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STUDIES ON THE EFFECT OF COOKING ON SELECTED METALS, OXALATE AND PHYTATE CONTENTS OF THE RAW AND COOKED LETTUCE FROM FIVE FARMS IN ETHIOPIA

BY AYNALEM LAKEW

JUNE, 2015

ADDIS ABABA, ETHIOPIA

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ADVISOR: PROF. B.S. CHANDRAVANSHI

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ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

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Declaration

I, the undersigned, declared that this is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged.

Name: Aynalem Lakew Asfaw
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Signature: ……

Place and date of submission: Research and Graduate Programs
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Abstract

The effect of cooking on mineral composition and anti-nutritional factors, oxalate and phytate, of lettuce grown in selected area of Ethiopia was investigated in this study. In addition, the effect of boiling at different time intervals on the mineral compositions, the bioavailability of Ca, Fe and Zn by the molar ratios of [Phy]:[Fe], [Phy]:[Zn], [Ca]:[Phy], ([Ca][Phy])/[Zn] were investigated. The mineral composition were found to be; 1557-3171, Ca; 13.8-14.7, Mg; 0.7-2.8, Zn; 21.4-123, Fe; 3.94-9.41, Mn; 0.39-1.19, Cu; ND-0.24, Co; and 1.46-2.63, Ni; in mg/100 g in the raw lettuce samples. They all show decreasing by boiling except Fe, Zn and Ca where they show a bit increment depending on boiling time interval. The anti-nutritional factor to mineral ratio tends to imply that the relative bioavailability of the minerals after boiling was found to be increased. These may present health-hazard potential, which in turn demands proper processing before consumption to eliminate the toxic effects of anti-nutritional factors.

Keywords: Effect of boiling, Bioavailability, Mineral composition, Anti-nutritional factors, Lettuce, Molar ratios.

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List of abbreviation and acronyms

AAS	Atomic Absorption Spectrometry
AAU	Addis Ababa University
FAO	Food and Agricultural Organization
g	gram
g/mol	gram per mole
ha	hectare
km	kilo meter
km^2	square kilometer
LSD	least significant difference
M	molar
mg/100 g	milligram per hundred gram
mg/day	milligram per day
mL	milliliter
mm	milimeter
^oC	degree Celsius
Ox	Oxalate
Phy	Phytate
ppm	parts per million
rpm	Revolution per minute
SD	Standard deviation
SPSS	Statistical and Presentational System Software
v/v	volume by volume
w/w	weight by weight

1. Introduction

1.1 Background of the study

Plant foods are the major staples of diets in developing countries like Ethiopia, in which the consumption of animal-source foods is often low because of economic and/or religious concerns. Vegetables constitute an important part of the human diet since they contain carbohydrates, proteins, as well as vitamins, minerals, and trace elements (Dastane, 1968). Until recently however, they did not constitute a major part of the Ethiopian diet, except during the fasting period. However, in recent years their consumption is increasing gradually, particularly among the urban community. This may be due to increased awareness on the food value of vegetables, as a result of exposure to other cultures or acquiring proper education.

Vegetables grown in the Addis Ababa area include: potato (*Solanum tuberosum* L.), Swiss chard (*Beta vulgaris* L. var. cicla), carrot (*Daucus carota* L.), cabbage (*Brassica oleracea* L. var. capitata), Ethiopian kale (*Brassica carinata* A. Br.), lettuce (*Lactuca sativa* L.), cauliflower (*Brassica oleracea* L. var. botrytis) and red beet (*Beta vulgaris* L. var. vulgaris). These are often grown on the embankments along the major rivers within Addis Ababa city itself and the neighboring towns (Itanna, 2002).

Lettuce (*Lactuca sativa* L.) belongs to the Composite (sunflower or daisy family). It is an annual plant native to the Mediterranean area. Cultivation may have started as early as 4500 BC, perhaps initially for the edible oil extracted from its seeds. Salad lettuce was popular with the Ancient Greeks and Romans. Cultivated lettuce was probably derived from the so called wild or prickly lettuce (*Lactuca sierriola*). The primitive forms of lettuce were loose and leafy. Firm heading forms became well developed in Europe by the 16th century. Oak leaved and curled-leaf types of various colors were described in the 16th and 17th centuries in Europe. There are 5 types of lettuce: (1) crisphead, (2) butterhead, (3) Cos or Romaine, (4) loose leaf or bunching and (5) stem lettuce (celtuce). Lettuce color for commercial cultivars varies from a yellow-green to dark red and all colors in between. Head lettuce grows best at 15 to 18 ºC (Lettuce Atlantic Provinces Vegetable Crops Production Guide, 2005). The two types of lettuce grown in Addis Ababa are shown in Figure 1.

(i) Red/brown loose leaf lettuce

(ii) Yellow green loose-leaf lettuce

Figure 1. The red/brown and green loose-leaf variety of lettuce.

Lettuce is currently an important crop with useful amounts of several nutrients including vitamins A and C; and minerals calcium and iron. Absorption capacity of heavy metals depends upon the nature of vegetables and some of them have a greater potential to accumulate higher concentrations of heavy metals than others. Lettuce is reported to accumulate more of heavy metals in humans through the edible portion (Intawongse and Dean, 2006). The source of heavy metal in plant is the environment in which they grow and their growth medium (soil) from which heavy metals are taken up by roots or foliage of plants (Okonkwo et al., 2005).

Plants grown in polluted environment can accumulate heavy metals at higher concentration causing serious risk to human health when consumed. Moreover, heavy metals are toxic because they tend to bioaccumulate in plants and animals, bioconcentrate in the food chain and attack specific organs in the body (Akinola et al., 2006). Heavy metals are one of a range of important types of contaminants that can be found on the surface and in the tissue of fresh vegetables (Bigdeli and Seilsepour, 2008). The degree of toxicity of heavy metals to human beings depends upon their daily intake and concentration of heavy metals and amount of vegetables consumed (Amoah et al., 2007).

All form of living matter requires many minerals for their life processes. The animal body requires seven minerals in relatively large amounts such as Ca, Na, Mg, K, P, Cl and S. These are called major minerals. And at least seven in trace amount these are Co, Cu, I, Fe, Mn, Mo and Zn. These are called minor minerals. The minor minerals are not less important than the major ones – all are needed for good health. The deficiency of these minerals means interruption of one or more of the above mentioned body mechanisms. Zn and Fe are two of the micronutrients that are most often deficient in developing countries, with children and women of reproductive age especially at risk of such deficiencies. In children Zn deficiency has been shown to poor growth, impaired immunity, and increased morbidity from common infectious disease and increased mortality. Zn deficiency arises to a large extent from impaired bioavailability of dietary Zn largely attributable to the high phytic acid content of diets. Fe deficiency is the most important cause of nutritional anemia. This arises from the low bioavailability of non-haem Fe caused by phytate and other anti-nutritional factors. Although some diets appear to contain sufficient iron, only a limited proportion of it is assimilated by the gastrointestinal mucus in humans, suggesting that the bioavailability of dietary iron is a major determinant of their status of the body rather than the total iron intake through the diet. When dealing with the absorption of nutrients, their bioavailability must be considered; bioavailability is defined as the proportion of the nutrient in the diet or the food that can be used by the organism (Fairweather-Tait, 1992). The chemical form of iron is an important factor in determining the amount of iron available for absorption. The amount of absorbed iron from food depends on many factors, including dietary ingredients, the source and content of iron in the diet and the body's need for iron (World Health Organization, 1983). Although intake of Ca in developing countries low, it is adequate to meet requirements. However, dietary Ca has been implicated in reducing the bioavailability of Fe both of non-haem and haem (Umeta, 2005).

Plant-based diets are often associated with micronutrient deficits, exacerbated in part by poor micronutrient bioavailability. The diet-related factors have a greater influence on the bioavailability of the micronutrients in plant foods, particularly Ca, Fe and Zn, than on the macronutrients. The absorption of Ca, Fe and Zn is particularly affected. The net effect on the nutrient bioavailability depends on the balance between factors that either inhibit or enhance nutrient absorption and/or utilization in the whole diet. Increasingly, the influence of both synthetic micronutrient fortificants and intrinsic micronutrients on micronutrient bioavailability must be considered (Gibson, 2006).

Phytate or phytic acid (myo-inositol hexakisphosphate) ($C_6H_{18}O_{24}P_6$) molecular wt. 660 g/mol is a naturally occurring compound in plants, where it represents the major storage form of phosphorus. Plant-food-based diets are rich in bioactive compounds, which are believed to be beneficial for the prevention of some chronic diseases. However, these diets are also rich in phytate; this compound can decrease the bioavailability of indispensable nutrients such as iron, zinc and calcium due to its high binding affinities to minerals and caused growth inhibition. Phytic acid exerts its inhibitory effect on the absorption of minerals by forming insoluble and indigestible complexes (Sirkka, 1997). Phytate in plant foods binds essential dietary minerals in the digestive tract, making them unavailable for absorption. It forms insoluble complexes with Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+} and as a result reduces the bioavailability of these essential minerals. Many animal feedings of plant food trials reveal that lower bioavailability of Zn, Ca, Mg, P and Fe are due to the presence of phytate. The main reason why phytate has been considered as an anti-nutrient, especially Zn and Fe deficiencies were reported as a consequence of high phytate intake (Greiner, 2006). A phytic acid intake of 4-9 mg/100 g dried weight is believed to decrease iron absorption by four-fold to five-fold in humans (Hurrel et al., 1992).

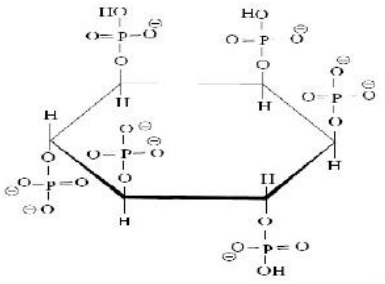

Figure 2. Molecular structure of phytate at neutral pH.

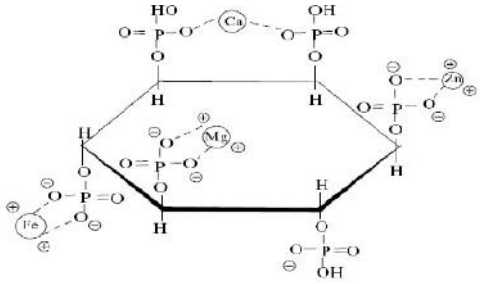

Figure 3. Molecular structure of phytate chelate at neutral pH.

Oxalic acid is a dicarboxylic acid which forms an insoluble calcium salt with a 1:1 molar stoichiometry. When this is formed in the intestine a fraction of dietary Ca is rendered unavailable for absorption. In view of this, the importance of the oxalate content of an individual plant product in limiting total dietary Ca availability is of significance only when the ratio, oxalate:Ca is greater than 1, since under these circumstances the oxalate has the potential to complex not only the Ca contained in the plant but also that derived from other food sources.

Oxalic acid and its salts occur as end products of metabolism on a number of plant tissues. When these plants are eaten they may have an adverse effect because oxalates bind Ca and other minerals while oxalic acid is a normal end product of mammalian metabolism. The consumption of additional oxalic acid may cause stone formation in the urinary tract when the acid is excreted in the urine. Soaking and cooking of foodstuffs high in oxalate reduces the oxalate content by leaching (Noonan, 1999).

Oxalate hinders mineral bioavailability. Higher content of oxalate can bind to Ca present in food, thereby rendering Ca unavailable for normal physiological and biochemical role such as the maintenance of strong bone, teeth, cofactor in enzymatic reaction, nerve impulse transmission and as clotting factor in the blood. The calcium oxalate, which is insoluble, may also precipitate around soft tissues such as the kidney, causing kidney stones. The loss of Ca leads to degeneration of bones, teeth and impairment of blood clotting process (Umaru, 2007).

Boiling may cause considerable skin rupture and facilitate the leakage of soluble oxalate into cooking water; this may be the possible reason to observed high reduction in oxalate level upon boiling (Bhandari, 2004). The content of the calcium oxalate changes as a result of processing. Although cooking proved to be most effective in terms of the reduction of total oxalate, water soluble minerals also leached out at the same time (Poeydomenge, 2007).

Recently effect of cooking temperature on mineral content and antinutritional factors of yam and taro grown in southern Ethiopia has been studied (Ayele et al., 2015). It has been reported that cooking increased the bioavailability of minerals by reducing the antinutritional factors phytate and oxalate in yam and taro (Ayele et al., 2015).

1.2 Nutritional values and health benefits of lettuce

The nutritional value of lettuce varies with the variety. Lettuce in general provides small amounts of dietary fiber, some carbohydrates, a little protein and a trace amount of fat. Depending on the variety, lettuce is a good source of vitamin A, vitamin K and potassium, with higher concentrations of vitamin A found in darker green lettuces. The vitamin A comes from beta carotene, whose yellow-orange color is hidden by green chlorophyll pigments. Beta carotene, of course, is converted to vitamin A in the human body (Marowa et al., 2007).

Lettuce extracts are sometimes used in skin creams and lotions for treating sunburn and rough skin and it has a number of uses as a medicinal herb. It also provides some dietary fiber (concentrated in the spine and ribs), carbohydrates, protein and a small amount of fat. With the exception of the iceberg type, lettuce also provides some vitamin C, calcium, iron and copper, with vitamins and minerals largely found in the leaf. Lettuce naturally absorbs and concentrates lithium (Hullin et al., 2007).

The high water content of lettuce (94.9%) creates problems when attempting to preserve the plant. It cannot be successfully frozen, canned or dried and must be eaten fresh. Lettuce also suffers from several viral diseases, including big vein which causes leaf distortion and ruffling in affected lettuce plants (Kennedy, 2012).

1.3 Statement of the problem

Despite the importance of information on chemical composition for the efficient using of lettuce for its major metals, the chemical composition of lettuce currently used in the main production area (areas which represent lettuce is dominantly harvested and distributed to the consumers) in Ethiopia, like Ziway, Akaki, Peacock, Sebeta and Kera, has not been extensively assessed and documented for any of the chemical compounds that are decisive for the major metals that are available to humans and the anti-nutritional factors which inhibiting on mineral availability of lettuce. Additionally, the level of beneficial chemical constituents has not been broadly assessed for the consumers.

1.4 Objectives

1.4.1 General objective

The objective of this study was to determine the levels of selected metals and the anti-nutrient factors and also the effect of cooking on minerals, oxalate and phytate contents of selected samples of lettuce (*Lactuca sativa*) cultivated in Ethiopia.

1.4.2 Specific objectives

(i) To determine the levels of selected metals (Na, K, Ca, Mg, Fe, Zn, Cu, Co, Cr, Mn, Ni, Cd, Pb) and the anti-nutritional factors (phytate and oxalate), of the raw and cooked lettuce (*Lactuca sativa*) collected from five different farms in Ethiopia.

(ii) To estimate the potentially inhibiting effect of phytate and oxalate on mineral availability of lettuce.

(iii) To get the optimum boiling time for the bioavailability of minerals.

(iv) To correlate the levels of the identified minerals and anti-nutrition factors in raw and cooked commercially available lettuce for their nutritional value.

(v) To compare the levels of minerals and the identified phytate and oxalate in lettuce with the reported values.

2. Materials and Methods

2.1 Instruments

Muffle furnace (Carbolite Astonlane, Hope, Sheffield, England), atomic absorption spectrophotometer (AA-6800 AAS Shimadzu), UV-Vis spectrophotometer (CECIL, CE 1021, 1000 series), centrifuge (DYNAC II centrifuge, clay adams, division of Becton Dikinson and Company, USA), Ohaus Adventurer Analytical balance, USA, hotplate (Wagtech UK hot plate), laboratory grinding mill (Kika-werke M 20), Stuart magnetic stirrer (Wagtech UK), flame photometer (Jenway PFP7) and desiccator (SCN Simax) were used.

2.2 Chemicals

Analytical reagent grade chemicals were employed for the preparation of all solutions. From standard metal ion solutions (1000 mg/L) which were purchased from the AAS Company Vorna Valley (ICP AAS standard), the desired concentration prepared by dissolving appropriate amounts of the nitrates in double-distilled water and were diluted daily for obtaining reference and working solutions.

Jenway flame photometer standards (1000 mg/L) for Na and K, sulfosalisalic acid, $FeCl_3.6H_2O$ (Merck, Germany), sodium phytate salt (phytic acid dodeca sodium salt hydrate water 10-15% product, Aldrich, USA), concentrated ammonia (about 33% w/w AR, Eurostar Scientific Ltd, Liverpool, UK), $CaCl_2.2H_2O$ (Merck, Germany), H_2SO_4 (Riedel-DeHaen, Germany), potassium permanganate (Merck KGaA, 64271 Darmstadt, Germany), HCl (37% Riedel-Dehaen, Sigma-Aldrich Chemicals GmbH, Germany) and HNO_3 (about 69% LR, Eurostar Scientific Ltd., UK) were used as received. Freshly prepared distilled deionized water was used in all experiments.

2.3. Sampling area description

The vegetable farms at Kera and Akaki are among the biggest farms in the capital Addis Ababa, where a substantial amount of vegetables is being harvested seasonally (Weldegebriel et al., 2012). These farms are irrigated with the wastewater from Kera and Akaki rivers, respectively. The Kera farm is situated in the southern part of the city very close to the Addis Ababa abattoir

and is irrigated with Kera River. Ziway farms use water from Lake Ziway. Peacock farm (also called Bulbula farm) is located on the east side of the road to Bole International Airport. This farm is irrigated by Kebena and Bulbula Rivers, which together form the Big Akaki River. Akaki farm is located in the south western part of Addis Ababa near Lake Aba Samuel in Sakelo village (Figure 4). Before several decades, the water from the rivers in the capital was clean. However, with the increase in the urban population and industrialization, the water has now become contaminated with various pollutants, among which are heavy metals. The vegetables grown at contaminated sites could take up and accumulate metals at concentrations that are toxic (Itanna, 2002). Lettuce samples were collected from populated and rural area and from Ziway area in the Main Ethiopian Rift. Ziway area was selected as a control point due to its location, which is away from the industrial activities. Populated area and rural area were compared to investigate for the concentrations of metals in the lettuce that are grown in metals rich soils in and around the city of Addis Ababa in order to understand the magnitude of metals in the edible part of the vegetables.

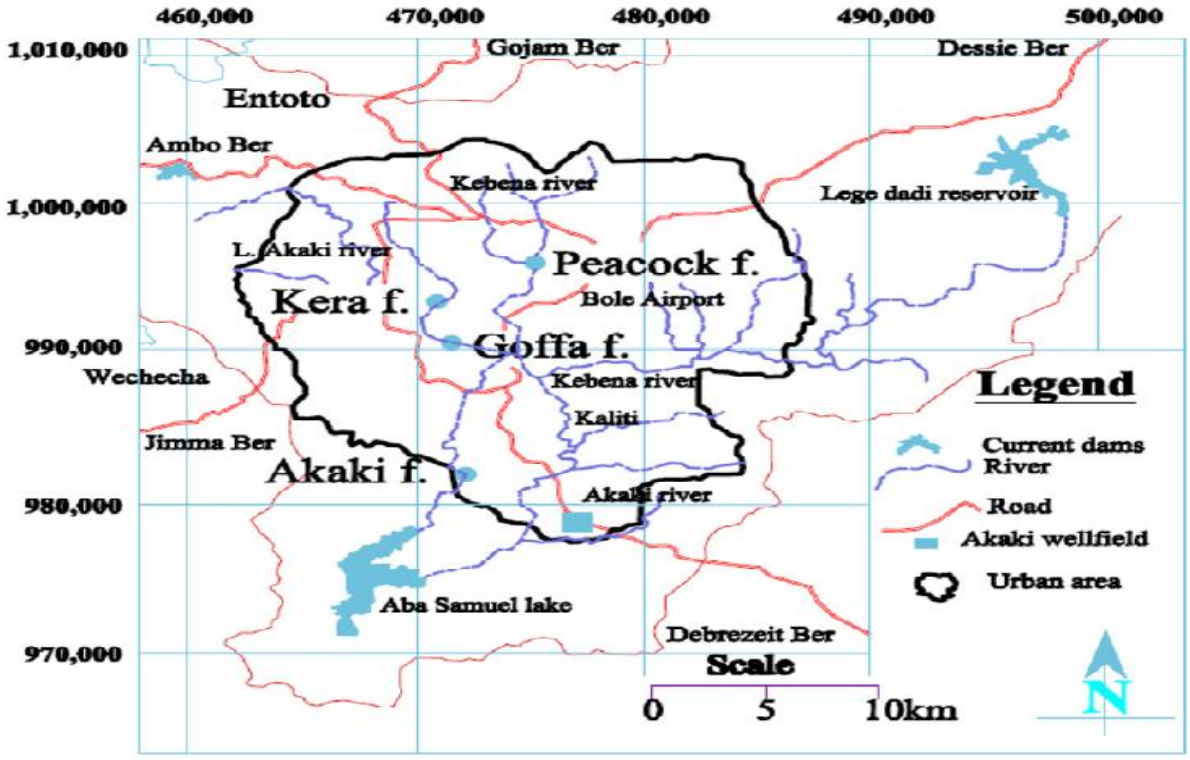

Figure 4. Sampling sites of study vegetable farms, Addis Ababa, Ethiopia (Weldegebriel, et al., 2012).

2.4 Sampling and sample preparation

To meet the objectives set, recently matured leaves of lettuce (*Lectuca sativa*) were collected from a total of five farms Akaki, Kera, Peacock, Ziway and Sebeta, and analyzed for metals, phytate and oxalate. The samples were handpicked then labeled and brought in plastic bags to Ethiopian Public Health Institute (EPHI). To avoid enzymatic degradation of phytate and oxalate, the samples were immediately washed with distilled water and subsequently rinsed with deionized water to eliminate all contaminants including air borne pollutants (Tiwari et al., 2011). By given five different time intervals (raw or 0, 30, 60, 90, and 120) seconds the samples were boiled at 93 ^{o}C, then the water was drained off. After treatment the hot samples were exposed to the air to allow surface water to evaporate (Bhandari, 2004). Immediate browning of the pieces was observed after boiling that may be due to enzymatic browning by polyphenol oxidase (Yemenicioglu, 1999).

All the raw and cooked samples were dried in an oven at 50 ^{o}C for overnight until it gets constant weight and ground to fine powder by using a laboratory grinding mill and sieved through a mesh size of 1 mm in diameter. Samples from each location were taken and analysed in triplicate (n = 3).

2.5 Boiling treatment

Distilled water was added to the 800 mL beaker and boiled to the boiling temperature of water. The water was boiled at 93 ^{o}C due to the high altitude, the lettuce were boiled for different time intervals at the required temperature in a beaker covered with watch-glass and then the water drained off. The temperature was regulated by a thermostat in the hotplate and measured with thermometer within each time intervals.

Each of the five sets homogenized lettuce samples (control or raw, boiled at 30, 60, 90 and 120 seconds at 93 ^{o}C) were analyzed in triplicate for their oxalate, phytate, and minerals (Ca, K, Na, Mg, Fe, Zn, Fe, Co, Cu, Mn, Ni, Cd and Pb).

2.6 Dry ashing for mineral content

Organic matter was burned off at as low a temperature as possible and inorganic material remaining was cooled and weighed. Heating was carried out in stages, first to drive off the water, then to char the product thoroughly and finally to ash at 450 ^{o}C in a muffle furnace.

The required numbers of crucibles were placed in a muffle furnace for 15 min. The crucibles were then removed from the furnace and cooled down in desiccators for about 1 hour, when cooled to room temperature, accurately weighed, to the nearest mg. Approximately 2.5 g of the sample were weighed into each crucible, and then placed on a hot plate under a fume-hood in slowly increasing temperature until smoking ceases. When the samples become thoroughly charred, the crucibles then placed in a muffle furnace, as near to centre as possible and ashed at 450 ^{o}C (fully ashed crucibles seen to be clean and white in appearance). Finally the crucibles were taken out of the muffle furnace placing immediately in a desiccators till cooled to room temperature and weighed.

2.7 Digestion for mineral determination

The ashes were digested with 5 mL of 6 M HCl at low temperature hotplate that cannot reach to boiling till it fully gets dried for about 2 h. 7 mL of 3 M HCl were added and heated on the hot plate until the solution just boils, cooled and filtered through a filter paper into a 50 mL volumetric flask retaining as much of the solids as possible in the dish. Then 5 mL 3 M HCl was added to the dishes and heated until the solution just boiled, cooled and filtered into the volumetric flask. The dishes were then washed with water and the washing filtered into the volumetric flask. The filter paper was washed thoroughly and the washing collected in the flask. Since Ca was to be determined 2.5 mL of 10% lanthanum chloride solution were added to the flask, finally diluted to the mark (50 mL) with de-ionized water. The blanks were prepared by taking the same amount of reagents through all steps.

The atomic absorption spectrophotometer was set according to the instructions. The absorbance of calibration solutions and the reagent blank solutions were measured. The calibration curve

was prepared for the required metal by plotting the absorption values against the metal concentration in mg/L, with r^2 ranging from 0.9998 to 1 for the standards. The mineral content was determined by AAS using air-acetylene flame (AOAC, 1990).

2.8 Determination of phytate

The phytate was determined by the reported method of Latta and Eskin (1980). 0.023 g of dried lettuce samples were extracted with 10 mL 2.4% HCl for 1 h in a mechanical shaker and centrifuged (3000 rpm/30 min). The clear supernatant, 3 mL was used for the phytate estimation. One milliliter of Wade reagent (1:1 ratio of 0.03 % solution of $FeCl_3 \cdot 6H_2O$ containing 0.3% sulfosalicylic acid in water) were added to 3 mL of the sample solution and the mixture then centrifuged. The absorbance at 500 nm was measured using UV-Vis spectrophotometer (the phytate concentration was calculated from the difference between the absorbance of the control (3 mL of water + 1 mL Wade reagent) and that of assayed sample. The concentration of phytate was calculated using phytic acid standard curve and results were expressed as phytic acids in mg per 100 g dry weight.

To prepare the phytic acid standard curve, a series of standard solution were prepared containing 5–40 g/mL phytic acid in water. Three milliliters of the standards then pipetted into 15 mL centrifuge tubes with 3 mL of water used as a zero level. To each tube was added 1 mL of the Wade reagent, and the solution then mixed on a vortex mixer for 5 s. The mixture then centrifuged for 10 min and the supernatant read at 500 nm by using water to zero the spectrophotometer.

2.9 Determination of oxalate

Oxalate was analyzed using the reported method of Iwuoha and Kalu (1994) in which the procedures involve three steps: digestion, oxalate precipitation and permanganate titration.

A 2 g sample of lettuce was suspended in 190 mL de-ionized water contained in a 250 mL volumetric flask; 10 mL of 6 M HCl was added and the suspension digested at the boiling point of water for 1 h that followed by cooling. Then filtered and made up to 250 mL.

Duplicate portion of 125 mL of the filtrate were measured in to a beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH_4OH solution drop wise until the test solution changes from salmon pink color to faint yellow color (pH 4-4.5). Each portion was then heated to 90 ^oC, cooled and filtered to remove precipitate containing ferrous ion. The filtrate was then again heated to 90 ^oC and 10 mL of 5% $CaCl_2$ solution was then added while being stirred constantly. After heating it was cooled and left overnight in refrigerator. The solution was then centrifuged at a speed of 2500 rpm for 5 min. The supernatant was decanted and the precipitate completely dissolved in 10 mL of 20 % (v/v) H_2SO_4 solution.

At this point the total filtrate resulting from digestion of 2 g of lettuce sample was made up to 300 mL. Aliquots of 125 mL of the filtrate were heated until near boiling, and then titrated against 0.05 M standard $KMnO_4$ solution to a faint pink color which persists for 30 s. The calcium oxalate content was then calculated.

After analysis of oxalate, the molar ratio of phytate and oxalate to calcium, zinc and iron were calculated to evaluate the effect of elevated levels of phytate and oxalate in the bioavailability of dietary minerals. As the ratios are the better indicators of the bioavailability than the amount of the minerals and the phytic acid in the diet (Omoruyi , 2007).

2.10 Recovery (validation of accuracy)

The raw sample whose minerals, phytate and oxalate was analyzed had been taken and known concentration of standard minerals, phytate and oxalate were added to them with the purpose of providing validation of accuracy of the procedure used. The spiked samples were then analyzed and the results of the samples containing the added amount of standards were compared to the expected increase in the parameter to be analyzed relative the control (Fruhbeck et al., 1995). The results are given in Tables 1-3. The percentage recoveries of the samples are between 91.2% and 98.5%, which are within the acceptable range (Mendoza et al., 2013).

Table 1. Recovery results of some of the minerals (Pb, Zn, Ni, Cd, Cr, and Cu).

Mineral	Amount in the food (mg/100 g)	Amount added (mg/100 g)	Total expected	Amount determined	Recovery value (%)
Pb	0.265±0.1	2.00	2.27	2.24±0.0	98.5
Cu	0.630±0.1	1.35	1.98	1.87±0.1	91.9
Zn	0.710±0.2	1.10	1.81	1.79±0.1	98.2
Ni	0.680±0.1	1.25	1.93	1.82±0.1	91.2
Cr	0.240±0.1	2.10	2.34	2.26±0.0	96.2
Cd	0.021±0.1	2.50	2.52	2.48±0.2	98.3

Table 2. Recovery results of phytate analysis.

Sample	Phytate in the food (mg/100 g)	Phytate added (mg/100 g)	Total phytate content (mg/100 g)	Phytate determined (mg/100 g)	Recovery (%)
Ziway control	175±1.8	50	225	223±0.6	96

Table 3. Recovery results of oxalate analysis.

Sample	Oxalate in the food (mg/100 g)	Oxalic acid added (mg/100 g)	Total oxalate content (mg/100 g)	Oxalate determined (mg/100 g)	Recovery (%)
Ziway control	11.4±0.79	5.0	16.4	16.3±0.21	98

2.11 Statistical analysis

Samples from each cultivar was taken and analyzed in triplicate (n = 3). Data obtained were subjected to multiple comparison tests using SPSS package, version 20. Mean separations were calculated by the general linear model procedures; post Hoc (LSD) multiple range tests with probability, $p < 0.05$. Statistical difference between the raw and cooked food was established using analysis of paired-samples T-test. All the results for minerals, oxalate and phytate were reported as mean value with their respective standard deviations.

3. Results and Discussions

This study provides data on the content of minerals such as: Zn, Fe, Ca, K, Na, Mg, Mn, Cu, Co Ni, Cd, and Pb, of lettuce grown in populated area and rural area of Ethiopia. Anti-nutritional factors phytate and oxalate of raw and boiled lettuce at different time intervals with boiling temperature of water are also reported. In Addition, the relative bioavailability of the minerals was determined by calculating molar ratios of antinutrient to the minerals.

Different cooking times studied have varied effect in reducing the levels of oxalate and phytate in lettuce. The reduction ranges of phytate on cooking were 19–24% in lettuce and the reduction range of oxalate on cooking were 63–72%. The reduction of these anti-nutrients levels on cooking is expected to enhance the mineral content of these vegetable if the relative rate of migration of minerals during boiling is less. This study indicated that the studied anti-nutritional factors, though showing a high concentration in raw lettuce, will not pose a problem in human consumption if the lettuces are properly processed. Consumption of such properly cooked vegetable may serve as an additional dietary source for the alleviation of malnutrition.

3.1 Ash and mineral content

Comparison of uncooked and cooked assessment revealed that cooking significantly reduced the ash contents in this study (Table 4). The percentage ash of the sample gives an idea about the inorganic content of the samples from where the mineral content could be obtained. Samples with high percentages of ash contents are expected to have high concentrations of various mineral elements, which are expected to speed up metabolic processes and improve growth and development (Bello, 2008). It is expected that processing of vegetables before freezing (washing, grinding, or cooking) causes statistically significant decrease in most constituents analyzed such as ash, K, Ca ,Mg, Na, Fe, Zn, Mn, Cu, and Ni (Kmiecik et.al., 2007).

The ash content of the boiled samples indicates the effect of boiling on the ash content of lettuce samples and it is markedly related with the boiling time and temperature. The result shows that

the mean ash content of the raw sample is significantly different at ($p < 0.05$) from boiled ones. This may be due to leaching of soluble minerals, damaging of cell wall of the plant cell resulted in leaching of not only the dissolved ones but also those dispersed in the solution (Lewu et.al., 2009). Accordingly the water absorb during boiling leading to dilution, hence it decreases the amount of the ash with boiling. But the raw sample is rich in the soluble minerals (Na and K leached during boiling).

Table 4. Ash, phytate, oxalate and moisture contents of (mean ± SD) raw and cooked lettuce samples in (mg/100 g) (n = 3).

Sample code	Phytate	Oxalate	% Ash	% Moisture
Akaki 0"	199±1.9	18.0±0.4	20.9±0.1	93.1±0.2
Akaki 30"	76.0±1.2	12.0±0.4	19.1±0.1	93.1±0.3
Akaki 60"	29.0±1.7	8.88±0.5	17.9±0.1	93.1±0.3
Akaki 90"	1.00±0.1	6.36±3.1	16.2±0.1	93.1±0.3
Akaki 120"	48.3±1.9	6.27±2.8	14.4±0.2	93.1±0.3
Peacock 0"	175±1.8	11.4±0.8	20.5±0.2	93.3±0.1
Peacock 30"	131±1.4	9.04±1.1	18.9±0.2	93.3±0.1
Peacock 60"	105±1.1	8.32±1.7	18.6±0.1	93.3±0.1
Peacock 90"	27.7±0.6	7.85±1.3	17.9±0.03	93.3±0.1
Peacock 120"	52.8±2.2	7.62±0.3	14.5±0.1	93.3±0.2
*Kera Br 0"	485±3.5	15.2±0.3	20.8±0.4	93.6±0.1
Kera Br 30"	182±6.3	9.09±0.3	17.9±0.03	93.6±0.2
Kera Br 60"	35.3±4.2	8.66±0.1	17.8±0.1	93.6±0.2
Kera Br 90"	20.8±1.5	5.13±0.4	16.2±0.1	93.6±0.2
Kera Br 120"	23.1±1.4	5.10±0.3	14.4±0.2	93.6±0.2
Ziway 0"	22.2±2.6	8.86±2.7	21.8±0.1	93.1±0.1
Ziway 30"	19.7±2.4	5.78±2.5	20.3±0.3	92.0±0.4
Ziway 60"	4.97±2.1	5.62±0.3	19.6±0.4	92.0±0.4
Ziway 90"	1.23±1.2	5.40±0.2	19.1±0.4	92.0±0.4
Ziway 120"	1.68±1.4	5.18±0.2	14.4±0.1	92.0±0.4
Sebeta 0"	13.7±1.7	10.6±0.7	20.3±0.3	93.8±0.2
Sebeta 30'	11.2±2.6	8.59±1.1	19.6±0.1	92.7±0.3
Sebeta 60"	5.69±4.9	5.54±1.9	19.3±0.2	92.7±0.3
Sebeta 90"	5.12±2.2	5.14±1.5	16.2±0.1	92.7±0.3
Sebeta 120'	7.36±6.3	4.95±3.2	14.5±0.1	92.7±0.3

Moisture content

From this assessment, the moisture content of the fresh lettuce collected from all sites was in the range 92.0-93.6% with the average value of 93.4%. The reported values of moisture contents are in the range 92-96. This variation may come from the weather or environment differences (Schwember A., Bradford K., 2011). The high water content of lettuce creates problems when attempting to preserve the plant it cannot be successfully frozen, canned or dried and must be eaten fresh (Kennedy, 2012).

3.2 Minerals

The treated samples result as raw and boiled at five different boiling time intervals 0, 30, 60, 90, 120 seconds, 93 ^{0}C of lettuce metals with standard deviation were given in Table 5a for (Fe, Mg, K, Na, Ca, Zn, Cu) and Table 5b (Pb, Co, Cr, Mn, Ni, Cd).

Table 5a. Mean mineral contents of lettuce metals with standard deviation (mg/100 g). Treated as raw and boiled at five different boiling time intervals 0, 30, 60, 90,120 seconds at 93 ^{0}C (n = 3).

Sample code	Fe	Mg	K	Na	Ca	Zn	Cu
Akaki 0"	105±0.13	14.7±0.17	7253±1.1	1770±3.7	2315±4.6	1.05±0.03	0.39±0.0
Akaki 30"	233±0.19	13.6±0.15	7418±1.6	1502±4.3	2561±4.2	1.06±0.04	0.38±0.02
Akaki 60"	231±0.13	13.7±0.15	7651±2.3	1682±2.8	2555±6.9	1.09±0.15	0.34±0.1
Akaki 90"	144±0.07	13.4±0.16	7701±0.9	1685±4.1	2526±5.7	1.55±0.0	0.24±0.2
Akaki 120"	144±0.16	13.4±0.08	7158±3.9	1383±4.4	2683±3.7	1.54±0.22	0.23±0.2
Ziway 0"	123±0.24	13.8±0.14	7487±3.9	2387±4.3	3171±4.6	2.19±0.0	0.63±0.0
Ziway 30"	147±0.21	13.4±0.20	7634±2.8	2231±1.5	4225±5.8	2.19±0.03	0.63±0.01
Ziway 60"	147±0.25	13.4±0.12	7554±1.0	2382±3.3	4218±5.2	2.27±0.1	0.68±0.1
Ziway 90"	143±0.30	13.5±0.03	7194±4.2	2375±1.5	2539±2.4	2.55±0.3	0.67±0.07
Ziway 120"	105±0.12	13.1±0.07	6329±2.1	1383±4.4	2318±3.7	1.8±0.4	0.60±0.02
*Kera Br 0"	33.2±0.31	13.8±0.22	8164±2.4	1244±1.8	2052±1.1	0.7±0.01	1.19±0.1
Kera Br 30"	39.8±0.22	16.5±0.17	7717±2.2	1293±2.6	2565±4.9	0.71±0.2	0.87±0.02
Kera Br 60"	39.7±0.21	16.5±0.17	7443±3.5	1237±0.82	1956±2.5	1.38±0.02	0.82±0.06
Kera Br 90"	25.2±0.70	14.9±0.16	7684±3.5	1286±3.2	2550±4.3	1.45±0.02	0.65±0.01
Kera Br 120"	22.4±0.60	13.1±0.17	6329±2.1	1383±4.4	2319±4.6	1.4±0.5	0.63±0.0
Peacock 0"	21.4±0.14	14.4±0.11	7431±3.8	3101±1.2	1557±5.1	2.8±0.01	0.63±0.0
Peacock 30"	28.6±0.15	14.3±0.17	7422±1.1	3105±4.1	1539±2.6	2.76±0.02	0.63±0.03
Peacock 60"	28.2±0.16	14.7±0.14	7331±3.2	2962±3.7	1844±5.2	2.82±0.2	0.57±0.01
Peacock 90"	26.2±0.14	14.1±0.13	7578±3.2	3454±3.5	2309±4.2	2.82±0.1	0.54±0.02
Peacock120"	24.8±0.12	13.1±0.17	6329±2.1	1444±3.4	2319±4.6	2.86±0.1	0.47±0.08
Sabeta 0"	35.3±0.13	14.7±0.19	8045±3.9	803±3.7	3043±5.6	2.48±0.6	0.95±0.1
Sebeta 30"	46.1±0.08	16.9±0.13	8875±4.0	862±5.1	3379±5.8	2.34±0.5	0.88±0.1
Sebeta 60"	46.7±0.87	15.2±0.14	8913±3.4	1394±2.4	3075±4.1	2.6±0.3	0.50±0.2
Sebeta 90"	45.5±0.12	15.5±0.15	8811±1.8	915±4.3	2748±6.6	2.39±0.2	0.53±0.2
Sebeta 120"	39.7±0.15	11.6±0.15	6317±4.0	891±4.1	2315±5.6	2.24±0.8	0.51±0.02

**Kera Br 0" = brown colour lettuce sample from kera.*

Sample code (30", 60", 90", 120") = boiling time intervals for 30, 60, 90, 120 seconds.

*Sample code 0" = uncooked or raw sample***.**

Table 5b. Mean mineral contents of lettuce metals with standard deviation (mg/100 g). Treated as raw and boiled at five different boiling time intervals 0, 30, 60, 90,120 seconds at 93 ^{0}C (n = 3).

Sample code	Pb	Co	Cr	Mn	Ni	Cd
Akaki 0"	0.198±0.01	0.24±0.02	4.69±0.04	9.41±0.08	2.34±0.15	0.021±0.01
Akaki 30"	0.194±0.01	0.16±0.01	4.49±0.22	9.06±0.8	2.23±0.16	0.018±0.01
Akaki 60"	0.188±0.01	0.011±0.0	4.48±0.22	9.03±0.08	2.22±0.16	0.015±0.01
Akaki 90"	0.159±0.02	0.009±0.0	1.11±0.08	5.8±0.05	0.95±0.02	0.011±0.01
Akaki 120"	ND	0.001±0.0	0.11±0.01	4.79±0.05	0.24±0.08	ND
Ziway 0"	0.149±0.01	ND	1.43±0.02	6.97±0.05	1.98±0.05	0.020±0.01
Ziway 30"	0.143±0.01	ND	1.39±0.31	5.49±0.05	1.93±0.12	0.019±0.0
Ziway 60"	0.126±0.02	ND	1.38±0.29	5.42±0.03	1.80±0.01	0.019±0.0
Ziway 90"	0.045±0.01	ND	1.35±0.21	5.24±0.01	1.10±0.11	0.017±0.0
Ziway 120"	0.040±0.01	ND	1.29±0.21	5.17±0.01	1.09±0.10	0.017±0.0
*Kera Br 0"	0.265±0.03	0.24±0.01	2.64±0.12	4.56±0.14	2.63±0.06	0.02±0.01
Kera Br 30"	0.230±0.03	0.193±0.0	2.56±0.28	4.51±0.04	2.62±0.14	0.016±0.0
Kera Br 60"	0.228±0.03	0.178±0.0	2.48±0.02	4.42±0.04	2.43±0.08	0.014±0.02
Kera Br 90"	0.221±0.03	ND	1.61±0.13	4.06±0.06	1.35±0.01	0.013±0.0
Kera Br 120"	0.213±0.03	ND	1.29±0.21	3.96±0.05	1.09±0.10	0.013±0.02
Pecokeck 0"	0.071±0.02	ND	1.29±0.03	4.02±0.06	1.76±0.05	0.002±0.01
Peacock 30"	0.069±0.02	ND	1.19±0.04	3.99±0.05	1.75±0.05	0.002±0.01
Peacock 60"	0.057±0.01	ND	1.14±0.31	4.01±0.01	1.23±0.07	0.003±0.0
Peacock 90"	0.053±0.02	ND	1.13±0.01	4.01±0.3	1.22±0.08	0.002±0.01
Peacock 120"	0.024±0.03	ND	1.09±0.21	3.52±0.04	1.19±0.10	0.001±0.01
Sebeta 0"	ND	ND	2.3±0.07	4.94±0.04	1.46±0.25	0.002±0.0
Sebeta30"	ND	ND	2.16±0.05	4.11±0.09	1.42±0.14	0.001±0.0
Sebeta 60"	ND	ND	2.13±0.06	4.16±0.05	1.37±0.06	0.001±0.0
Sebeta 90"	ND	ND	2.12±0.3	3.51±0.02	1.30±0.16	0.001±0.0
Sebeta 120"	ND	ND	1.11±0.21	3.17±0.01	1.19±0.03	0.001±0.0

ND = not detected (below the detection limit).

Calcium: The effect of boiling on the calcium content did not show significant difference ($p < 0.05$) between the raw and boiled lettuce at all time intervals. The levels of calcium were highest in lettuce both in the raw and boiled. It was also observed that the samples show increment of calcium content during boiling. The samples boiled for 120 second (2 min) showed decrement of calcium content in boiling that may be due to the expected leaching of calcium salts when the cell wall is damaged during boiling then drained with the boiling water.

The Ca:Phy molar ratio in all cases are greater than 6:1 (Table 6), that shows the good bioavailability of Ca (Wise, 1983). Wise suggested that the solubility of the phytates and that the proportion of Zn bound in a mineral complex in the intestines depend on the levels of Ca. In his model, phytate precipitation is not complete until dietary Ca:Phy molar ratios attain a value of approximately 6:1 and phytate precipitation is incomplete, means some of the dietary Zn remains in solution. The proportion remaining in solution increases with decreasing Ca:Phy molar ratios (Bhandari, 2004). In this study almost all results are in the required value as mentioned earlier. The synergetic effect of secondary cation, i.e. Ca on Zn is not expected.

Zinc: The mean Zn content shows significant difference ($p < 0.05$) between the raw and all the cooked samples for four boiling time intervals. Boiling to the three time intervals, 30 s, 60 s and 90 s show an increasing concentration of Zn but it decreases at 120 s for all the samples, this may be because of leaching of the minerals during boiling. Similarly Phy:Zn ratio ranges from 0.55-7.92 mg/100 g, except for the brown lettuce with 68.6 mg/100 g, and for akaki 18.8 mg/100 g (Table 6). Phy:Zn molar ratio shows the relative increment of the denominator (the mineral) as it is reported (Mark et al., 2000). High dietary phytate content that compromises zinc nutrient is thought to be a major problem among children of the developing world. For the lesser bioavailability of Zn to happen Phy:Zn molar ratio has to be greater or within 10–15 (Lestienne et al., 2005) . But no values fell in this range so that phytate induced Zn deficiency is not expected.

Iron: The phytate/iron molar ratios are used to predict the inhibitory effect on the bioavailability of minerals. A phytate/iron molar ratio > 1 is regarded as indicative of poor iron bioavailability (Bhandari, 2004). The mean Fe content shows significant difference ($p < 0.05$) between the raw and all the four boiling time intervals.

The content of iron shows increment during boiling in the three time interval, 30 s, 60 s and 90 s but it decreases at 120 s. This may be due to the oxidation state of iron, i.e. Fe^{+3} is less soluble than Fe^{+2}. However, this needs a further study for in which oxidation state does Fe exists in the lettuce samples. It shows decrement in boiling at 120 s for all samples this may be because of leaching of the minerals during boiling. The molar ratio of Phy:Fe (Table 6) were less than 0.16 which is indication of the bioavailability (Gibson, 2006). In all boiling time intervals except the Kera brown lettuce with Phy:Fe molar ratio 1.24, though it shows progress in boiling for most of the samples.

Sodium and potassium: Effect of boiling on the mean sodium and potassium content did not shows significant difference at the $p < 0.05$ level between raw and cooked lettuce. The result shows that lettuce is a better source of sodium and potassium. Loss of sodium and potassium during boiling observed 30–55% with respect to the raw samples; this may be due to the fact that salts of sodium and potassium are more soluble in water (Noonan and Savage, 1999).

Magnesium: The levels of magnesium were in the range 13.77-14.74 mg/100 g min the raw lettuce. Effect of boiling on the mean magnesium content did not shows significant difference at the $p < 0.05$ level between raw and cooked lettuce.

Loss of magnesium during boiling is not as such pronounced in lettuce when compared to the loss of other minerals like sodium. A loss of 4–5% of magnesium occurs as it boils to 120 s. This might be due to the fact that magnesium oxalate is less soluble than the potassium and sodium salts (Poeydomenge, 2007).

Manganese: The results showed that lettuce is a good source of Mn; it was in the range 4.02-9.41 mg/100 g in the raw sample. Manganese content show significant difference at ($p < 0.05$) in the raw and boiled in all the four different time intervals.

Boiling resulted in loss of 51–88% of manganese in lettuce samples that may be due to leaching of the mineral to the boiling water.

Copper, chromium, and cobalt: These are found in trace in lettuce except chromium in the samples from Akaki and Kera with the value of (4.69 and 2.64 mg/100 g), respectively, which has relatively higher content due to the pollution of the irrigation rivers from the industrial waste.

These metal content shows decreasing with increasing boiling time interval from 20–52% that may be due to leaching of some parts with the boiling water. In addition as Cu is component of tyrosynate (polyphenol oxidase), the boiling temperature may damage the enzyme, thereby the copper is cleaved and drained with the boiling water. Cobalt contents also shows decreasing trend as most of the minerals decreased in the range 34.5–55% after boiling and that may also be due to leaching of parts of cobalt with the boiling water and degradation of vitamin B_{12}. Boiling may cleave the cobalt free to be drained with the boiling water.

Nickel, cadmium and lead: Nickel(II) under various conditions could either activate or inhibit several enzymatic reactions, which are considered to be of crucial importance in humans and other animals, and that interference with these reactions could have severe deleterious effects (Brunton et al., 2011). Taking a very small amount nickel result in growth depression, anaemia, ultra structural changes in liver and impaired reproduction (Mertz, 1981).

Lead results ranges from 0.075 to 0.265 mg/100 g and cadmium was from 0.002 to 0.02 mg/100 g which is less than compared to the concentration reported in the literature (Pb 1.01–1.59 $mgkg^{-1}$ in lettuce. Cadmium ranged from 0.05–0.13 $mgkg^{-1}$ to 0.44–0.81 $mgkg^{-1}$ in lettuce (Davies and Olpin, 1979). The negligible amounts of Pb and Cd indicate that Ethiopian lettuce is practically free from these two toxic metals.

3.3 Phytate

The molar ratio of oxalate to concurrent minerals has been used as a measure to the availability of oxalate for absorption. Molar ratios of oxalate to minerals greater than 2 and phytate to minerals greater than 0.24 have been reported as hazardous (Adeyeye et al., 2000). This study aimed to investigate the molar ratio of oxalate and phytate to concurrent minerals in common plant materials in order to assess the availability of oxalate for absorption.

Table 4 shows the phytate contents in raw and cooked samples of lettuce. The levels of

phytate in raw lettuce were (13.7-485) mg/100 g from Sebeta and brown lettuce from Kera, respectively, in the raw lettuce samples among the studied samples. The results indicate that phytate content was decreased with the boiling treatments (Figure 1). Phytate shows considerable loss during boiling of lettuce. The effect shows significant difference within treatments (different boiling time intervals) at $p < 0.05$. Loss of phytate from lettuce samples ranged from 32-70% with the average loss from boiling to be 20%.

Table 6. Values of (Ca, Zn, Fe) mmol/100 g and the [Phy]:[Fe], [Phy]:[Zn], [Ca]:[Phy], ([Ca][Phy])/[Zn] molar ratios of lettuce samples

Sample code	Ca mmol/100g	Phytate mmol/100g	[Ca]:[Phy] molar ratio	Zn mmol/100g	[Phy]:[Zn] molar ratio	Fe mmol/100g	[Phy]:[Fe] molar ratio	([Ca][Phy])/ [Zn]
Akaki 0"	57.9	0.302	192	0.016	18.8	18.8	0.16	1089
Akaki 30"	63.5	0.115	552	0.015	7.84	7.84	0.03	498
Akaki 60"	63.3	0.045	1415	0.017	2.68	2.68	0.01	170
Akaki 90"	63.1	0.017	3789	0.024	0.70	0.70	0.01	44.4
Akaki 120"	66.3	0.073	907	0.024	3.11	3.11	0.03	206
Pecokeck 0"	78.8	0.265	297	0.033	7.92	7.92	0.12	625
Peacock 30"	105.2	0.198	531	0.033	5.92	5.92	0.08	623
Peacock 60"	105	0.160	658	0.035	4.60	4.60	0.06	483
Peacock 90"	63.4	0.042	1511	0.039	1.08	1.08	0.02	68.2
Peacock120"	57.9	0.080	723	0.028	2.91	2.91	0.04	168
Kera Br 0"	51.6	0.734	70.3	0.011	68.6	68.6	1.24	3542
Kera Br 30"	64.1	0.276	233	0.011	25.4	25.4	0.39	1627
Kera Br 60"	48.8	0.053	913	0.021	2.53	2.53	0.07	124
Kera Br 90"	63.7	0.032	2019	0.022	1.42	1.42	0.07	90.8
Kera Br 120"	57.9	0.035	1652	0.021	1.64	1.64	0.09	94.7
Ziway 0"	39.4	0.034	1172	0.043	0.79	0.79	0.09	31.0
Ziway 30"	38.7	0.03	1296	0.042	0.71	0.71	0.08	27.3
Ziway 60"	46.3	0.008	6148	0.043	0.17	0.17	0.02	8.09
Ziway 90"	57.5	0.002	30861	0.05	0.04	0.04	0.0004	2.13
Ziway 120"	57.9	0.003	22736	0.044	0.06	0.06	0.0001	3.37
Sebeta 0"	76.8	0.021	3691	0.038	0.55	0.55	0.03	42.2
Sebeta 30'	83.9	0.017	4937	0.066	0.26	0.26	0.01	21.5
Sebeta 60"	76.3	0.009	8849	0.04	0.22	0.22	0.01	16.5
Sebeta 90"	67.8	0.008	8737	0.082	0.09	0.09	0.01	6.38
Sebeta 120'	57.9	0.011	5189	0.04	0.28	0.28	0.02	16.0

The apparent decrease in phytate content during cooking may be partly due either to the formation of insoluble complexes between phytate and other components, such as phytate-protein and phytate-protein-mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphates (Bhandari, 2004). Phytate may reduce the bioavailability of dietary zinc by forming insoluble mineral chelates at a physiological pH. The formation of the chelates depends on relative levels of both zinc and phytic acid (Davies and Olpin, 1979).

The importance of a food stuffs as a source of dietary zinc depends on both the total zinc content and the level of other constituents in the diet that affect zinc bioavailability. Hence, the Phy:Zn molar ratio is considered as a better indicator of zinc bioavailability than total dietary phytate levels alone. The critical Phy:Zn molar ratio may also depend on dietary calcium levels. A kinetic synergism exists between the calcium and zinc ions resulting in a Ca:Zn:Phy complex which is less soluble than phytate complexes formed by either ion alone (Mathew and Saleh, 2006).

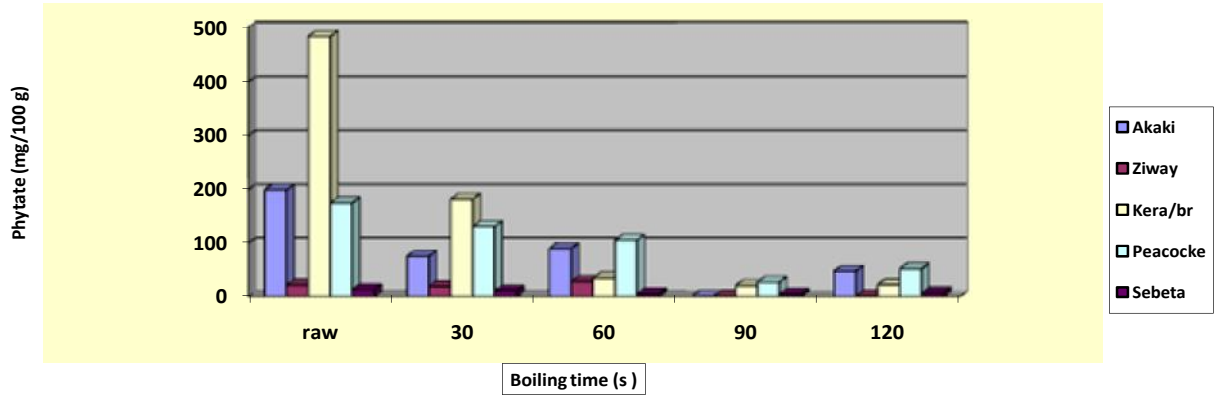

Figure 5. Phytate reduction progress against boiling time in lettuce (phytate in mg/100 g vs boiling time interval in seconds at 93 ^{o}C).

3.4 Oxalates

Oxalate contents of raw and cooked lettuce samples are shown in Table 7. The oxalate content in

the raw samples varied greatly between sites and ranged from 8.66 to 17.99 mg/100 g for Kera brown and Akaki raw lettuce, respectively. The boiled oxalate content decreased in the Kera brown and Akaki raw lettuce from 5.1 to 6.27 mg/100 g, respectively, i.e. (34.9-59.9%).

Oxalate content shows significant difference at ($p < 0.05$) in the raw and boiled samples in all the four different time intervals. It shows decrement of 34.9-59.9% in lettuce. This may be due to the fact that boiling damage the cell wall and oxalate leached and drained off with the boiling water. More oxalate loss is not expected if the boiling water is not drained off (Noonan and Savage, 1999). The higher percentage of oxalate reduction during boiling may also be due to its solubility in boiling water. Boiling may cause considerable skin rupture and facilitate the leakage of soluble oxalate into cooking water. This may be the possible reason to observed high reduction in oxalate level upon boiling (Bhandari, 2004).

The reduced oxalate content in cooked samples could have positive impact on the health of consumers. The reduction of oxalate levels on cooking is expected to enhance the bioavailability of essential dietary minerals of the lettuce and reduce the risk of kidney stones occurring among consumers. The data on the progress of availability of minerals from reduction of oxalate content after boiling is given in Table 7. The oxalate to mineral molar ratio 0.004 to 0.001 shows increment of available calcium.

Table 7. Oxalate contents (mmol/100 g) and [Ox]:[Ca] molar ratios of lettuce

Sample code	Oxalate (mg/100 g)	Oxalate content (mmol/100 g)	Calcium content (mmol/100 g)	[Oxalate] : [Calcium] molar ratio
Akaki 0"	18.0±0.4	0.204	57.9	0.004
Akaki 30"	12.0±0.4	0.136	63.5	0.002
Akaki 60"	8.88±0.5	0.101	63.3	0.002
Akaki 90"	6.36±0.1	0.072	63.1	0.001
Akaki 120"	6.27±0.9	0.071	66.3	0.001
Peacock 0"	11.4±0.8	0.130	78.8	0.002
Peacock 30'	9.04±1.1	0.103	105	0.001
Peacock 60"	8.32±1.7	0.095	105	0.001
Peacock 90"	7.85±1.2	0.089	63.4	0.001
Peacock 120"	7.62±0.2	0.087	57.9	0.001
Kera Br 0"	15.2±0.3	0.173	51.6	0.003
Kera Br 30"	9.09±0.3	0.103	64.1	0.002
Kera Br 60"	8.66±0.1	0.098	48.8	0.002
Kera Br 90"	5.13±0.4	0.058	63.7	0.001
Kera Br 120"	5.10±0.3	0.058	57.9	0.001
Ziway 0"	8.86±2.7	0.101	39.4	0.003
Ziway 30"	5.78±2.5	0.066	38.7	0.002
Ziway 60"	5.62±0.3	0.066	46.3	0.001
Ziway 90"	5.40±0.2	0.061	57.5	0.001
Ziway 120"	5.18±0.2	0.059	57.9	0.001
Sebeta 0"	10.6±0.7	0.120	76.8	0.002
Sebeta30'	8.59±1.1	0.098	83.9	0.001
Sebeta 60"	5.54±1.9	0.063	76.3	0.001
Sebeta 90"	5.14±4.5	0.058	67.8	0.001
Sebeta 120'	4.95±3.2	0.056	57.9	0.001

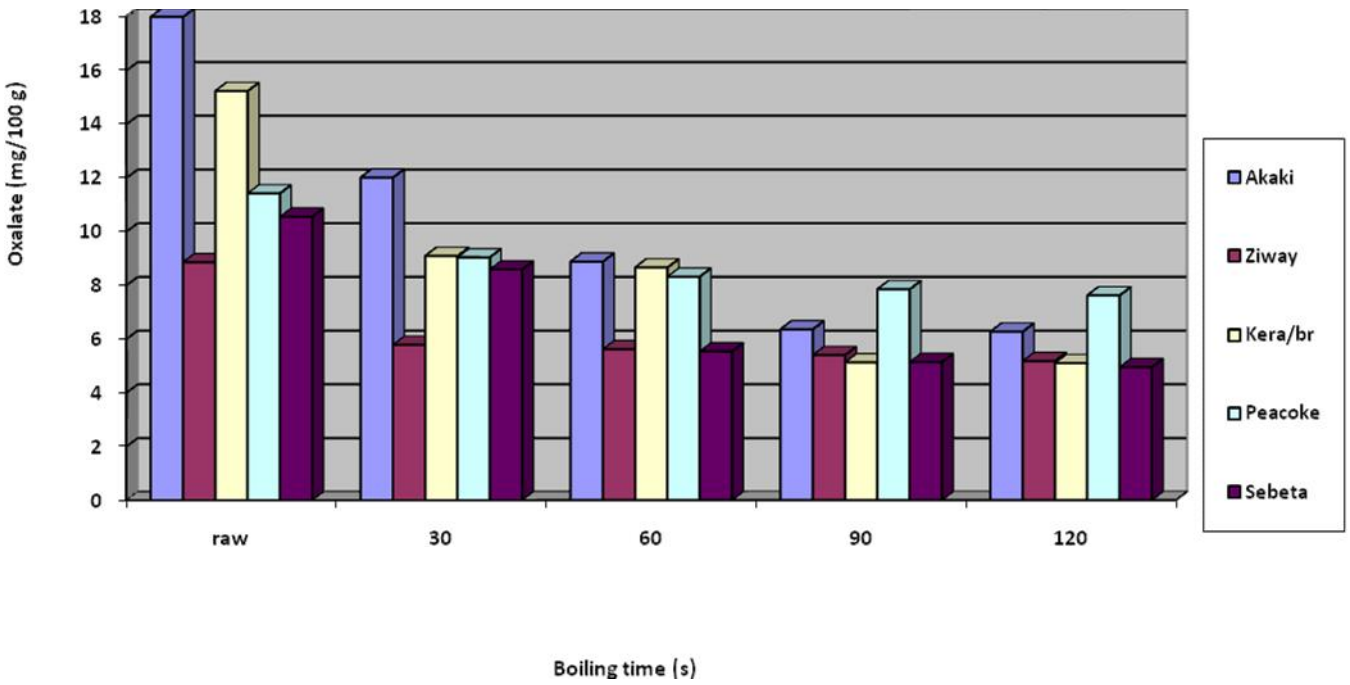

Figure 6. Oxalate reduction trend of lettuce samples through different time intervals of boiling.

3.5 Comparison of mineral, phytate and oxalate contents of lettuce from with that of other countries of the world

It has been observed that the mineral composition of a food crop is directly related to its genetic origin, geographical source and soil conditions (Mathew and Saleh, 2006). Therefore, comparison of mineral, phytate and oxalate contents of lettuce from Ethiopia with that of other countries of the world has been made and presented in Tables 8. The data in tables show that there is wide variation in the mineral, phytate and oxalate contents in Ethiopian lettuce.

Chemical composition of lettuce: The chemical composition (mineral contents, phytate, oxalate, etc.) of lettuce reported in the literature from different parts of the world ranged as follows: content of minerals in–lettuce as reported by (Koudela, 2008): potassium (2,394–6,477 mg/kg), sodium (39–223

mg/kg), calcium (200–755 mg/kg), magnesium (110–413 mg/kg) and the composition for the wild lettuce indicated in Table 8 (Arawande et al., 2013).

Table 8. Mineral composition of lettuce from other countries of the world.

Concentration of element (mg kg^{-1} wet mass)								
Farm	Cd	Co	Cr	Cu	Pb	Mn	Ni	Zn
Peacock	0.17	0.057	1.35	0.80	0.17	2.84	0.51	4.90
Kera	0.66	0.26	1.05	1.63	0.37	8.56	0.30	9.90
Goffa	0.34	0.64	0.05	5.17	0.27	8.08	1.15	13.77
Akaki	0.38	0.36	0.29	3.42	0.31	8.05	0.45	11.40
Kwadon, Gombe State Nigeria[1]	1.9	-	0.2	0.61	0.56	-	-	2.65
wild lettuce (mg kg^{-1} dry matter)[2]	-	-	-	0.20±0.01	0.21±0.0	0.20±0.0	-	0.10±0.0
Maximum limit of metals	0.2	50	2.3	73	0.3	500	67	99.40

Source: Ayers and Westcot, FAO (1985), (Singh et al., 2010)[1], (Arawande et al., 2013)[2].

The study conducted on concentrations of trace metals in leaves of some vegetables grown in Addis Ababa are given in Table 8 (Itanna and Olsson, 2004).

The trend of average metal accumulation of lettuce in all the farms was Zn > Mn > Cu > Cr > Ni > Cd > Co > Pb. Lettuce from Goffa farm was the highest accumulator of Co, Cu, Ni and Zn and that from Kera farm was the highest accumulator of Cd, Pb and Mn as given in Table 8. When the accumulation of metals in lettuce was compared with the previous work, only Cd in lettuce from Kera and Peacock farms and Cr from Peacock of the present study showed higher results. The other metals showed lower metal concentrations (Itanna, 1998; Rahlenbeck et al., 1999). Other reports also indicated that metal accumulations in lettuce showed some similarities with this report, which might be due to the genotypic behavior of this vegetable (Mohammed et al., 2003; Santos et al., 2004). Unlike the other vegetables, lettuce which is consumed raw may pose greater health risk (Weldegebriel et al., 2012).

Assessment of heavy metals accumulated in wastewater irrigated soils and lettuce in Kwadon, Gombe State Nigeria have the following result in Table 8 (Ibrahim et al., 2014). The concentration of Cu ranged from 0.43 to 0.83; Zn from 2.20 to 3.60; Fe, from 2.60 to 3.03; Pb, from 0.09 to 0.22 $mgkg^{-1}$ and with a mean value of 0.61, 2.65, 0.56, 1.90 and 0.20 $mgkg^{-1}$ dried weight of plant, respectively. These values were lower than maximum permissible limits of India with the exception of Cd (Singh et al., 2010).

Quantitative phytochemical content in African lettuce is presented in Table 9 (Arawande et al., 2012). The oxalate content is less than 750 mg/100 g established toxic level (Birgitta and Caroline, 2000). The low content of oxalate will disallow its binding tendency with calcium that will be available for normal physiological and biochemical roles in the body thereby reducing the risk of renal calcium absorption (Osagie, 1998).

Table 9. Quantitative phytochemical content in African lettuce.

	African lettuce
Oxalate (mg/kg)	18.5±0.2
Phytates (mg/kg)	116±1.14

4. Conclusions

This study found that lettuce contains significant amounts of major and trace minerals. The cooking/boiling times at 60 and 90 s of lettuce is the optimum time to get a significant difference in the samples for the bioavailability of minerals. At 30 s no significant difference due to insufficient time to break the cell wall and to release the minerals. At 120 s, the metals concentration decreases due to the total loss of minerals because the cell wall is broken down further to occur dissolution to the water and discarded, though the bioavailability decreases. Therefore the lettuce must be boiled between 60 to 90 s (1:00-1:30 min) at the boiling temperature of water, i.e. 93 ^{0}C to effectively decrease the phytate and oxalate content and to deserve the minerals of lettuce.

In general the study results indicated that lettuce is good sources of minerals with high predicted bioavailability for Ca, Fe and Zn if processed properly, i.e. 93 ^{0}C, for 1:00 to 1:30 min, not to miss the minerals drained with the boiling water.

5. References

Adeyeye, E.I.; Arogundade, L.A.; Akintayo, E.T.; Aisida, O.A.; Alao, P.A. Calcium, zinc and phytate interrelationships in some foods of major consumption in Nigeria. *Food Chem.* **2000**, 71: 435– 441.

Amoah, P.; Drechsel, P.; Abaidoo, R.C.; Henseler, M. Irrigated urban vegetable production in Ghana: Pathogen contamination in farms and markets and the consumer risk group. *J. Water Health* **2007,** 5(3): 455 - 466

Akinola, M.O., Njoku, K.L., Ekeifo, B.E., Determination of lead, cadmium and chromium in the tissue of an economically important plant grown around a textile industry at Ibeshe, Ikorodu area of Lagos State, Nigeria. *Advances in Environ. Biol.* **2008, 2**(1): 25–30.

AOAC (Association of Official Analytical Chemists) *Official Methods of Analysis*, 14th Edn., Association of Official Analytical Chemists, Washington DC, **1990**.

AOAC (Association of Official Analytical Chemists) *Official Methods of Analysis*, 14th Edn., Association of Official Analytical Chemists, Washington DC, **2000**.

Ayele, E.; Urga, K.; Chandravanshi, B.S. Effect of cooking temperature on mineral content and antinutritional factors of yam and taro grown in southern Ethiopia. *Int. J. Food Eng.* **2015**, DOI: 10.1515/ijfe-2014-0264.

Bello, M.O.; Falade, O.S.; Adewusi, S.R.; Olawore, N.O. Studies on the chemical compositions and anti nutrients of some lesser known Nigeria fruits. *Afr. J. Biotechnol.* **2008**, 7: 3972– 3979.

Bhandari, M.R.; Kawabata, J. Cooking effects on oxalate, phytate, trypsin and amylase inhibitors of wild yam tubers of Nepal. *J. Food Comp. Anal.* **2004**, 19: 524–530.

Bigdeli, M., Seilsepour, M., Investigation of metals accumulation in some vegetables irrigated with waste water in Shahre Rey-Iran and toxicological implications. *American-Eurasian J. Agric. Environ. Sci.* **2008,** 4 (1): 86–92.

Birgitta, G.; Caroline, G. Exploring the potential of indigenous wild plants in South Sudan. In Proceedings of a workshop held in Lokichoggio, Kenya **2000**, pp 22-25.

Brunton, L.; Chabner, B.; Knollman, B. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 12th Ed, Amazon, **2011**.

Dastane, N.G. Use of brackish waters in horticulture water quality. *Bulletin* **1987**, 12: 64–71.

Davies, N.T.; Olpin, S. Studies on the phytate:Zn molar content in diet as determinant of Zn availability to young rats. *Brit. J. Nutr.* **1979**, 41: 591–603.

Fairweather-Tait, S.J. Bioavailability of trace elements. *Food Chem.* **1992**, 43: 213–217.

Fruhbeck, G.; Alonso, R.; Marzo, F.; Santidrian, S. A modified method for the indirect quantitative analysis of phytate in food stuffs. *Anal. Biochem.* **1995**, 225: 206–212.

Gibson, R.S.; Perals, L. Improving the availability of nutrients in plant foods at the house hold level. *Proc. Nutr. Soc.* **2006**, 11:60–168.

Greiner, R.; Konietzny, U. Phytase for food application. *Food Technol. Biotechnol.* **2006**, 44: 125–140.

Hurrel, R.F.; Juillert, M.A.; Reddy, M.B.; Lynch, S.R.; Dassenko, S.A.; Cook, J.D. Soy protein, phytate and iron absorption in humans. *J. Clin. Nutr.* **1992**, 56: 573–578.

Intawongse, M.; Dean, J.R. Uptake of heavy metals by vegetable plants grown on contaminated soil and their bioavailability in the human gastro-intestinal tract. *Food Addit. Contam.* **2006**, 23 : 36-48.

Itanna, F. Metals in leafy vegetables grown in Addis Ababa and toxicological implications. *Ethiop. J. Health Dev.* **2002**, 16: 295–302.

Iwuoha, I.; Kalu, A. Calcium oxalate and physicochemical properties of cocoyam (*Colocasia esculenta* and *Xanthosoma sagittfolium*) tuber flours as affected by processing. *Food Chem.* **1994**, 54: 61–66.

Kennedy. G.; Nantel, G.; Shetty, P. The scourge of "hidden hunger": global dimensions of micronutrient deficiencies. *Food Nutr. Agric.* **2003**, 32: 8–16.

Kmiecik, W.; Lisiewska, Z.; Korus, A. Retention of mineral constituents in frozen brassicas depending on the method of preliminary processing of the raw material and preparation of frozen products for consumption. *Eur. Food Res. Technol.* **2007**, 224: 573–579.

Lestienne, I.; Icard-Verniére, C.; Mouquet, C.; Picq, C.; Tréche, S. Effects of soaking whole cereal and legume seeds on iron, zinc and phytate contents. *Food Chem.* **2005**, 89: 421–425.

Lettuce Atlantic Provinces Vegetable Crops Production Guide 2005. *Publication No. 1400A*, 2005, p. Agdex No. 250/600.

Lewu, M.N.; Adebola, P.O.; Afolayan, A.J. Effect of cooking on the proximate composition of the leaves of some accessions of *Colocasia esculenta* (L.) Schott in KwaZulu-Natal Province of South Africa. *Afr. J. Biotechnol.* **2009**, 8: 1619–1622.

Mark, J.M.; Hotz, C.; Nancy, F.K.; Rosalind, S.G.; Jamie, E.W.; Arnold, T. Dietary phytate reduction improves zinc absorption in Malawian children recovering from tuberculosis but not in well children. *J. Nutr.* **2000**, 130: 2959–2964.

Mathew, K.B.; Saleh, B.B. Selected physicochemical properties of flour from the root of African fan palm (*Borassus aethiopum*). *Int. J. Food Prop.* **2006**, 9: 701–713.

Mertz, W. The essential trace elements. *Science* **1981**, 213: 1332–1338.

Mendoza, H.H.; Mejuto, M.; Cardona, I.; Álvarez, A.G.; Millán, R; Yllera, A. Optimization and validation of a method for heavy metals quantification in soil samples by inductively coupled plasma sector field mass spectrometry (ICP-SFMS) *Am. J. Anal. Chem.* **2013**, 4: 9–15

Noonan, S.C.; Savage, G.P. Oxalate content of foods and its effect on humans. *Asia Pac. J. Clin. Nutr.* **1999**, 8: 64–74.

Okonkwo, N.C.; Igwe, J.C.; Onwuchekwa, E.C. Risk and health implications of polluted soils for crop production. *Afr. J. Biotechnol.* **2005,** 4(13): 1521– 1524.

Omoruyi, F.O.; Dilworth, L.; Asemota, H.N. Anti-nutritional factors, zinc, iron and calcium in some caribbean tuber crops and the effect of boiling or roasting. *Nutr. Food Sci.* **2007**, 37: 8–15.

Osagie A.U. Antinutritional factors in nutritional quality of plant foods. Post Harvest Research Unit, University of Benin, Benin City **1998,** pp2221-244.

Poeydomenge, G.Y.; Savage, G.P. Oxalate content of raw and cooked purslane. *J. Food Agric. Environ.* **2007**, 5: 124–128.

Sandberg, A.S. Phytate hydrolysis by phytase in cereals; effects on in vitro estimation of iron availability. *J. Food Sci.* **1991**, 56: 1330-1333.

Schwember, A. Bradford, K. Oxygen interacts with priming, moisture content and temperature to affect the longevity of lettuce and onion seeds. *Seed Sci. Res.* **2011**, 21, 175–18.

Singh, G.; Kawatra, A. Sehgal, S. Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Foods Human Nutr.* **2001**, 56: 359–364.

Singh, K.P.; Mohon, D.; Sinha, S. Dalwani. R. Impact assessment of treated/untreated wastewater toxicants discharge by sewage treatment plants on health, agricultural and environmental quality in waste water disposal area. *Chemosphere* **2004**, 55: 227–255.

Sirkka P. Myoinositol phosphates: analysis, content in foods and effects in nutrition. *Lebensm Wiss Technol.* **1997**, 30: 633–647.

Tiwari, K.K.; Dwivedi, S.; Mishra, S.; Srivastava, S.; Tripathi, R.D.; Singh, N.K.; Chakraborty, S. Phytoremediation efficiency of *Partulaca tuberosa* Rox and *Partulaca oleracea* L., naturally growing in an industrial effluent irrigated area in Vadodra, Gujrat, India. *Environ. Monit. Assess.* **2008**, 147: 15–22.

Tiwari, K.K.; Singh, N.K.; Patel, M.P.; Tiwari, M.R.; Rai, U.N. Metal contamination of soil and translocation in vegetables growing under industrial wastewater irrigated agricultural field of Vadodara, Gujarat, India. *Ecotoxicol. Environ. Saf*. **2011**, 74(6): 1670–1677.

Umaru, H.A.; Adamu, R.; Dahiru, D.; Nadro, M.S. Levels of antinutritional factors in some wild edible fruits of Northern Nigeria *Afr J. Biotechnol.* **2007**, 6: 1935-1938.

Umeta, M.; West, C.E.; Fufa, H. Content of zinc, iron, calcium and their absorption inhibitors in foods commonly consumed in Ethiopia. *J. Food Comp. Anal.* **2005**, 18: 803–817.

Wise, A. Dietary factors determining the biological activities of phytate. *Nutr. Abstr. Rev. Clin. Nutr.* **1983**, 53: 791–806.

Weldegebriel, Y.; Chandravanshi, B.S.; Wondimu, T. Concentration levels of metals in vegetables grown in soils irrigated with river water in Addis Ababa, Ethiopia. *Ecotoxicol. Environ. Saf.* **2012**, 77: 57–63.

World Health Organization ,WHO. Toxicological evaluation of certain food additives and contaminants. Food Additives Series No. 18/571. WHO, Geneva, **1983**.

Yemenicioglu, A.; Ozkan, M.; Cemeroglu, B. Some characteristics of polyphenol oxidase and peroxidase from taro (*Colocasia antiquorum*). *J. Agric. Forestry* **1999**, 23: 425–30.

Appendix I

Minerals of Lettuce

Multiple Comparisons

Dependent Variable	(I) Cookingtime	(J) Cookingtime	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
lead	0sec	30sec	-0.0004	0.004725	0.933	-0.01026	0.00946
		60sec	-0.002	0.004725	0.677	-0.01186	0.00786
		90sec	0.0002	0.004725	0.967	-0.00966	0.01006
		120sec	0.0046	0.004725	0.342	-0.00526	0.01446
chromium	0sec	30sec	-0.01	0.04861	0.839	-0.1114	0.0914
		60sec	-0.032	0.04861	0.518	-0.1334	0.0694
		90sec	0.06	0.04861	0.231	-0.0414	0.1614
		120sec	0.082	0.04861	0.107	-0.0194	0.1834
Zinc	0sec	30sec	-0.002	0.03552	0.956	-0.0761	0.0721
		60sec	-0.016	0.03552	0.657	-0.0901	0.0581
		90sec	-0.042	0.03552	0.251	-0.1161	0.0321
		120sec	-0.02	0.03552	0.58	-0.0941	0.0541
Iron	0sec	30sec	-2.632	3.21739	0.423	-9.3434	4.0794
		60sec	-2.882	3.21739	0.381	-9.5934	3.8294
		90sec	-1.074	3.21739	0.742	-7.7854	5.6374
		120sec	-1.622	3.21739	0.62	-8.3334	5.0894
calcium	0sec	30sec	-19.792	31.39721	0.536	-85.2854	45.7014
		60sec	-29.104	31.39721	0.365	-94.5974	36.3894
		90sec	4.278	31.39721	0.893	-61.2154	69.7714
		120sec	-4.116	31.39721	0.897	-69.6094	61.3774
copper	0sec	30sec	-0.00042	0.00267	0.877	-0.00599	0.00515
		60sec	-0.00396	0.00267	0.154	-0.00953	0.00161
		90sec	-0.00152	0.00267	0.576	-0.00709	0.00405
		120sec	-.005805*	0.00267	0.042	-0.01137	-0.00024

		30sec	.	0.08352	0.637	-0.2142	0.1342
		60sec	-0.062	0.08352	0.467	-0.2362	0.1122
manganese	0sec	90sec	0.022	0.08352	0.795	-0.1522	0.1962
		120sec	0.01	0.08352	0.906	-0.1642	0.1842

Multiple Comparisons

		30sec	-0.008	0.07525	0.916	-0.165	0.149
		60sec	-0.1	0.07525	0.199	-0.257	0.057
		90sec	-0.026	0.07525	0.733	-0.183	0.131
magnesium	0sec	120sec	0.038	0.07525	0.619	-0.119	0.195
		30sec	0.001	0.003542	0.781	-0.00639	0.00839
	0sec	60sec	0.004	0.003542	0.272	-0.00339	0.01139
		90sec	0.0062	0.003542	0.095	-0.00119	0.01359
cobalt		120sec	0.0062	0.003542	0.095	-0.00119	0.01359
	0sec	30sec	-0.008	0.02732	0.773	-0.065	0.049
nikel		60sec	-0.018	0.02732	0.518	-0.075	0.039
		90sec	0.038	0.02732	0.18	-0.019	0.095
		120sec	0.048	0.02732	0.094	-0.009	0.105
cadmium	0sec	30sec	0.002	0.005815	0.734	-0.01013	0.01413
		60sec	0.0018	0.005815	0.76	-0.01033	0.01393
		90sec	0.0018	0.005815	0.76	-0.01033	0.01393
		120sec	0.006	0.005815	0.314	-0.00613	0.01813
sodium	0sec	30sec	4.816	35.93009	0.895	-70.1329	79.7649
		60sec	-9.396	35.93009	0.796	-84.3449	65.5529
		90sec	-1.916	35.93009	0.958	-76.8649	73.0329
		120sec	34.994	35.93009	0.342	-39.9549	109.9429
potassium	0sec	30sec	9.13	45.14742	0.842	-85.0459	103.3059
		60sec	-22.766	45.14742	0.62	-116.942	71.4099
		90sec	24.238	45.14742	0.597	-69.9379	118.4139

	120sec	56.48	45.14742	0.225	-37.6959	150.6559

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	phytatraw - phytatecooked	139.60950	159.57225	35.68144	64.92739	214.29161	3.913	19	.001
Pair 2	oxalate_raw - oxalate_cooked	5.78350	3.12384	.69851	4.32150	7.24550	8.280	19	.000
Pair 3	zn_raw - zn_cooked	-.58250	.81532	.18231	-.96408	-.20092	-3.195	19	.005
Pair 4	fe - fe_cooked	-27.71350	41.21337	9.21559	-47.00195	-8.42505	-3.007	19	.007
Pair 5	Ca - Ca_cooked	-179.93750	532.76154	119.12910	-429.27758	69.40258	-1.510	19	.147
Pair 6	Cu - Cu_cooked	-.10500	.39230	.08772	-.28860	.07860	-1.197	19	.246
Pair 7	Mn - Mn_cooked	.69700	1.39604	.31216	.04363	1.35037	2.233	19	.038
Pair 8	Mg - Mg_cooked	-.62150	2.82506	.63170	-1.94367	.70067	-.984	19	.338
Pair 9	Co - Co_cooked	.06855	.10183	.02277	.02089	.11621	3.010	19	.007
Pair 10	Na - Na_cooked	133.60600	460.38944	102.94621	-81.86289	349.07489	1.298	19	.210
Pair 11	K - K_cooked	215.32000	823.31747	184.09938	-170.00444	600.64444	1.170	19	.257
Pair 12	Pb - Pb_cooked	.01895	.06567	.01468	-.01178	.04968	1.291	19	.212
Pair 13	Cr - Cr_cooced	.36500	1.38125	.30886	-.28144	1.01144	1.182	19	.252
Pair 14	Ni - Ni_cooked	.24700	.67465	.15086	-.06875	.56275	1.637	19	.118
Pair 15	Cd - Cd_cooked	.00295	.00496	.00111	.00063	.00527	2.661	19	.015
Pair 16	ash - ashcooked	3.52000	2.03019	.45396	2.56984	4.47016	7.754	19	.000

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	phytatraw & phytatecooked	20	.445	.049
Pair 2	oxalate_raw & oxalate_cooked	20	.428	.060
Pair 3	zn_raw & zn_cooked	20	.729	.000
Pair 4	fe & fe_cooked	20	.821	.000
Pair 5	Ca & Ca_cooked	20	.658	.002
Pair 6	Cu & Cu_cooked	20	.004	.988
Pair 7	Mn & Mn_cooked	20	.784	.000
Pair 8	Mg & Mg_cooked	20	.111	.640
Pair 9	Co & Co_cooked	20	.544	.013
Pair 10	Na & Na_cooked	20	.826	.000
Pair 11	K & K_cooked	20	.208	.379
Pair 12	Pb & Pb_cooked	20	.807	.000
Pair 13	Cr & Cr_cooced	20	.373	.105
Pair 14	Ni & Ni_cooked	20	.407	.075
Pair 15	Cd & Cd_cooked	20	.859	.000
Pair 16	ash & ashcooked	20	.173	.465

Appendix II

Phytate and Oxalates of Lettuce

Multiple Comparisons

		95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Lower	Upper			
Pair 1	phytatraw - phytatecooked	64.92739	214.29161	3.913	19	.001
Pair 2	phytatraw - oxalate_cooked	90.23836	253.78064	4.403	19	.000
Pair 3	phytatraw - zn_cooked	94.37209	259.14091	4.491	19	.000
Pair 4	phytatraw - fe_cooked	-1.76266	177.54366	2.052	19	.054
Pair 5	phytatraw - Ca_cooked	-2764.79372	-2110.43328	-15.594	19	.000
Pair 6	phytatecooked - zn_cooked	13.96245	60.33155	3.354	19	.003
Pair 7	phytatecooked - fe_cooked	-86.05086	-17.38714	-3.153	19	.005
Pair 8	phytatecooked - Ca_cooked	-2879.35548	-2275.09052	-17.854	19	.000

Dependent Variable	(I) Cookingtime	(J) Cookingtime	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
		30sec	0.11	0.09392	0.255	-0.0859	0.3059
		60sec	.21400*	0.09392	0.034	0.0181	0.4099
		90sec	.26000*	0.09392	0.012	0.0641	0.4559
Phyatate	0sec	120sec	.21000*	0.09392	0.037	0.0141	0.4059

		30sec	2.52400*	0.89784	0.011	0.6511	4.3969
		60sec	3.49400*	0.89784	0.001	1.6211	5.3669
	0sec	90sec	4.41400*	0.89784	0	2.5411	6.2869
oxalate		120sec	4.51600*	0.89784	0	2.6431	6.3889