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Addis Ababa University School of Graduate Studies

Food Science and Nutrition Program

**Study on The Effect of Traditional Cooking of Leafy Vegetables on the Content of
Vitamin C, Essential Minerals and Antioxidant Activity**

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LIST OF ABBREVIATION

AA	-	Ascorbic Acid
AAS	-	Atomic Absorption Spectroscopy
AMP	-	Adenosine Monophosphate
ANOVA	-	Analysis of variance
AOA	-	Antioxidant Activity
AOAC	-	Association of official Analytical chemistry
ATP	-	Adenosine Triphosphate
Ca	-	Calcium
CVD	-	Cardio Vascular Disease
DNA	-	Deoxyribonucleic Acid
DNPH	-	Dinitrophenyl Hydrazine
DPPH	-	A standard 1, 1-Diphenyl-2-picrylhydrazyl
FAO	-	Food and Agriculture Organization
Fe	-	Iron
HIF	-	Hypoxia Inducible Factors
HNO ₃	-	Nitric acid
H ₂ SO ₄	-	Sulphuric Acid
K	-	Potassium
LV	-	Leafy Vegetables
Mg	-	Magnesium
MDD	-	Micronutrient Deficiency Disease
NADP ⁺	-	Nicotinamide Adenine Dinucleotide Phosphate
NCD	-	Non Communicable Disease
RDA	-	Recommended Daily Allowance
ROS	-	Reactive Oxygen Species
RNA	-	Ribonucleic Acid
SNNPR	-	Southern Nations, Nationalities and people's Region
SPSS	-	Statistical package for social sciences
SOD	-	Superoxide Dismutases
TCA	-	Tri Carboxylic Acid
UV- VIS	-	Ultra-Violet Visible Spectrophotometer

WFP - World Food Program
WHO - World Health Organization
Zn - Zinc

ABSTRACT

Leafy vegetables are the fresh and edible portions of herbaceous plants which are important in nutrition, especially as a favorable influence on the functions of the physiology of human. This study was carried out to assess the content of vitamin c, mineral composition and total antioxidant activity of fresh and traditionally cooked LV which are commonly used as food in Ethiopia. Four leafy vegetables were subjected to nutrient analysis. The Vitamin C contents of raw LV ranged from 80.7mg/100g (Ye,abesha gomen) – 18.41mg/100g(Swiss chard) and the vitamin C content of traditionally cooked LV ranged from 24.616mg/100g-0.597mg/100g. Ye,abesha gomen(B.carinata) had a significantly ($P \leq 0.05$) higher vitamin C content than all the tested samples and Swiss chard had the lowest vitamin C content. In raw LV the micronutrients including Fe and Zn were found to be in the range, 9.17-3.9(Fe) and 0.34-0.24(Zn) ppm respectively. The results of macronutrients obtained in raw LV having values of Ca (1.91-1.80.3ppm) and Mg (2.45-1.99 ppm). The results indicate that all these vegetables Ye,abesha gomen, Yeguragae gomen, Cabbage and Swiss chard have the potential to provide essential nutrients to the human beings. Traditional cooking of LV caused significant ($P \leq 0.05$) reductions in the Ca, Zn, Fe and Mg contents and caused significant ($P \leq 0.05$) losses of Vitamin C. The result of the study revealed that longer time of cooking (higher than 10 min) caused negative impact by reducing nutritive value. The registered losses at 10 min were as follow: ash (88.38– 82.0 %), minerals: Ca (1.88-1.66), Mg (2.32-1.84), Fe (8.87-3.76) and Zn (0.33-0.22) and vitamin C (24.616–1.44). Furthermore at 30 min, the residual contents of minerals were: calcium (1.83 – 1.49 ppm), magnesium (2.16 – 1.69 ppm), iron (8.36 – 3.09ppm) and zinc (0.29 – 0.19 ppm). Traditional cooking of LV for 30min significantly affect the total antioxidant activities (61.07 ± 0.88 - 4.33 ± 0.21) and vitamin C (16.03 ± 0.23 - 0.597 ± 0.04). All these results suggest that the recommended time of traditional cooking must be less than 10 min for the studied LV in order to contribute efficiently to the nutritional requirement and to the food security of Ethiopian population.

Key words: Leafy vegetables, vitamins, minerals, DPPH, traditional cooking

1. INTRODUCTION

1.1 Background

Vegetables are the fresh and edible portions of herbaceous plants. They are important food and highly beneficial for the maintenance of health and prevention of diseases(Eintenmiller and Landen 1999)

Vegetables are important in nutrition, especially as a favorable influence on the functions of the physiology of human organism. Most vegetables are rich in essential oils, glycosides, pigments etc, which stimulates appetite. The large number of species and varieties, vegetables are raw materials for preparing a variety of foods, thus improving range enriching food(Hanif et al. 2006)

Vegetables constitute an important part of the human diet since they contain various vitamins, minerals, and trace elements. Until recently, however, they did not constitute a major part of the Ethiopian diet, except during the fasting period. However, since recent years their consumption is increasing gradually, particularly among the urban community. This is due to increased awareness on the food value of vegetables, as a result of exposure to other cultures and acquiring proper education(Sveto et al. 2007).

The term vegetable includes plants and their parts (leaves, roots, shoots, flowers, seed, and fruits) other than ripe fruits and seeds that are eaten after cooking. In popular sense, the term vegetable applies to those plants or parts that are eaten with the meal and commonly salted and boiled or used for desert and salads. This food of plant origin contains many bioactive compounds and thus serves as an important source of minerals, vitamins and certain hormone precursors in addition to protein and energy sources. Vegetables form an essential component of the meal by providing vitamins, minerals such as iron, calcium, magnesium, zinc and other important nutrients for human health(Kala and Prakash 2004).

Green leafy vegetables constitute an indispensable constituent of human diet in Africa generally and west and east African in particular. Apart from the variety which they add to the menu they are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, minerals, vitamins, fiber and other nutrients which are usually short supply in daily diet(Wilson III, Shaw and Knight Jr 1982).

Braceasae family (green leafy vegetables) occupies an important place among the food crops as these provide adequate amounts of many vitamins, and minerals for humans. They are valuable

sources of nutrients especially in rural areas of Ethiopia where they contribute substantially to nutrients, antioxidant, vitamins, dietary fiber, energy and certain hormone precursors, which are usually in short supply in daily diet (Sciaccia 2004).

Food quality often deals only with influences of primary production and industrial processing. Food preparation at home as final step of the chain has also great influence on quality determine parameters like sensory attributes and the content of antioxidants, vitamins and minerals. It can change the quality of food both in a positive and negative way (SCHNEPF and DRISKELL 1994).

Vegetables are well represented in the composition of antioxidants, vitamins and numerous minerals such as those of Ca, Fe, Cu, P, Zn, Cl, Na and others. The dominant basic elements in plants and vegetables are Ca, K, Mg, Fe, Na. These provide alkalizing effects, neutralizing the acidity produced by other foods, especially those of animal origin (Gupta et al. 2005).

Minerals are very important and essential ingredients of diet required for normal metabolic activities of body tissues. Out of 92 naturally occurring minerals 25 are present in living organisms. They are constituent of bones, teeth, blood, muscle, hair and nerve cells. Vitamins cannot be properly assimilated without the correct balance of minerals.

Vitamins are organic compounds occurring in natural foods especially in vegetables either as such or as utilizable 'precursors'. Vitamins are needed for maintenance of skin muscles membranes, bone, teeth, vision and reproductive. They help body to absorb calcium and phosphorous needed for bone growth and maintenance (Frossard et al. 2000).

Antioxidants are chemicals substances that donate an electron to the free radicals and convert it to a harmless molecule. Antioxidants intercept free radicals and protect cells from the oxidative damage that leads to aging and disease (Alscher, Donahue and Cramer 1997).

Consumption of numerous types of edible plants as sources of food could be beneficial to nutritionally marginal population especially in developing countries where poverty and climate is causing havoc to rural population. The average daily intake of leafy vegetables among rural families which might be due to an easy availability of these vegetables in rural area (Ogle, Hung and Tuyet 2001).

Brassica oleracea, Beta vulgaris and Brassica carinata are most commonly used green leafy vegetables which are known by their high content of vitamins and minerals. Four green leafy

vegetables which are commonly consumed are used to analyze the effect of traditional cooking methods on the content of nutrients. Antioxidants, water soluble vitamin (vitamin C)/ Ascorbic acid/ and essential minerals are analyzed

Fresh vegetables are important foods both from an economic and nutritional point of view and vegetable of all types are valuable part of our diet. They play an important part in maintaining general good health owing to the presence of mineral element, antioxidant and vitamin., no food stored in the leaves; it is only produced there, therefore little protein or carbohydrate is found in green vegetables. The greener the leaf the larger the quantity of vitamins and minerals present. All these substances help to build teeth, protect the body from disease and regulate the body process on which vitality and good health depend(van Rensburg et al. 2007).

In Ethiopia, unlike other countries, where green leafy vegetables are usually consumed in their unprocessed forms, green leafy vegetables are usually used in their daily diet in their cooked form. The vegetables can also be consumed as whole or mixed with other staple after cooking and processing in different ways. Particularly in Gurage Zone vegetables are consumed after cooking and mixed with meat(gomen besiga) and cheese which is known by 'ye,gomen kitfo' specially during holiday, like Meskel

However, there is limited information with regards to the effect of cooking which causes considerable losses in antioxidants, vitamins, minerals and dietary fiber values of green leafy vegetables. Therefore, the purpose of this study is to conduct investigation on the influence of traditional cooking methods on the content of antioxidants, vitamins and minerals

1. 2 Statement of the problem

Vegetable is an important contributor to the daily human well –being. vegetable also the major contributor of edible plants to human health, they have always been thought to have large amount of vitamins and minerals like folic acid, vitamin A and vitamin C which are usually responsible for more subtle felling of daily well being and protection from long term degenerated disease(Hanif et al. 2006).

Many researches revealed that cooking affects the nutritional content of vegetables like vitamins and minerals but only few on the traditional cooking processes. A study on the effect of traditional cooking on the content of vitamins, minerals and antioxidants therefore be a great interest, because

the knowledge provided will help to orient the consumer to reduce the losses of nutrient that may occur during traditional cooking process. The data on nutritional content of vegetables on different traditional cooking time may help to select specific time of cooking vegetables to retain nutrients.

Specifically in the effect of traditional cooking of vegetables like Brassica carinata/Ye,abesha gomen/, Brassica carinata cpita/yegurage gomen/, Brassica oleracea and Beta vulgaris/Swiss chard/ on the content of vitamins, minerals and antioxidants, no comparative studies were conducted. Therefore it is imperative to research and find out optimum loss of vitamins, minerals and antioxidants that occur during traditional cooking process of vegetables that commonly consumed in the society.

1. 3 Significance of the study

Generally, preparations of vegetables at home are based on test preference and convenience rather than retention of nutrients and health promoting compounds.

- This study therefore will provides an empirical data about the nutritional composition of vegetables among traditional cooking methods
- It provides a baseline for further analysis of other species in the area.
- Provide information on how to retain nutrients during traditional cooking process.
- It contributes important information to identify the gap and undertake further study on leafy vegetable. Moreover, this study will help the consumer, nutritionists, agricultural research institutes and scientists to give an emphasis in the future on traditional cooking processes.

1.4 Hypothesis

Traditional cooking process of commonly consumed leafy vegetable tends to contribute to significant loss of antioxidants, vitamin C and essential minerals.

1.5 Objectives

1.5. 1 General objective of the study

To assess the nutritional content of the raw and traditionally cooked leafy vegetables which are commonly consumed in Ethiopia

1. 5. 2 Specific Objectives

- To evaluate the effect of traditional cooking of leafy vegetables in clay pots relative to other cooking methods.
- To determine the effect of traditional cooking on nutritional composition of vegetables

- To estimate the content of Antioxidant activities, vitamin C and minerals compositions of traditionally cooked vegetables
- To study the effect of traditional cooking time of different leafy vegetables on the content of Antioxidant, vitamin C and minerals which are commonly consumed by the society.

2. LITERATURE REVIEW

2. 1. Vegetable Description

The term vegetable include plants and their parts (leave, roots, shoots, flowers, seeds, fruits) other than ripe fruits and seeds that are eaten after cooking. In a popular sense, the term vegetable applies to those plants or parts that are eaten with the main course of a meal and commonly salted and boiled or used for desert and salads (Abbott et al. 2010). Vegetables are the fresh and edible portions of herbaceous plants. They are important food and highly beneficial for the maintenance of health and prevention of diseases. Vegetable contains many bioactive compounds and thus serves as an important source of minerals, vitamins and certain hormone precursors in addition to protein and energy source(Potter and Steinmetz 1995)

2. 2 Types of vegetables and their uses

Vegetables naturally grouped in to three; 1) Green leafy vegetables for examples pumpkin, amaranth, cabbage, Swiss chard which have high health value. 2) Roots and tubers for example potatoes, carrots, yam, and 3) other vegetables like beans and peas(Puupponen-Pimiä et al. 2003)

Vegetable is an important contributor to human well being. They are usually responsible for more subtle felling of dally well being and for protection from long term degenerated disease(Moore and Diez Roux 2006)

Green leafy vegetables constitute an indispensable constituent of human diet in Africa in general. Apart from the Varity which they add to the menu, they are venerable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diet. Green leafy vegetable act as a butter and maintains the proper alkalinity of the blood by balancing acidic producing foods like meat(Afolayan and Jimoh 2009)

2. 3 Leafy vegetables in Ethiopia

Agriculture is the mainstay of the Ethiopian economy, contributing 43% of the gross domestic product, providing 85% of export revenue and employing over 86% of the population. Ethiopia has highly-diversified agro-ecological conditions which are suitable for the production of various types of fruit and vegetables(Ayana et al. 2014).

Indeed, Ethiopia is endowed with diverse agro-ecologies suitable for the production of different categories of vegetables. Tropical, sub-tropical and temperate vegetables are produced in the

lowlands (<1500 meters above sea level), midlands (1500-2200 masl), and highlands (>2200 masl), respectively (Block et al. 2008). The development of the vegetable sub-sector is one of the priority areas in the agricultural development strategy of Ethiopia. However, the contribution of horticultural crops both to the diet and income of Ethiopians is insignificant (Ayana et al. 2014)

Ethiopians consume on average 97g of fruit and vegetables per day. Cereals contribute about 75% of the Ethiopian diet. Pulses are a source of protein and widely consumed. The main constraint with regard to fruit and vegetable production is that, because of market and food security concerns, rural farmers prefer to produce cereals and pulses. Other constraining factors include low production and productivity, lack of adequate pest control, poor soil fertility management practices, lack of attention to product quality and prevention of physical damage, as well as the lack of storage and packaging facilities

Several vegetable species abound in the world. Green leafy vegetables constitute an indispensable constituent of human diet in Africa generally and West Africa and East Africa in particular (Block et al. 2008). In addition, green leafy vegetables are used in the diets of postpartum women during which time it is claimed that they aid the contraction of the uterus. (Smith and Eyzaguirre 2007)

In Ethiopia, on average more than 2,399,566 tons of vegetables and fruits are produced by public and private commercial farms, this is estimated to be less than 2 percent of the total crop production (Dawit 2014)

According to recent information obtained from the Central Statistics Authority, the total area under fruits & vegetables is about 12,576 hectares in 2011. Of the total land area under cultivation in the country during the same year, the area under fruits and vegetables is less than one per cent (i.e. 0.11%), which is insignificant as compared to food crops (Setegn 2015).

Several green leafy vegetables are grown in the country. In this study only green leafy vegetables, swiss chard (*Beta vulgaris*), yegurage gomen (*Brassica carinata*), ye, abesha gomen (*Brassica carinata*) and cabbag (*Brassica oleracea*) which are commonly grown and widely used in the country are selected

2. 4. Ye, abesha gomen and Ye,guragae gomen(*B.carinata*)

Ye, abesha gomen and Ye,guragae gomen are a leafy crop which are like rape and kale. These vegetables have sharp garlic like odor when cut. These vegetables are common in most African countries including Zambia, Kenya, Tanzania and Botswana where mainly used as a leafy crop. However, in developed countries these are used for oil production. Studies done in Botswana have revealed that in terms of leaf yield, Ethiopian mustard compares well to rape and kale. When the plant starts flowering, the leaf area start reduce therefore making the leaves not marketable. One advantage of Ethiopian mustard compared to rape and kale is that it produces seeds to overcomes problem of seed availability and affordability.



Figure 2.1: Abyssinian cabbage/ (*B. carinata*)

The kale (*Brassica carinata* L. var. *acephala*), is classified as *Brassicaceae*, known as cultivator plant from ancient times, and notices about its cultivation in Poland are as old as XIVth century In East Africa (except Ethiopia) *Brassica carinata* leaves (aptly called Loshu) is an important leafy vegetable in East and Southern Africa. It is consumed fresh or partially fried or stewed with other

vegetables (spinach and spices). It is mostly grown in small farm holdings or in kitchen garden in Tanzania, Malawi and Zambia and to a lesser extent in Zimbabwe. Fresh leaves are sold in nearby and distant markets. Within season glut and low market demand are serious challenges in production. To date no statistical data on production and marketing are known. The utilization of the Ethiopian mustard leaves appears to be declining because of higher yielding leaf cabbage (*Brassica oleracea*) and leaf mustard (*Brassica juncea*) (Van der Vossen and Mkamilo 2007).

Similarly, information on the nutritional composition of *Brassica carinata* leaves is limited, but it is probably comparable to *Brassica juncea* (Van der Vossen and Mkamilo 2007). Ethiopian mustard lacks bitterness, but rich in vitamins C and K, beta carotene and calcium, as well as cancer-fighting anti-oxidants (Adeniji and Aloyce 2014). It produces more leaves per plant than kale and a few leaves can be removed at a time for home consumption, allowing the rest of the plant to continue to grow. The crop has erect, annual or occasionally biennial or perennial herb up to 200 cm tall, usually branched, glabrous to slightly hairy at stem and petiole bases, slightly galls; taproot strong, leaves are alternate and usually simple (Adeniji and Aloyce 2014). Propagation is by seed, when grown for the leaves, seed broadcast in the nursery beds is widely practiced. Seedbeds mixed with organic manures are usually raised above the soil to reduce the incidence of damping off. The field spacing is about 35 – 40 cm within and 50 – 60 cm between rows, depending on the plant size.

Fresh market production of Ethiopian mustard leaves is usually under monoculture, the whole plants are harvested by uprooting 5 - 6 weeks after sowing in seed beds. The average leaf and shoot yield of 35 t/ha on farmers' field and 50 – 55 t/ha on research stations depending on production season and cultivar (Adeniji and Aloyce 2014). Leaves are perishable and wilt or become yellow when left on the shelf for more than a day; therefore farmers harvest small quantities at a time and sell them in nearby or distant markets. To retain freshness, the leaves are kept moist inside a bag that is left in the shade or in a cool place. When the product is offered as whole plants with roots, traders place the roots in water and plants can thus be kept for a few days. In Ethiopia Ethiopian mustard leaves are important component of vegetable diet, sold in grocery stores and retail outlets throughout the country. Cultivation takes place throughout the year wherever water is not limiting. Plant growth slows during the cold season compared to the warm season.

Ye, abesha gomen finds favorable growing conditions in Ethiopia, and because its morphological characteristics can be available as fresh for prevailing part of the year. Its cultivation demands a lot of water, but still the plant is resistant to drying

2. 5. Swiss chard (*Beta vulgaris*)

Swiss chard belong the beet family of vegetables and has been cultivated in Europe for thousands of years. Due to its extensive cultivation in Switzerland it has been referred to as Swiss chard. In South Africa it is often called spinach and has been a substitute for spinach. It has thicker leaves and is higher yielding than true spinach. Technically Swiss chard is not spinach and is more closely related to beetroot than it is to spinach. Swiss chard is a very good source of vitamin A, K, C, magnesium (Mg) and manganese (Mn)



Figure 2.2: Swiss chard (*Beta vulgaris*)

Swiss chard is considered to be one of the healthiest vegetables available, and is a valuable addition to a healthy diet (like other green leafy vegetables)(Bakry et al.)

Fresh young chard can be used raw in salads. Mature chard leaves and stalks are typically cooked; their bitterness fades with cooking, leaving a refined flavor which is more delicate than that of cooked spinach. Chard has a slightly bitter taste and is used in a variety of cultures around the world, including Arab cuisine. Wikipedia, the free encyclopedia Swiss chard (*Beta vulgaris* L. var. *cycla* L.) is a leafy vegetable highly valued because it is available year round and for the nutritional properties of its leaves which contain considerable amounts of vitamin C, potassium, calcium and magnesium(de COSTA et al. 2003).

Swiss chard is high in vitamins A, K and C, with a 175 g serving containing 214%, 716%, and 53%, respectively, of the recommended daily value (Jump up, 2012). It is also rich in minerals, dietary fiber and protein(Bakry et al.)

Nutritionally, Swiss chard deserves to be highly valued, as it is an excellent source of many core nutrients as well as phytochemical, it contains high levels of lutein and zeaxanthin. Multi-coloured stalks also contain betalains, which have strong antioxidant activity. Other phenolics in Swiss chard, such the flavonoid kaempferol, are also important antioxidants .Its slightly bitter taste is

perhaps to blame for its lack of popularity, but if served mixed with other sweeter vegetables, it can provide an interesting contrast in flavor. Swiss chard, its nutritional profile is very similar to that of its close relative, spinach. Besides its many nutrients, it too contains anti-nutritive oxalates. Swiss chard is particularly rich in vitamin E. In addition, it provides useful amounts of a wide range of minerals(Hedges and Lister 2007).

Phytochemical screening of Swiss chard has revealed the presence of some fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids), phospholipids, glycolipids polysaccharides), folic acid, ascorbic acid and pectin(Secchi 1963).

Swiss Chard (Chenopodiaceae; *Beta vulgaris* subsp. *cycla*) has been indicated in folk medicine as a hypoglycemic, anti-inflammatory and hemostatic herb. It has been proposed that another cultivar *Beta vulgaris*, the beet root (*Beta vulgaris rapacea*) has anticancer activity(Patkai, Barta and Varsanyi 1997).

Chard may decrease blood sugar by increasing insulin secretion from B cells of the pancreas. We can say that the chard extract acts like an insulinogenic agent in induced diabetic rats. As a result, it can be assumed that plant therapy can provide blood glucose homeostasis and can cause regeneration of B cells of endocrine pancreas(Bolkent et al. 2000).

Swiss chard can be produced under mild to cool conditions. It is advisable not to plant Swiss chard under conditions where frost is prevalent during the winter. Swiss chard can tolerate light frosts but ongoing frost may damage the plant and suppress optimal production. Under hot conditions Swiss chard grows well, but extreme hot weather may cause problems producing leaves with good quality. Hot conditions are also not conducive to the hold ability of the final product if this is not managed properly(Hadfield 2001).

2. 6. Cabbage (*Brassica oleracea*)



Figure 2.3: cabbage(*Brassica oleracea*)

Cabbage vegetables like *Brassica* group are perceived as very valuable food products. The research conducted, in many countries, corroborated the nutritive value, high antioxidant and pro-healthy potential of these vegetables. These vegetables are a precious source of fiber, mineral compounds, vitamin α -tokoferol and carotenoids (β -carotene, lutein). They are abundant of polyphenolic compounds and contain 15-20 different glucosinolates like compounds(Sikora and Bodziarczyk 2012).

2. 7. Chemical composition of Leafy Vegetable

Leafy Vegetable has always been thought to have large amount of vitamins and minerals like folic acid, vitamins A and vitamin C. vegetables also have starch and cellulose present in them they supply high amount of water and mineral elements and the mineral elements in them contribute to the alkaline substance in the body and this enables them to maintain the acid base balance of the body(Aletor, Oshodi and Ipinmoroti 2002)

Green leafy vegetables are fair source of protein and good source of vitamins and also minerals some of vitamins and also minerals some contains anti-nutrient that interfere with the absorption of some nutrients in diet(Odhav et al. 2007)

Swiss chard and cabbage are the most commonly consumed green leafy vegetable in house hold. Brassica vegetables belong to brassicacea family and include different genus of Abyssinian cabbage, broccoli, cabbage, cauliflower and other. These vegetable posses both antioxidant and ant-carcinogenic properties(Van Duyn and Pivonka 2000). Variation in the antioxidants content of brassica vegetable such as vitamins, carotenoids, flavonoids and other phenolic compounds is

caused by many factors: variety maturity at harvest, growing conditions, soil state and conditions of post harvest storage(Afolayan and Jimoh 2009).

2. 8. Nutritional importance of leafy vegetables

Wild food plants play a very important role in the livelihoods of rural communities as an integral part of the subsistence strategy of people in many developing countries. Locally available wild food plants serve as alternatives to staple food during periods of food deficit, are a valuable supplement for a nutritionally balanced diet and are one of the primary alternative sources of income for many poor rural communities(Gockowski et al. 2003). Millions of people in many developing countries do not have enough food to meet their daily requirements and a further more people are deficient in one or more micronutrients (Gockowski et al. 2003). In most cases rural communities depend on wild resources including wild edible plants to meet their food needs in periods of food crisis. The diversity in wild species offers variety in family diet and can contribute to household food security. A research compiled a comprehensive nutrient report of wild vegetables consumed by the first European farmers, and nearly all the species had significant amounts of several micronutrients such as copper, magnesium, zinc, iron, vitamin E, carotenoids and vitamin C(Hanif et al. 2006). Turan et al (2003) reported that the potassium, calcium, magnesium and protein contents of wild vegetables in Turkey were all higher than cultivated species(Turan et al. 2003). The cultivated species analyzed and compared to the wild vegetables were spinach, pepper, lettuce, and cabbage. Concentrations of iron, manganese, zinc and copper were similar in both vegetable types. Studies conducted in South America have confirmed the importance of wild vegetables as sources of micronutrients(Flyman and Afolayan 2006). Studies conducted on wild South African vegetables in Tanzania underscored the wild plants' significant contribution as sources of micronutrients. However, the nutritional quality of four wild vegetables analyzed in Ghana was found to be in the same range as conventional vegetables (Flyman and Afolayan 2006)

Many researches showed that wild plants are essential components of many Africans' diets, especially in periods of seasonal food shortage. A study conducted in Zimbabwe revealed that some poor households rely on wild plant foods as an alternative to cultivated food for a quarter of all dry season meals(Muchuweti et al. 2009).

2. 9. Vegetables and Health

Non communicable diseases (NCDs), especially cardiovascular diseases (CVDs), cancer, obesity and type 2 diabetes mellitus, currently kill more people every year than any other cause of death.

Four factors in the epidemiology of these diseases – poor diet, physical inactivity, tobacco and alcohol use – are of overwhelming importance to public health(Schmidt et al. 2011).

Fruit and vegetables are an important component of a healthy diet and, if consumed daily in sufficient amounts, could help prevent major diseases such as CVDs and certain cancers. According to The World Health Report 2002, low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide. Overall it is estimated that up to 2.7 million lives could potentially be saved each year if fruit and vegetable consumption was sufficiently increased. Recommendations in this direction tend to complement and reinforce other valid messages based on the long known health benefits of consuming vegetables and fruit as dietary sources of fiber, vegetable proteins and protective micronutrients(Organization 2002). The recent Joint FAO/WHO Expert Consultation on diet, nutrition and the prevention of chronic diseases, recommended the intake of a minimum of 400g of fruit and vegetables per day (excluding potatoes and other starchy tubers) for the prevention of chronic diseases such as heart disease, cancer, diabetes and obesity, as well as for the prevention and alleviation of several micronutrient deficiencies, especially in less developed countries. The recommendation thus adds to the already strong case for the health benefits to be gained from the consumption of fruit and vegetables and paves the way for concrete action advocating increased consumption of these commodities(Who and Consultation 2003).

Human health is very important to our survival. Vitamins help the human to maintain a healthy diet. They serve as essential components of the specific coenzymes participating in metabolism and other specialized activities. Among the vitamins, vitamin C (ascorbic acid) is an essential micronutrient required for normal metabolic function of the body(Naidu 2003)

Micronutrient deficiency diseases (MDD) are widespread and affect large numbers of people in developing countries. It is estimated that one in every three people in the world is at risk for one or more micronutrient deficiencies, while approximately 2 billion people world-wide suffer from some kind of micronutrient deficiency, causing a wide array of disorders and increasing the risk of death, disease and disability(Joint and Organization 2005). Vitamin (an important micronutrient), is essential for most of the body's functions and is required by the body in small amounts for metabolism, to promote health and proper growth especially among children. Vitamin deficiency diseases do not only occur in poverty stricken communities; in affluent societies, deficiency diseases also occur often as the result of poor choice of food or unhealthy eating habits(Brigelius-Flohe and Traber 1999)

Non communicable diseases (NCDs), especially cardiovascular diseases (CVDs), cancer, obesity and type 2 diabetes mellitus, currently kill more people every year than any other cause of death. Four factors in the epidemiology of these diseases – poor diet, physical inactivity, tobacco and alcohol use – are of overwhelming importance to public health(Ng et al. 2011).

Vegetables contain both essential and toxic elements over a wide range of concentrations. The concentration of these elements is a function of the concentrations in the soil in which the vegetable is planted (Liu, Probst and Liao 2005)

Many studies have shown that diets rich in fruits or vegetables are protective against diseases and populations that consume such diets have the higher plasma antioxidant status and exhibit a lower risk of non communicable diseases(Song et al. 2013)

Vitamin C plays an important role as a component of enzymes involved in the synthesis of collagens and carnitine. Vitamin C is the major water – soluble antioxidant within the body. It lowers blood pressure and cholesterol level. Not only does a vitamin C intake markedly reduce the severity of a cold, it also effectively prevents secondary viral or bacterial complications. Numerous analysis have shown that an adequate intake of vitamin C is effective in lowering the risk of developing breast cancer, cervix, colon, rectum, lung, mouth, prostate and stomach. This vitamin is especially plentiful in fresh fruit, in particular citrus fruit, and vegetables(Wilkinson et al. 2005).

2. 10. Vitamins

Vitamins are organic compounds that occur naturally in plant and animal tissues. They are necessary in very small amounts in the diet to promote growth and maintain health and life. Essentially, vitamins make it possible for other nutrients to be digested, absorbed, and metabolized by the body. It is important to remember that since vitamins do not contain calories, they provide no energy. Vitamins act as catalysts—they increase the speed of chemical reactions without being used up by the reaction. This explains why vitamins are only needed in miniscule amounts(Buettner and Jurkiewicz 1996).

Vitamins are organic compounds occurring in natural foods especially in vegetables either as such or as utilizable precursors. Vitamins help body to absorb Ca and P, needed for bone growth and maintenance. Vitamins are involved in blood clotting normal functioning of nervous system and endocrine glands. They are also needed for metabolism of macromolecules(Hall 2010).

Vitamins are essential nutrients that are necessary in small amounts for various functions in the human body. Based on solubility vitamins are grouped in to two, fat soluble vitamins and water soluble vitamins: water soluble (such as B – complex and C vitamins) and fat soluble (such as vitamin A, D, E, and K)(Delgado-Zamarreno et al. 2002).

2. 11. Water soluble vitamins

Water soluble vitamins are a broad group of organic compounds that are minor, but essential, constituents of food required for normal growth, self-maintenance, and functioning of human and animal bodies. The water soluble vitamins includes vitamin C and the B complex(Li and Chen 2001).

2. 11. 1. B-complex Vitamins

Eight of the water-soluble vitamins are known as the vitamin B-complex group: thiamin (vitamin B1), riboflavin (vitamin B2), niacin (vitamin B3), vitamin B6 (pyridoxine), folate (folic acid), vitamin B12, biotin and pantothenic acid. The B vitamins are widely distributed in foods, and their influence is felt in many parts of the body. They function as coenzymes that help the body obtain energy from food. The B vitamins are also important for normal appetite, good vision, and healthy skin, nervous system, and red blood cell formation(Millen et al. 2007).

Among the B group of water- soluble vitamins, thiamine (B1), riboflavin(B3) and pyridoxine(B6) are the most important. They play different specific and vital functions in metabolism and their lack or excess produces specific diseases. Water soluble vitamins are relatively unstable and can be lost during processing and storage of food. The concentration of water soluble vitamins is regulated by the kidneys. Any intake in excess of the body's need is excreted in the urine. Fat soluble vitamins, however, are stored in the body, and excessive intakes (especially of vitamins A and D) can lead to toxicity. Some water soluble vitamins and most minerals can also be toxic in large amounts(Kennedy et al. 2011).

2.11.2. Vitamin C (Ascorbic acid)

Vitamin C (L-Ascorbic acid) is water-soluble vitamin with strong reducing action and it is an important co-enzyme for internal hydroxylation reaction. Vitamin C is found in both reduced form (ascorbic acid) and oxidized form (dehydroascorbic acid). It is widely used food additive with many functional roles, many of those are based upon its oxidation-reduction properties. Functional roles include its use as: a nutrition food additive, antioxidant, reducing agent, stabilizer, modifier, color stabilizer(Davey et al. 2000)

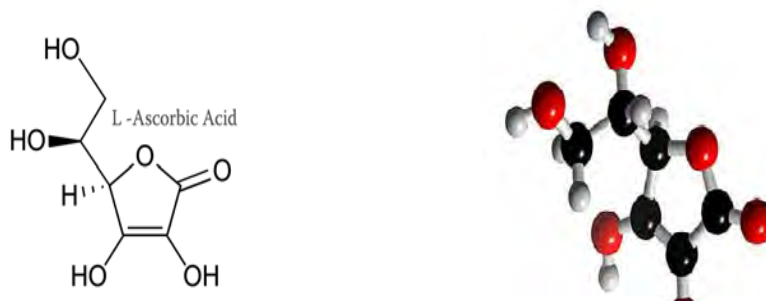


Figure2.4: the chemical structures of vitamin C.(Rahman et al. 2005)

The body needs vitamin C, also known as ascorbic acid or ascorbate, to remain in proper working condition. Vitamin C benefits the body by holding cells together through collagen synthesis; collagen is a connective tissue that holds muscles, bones, and other tissues together. Vitamin C also aids in wound healing, bone and tooth formation, strengthening blood vessel walls, improving immune system function, increasing absorption and utilization of iron, and acting as an antioxidant(Kaur and Kapoor 2002).

Vitamin C also plays an important role in the synthesis of the neurotransmitters, norepinephrine. Neurotransmitters are critical to brain function and are known to affect mood. In addition, vitamin C is required for the synthesis of carnitine, a small molecule that is essential for the transport of fat into cellular organelles called mitochondria, where the fat is converted to energy. Research also suggests that vitamin C is involved in the metabolism of cholesterol to bile acids, which may have implications for blood cholesterol levels and the incidence of gallstones(Harrison and May 2009)

Vitamin C is also a highly effective antioxidant. Even in small amounts vitamin C can protect indispensable molecules in the body, such as proteins, lipids, carbohydrates, and nucleic acids, from damage by free radicals and reactive oxygen species that can be generated during normal metabolism as well as through exposure to toxins and pollutants. Vitamin C may also be able to

regenerate other antioxidants such as vitamin E. One recent study of cigarette smokers found that vitamin C regenerated in vitamin E from its oxidized form(Naidu 2003). Since our bodies cannot produce or store vitamin C, an adequate daily intake of this nutrient is essential for optimum health. Vitamin C works with vitamin E as an antioxidant, and plays a crucial role in neutralizing free radicals throughout the body. An antioxidant can be a vitamin, mineral, or a carotenoid, present in foods, that slows the oxidation process and acts to repair damage to cells of the body. Studies suggest that vitamin C may reduce the risk of certain cancers, heart disease, and cataracts (Cadet and Brannock 1998).

2.12. Minerals

Vegetables are good source of minerals. Minerals are inorganic substances, present in all body tissues and fluids and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Minerals are chemical constituents used by the body in many ways. Although they yield no energy, they have important roles to play in many activities in the body. Every form of living matter requires these inorganic elements or minerals for their normal life processes(Gupta et al. 2005)

Minerals may be broadly classified as macro (major) or micro (trace) elements. The third category is the ultra trace elements.(Afolayan and Jimoh 2009)

The macro-minerals include calcium, phosphorus, sodium and potassium, while the micro-elements include iron, copper, cobalt, potassium, magnesium, iodine, zinc, manganese, molybdenum, fluoride, chromium, selenium and sulfur. The macro-minerals are required in amounts greater than 100 mg/dl and the micro-minerals are required in amounts less than 100mg/dl. The ultra trace elements include boron, silicon, arsenic and nickel which have been found in animals and are believed to be essential for these animal(Bangash et al. 2011).This study focused only on calcium(Ca), iron(Fe), Zinc(Zn), Magnesium(Mg)

2. 12. 1 Calcium

Calcium is the most common mineral in the human body. About 99% of the calcium in the body is found in bones and teeth, while the other 1% is found in the blood and soft tissue. Calcium levels in the blood and fluid surrounding the cells must be maintained within a very narrow concentration range for normal physiological functioning. The physiological functions of calcium are so vital to survival that the body will demineralize bone to maintain normal blood calcium levels when

calcium intake is inadequate. Thus, adequate dietary calcium is a critical factor in maintaining a healthy skeleton(Power et al. 1999)

Calcium is a major structural element in bones and teeth. The mineral component of bone consists mainly of hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ crystals, which contain large amounts of calcium and phosphate(Power et al. 1999) Bone is a dynamic tissue that is remodeled throughout life. Bone cells called osteoclasts begin the process of remodeling by dissolving or restoring bone. Bone-forming cells called osteoblasts Then synthesize new bone to replace the bone that was resorbed. During normal growth, bone formation exceeds bone resorption. Osteoporosis may result when bone resorption chronically exceeds formation (Peters and Martini 2010)

Calcium plays a role in mediating the constriction and relaxation of blood vessels, nerve impulse transmission, muscle contraction, and the secretion of hormones like insulin(Adongo et al. 2012). Excitable cells, such as skeletal muscle and nerve cells, contain voltage-dependent calcium channels in their cell membranes that allow for rapid changes in calcium concentrations. For example, when a muscle fiber receives a nerve impulse that stimulates it to contract, calcium channels in the cell membrane open to allow a few calcium ions into the muscle cell. These calcium ions bind to activator proteins within the cell, which release a flood of calcium ions from storage vesicles inside the cell. The binding of calcium to the protein, troponin-c, initiates a series of steps that lead to muscle contraction(Qureshi et al. 2014)

The binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen to provide energy for muscle contraction(Clapham 2007) Calcium is necessary to stabilize a number of proteins and enzymes, optimizing their activities. The binding of calcium ions is required for the activation of the seven vitamin K-dependent clotting factors in the coagulation cascade. The term, coagulation cascade, refers to a series of events, each dependent on the other that stops bleeding through clot formation(Despotis et al. 1999)

2. 12. 3 Iron (Fe)

Iron has the longest and best described history among all the micronutrients. It is a key element in the metabolism of almost all living organisms. In humans, iron is an essential component of hundreds of proteins and enzymes(Qureshi et al. 2005). Haeme is an iron-containing compound found in a number of biologically important molecules. Hemoglobin and myoglobin are haeme-containing proteins that are involved in the transport and storage of oxygen. Hemoglobin is the primary protein found in red blood cells and represents about two thirds of the body's iron. The vital role of hemoglobin in transporting oxygen from the lungs to the rest of the body is derived from its unique ability to acquire oxygen rapidly during the short time it spends in contact with the lungs

and to release oxygen when needed during its circulation through the tissues. Myoglobin functions in the transport and short-term storage of oxygen in muscle cells, helping to match the supply of oxygen to the demand of working muscles(Reinke et al. 2012). Cytochromes are haeme-containing compounds that have important roles in mitochondrial electron transport; therefore, cytochromes are critical to cellular energy production and thus life. They serve as electron carriers during the synthesis of ATP, the primary energy storage compound in cells. Cytochrome P450 is a family of enzymes that functions in the metabolism of a number of important biological molecules, as well as the detoxification and metabolism of xenobiotics. Non-haeme iron-containing enzymes, such as NADH dehydrogenase and succinate dehydrogenase, are also critical to energy metabolism(Chipurura 2010)

Catalase and peroxidases are haeme-containing enzymes that protect cells against the accumulation of hydrogen peroxide, a potentially damaging reactive oxygen species (ROS), by catalyzing a reaction that converts hydrogen peroxide to water and oxygen. As part of the immune response, some white blood cells engulf bacteria and expose them to ROS in order to kill them. The synthesis of one such ROS, hypochlorous acid, by neutrophils is catalyzed by the haeme-containing enzyme myeloperoxidase(Prasad, Kodliwadmth and Kodliwadmth 2007). Inadequate oxygen, such as that experienced by those who live at high altitudes or those with chronic lung disease, induces compensatory physiologic responses, including increased red blood cell formation, increased blood vessel growth, and increased production of enzymes utilized in anaerobic metabolism. Under hypoxic conditions, transcription factors known as hypoxia inducible factors (HIF) bind to response elements in genes that encode various proteins involved in compensatory responses to hypoxia and increase their synthesis. Research indicates that an iron dependent prolyl hydroxylase enzyme plays a critical role in regulating HIF and, consequently, physiologic responses to hypoxia(Hagen 2012). When cellular oxygen tension is adequate, newly synthesized HIF α subunits are modified by a prolyl hydroxylase enzyme in an iron-dependent process that targets HIF α for rapid degradation. When cellular oxygen tension drops below a critical threshold, prolyl hydroxylase can no longer target HIF α for degradation, allowing HIF α to bind to HIF β and form an active transcription factor that is able to enter the nucleus and bind to specific response elements on genes. Ribonucleotide reductase is an iron-dependent enzyme that is required for DNA synthesis. Thus, iron is required for a number of vital functions, including growth, reproduction, healing, and immune functions(Saab-Rincón and Valderrama 2009)

2. 12.4 Zinc (Zn)

Zinc is an essential trace element for all forms of life. Clinical zinc deficiency in humans was first described in 1961, when the consumption of diets with low zinc bioavailability due to high phytic acid content was associated with "adolescent nutritional dwarfism" in the Middle East(Jeejeebhoy 2009). Since then, zinc insufficiency has been recognized by a number of experts as an important public health issue, especially in developing countries. Numerous aspects of cellular metabolism are zinc-dependent. Zinc plays important roles in growth and development, the immune response, neurological function, and reproduction. On the cellular level, the function of zinc can be divided into three categories namely, catalytic, structural and regulatory(Frassinetti et al. 2006)

Nearly 100 different enzymes depend on zinc for their ability to catalyze vital chemical reactions. Zinc-dependent enzymes can be found in all known classes of enzymes(Sharma et al.). Zinc plays an important role in the structure of proteins and cell membranes. A fingerlike structure, known as a zinc finger motif, stabilizes the structure of a number of proteins. For example, copper provides the catalytic activity for the antioxidant enzyme copper-zinc superoxide dismutase (CuZnSOD), while zinc plays a critical structural role. The structure and function of cell membranes are also affected by zinc. Loss of zinc from biological membranes increases their susceptibility to oxidative damage and impairs their function(Marian and Sacks 2009).

Zinc finger proteins have also been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling and has been found to influence hormone release and nerve impulse transmission. Zinc has been found to play a role in apoptosis, a critical cellular regulatory process with implications for growth and development, as well as a number of chronic diseases(Shinozaki, Yamaguchi-Shinozaki and Seki 2003)

2.12.5. Magnesium

Magnesium plays important roles in the structure and the function of the human body. The adult human body contains about 25 grams of magnesium. Over 60% of all the magnesium in the body is found in the skeleton, about 27% is found in muscle, 6% to 7% is found in other cells, and less than 1% is found outside of cells(Andzouana and Mombouli 2012).

The metabolism of carbohydrates and fats to produce energy requires numerous magnesium-dependent chemical reactions. Magnesium is required by the adenosine triphosphate (ATP)-synthesizing protein in mitochondria. ATP, the molecule that provides energy for almost all metabolic processes, exists primarily as a complex with magnesium(Szentmihályi et al. 2012).

Magnesium is required for a number of steps during nucleic acid and protein synthesis. Several enzymes participating in the synthesis of carbohydrates and lipids require magnesium for their activity. Glutathione, an important antioxidant, requires magnesium for its synthesis(Hartwig 2001). Magnesium plays a structural role in bone, cell membranes, and chromosomes(Mornet et al. 2001) Magnesium is required for the active transport of ions like potassium and calcium across cell membranes. Through its role in ion transport systems, magnesium affects the conduction of nerve impulses, muscle contraction, and normal heart rhythm(Schatzmann and Vincenzi 1969) Cell signaling requires MgATP for the phosphorylation of proteins and the formation of the cell-signaling molecule, cyclic adenosine monophosphate (cAMP). cAMP is involved in many processes, including the secretion of parathyroid hormone from the parathyroid glands. Calcium and magnesium levels in the fluid surrounding cells affect the migration of a number of different cell types. Such effects on cell migration may be important in wound healing(Nadler et al. 2001)

2.13. Anti-Oxidant

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants(Devasagayam et al. 2004)

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid proxy and thus inhibit the oxidative mechanisms that lead to degenerative diseases(Prakash, Rigelhof and Miller 2001)

Dietary antioxidants protect against free radicals such as reactive oxygen species in the human body Free radicals are known to be a major contributor to degenerative diseases of aging .Fruit and vegetables are good sources of natural antioxidants such as vitamins, carotenoids, flavonoids and

other phenolic compounds. Due to the detection of many bioactive compounds in food with possible antioxidant activity, there has been increased interest in the relationship between antioxidant and disease risks. Epidemiological studies have shown a strong and consistent protective effect of vegetable consumption against the risk of several age-related diseases such as cancer, cardiovascular disease, cataract and macular degeneration(Liu 2003).

2.13.1. Classes of antioxidants

Antioxidants are divided into two major classes, namely endogenous antioxidants and exogenous antioxidants(Carocho and Ferreira 2013)

2.13.1.1 Endogenous antioxidants

Three groups of enzymes play important roles in protecting cells from oxidative stress. Firstly, superoxide dismutases (SOD) are enzymes that catalyze the conversion of two superoxides to hydrogen peroxide and oxygen. Hydrogen peroxide is substantially less toxic than superoxide. The detoxifying reaction catalyzed by SOD is ten thousand times faster than the uncatalyzed reaction.

SODs are metal-containing enzymes that depend on bound manganese, copper or zinc ion for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms are predominant in cytoplasm. SODs are inducible enzymes, with exposure of bacteria or vertebrate cells to higher concentrations of oxygen resulting in rapid increases in the concentration of SOD. Secondly, catalase, found in peroxisomes in eukaryotic cells, degrades hydrogen peroxide to water and oxygen, and hence completes the detoxification reaction started by SOD. Finally, glutathione peroxidase, a group of enzymes which are the most abundant contain selenium and like catalase, degrade hydrogen peroxide. Glutathione is the most important intracellular defense against damage by reactive oxygen species(Lambert and Brand 2009). The cysteine on the glutathione molecule provides an exposed free sulphhydryl group that is very reactive, providing an abundant target for radical attack. Reaction with radicals oxidizes glutathione but the reduced form is regenerated in a redox cycle that involves glutathione reductase and the electron acceptor NADPH. In addition to the three enzymes above, glutathione transferase, ceruloplasmin, hemoxygenase may participate in enzymatic control of oxygen radicals and their products(Chipurura 2010).

2.13.1.2. Exogenous antioxidants

The three common exogenous antioxidants are vitamin E, vitamin C/ ascorbic acid/ and glutathione. Vitamin E is the major lipid-soluble antioxidant and plays an important role in protecting membranes from oxidative damage. The primary activity of vitamin E is to trap peroxy radicals in cellular membranes and consequently prevent lipid peroxidation of the membranes(Liebler 1993).

Vitamin C is a water-soluble antioxidant that can reduce radicals from a variety of sources. Vitamin C participates in recycling vitamin E radicals. Vitamin E radicals are generated when the vitamin trap peroxy radicals in cellular membranes. Vitamin C also functions as a pro-oxidant under certain circumstances and sometimes produces oxygen by-products of metabolism that can cause damage to cells(Nag 2009)

In addition to vitamin E and vitamin C, phenolic compounds can function as antioxidants. The antioxidant properties of some plant extracts have been attributed partially to their phenolic compound contents(Tripoli et al. 2005)

2.13.2. Antioxidant extraction processes

Plants contain a wide spectrum of metabolites, as many as 200 000 different compounds (Fiehn *et al.*, 2002), although not every metabolite occurs in every species. These metabolites represent many different classes of compounds and their derivatives such as amino acids, fatty acids, carbohydrates, and organic acids. The physical chemical properties of the metabolites are highly variable, therefore appropriate extraction protocols have to be chosen, as the optimum extraction conditions differ widely for different types of compounds.

2.13.3 Homogenization and solvent extraction

The plant tissue must be homogenized properly in order to extract plant metabolites efficiently. Various techniques such as grinding with a mortar and pestle together with liquid nitrogen, milling in vibration mills with chilled holders, homogenization with a metal pestle connected to an electric drill (Edlund *et al.*, 1995) and ultra-turrax devices(Orth *et al.*, 1999) are available.

The degree of homogenization determines the efficiency at which the solvent can penetrate the tissue, and therefore strongly influences the length of time required for solvent extraction. The most common way to extract metabolites is to shake the homogenized plant tissue at low or high temperatures in organic solvents, or mixtures of solvents (Fiehn *et al.*, 2000). Methanol, ethanol, and water are the solvents mostly used for extracting polar metabolites, whereas chloroform is the most common solvent for non-polar ones (Roger, 1999).

2.14. The effect of different cooking methods vegetables on vitamins minerals and antioxidants

Vegetables are rich sources of essential vitamins minerals, fibers and disease fighting photochemical which the human body needs to maintain good health. Some vegetables can be taken raw but most are commonly cooked before being consumed. Generally preparations of vegetables at home are based on taste preference and convenience rather than retention of nutrients and health promoting compounds(Igwemmar, Kolawole and Imran 2013)

Cooking is responsible for losses of vitamins and minerals in foods. However the bioavailability of some minerals, for example iron, may be increased by cooking(Hallberg 1981). Loss of vitamins and minerals from vegetables is mainly because of extraction into the cooking liquid rather than their destruction(Lešková et al. 2006)

There are different kinds of cooking methods such as boiling, steaming, stir frying, blanching etc. In which all affects the nutrient content of the food. Like other African countries in the rural area of Ethiopia vegetables such as spinach, cabbage, paper, broccoli, and salad are the source of nutrients which are usually consumed after cooking(Ayele and Peacock 2003)

Cooking losses of vitamins and minerals are depend upon degree of heating, leaching in to the cooking medium, surface area exposed to water and oxygen, and cooking time(Lešková et al. 2006)

Blanching which is an important pre-processing heat-treatment of vegetable destined for freezing, canning or dehydration inevitably causes separation and losses of water soluble nutrients (minerals, water soluble vitamins and sugars. Balancing at 88°C stop all life process, inactivates enzymes fixes green color and removes certain harsh flavors lemon in vegetable(Puupponen-Pimiä et al. 2003)

Methods, temperature and duration of cooking may also effect significantly on the nutritive value of vegetables. Some of the important nutrients such as ascorbic acid and folic acid which are susceptible to oxidation are readily oxidized by brisk cooking minerals are also affected by high temperatures in some other cases flavor may be lost by brisk cooking. Excessive cooking may also cause an adverse effect on the digestibility of the vegetables(Song and Thornalley 2007)

3. MATERIALS AND METHODS

3.1 Sample Collection Area

The samples were collected in Sodo woreda, Gurage Zone of SNNPR State, south Ethiopia. It is located in the Western part of central Ethiopia and at the same time it is the Northern tip of the region. It is bounded with Hadiya Zone and Yame special woreda in the south and south west respectively. The Northern and Eastern portion are sharing boarder with Oromia state. The Gurage people occupy Sothern most area of the central plateau, about 150km southwest of Addis Ababa, mainly semi mountainous country with high land forest.

The climate in the Zone is of three conditions. These are dega, weinadega and kola. The dega section is connected with the distribution of the Gurage Chain Mountains. Most of the Zone lines in the weinadega division. The distribution of rain fall and temperature mainly flows this pattern. The highest rain fall record is 1600mm per year, while the lowest was recorded as 700mm per year. The highest and lowest temperature record is 32 and 15 degree celicious respectively.

Sodo is one of the 13 wordas in Gurage Zone. The capital city of the woreda is Buei and is 103km from Addis Ababa.

The majority of the woreda kebeles is mountainous and has a good weather condition to cultivate vegetables for sale or self consumption.

3.2. Sample Collection

For this study green leafy vegetables were selected. The green leafy vegetables Ye,abesha gomen(*Brassica carinata*),cabbage(*Brassica oleracea*), ye,gurage gomen(*Brassica carinata*) and Swiss char (*Beta vulgaris*) were purchased from the local market of Buei and Damu Gent Rebu Gebeya, in which most of the farmers sell their products.

3.3 Sample preparation and cooking treatment

Four different vegetables purchased and collected from the local market were washed with tap water to remove all dust and soil. The inedible parts were manually removed with the help of a sharp knife. All the vegetables(namely, Ye,abesha gomen(*Brasica carinata*), cabbage(*Bracica Oleracea*), yegurage gomen (*brassica carinata*) and Swiss chard(*Beta vulgaris*) of 1kg of each chopped in to almost equal small pieces, thoroughly mixed homogeneity and divided into three portions for cooking at (raw, 10min, 30min). Cooking were done by smearing the vegetables in 1litter boiling water at 90⁰c in clay pot and covered by clay lid. The cooked vegetable leaving to

cool at room temperature. One part for each vegetable was reserve for raw analysis. The sample were cooled in ice water and stored at -5°c for three day before they are transported to the laboratory for vitamin and mineral analysis.

3.4 Vitamin analysis

3.4.1 Extraction for vitamin C (Ascorbic acid) analysis

This was carried out using the Colorimetric Analytical method since it gives an accurate analysis of vitamin C content than the other methods of analysis. Into a beaker containing 50 ml of 4% Oxalic Acid solution, 5 g of the ground sample was placed and swirled. The solution containing the ground sample was filtered using filter paper and a funnel over another beaker in order to collect the liquid. An aliquot (10 ml) of the collected liquid was taken using a 10 ml pipette into 50 ml volumetric flask and Bromine water was added drop wise with constant mixing till the extract turned orange-yellow due to excess bromine water. Bubbles were expelled by blowing air into the flask containing the liquid.

The volume was made up to the 50 ml mark on the flask with 4% Oxalic Acid solution. Similarly, 10 ml of the stock vitamin C solution was also brominates to obtain an orange-yellow color. A volume of 1 ml of the liquid in the 50 ml volumetric flask was taken using a 1 ml volume pipette into a clean test tube. The volume of the test tube was made to 4 ml by the addition of 1 ml each of Thiourea solution, 2,4-Dinitrophenyl Hydrazine (DNPH) reagent and distilled water. The content of the test tube was thoroughly mixed and incubated in a water bath at 37°C for 3 hours. The solution in the test tube was allowed to cool and 7 ml of 80% Sulfuric acid was added to make up the volume to 11 ml. This was done for all the samples to be analyzed.

3.4.2 Determiration of vitamin C (Total Ascorbic acid)

The absorbance of the extract was measured using a spectrophotometer (UV mini 1240) at a wavelength of 515 nm. A cuvette containing the prepared blank solution was used to calibrate the spectrophotometer to the point zero. Samples of the extract were placed into the cuvette and readings were taken when the figure in the display window became steady. The spectrophotometer was blanked each time with the prepared blank solution before the readings were taken. The operation was repeated three times for each sample and the average readings were recorded. The absorbance obtained was extrapolated on a vitamin C standard curve. Each value of the vitamin C obtained from the standard curve was put into the equation below to determine the final concentration. About 1g of the sample was weighed, macerated with 10mls of 0.4% oxalic acid in a

test tube for 10 minutes, centrifuged for 5 minutes and the solution filtered. 1ml of the filtrate was transferred into a dry test tube in duplicates, 9mls of 2, 6-dichlorophenol indophenols was added and absorbance was taken at 15 seconds and 30 seconds interval at 520nmorbic

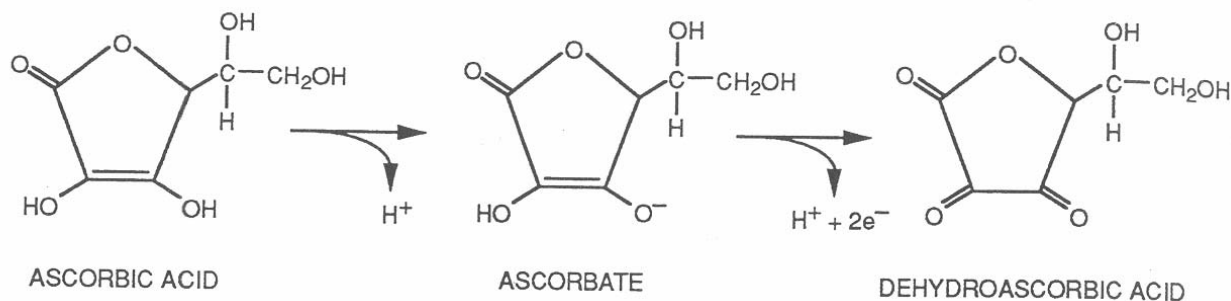


Figure3.1. Ascorbic acid and its oxidation to ascorbate and dehydroascorbic acid(Davey et al. 2000)

Calculation

$$\text{VC concentration} = \frac{\text{mg AA}/100\text{g} [(A_s - A_b) * 10]}{[A_{10\mu\text{g}}\text{Std} - A_b]}$$

Where A_s _ Absorbance of samples
 A_b _ Absorbance of blank
 $A_{10\mu\text{g}}\text{Std.}$ _ Absorbance of 10 μg AA standard

3.5. Antioxidant analysis

Plant Materials The leaves were washed, cooked and then milled into coarse powder by mortar and mill. Four plant materials, which were tested for their antioxidant activity, were selected

3.5.1 Antioxidant extraction

Milled leafy vegetable (2.5 g) of each sample were extracted with 25 ml of 99% methanol, by stirring and sonicating for 20 min. The procedure was repeated twice. The supernatant was pooled and stored at 4°C. The concentrated sample was used as a sample extract for estimation of total antioxidant.

A simple method that has been developed to determine the antioxidant activity of foods utilizes the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical. The structure of DPPH and its reduction by an antioxidant are shown below. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to yellow as the molar absorptive of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form

the reduced DPPH-H. The resulting decolonization is stoichiometric with respect to number of electrons captured. Antioxidant compounds may be water-soluble, lipid soluble, insoluble, or bound to cell walls. Hence, extraction efficiency is an important factor in quantification of antioxidant activity of foods. Trolox (as the reference standard) and the sample are reacted with DPPH solution in methanol/water for four hours at 35°C in a vessel mounted on a rotary shaker and the absorbance changes are measured at 517 nm. The quantity of sample necessary to react with one half of the DPPH is expressed in terms of the relative amount of Trolox reacted. Antioxidant activity of a sample is expressed in terms of micromole equivalents of Trolox (TE) per 100 grams of sample, or simply Trolox units per 100 gm or TE/100g. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (20, 40, 80,120, 150 and 200 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer.

3.5.3 DPPH* (2, 2'-diphenyl-1 - picrylhydrazyl) free radical scavenging assay

DPPH* method is used to study the scavenging activity of antioxidants in vegetables and oils. It is seemed to be endowed with good antioxidant properties. This method is based on the reduction of a methanol solution of DPPH*in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H (Soler *et al.*, 2000). The samples are prepared by dissolving extracted vegetable sample to methanol and taken (20, 40, 80,120 150 and 200 µg/ml) 2 ml of a methanol added to DPPH• free radical (Brand *et al.*, 1995). The reaction mixture is shaken by cyclo-mixer and then kept in the dark for 30 min under ambient conditions.

This transformation results is a change in color from purple to yellow, which has been measured spectrophotometrically by using UV-Spectrophotometer. The disappearance of the purple color change at 517 nm is observed.

3.7 Mineral Analysis

3.7.1 Samples Preparation for Mineral Analysis

2.5g of the powdered sample was weighed into a crucible and gently heated over a hot plate until it charred. The charred sample with the crucible was transferred into a muffle furnace at about 550°C and left for about 5 hours. About this time it had turned to white ash. It was cooled first at room temperature and then in a desiccators. 7ml of 6N HCl was added to each crucible and heated until semi-dry on a hot plate in a fume cupboard. 15ml of 3N HCl added to each crucible and boiled. After cooling, the boiled mixture was then filtered into a 50ml flask and then 10ml of 3N HCl was added in to the crucible and heated for 5min. This mixture was then cooled, filtered and rinsed into 50ml volumetric flask. The crucible was washed several times with distilled water filtered and rinsed in to volumetric flask to made up mark [9]. The extract was prepared in triplicates. The concentrations of calcium (Ca), Zink(Zn),Iron(Fe), magnesium (Mg) and nickel(Ni) in the solutions were determined using Atomic Absorption Spectrophotometer, AAS (Idris et al. 2011)

3.8 Statistical Analysis

All data on total antioxidant activity, vitamin C and minerals are the average of triplicate. To examine the total antioxidant activity, concentration of vitamin C and composition of selected minerals of raw and traditionally cooked leafy vegetables graphs are used. The data were recorded as mean \pm SD and analyzed by SPSS (version 15) and tabulated in Table. One-way analysis of variance is performed by ANOVA procedures. Significant differences between means are determined by Duncan's multiple range tests, p-Values <0.05 are regarded as significant.

4. RESULTS AND DISCUSSION

4.1. Total antioxidant activity

Total antioxidant activity of tested leafy vegetables decreased during cooking methods compared to the values for the fresh ones. Traditional Cooking was found to lead to reductions in the antioxidant activity for the tested vegetables, with more cooking time causing a more preformed effect. Antioxidants are compounds that can delay or prevent the oxidation of lipids or other molecules by inhibiting the initiation or propagation of an oxidizing chain reaction and by many other mechanisms and thus prevent diseases(Panda 2012). The antioxidant activity (AOA), expressed as percent inhibition of oxidation, ranged from as high as $87.4 \pm 0.61\%$ to as low as $4.33 \pm 0.21\%$. Cooking these vegetables for both ten and thirty minutes decreased their AOA with the latter recording the least value for each of the cooking times as shown in Table 1.

Table 1 DPPH Scavenging activities of four leafy vegetables in methanol extracts

Sample Concentration	AA Standard	Ye, Abesha Gomen / B. Carinata/			Ye, Gurage Gomen / B. Carinata/			Tekel Gomen/B.oleraceae/			Swiss chard/Beta Vulgaris/		
		Fresh	10min. Cooked	30min. Cooked	Fresh	10min. Cooked	30min. Cooked	Fresh	10min. Cooked	30min. Cooked	Fresh	10min. Cooked	30min. Cooked
20	9.76±1.96 ^d	35.79±0.58 ^k	28.22±0.39 ⁱ	17.18±0.38 ^g	34.08±0.22 ^j	25.59±0.74 ^h	14.26±0.15 ^f	13.01±0.39 ^e	10.01±0.26 ^d	6.92±0.53 ^b	10.51±0.36 ^d	8.22±0.31 ^c	4.33±0.21 ^a
40	21.38±1.69 ^e	40.84±0.46 ^j	32.62±0.43 ^h	22.18±0.67 ^e	39.78±0.27 ⁱ	31.48±0.58 ^g	18.65±0.05 ^c	29.22±0.69 ^f	20.03±0.28 ^d	14.17±0.46 ^b	21.41±1.33 ^c	24.61±0.82 ^b	10.85±0.48 ^a
90	52.58 ± 1.02 ^j	53.46±0.45 ^j	43.84±1.25 ⁱ	29.92±0.14 ^{ef}	52.7±0.19 ^j	39.98±0.52 ^h	29.04±0.34 ^e	38.02±0.29 ^g	30.45±0.62 ^f	21.32±0.30 ^c	25.95±1.62 ^d	17.24±0.35 ^b	12.99±0.33 ^a
120	94.44± 0.07 ⁱ	63.08±0.62 ^e	55.32±0.61 ^j	40.76±0.39 ^g	60.37±0.04 ^k	50.18±0.77 ⁱ	38.7±0.15 ^e	45.8±0.16 ^h	39.62±0.43 ^f	28.84±0.47 ^c	31.31±0.29 ^d	22.89±0.38 ^b	16.17±0.21 ^a
150	95.27 ± 0.43 ^j	73.82±0.33 ^j	61.72±0.52 ^j	51.17±0.91 ^g	70.44±0.16 ⁱ	57.08±0.34 ^h	48.83±0.97 ^f	52.09±1.19 ^g	46.15±0.61 ^e	35.94±0.49 ^c	39.29±0.56 ^d	28.20±0.27 ^d	19.41±0.80 ^a
200	96.88 ± 0.05 ⁱ	87.4±1.61 ^h	76.79±0.88 ^g	61.07±0.73 ^e	86.21±1.15 ^h	75.4±0.87 ^g	58.12±0.34 ^d	66.66±0.94 ^f	52.27±1.06 ^c	45.68±1.11 ^b	57.35±1.75 ^d	44.03±0.74 ^b	30.4±30.73 ^a

- All the values are mean ± standard deviation of triplicates determination
- Means followed by different letter across the same raw have significant difference (P < 0.05)

Mean antioxidant activity of fresh leafy vegetables, Ye,abesha gomen > Ye,guragae gomen > Tikle gomen(cabbage) > Swiss chard at mg/100g were 87.4 %, 86.2%, 66.66% and 57.35% respectively. Results of ANOVA analysis indicated that antioxidant activity of Abyssinian cabbage is significantly higher ($p < 0:05$) than the rest of the other leafy vegetables. A high antioxidant activity of Abyssinian cabbage may be attributed to the nutrient antioxidants found in these vegetables. According to (Ismail, Marjan and Foong 2004),swamp cabbage and spinach have high contents of carotenoids, ascorbic acid and a tocopherol and these might have been the main factors that contribute to the high antioxidant activity. The low antioxidant activity of cabbage and Swiss chard was in agreement with(Nithiyantham, Varadharajan and Siddhuraju 2012). As shown in table1, the mean antioxidant activities of the 10-min and 30min traditionally cooked leafy vegetables were similar to the fresh vegetable, Ye,abesha gomen> Ye,guragae gomen > cabbage > Swiss chard (76.79, 75.4%, 52.27% and 44.03% respectively). As shown in the table1 above, high sample concentration increases % of inhibition. Heat treatments affect the antioxidant activity of vegetables and in many cases have been observed lower antioxidant capacity in processed samples versus raw vegetables(Podsędek 2007). During the conventional and microwave cooking for 10min, leafy vegetables retained about 35% of total antioxidant activity measured by DPPH method(Zhang and Hamazu 2004). Similarly, according to Lin and Chang(El-Din et al. 2013),the extract from broccoli cooked for 10 min at 50°C showed scavenging activity toward DPPH radicals of 31% DPPH index of cauliflower decreased by 23%, but in case of cabbage increased by 9% during blanching in water(Rodriguez-Amaya 2001). Other researchers indicated that processing caused no change to antioxidant activities of fruits and vegetables. The differences of the results obtained from this study compared to the other findings may have been due to the differences in the extraction methods. Antioxidant activity of vegetable extracts also depends on the type and polarity of the extracting solvent, the isolation procedures and purity of active compounds, as well as the assay techniques and substrate used(Ismail et al. 2004). In the study conducted on the type of solvent used to extract vegetable sample, less polar solvents provided slightly more active extracts than mixtures with ethanol or methanol, or methanol alone(Ismail et al. 2004). The extraction solvent in this study was 99% methanol while 100% acetone was used by Cao et al. (1996). The result reported by Cao et al. (1996), microwave cooking on cabbage and kale did not show any significant difference in antioxidant activity which was contradict with the result found in this study.

4. 2. Vitamin C in Extracts of Vegetables

The mean and standard deviations of vitamin C content of raw and traditionally cooked leafy vegetable was presented in (table 2). It shows the vitamin C content of fresh and cooked leafy vegetables with respect to time. It was shown that vitamin C content of the raw and traditionally cooked leafy vegetable significantly different from each other. The result shows that high percentage of vitamin C was lost during extraction at 30minute of cooking but with low vitamin C in the extract at 10 minutes.

Table 2 Vitamin C concentration of four leafy vegetables commonly consumed in Ethiopia (mg/100g)

Vegetables species	Cooking time	Concentration
Ye,abesha gomen	Raw	80.7± 0.53 ^b
	10min	24.62±0.62 ^e
	30min	1.09±0.12 ^a
Ye,guragae gomen	Raw	78.20±0.59 ^g
	10min	28.09±0.36 ^f
	30min	2.43±0.10 ^b
Cabbage(B.oleraceae)	Raw	24.62±0.46 ^e
	10min	18.64±0.63 ^d
	30min	16.03±0.23 ^c
Swiss chared(B.vulgares)	Raw	18.41±0.34 ^d
	10min	1.44±0.08 ^a
	30min	0.60±0.04 ^a

- All the values are mean ± standard deviation of triplicate determinations
- Means within the same column with different letter superscripts indicate significant differences (p< 0.05)

Averagely, table 2 shows that for most of the leafy vegetables, low vitamin C was lost at 10 minutes. This invariably means that the vegetables are best consumed fresh or cooked for 10 minutes or less because very little vitamin C will be lost to the extract and higher percentage will be in the cooked leaves left for consumption. Loss as a result of cooking is justified since vitamin C is water-soluble and heat labile (Olayiwola, Oyeleke and Adeoye 2012). Olayiwola and Oyeleke (2012), explained in their study that as time of boiling increases vegetables are subjected to denaturation, further heating may tend to bring the level to zero (Olayiwola et al. 2012). The results obtained are in accordance with what was obtained by Davey *et al.* (2000).

For vitamin C content, a significant reduction of Ye, abesha gomen ($80.7 \pm 0.53 - 1.09 \pm 0.11$), Ye, guraga gomen ($78.19 \pm 0.58 - 2.43 \pm 0.10$), Tikle gomen (cabbage) ($24.62 \pm 0.45 - 16.03 \pm 0.23$) and Kosta ($18.41 \pm 0.34 - 0.59 \pm 0.04$) was highlighted at 30 min cooking process (table 2). These values support the results obtained for other studies which indicate losses up to 66 % in cooked vegetables (Oulai et al.). It is important that ascorbic acid is a water-soluble antioxidant that promotes absorption of soluble iron by chelating or by maintaining the iron in the reduced form (Fang, Yang and Wu 2002). With regard to the drastic decrease of vitamin C during cooking, consumption of cooked leafy vegetables may be supplemented with other sources of vitamin C such as fruits to cover the daily need for humans (40 mg/day) as recommended by food and agriculture organization (Joy et al. 2014).

4.3 Mineral composition

The ash and mineral compositions of the raw and traditionally cooked leafy vegetables examined in this study are presented in (Table 3). It was shown that the mineral content of the raw and traditionally cooked leafy vegetable were significantly different from each other. The result shows that traditional cooking at 10min and 30min of leafy vegetables resulted in reduction of mineral concentration.

The ash content of the studied raw leafy vegetables ranged from *Ye, gurage gomen* (89.84 ± 0.43) to *Swiss chard* (85.88 ± 0.67), at 10min of cooking ranged from 88.38 ± 0.51 % (*ye, gurage gomen*) to 82.00 ± 0.31 % (*Swiss chard*) and at 30min of cooking (85.77 ± 0.49 (*ye, gurage gomen*) to (77.85 ± 0.62 (*kosta*)). These values were closed to 76.43 ± 0.12 % and 79.86 ± 0.12 % after 30 min of cooking for the wild leafy vegetables studied in South Africa. The decrease rate of ash content during cooking time of 10 min and 30min in the studied leafy vegetables may be a result of minerals leaching into the boiling water.

Table 3 Ash and Mineral composition of four leafy vegetables commonly consumed in Ethiopia

Vegetable type	Cooking time	Ash	Minerals			
			Ca	Mg	Fe	Zn
Ye, Abesha Gomen / B. Carinata/	Fresh	88.63 ± 0.98 ^g	1.907 ± 0.012 ^f	2.24 ± 0.048 ^h	9.171 ± 0.051 ⁱ	0.31 ± 0.01 ^f
	10 min Cooked	87.29 ± 0.58 ^f	1.84 ± 0.021 ^d	2.09 ± 0.051 ^e	8.50 ± 0.072 ^f	0.28 ± 0.005 ^f
	30 min Cooked	84.89 ± 0.54 ^d	1.83 ± 0.472 ^d	2.05 ± 0.009 ^a	8.367 ± 0.038 ^e	0.27 ± 0.004 ^d
Ye, Gurage Gomen / B. Carinata/	Fresh	89.84 ± 0.7 ^h	1.803 ± 0.025 ^d	2.12 ± 0.045 ^g	8.94 ± 0.044 ^h	0.33 ± 0.009 ^g
	10 min Cooked	88.38 ± 0.51 ^g	1.667 ± 0.055 ^d	2.07 ± 0.109 ^f	8.32 ± 0.100 ^e	0.31 ± 0.005 ^f
	30 min Cooked	85.77 ± 0.49 ^{de}	1.496 ± 0.021 ^a	1.901 ± 0.035 ^b	8.02 ± 0.10 ^d	0.28 ± 0.003 ^d
Tekel Gomen/Boleracae/	Fresh	86.66 ± 0.45 ^{ef}	1.83 ± 0.01 ^d	1.99 ± 0.05 ^e	3.9 ± 0.052 ^c	0.24 ± 0.012 ^c
	10 min Cooked	81.52 ± 0.56 ^c	1.76 ± 0.006 ^e	1.84 ± 0.044 ^d	3.76 ± 0.111 ^b	0.22 ± 0.005 ^b
	30 min Cooked	80.22 ± 0.89 ^b	1.66 ± 0.025 ^b	1.69 ± 0.032 ^c	3.09 ± 0.066 ^a	0.19 ± 0.007 ^a
Swiss chard/Beta Vulgares/	Fresh	85.88 ± 0.67 ^{de}	1.94 ± 0.015 ^h	2.45 ± 0.014 ^d	8.88 ± 0.59 ^h	0.34 ± 0.006 ^g
	10 min Cooked	82.00 ± 0.31 ^c	1.88 ± 0.015 ^e	2.32 ± 0.07 ^a	8.87 ± 0.031 ^g	0.311 ± 0.009 ^f
	30 min Cooked	77.85 ± 0.62 ^a	1.83 ± 0.015 ^d	2.16 ± 0.044 ^a	8.32 ± 0.106 ^e	0.29 ± 0.002 ^e

- Value Represents means ± SD, n = 3
- Means in the same column with different letter superscripts indicate significant differences (p<0.05).
- Means in the same column with the same letter superscripts indicate no significant differences (p<0.05).

Mineral composition of raw and cooked leafy vegetables used in this study shown in table3. Based on this finding leafy vegetables are good source of calcium. The concentration of calcium in raw leafy vegetable is in the order of *Swiss Chared/Beta vulgaris*/ 1.94 ± 0.02), *Ye, abesha gomen*(*B. carinata*/ 1.91 ± 0.01), Tikle gomen/*B. oleraceae*/ 1.83 ± 0.01) and *Simaro*(*B. carinata*/ 1.81 ± 0.03). Calcium is a major factor sustaining strong bones and plays a part in muscle contraction and relaxation, blood clotting, synaptic transmission and absorption of vitamin B12. The relatively high content of calcium (1.94 ± 0.02) in Swiss chard suggests that it may be of therapeutic value in hypocalcaemic state like osteoporosis. Calcium is associated for growth and maintenance of bones, teeth and muscles(Turan et al. 2003). Traditional cooking for 10min and 30min significantly affects the calcium content in leafy vegetables. Its low contents in traditionally cooked leafy vegetables suggest a low intake by vegetarians that must seek alternative sources to meet their needs for calcium. This is important because the cells need calcium and more than 99% of calcium in the body is used as a structural component of bones and teeth. This represents about 40% of all the minerals present in the body(Mepba, Eboh and Banigo 2007).

As concern of magnesium, the highest concentration of (2.45 ± 0.04) was found in raw Swiss chard (*Beta vulgaris*), while the lowest magnesium occurred in (1.99 ± 0.05)Cabbage(*B. oleraceae*). This value is very close to the result found in Nigerian leafy vegetables (3.69 mg/100 g). The magnesium content of leafy vegetables at 10min of cooking was ranged from (2.32 ± 0.07 - 1.84 ± 0.04) and at 30min of cooking was (2.16 ± 0.04 - 1.69 ± 0.03). Magnesium content of leafy vegetables studied in Nigeria was high). Magnesium is known to prevent cardiomyopathy, muscle degeneration, growth retardation, congenital malformations and bleeding disorders(Chaturvedi, Shrivastava and Upreti 2004). Magnesium is widely distributed in plant and animal foods and geochemical and other environmental variables rarely have a major influence on its content in foods(Mohammed and Sharif 2011). Magnesium content the leafy vegetables used in this study was found to be lower than that of Purslane (101 mg/100 g) but higher than that of kale (1.68mg/100g).

Ye, gurage gomen(*B. carinata*) ,*Ye, abesha gomen* (*B. carinata*),Tikle gomen (*B. oleraceae*) and *Swiss Chared*(*B. vulgaris*) of raw leaves have iron and zinc with mean concentration of (8.94 and 0.33),(9.17and0.31),(3.9 and 0.24) and (8.88 and 0.34)ppm respectively.

The residual contents of micro minerals at 10min of cooking were: iron (8.87 ± 0.03 - 3.76 ± 0.11) and zinc (0.31 ± 0.01 - 0.22 ± 0.01) and at 30min of cooking were: iron(8.36 ± 0.04 - 3.09 ± 0.07) and

zinc(0.29 ± 0.00 - 0.19 ± 0.01). These observed reductions may be due to leaching of the mineral compounds into the boiling water(Lola 2009).

Iron level of all the studied raw and cooked leafy vegetables were higher (3.09 mg/100 g) than the FAO/WHO recommended dietary allowance for males (1.37 mg/day) and females (2.94 mg/day)(WHO 2009). (Hall 2009), also reported a lower iron value (1.70 mg/100 g) for leafy vegetables. Iron has been reported as an essential trace metal and plays numerous biochemical roles in the body, including oxygen binding in hemoglobin and acting as an important catalytic center in many enzymes, for example, the cytochrome (Nicodemus 2013). It is estimated that 2 billion of the world's population (largely in developing countries) have marked iron deficiency anemia. This in turn limits work performance and leads to impaired performance in mental and motor tests in children (Bouis 2003). In infants, children and adolescents, in addition to basal losses, iron is also required for growth of the tissues and organs and for the expanding red blood cell mass. Assuming average endogenous iron losses in adults are 1.0 mg/day in men and 1.7 mg/day in women, the estimated average dietary requirement for iron are 6.7 and 11.4 mg/day in men and women respectively(Emebu and Anyika 2011). Thus, the use of the leafy vegetables in the diet can furnish the diet with iron sufficient enough to meet the daily requirement for the nutrient.

Zinc content of both raw cooked Brassica and charred leaves (0.19 - 0.34mg/100 g) was observed to be closed to the value (0.44 mg/100 g) as reported by(Hall 2009). The zinc content of raw Brassica and chard leaves was also found to be lower than that of other vegetables like "broccoli" (5.20mg/100 g), "red cabbage" (3.81 mg/100 g) and "kale" (12.00 mg/100 g). FAO/WHO, reported that zinc is an essential component of a large number (>300) of enzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrient(Organization 1999). s(Basu et al. 2014). Zinc stabilizes the molecular structure of cellular components and membranes and contributes in this way to the maintenance of cell and organ integrity. Furthermore, zinc has an essential role in polynucleotide transcription and thus in the process of genetic expression. Its involvement in such fundamental activities probably accounts for the essentiality of zinc for all life forms. Zinc plays a central role in the immune system, affecting a number of aspects of cellular and humoral immunity(Shankar and Prasad 1998). The Required Daily Allowance (RDA) of zinc for infants, children, adolescents and adult males and females ranges between 2.0 mg/100 g to 11 mg/100 g(Brown, Wuehler and Peerson 2001).

Considering, the recommended dietary allowance (RDA) for both macro and micro minerals (Hawkesworth et al. 2010): calcium (1000 mg/day), magnesium (400 mg/day), iron (8 mg/day) and zinc (6 mg/day), consumption of 10 min cooked leafy vegetables could cover at least 50% RDA.

Data on mineral analysis (Table 3) indicate that traditional cooking of leafy vegetable for 10min and 30min caused significant ($P \leq 0.05$) reductions in the Ca, Mg, Fe, and Zn contents of the vegetables. Similarly, (Kunyuru 2014), observed significant ($P \leq 0.05$) reductions in Ca, Mg, Zn and Fe contents of cooked brassica leaves. Cooking caused a significant reduction of the Fe, and Zn contents of cabbage leaf that were used in soup preparation (Oulai et al.). The result for mineral analysis of the vegetables suggests consumption of large quantities of cooked leafy vegetables to meet the Recommended Daily Allowance (RDA) for minerals (Hunt 1996).

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

Leafy vegetables are good sources of vitamin C. The vitamins are however lost during cooking since the vitamin is a water soluble vitamin. As the time of cooking increases, the level of vitamin C lost to the extract increases thereby leaving a lower vitamin C in the leaves left for consumption. From the results, Swiss chard/*Beta vulgaris*/ had the highest reduction of 85% in vitamin C, with *Ye, gurage gomen/Brassica carinata/* recording the least (42%) reduction in vitamin C among the vegetables. The result of the study revealed that the different species of green leafy vegetable contain appreciable quantity of minerals and antioxidant activity. Traditional cooking has inevitable consequences on the antioxidant activity and minerals content of the leafy vegetables. From the study it is concluded that the antioxidant capacities of the four leafy vegetables which are widely used by the country are considered as good sources of antioxidants as observed in DPPH scavenging assay. Among all ye, abesha gomen has the highest antioxidant activity. Other three leafy vegetables like *ye, gurage gomen, cabbage and Swiss chard* are also equally potent proves its wide use as food in the daily diet of the country with nutritional and therapeutic value.

The minerals contained within the leafy vegetables show varying degrees of stability when the vegetables are traditionally cooked. In comparison to vitamins and antioxidant activities, minerals have greater stability and their contents shows slight reduction due to cooking. The results indicated that all these vegetables have the potential to provide essential nutrients to the human beings. The study of LV revealed that they are good sources for macro- and micro-minerals. If they are used raw all of the LV used in this study are rich source of calcium, magnesium, iron and zinc. So it can be concluded that regular consumption of these LV can meet the nutritional requirement to overcome the micronutrient malnutrition at minimum cost. This study also indicated that the content of antioxidants in these vegetables varies significantly between and within their subspecies.

5.2. RECOMMENDATION

The following recommendations should be taken into consideration:

- ✓ Traditional cooking of *leafy vegetables* affects the content of antioxidant, vitamin C and minerals. It implies that they are best consumed as fresh or cooked for less than 10 minute.
- ✓ Since cooking of most vegetables is tends to reduce the vitamin C content of the vegetable, it is therefore advisable to add fruits to ones daily meal alongside vegetables to meet up with the daily reference intake.
- ✓ From the result for mineral analysis of the leafy vegetables it is recommended that to consume large quantities of cooked leafy vegetables to meet the Recommended Daily Allowance (RDA) for minerals.
- ✓ The studied leafy vegetables are consumed by the society almost in cooked form. Specially to prepare “yegomen kitfo” during holiday they cooked for longer time and extract the liquid part which may contain the essential minerals that may leach during cooking. Therefore, it is recommended that to consume the cooked leafy vegetables before extraction of the liquid.

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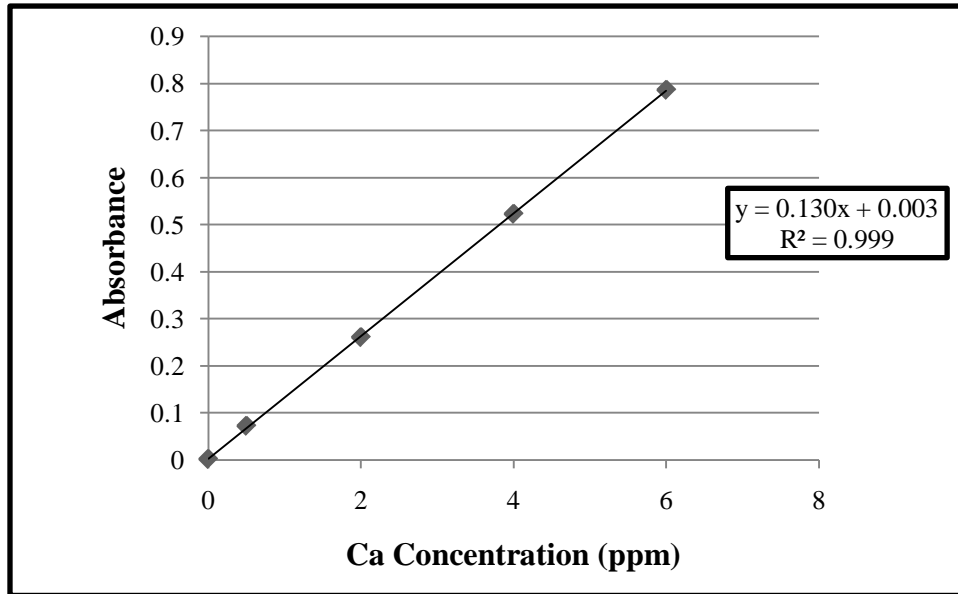
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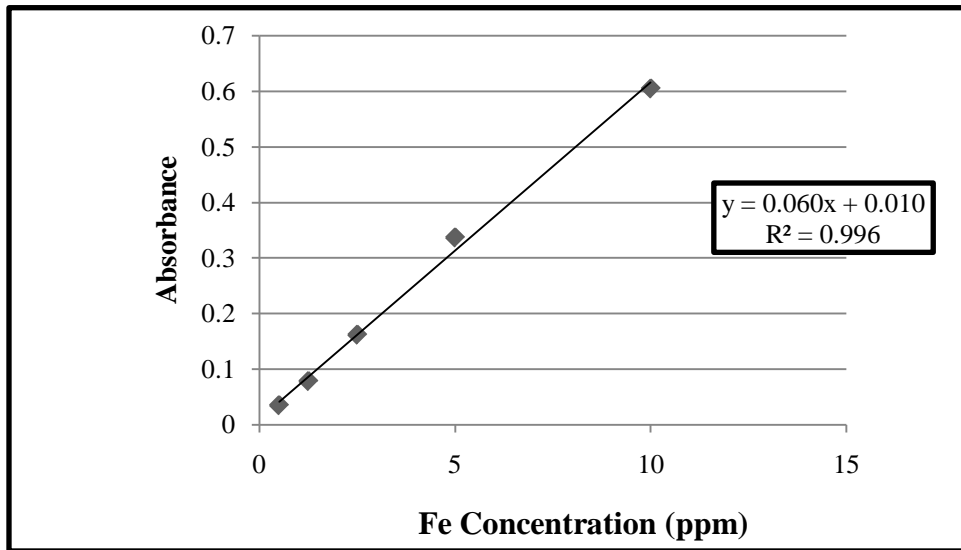
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APPENDICES

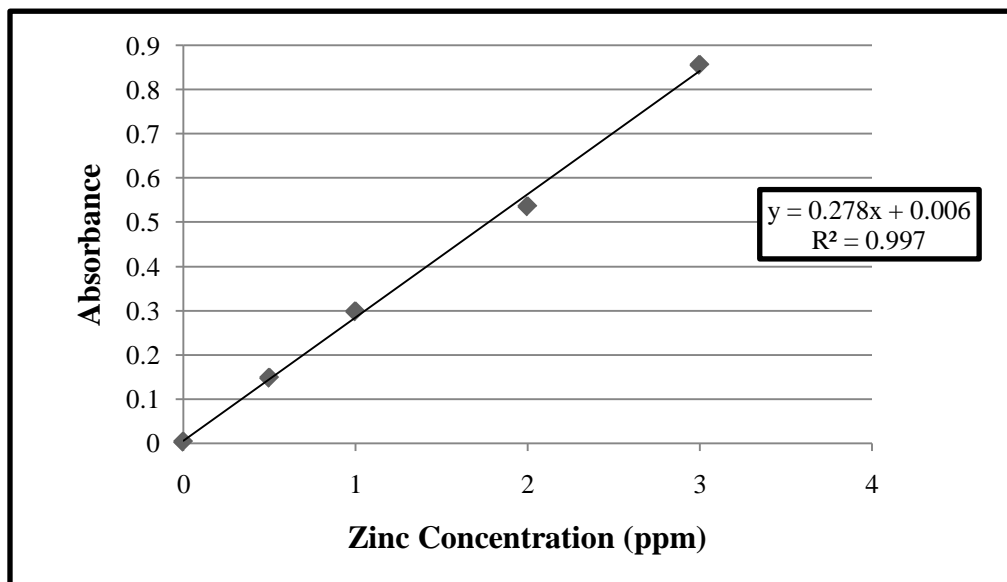
Appendix 1. Calibration Curve for Calcium (Ca)



Appendix 2. Calibration Curve for Iron (Fe)



Appendix 3. Calibration Curve for Zinc (Zn)



DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for any degree in this or any other institution and that all sources of materials used in this thesis have been duly acknowledged.

Candidate: **Habtamu Kassa**

Signature _____

This thesis has been submitted for examination with my approval as a University advisor. In addition, I declare that this thesis is the original work of my student and has been done under my supervision.

Advisor: **Kelbessa Urga (Associate professor)**

Signature_____

APPROVAL

This thesis has been approved by the examining board:

	<u>Signature</u>	<u>Date</u>
1.	_____	_____
2.	_____	_____
3.	_____	_____

Place and Date of Submission: Addis Ababa University School of Graduate Studies

Centre for Food Science and Nutrition

Addis Ababa, Ethiopia

June 3, 2016