83
Fe	9.17-11.91	0.63-61.7	62, 77, 78, 81, 83
Pb	0.142-0.176	nd-0.5	62, 77, 83
Mn	6.7-8.2	7.41-19.0	62, 79, 83
Ni	0.120-0.244	0.24	82
Zn	8.62-10.03	6.11-58	62, 81, 82, 83, 84

All the metals analyzed in this project are in close agreement with values of the lower range in the respective literatures. This close relationship between the results in this study and the literature values indicate the reliability of the present analysis on lentil.

5 STATISTICAL ANALYSES

Differences between the mean values of the various samples obtained in this study were evaluated by student's paired *t*-test. Linear regression statistical test and correlation analysis were performed for the calculation of the slope (m), and correlation coefficient (R) of the regression line as shown in table 4. Statistical analysis was based on triplicate measurements of all samples.

In pairwise student's t-test, the term on the right side of equation 2 is computed using t-test values for the particular confidence level desired. The number of degrees of freedom for finding the t value is $(N_1 + N_2) - 2$, where N_1 and N_2 are number of replicate measurements of sample 1 and sample 2 respectively

$$x_1 - x_2 = t\, S_d[(N_1 + N_2)/ N_1N_2]^{1/2} \quad \text{----------(2)}$$

If the experimental mean difference, $x_1 - x_2$, is smaller than the computed value, no significant difference between the two means is demonstrated. An experimental difference greater than the value computed from *t* indicates that there is a significance difference between the means.

Table 10 Pairwise comparison between mean values of various lentil samples by student's *t*-test at the 95 % confidence level

Samples	parameters* for comparison	Metals						
		Cd	Co	Cu	Fe	Mn	Ni	Zn
Dejen, Molale	D (m)	0.0009	0.016	0.019	0.01	0.012	0.076	0.066
	t_p	0.002	0.026	0.015	1.04	0.34	0.023	0.18
	conclusion	NS	NS	S	NS	NS	S	NS
Dejen, Boset	D (m)	0.001	0.008	0.38	1.55	0.89	0.023	1.00
	t_p	0.0007	0.014	0.15	1.00	0.72	0.025	0.41
	conclusion	S	NS	S	S	S	NS	S
Molale, Boset	D (m)	0.0012	0.024	0.19	1.55	0.88	0.10	1.1
	t_p	0.0008	0.029	0.096	0.39	0.76	0.008	0.18
	conclusion	S	NS	S	S	S	S	S

** D (m): differences between means,* $t_p = tS_d[(N_1+N_2)/N_1N_2]^{1/2}$ *at 95 % confidence level* ($N_1=N_2=3$), *S: significant, NS: not significant*

As shown in table 12, student's t-test at 95 % confidence level indicated that there were significant differences between the mean values of Dejen and Molale samples in copper and nickel. Significant differences were observed between Dejen and Boset samples in most metals except cobalt and nickel. Molale and Boset samples showed significant differences in all metals except cobalt.

6 CONCLUSIONS AND RECOMMENDATION

An efficient and simple digestion procedure was developed for the analysis of lentil flour and validated by the standard addition method. The optimized digestion procedure allowed the use of mixtures of small volumes of HNO_3, $HClO_4$ and H_2O_2 (4:1:1 v/v) respectively, leading to reduced blank contamination and lower method detection limit.

The levels of six essential and two toxic metals were determined in lentil samples collected from different parts of the country by FAAS method. A pairwise student's *t*-test at the 95 % confidence level revealed that there were no significant differences between the mean values of the mineral contents of lentil samples from Dejen and Molale. It also indicated that the random errors associated in sample preparation and measurement steps are not significant indicating the reliability and applicability of the proposed method for these samples.

Although the data obtained in this study is not sufficient to draw authoritative conclusions about the metal contents of lentil in Ethiopia, it will provide base line information for further investigation. It also gives good awareness for those people who have little concern about the nutritional status of lentils.

To draw remarkable conclusions about the nutritional values of lentils, further investigations including the physical and chemical composition of soils, the applications of fertilizers, herbicides and insecticides, agro-climatic conditions, harvest and storage mechanisms and transport systems are needed.

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Declaration

This project is my original work. It is not presented for a degree in this and other universities. All resources and materials used for this project work are duly acknowledged.

Sintayehu Lehse Kitaw

The project has been submitted for examination with my approval as a university advisor.

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June 2010