ADDIS ABABA UNIVERSITY

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Commonly used processing methods on Kale (*Brassica Carinata*) in rural parts of Ethiopia: Effect on proximate, mineral, vitamin C and β-carotene composition

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DECLARATION

I, the undersigned, declare that this is original work and has never been presented in any other University as well as research institutes and all sources of materials used in this thesis have been fully acknowledged. This paper has never been submitted to and/or presented in any other University, college or institution in the candidature of any other degree, diploma, or certificate.

Candidate: Tsion Yemane 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: Dr. Paulose Getachew Signature_____

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LIST OF ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AMD	Age-related macular degeneration
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BHT	Butylated hydroxytoluene
DNA	Deoxyribonucleic acid
DNPH	Dinitrophenylhydrazine
ENNS	Ethiopian National nutrition strategy
FAO	Food and Agricultural Organization
FDA	Food and Drug Administration
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonization
IOM	Institute of Medicine
LOD	Limit of detection
LOQ	Limit of quantification
Masl	Mater above sea level
MeOH	Methanol
ODS	Operational data store
PE	Petroleum ether
ppb	Parts per billion
ppm	Parts per million
PTFE	Polytetrafluoroethylene
PVAC	Pro vitamin A Carotenoid
QDA	Statistical Analysis System
RDA	Recommended daily allowance
RSD	Relative Standard Deviation
SAS	Qualitative Descriptive Analysis
SNNPR	Southern Nations, Nationalities and People's Region
SOD	Superoxide dismutase

sp.gr	Specific gravity
SPSS	Statistical package for service solution
t-AC	*trans* Alpha Carotene
t-BC	*trans* β-Carotene
TCA	trichloroacetic acid
TuMV	Turnip mosaic virus
UV VIS	Ultraviolet visible
UV	Ultraviolet
VAD	Vitamin A deficiency
VAD	Vitamin A deficiency
WHO	World Health Organization

ABSTRACT

Vitamin A deficiency contributes to high morbidity and mortality rates in developing countries and remains a serious public health problem. Dietary sources and adequacy of provitamin A continue to be a major concern in areas where animal source foods are very expensive. A major proportion of vitamin A activity is accounted for β-carotene, which is widely found in green leafy vegetables and fruits. Among green leafy vegetables, Kale (Brassica carinata) (n =17), commonly known as Abyssinian mustard, has been selected for this study. It has been grown in and around Ethiopia for centuries and a preliminuteary questionnaire was administrated from four villages of two regions: Oromia (Zway and Meki) and SNNPR (Bolososore and Butajira) within 48 households. The evaluation was carried out for KAP assessments and other questions related to vegetable consumption and processing types. Based on the assessment it was found that 84.9% of household members consumed Kale with the frequency of 4-6 out of 7 days. Following this assessment, a processing flowchart was developed to assess the macro and micronutrients, vitamin C and oxalate particularly β-carotene by using HPLC within 10-minute cooking interval for about 2 hours based on the practice applied in rural areas of Ethiopia. This was to evaluate whether the rural community processing method retains the nutrients. AAS was used for the analysis of minerals, and UV VIS Spectrophotometer was used for vitamin C analysis. Based on the findings, raw Kale contained enough amounts of macro and micronutrients. However the result illustrates that a high percentage of vitamin C were lost during process II, 68.5% and 54.89% of during process I. Content of β-carotene in μg/100g were very higher in 20 minute 3519.25 ± 54.29 and 30 minute 3485.54 ± 4.09 with the retention of 98.2% and 97.28 respectively; along processing significant difference ($p<0.05$) was shown until 66.38% loss counted by 120 minute cooking. The assessment showed that Kale was consumed by the society almost in an over-cooked form which leads to a considerable loss of vitamins and pro vitamins. Therefore, it is recommended to consume the cooked leafy vegetables at 20-30 minutes with sensory acceptability to keep its nutrients and advisable to eat kale before extracting the liquid. Hence, consumption of moderately cooked and sufficient amount of Kale is recommended as the preferred intervention in the reduction of Vitamin A deficiency.

Keywords: - *Vitamin A deficiency, β-carotene, kale (Brassica carinata)*

1. INTRODUCTION

1.1. Background and Justification

Micronutrients are the collective term applied to essential vitamins and trace minerals. They are essential nutrients for human beings and required in small amounts to make the body to produce hormones, enzymes and other essential substances for proper growth and development. Even though they are needed in small amounts, consequences of their absence are severe and can lead to infectious diseases, physical and mental impairments, increased prevalence and severity of infection and increased mortality rates (Ottawa and UNICEF 2004, Tomkins, 2000).

Inadequate intake of them is now recognized as an important contributor to the global burden of disease through increased rates of illness and death from infectious diseases, and of disability such as mental impairment. Severe micronutrient deficiency causes clinical manifestations in humans that are also demonstrable in animal experiments using selectively restricted diets. Mild to moderate deficiencies also have important consequences for human health. Deficiencies of some micronutrients are highly prevalent in low- and middle-income countries and may affect the risk of illness or death from infectious diseases by reducing immune and non-immune defenses and by compromising normal physiology or development (Black, 2003).

Vitamin A is a fat-soluble molecule found in animal products which can be found in the body in three main active forms that are retinol, retinal and retinoic acid, collectively, these compounds are known as a retinoid. The cells in the body can convert retinol and retinal to the other active forms of vitamin A as needed. When vitamin A intake is below required levels, a number of manifestations collectively known as vitamin A deficiency disorders occur (Kapil & Bhavna, 2002; Tomkins, 2000).

In developing world VAD remains a serious public health problem, therefore, dietary sources and adequacy of provitamins A continue to be a major concern, especially where animal-based foods are very expensive. Therefore, a potential compound with a provitamin A activity are carotenoids of the more than 600 carotenoids now known,

about 50 would be precursors of vitamin A based on structural considerations (Rodriguez-Amaya, 1997). Among the carotenoid precursors of vitamin A, a major proportion of vitamin A activity is accounted for by β-carotene, which is widely distributed in green leafy vegetables, and fruits (Veda et al., 2010).

Results demonstrate that green leafy vegetables are good sources of minerals and vitamins, especially they are a good source of provitamin A, (Raju et al., 2007). Studies done in different countries showed that dark green, leafy vegetables figure as the most common rich sources of provitamin A. Relatively easy to produce, and available practically all year round, inexpensive and accessible sources of provitamin A for most of the developing world (Rodriguez-Amaya, 1997).

Vegetables are a significant component of the Ethiopian diet, and traditional vegetable species are particularly important. These traditional vegetables have been relatively neglected (Asfaw, 1997). In Ethiopia, unlike other countries, green leafy vegetables are usually consumed in the form of processed in different ways; However, there is limited information with regards to the effect of cooking on nutritional loss. Therefore, the purpose of this study was to see the influence of traditional cooking methods on vitamins and minerals.

1.2. Statement of the Problem

Most vegetables are commonly cooked before being consumed. Preparations of vegetables at home are based on taste preference and convenience rather than retention of nutrients and health-promoting compounds. Knowledge on processing effect is very limited. Currently, animal-based foods are very expensive, as a result vitamin A deficiency become one of the major avoidable public health problems worldwide and it is the most important factors that contribute to the high morbidity and mortality rates.

In Ethiopia, there are a number of green vegetables which are a vast source of phytochemicals like carotenoids which account for 82% of dietary vitamin A intake in developing countries. However, cooking, serving and way of consumptions are highly affecte provitamine intake. In Ethiopia, unlike other countries, green leafy vegetables are usually consumed in different ways, However, there is limited information with regards to the effect of cooking causes considerable losses in macronutrient and micronutrient particularly vitamins and provitamins.

1.3. Objectives

1.3.1. General Objective

To evaluate the proximate, mineral, vitamin C and β-carotene composition in commonly consumed dark leafy vegetable upon various processing methods used in rural parts of Oromia and SNNPR, Ethiopia.

1.3.2. Specific Objectives

a. Asses processing method of commonly used dark leafy vegetable in rural parts of Ethiopia.
b. Determine proximate, mineral, vitamin C and β-carotene composition and their changes during processing with sensory parameter.

2. LITERATURE REVIEW

2.1. Micronutrient Deficiencies

Micronutrient deficiencies were unlikely features of humankind's early existence. As a hunter-gatherer flesh foods were the primary food sources like wild fruits and vegetables that provided a quality-rich diet. Most likely deficiency problems emerged and sever as lifestyles gradually changed toward more stationary living and dependence on subsistence agricultural. Flesh foods become significantly replaced by cultivated cereals, fruits and vegetables, which almost certainly introduced bioavailability problems for several micronutrients, including provitamin A carotenoids from plant sources (Underwood, 2004) and among different micronutrient deficiency, vitamin A deficiency become very dominant and responsible for millions death.

2.2. Vitamin A

Vitamin A is a fat-soluble molecule found in animal products. After absorption from the gut, retinol is transported to the liver, where it is stored. Under normal conditions, 95% of all vitamin A is stored in the liver. In well-fed adult populations, such stores can sustain bodily needs for a year or more and make liver the most concentrated dietary source of preformed vitamin A. When needed, vitamin A is released from the liver as retinol, firmly attached to its specific carrier protein ('retinol-binding protein', RBP) RBP acts as a vitamin shuttle, moving retinol from the liver to its target cells, where the complex binds to cellular RBP receptors; the retinol enters the cell, while the plasma RBP is recycled and metabolized (Sommer, 2001).

Vitamin A is a fat-soluble vitamin which can be found in the body in three main active forms that are retinol, retinal and retinoic acid, collectively, these compounds are known as retinoid. The cells in the body can convert retinol and retinal to the other active forms of vitamin A as needed. In the body the conversion of retinol to retinal is reversible; whereas the further conversion of retinal to retinoic acid is irreversible. Foods derived from animals provide compounds retinyl esters that are readily digested and absorbed as retinol in the intestine. Foods derived from plants provide carotenoids, some of which

have vitamin A activity. The carotenoids with the greatest vitamin A activity is accounted by β-carotene which can be split into forms retinol in the intestine and liver (Whitney & Rolfes, 2008).

2.2.1. Source of vitamin A

Animal-based foods are good sources of vitamin A like, fish, milk and milk products, eggs, (van der Beek, 1991), egg yolk, and, breast milk (Sommer, 2001). Only the wealthier part of the population gets a substantial part of their vitamin A from animal sources (Khan, 2006). also green leafy vegetables and fruits (Khan, 2006) as a form of Carotenes (Provitamin A), particularly β-carotene, are produced by a wide range of plants, particularly colored fruits (papaya, mango) and vegetables (dark green leafy vegetables, red palm oil) (Sommer, 2001), Carrots, dark green leafy vegetables, tomatoes, oranges (van der Beek, 1991).

2.2.2. Significance of vitamin A

The three forms of Vitamin A carry out specific functions; Retinal is active in vision and it's also an intermediate in the conversion of retinol to retinoic acid. Retinoic acid acts like a hormone, regulating cell differentiation, growth, and embryonic development. Retinol supports reproduction and it is a major transport and storage form of Vitamin A (Whitney & Rolfes, 2008). According to its significance, its recommended intake based on the group is as follow.

Table 1: Recommended intakes of vitamin A, by age group (FAO & WHO, 2005)

Group (μg RE/day)	Age	Mean requirements	Recommended safe intake μg/day
Infants and children	0-6 months	180	375
	7-12 months	190	400
	1-3 years	200	400
	4-6 years	200	450
	7-10 years	250	500
Male	11-12	500	1000
	13-15 and adult	600	1000
Adolescent	10-18 years	330-400	600
Adults: Female	19-65 years	270	500
Male	19-65 years	300	600
Pregnant women	-	370	800
Lactating mother	-	450	850

2.2.3. Vitamin A deficiency

Clinical manifestations of vitamin A deficiency, but not their cause, were recorded as early as 1500 B.C. The foundations of modern nutritional science were founded between 1910 and 1920 by the discovery of micronutrients, dietary constituents required in small amounts to promote and maintain a healthy, normal individual. Vitamin A was one of the first of this new class of essential nutrients to be recognized. Newborn animals made vitamin A deficient soon failed to grow, became septic, developed classical 'drying' of the eyes (xerophthalmia) ending in blindness, and died prematurely. By 1920, vitamin A deficiency, then common among poorer populations around the world, was recognized as responsible for these dramatic ocular changes and as an important cause of blindness in children (often in orphanages) and adults (Brazilian slaves, Russian peasants during the fasting month of Lent, and impoverished Chinese army recruits and university students). It took another 65 years before other clinical manifestations observed in laboratory animals (particularly increased the severity of infectious episodes and attendant mortality) were recognized in humans (Sommer, 2001).

2.2.5. Prevalence of vitamin A in Ethiopia

Vitamin A is an essential micronutrient for the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune function, and reproduction (Zewditu et al., 2010). As different studies show vitamin A deficiency is a major health problem in Ethiopia. Several factors are contributing to vitamin A deficiency, mostly inadequate intakes of the nutrient takes the major cause (Demissie, Ali, & Zerfu, 2009). However, in Ethiopia adequate intakes of vitamin A rich food are not more than enough. At the national level, the prevalence of inadequate intakes is 82% in women of childbearing age and 90% in urban males. Urban women have a slightly higher prevalence of inadequate intakes than their rural counterparts (90% urban, 79% rural). Women in SNNPR and Gambella had the lowest prevalence of inadequate intakes (41% and 56% respectively), compared to the following regions where almost all (>90%) women had inadequate intakes of vitamin A: Tigray, Amhara, Somali, Harari, Addis Ababa, Dire Dawa (Kebede et al., 2013).

According to the Ethiopian national micronutrient survey report, which is performed by using serum retinol as vitamin A biomarker, at national level 13.9% of preschool age children (6-59 months), 10.9% of school-age children (5-14 years), and 3.4% non-pregnant women (15-49 years) have the vitamin A status below the recommended limit of serum retinol (<0.07μmol/L). Among the regions, preschool school children who live in Harari have the highest prevalence of vitamin A deficiency 21.0% as compared to other regions. The school-age children who live in Harari also have the highest prevalence of 25% compared to other regions. The prevalence of vitamin A deficiency in Tigray, Oromia, Somalia, SNNPR, Harari, Addis Ababa, and Dire Dawa can also be considered as a mild public health problem of women of reproductive age (Zerfu et al., 2016). Therefore conducting research on mitigation of vitamin A deficiency could contribute to the reduction of vitamin A related disease.

2.2.5. Factors associated with vitamin A deficiency

Usually, vitamin A deficiency develops in an environment of ecological, social and economic deprivation, in which the key risk factors for vitamin A deficiency are diet

which is low in sources of vitamin A (i.e. dairy products, eggs, fruits, and vegetables), poor nutritional status, and high rate of infections, in particular, measles and diarrheal diseases (Bao & Chang, 1994; Bartholomew & Ogden, 1990).

The best sources of vitamin A are animal source foods, in particular, liver, eggs and dairy products, which contain vitamin A in the form of retinol, i.e. in a form that can be readily used by the body. It is not surprising then that the risk of vitamin A deficiency is strongly inversely related to intakes of vitamin A from animal source foods. In fact, it is difficult for children to meet their requirements for vitamin A if their diet is low in animal source foods (Balcha, 2001).

When vitamin A intake below the required level, the numbers of manifestations collectively known as vitamin A deficiency disorder occur, this is the result of different factor like diseased livers, as in cirrhosis, may fail to store adequate amounts of vitamin A, or fail to synthesize adequate amounts of RBP. Under these conditions, bodily needs may not be met despite 'adequate' dietary vitamin A intake. Severe protein deprivation, by preventing the synthesis of adequate levels of RBP, will have the same effect (Sommer, 2001).

2.2.6. Improving vitamin A status

Improving vitamin A status reduces mortality among older infants and young children and reduces mortality related to pregnancy also reduces the prevalence of severe illness and clinic attendance among children (Tomkins, 2000). To increase the vitamin A intake in the population, several approaches are possible like supplementation which provide micronutrients usually in the form of pills, capsules or syrups it has the advantage of being capable of supplying an optimal amount of a specific nutrient or nutrients in a highly absorbable form to control in individuals or population groups that have been identified as being deficient but this kind of solution does not provide the overall long-term economic benefits and sustainability that food-based approaches can deliver (Allen et al., 2006).

Therefore food-based approaches promote the consumption of foods that are naturally rich in micronutrient or are enriched foods through fortification is the best solution to tackle such problems (Thompson, 2011), therefore for many developing countries where vitamin a deficiency is very high, a food-based approach using foods naturally rich in vitamin A and other micronutrients is preferable because fruits and vegetables provide 70- 80% of the total vitamin A intake due to their high content of provitamin A carotenoids. Thus, increased consumption of plant provitamin A-rich foods should be encouraged (Khan, 2006), therefore using vegetables are a good source of vitamin and minerals.

Dietary diversification, which is central to food-based approaches, can meet these needs. This approach includes assessing dietary consumption, expanding and diversifying food production, improving food processing, preservation, storage and marketing, and improving food preparation. This strategy has to be supported with a nutrition education program. As diets in developing countries do not just lack a single micronutrient, but a wide range of them, strategies should work towards enhancing the total energy and micronutrient intake, in addition to paying attention to the bioavailability of the ingested micronutrients (Tontisirin et al., 2002).

Diets in developing countries generally lack many nutrients, including energy (inadequate amounts of food), so that strategies need to also emphasize an increase in total food intake, in addition to a greater variety. Agricultural and food policies tend to be oriented to primary agricultural productions, but they could also be formulated to promote and support home gardens and small livestock production for the explicit purpose of increasing the household consumption of micronutrient-rich foods (Tontisirin et al., 2002).

Food fortification is a supportive link to the sustainable long-term dietary change in populations. A frequent problem with fortified foods, however, is that some target populations, particularly those located far from urban areas; do not have access to centrally-processed fortified foods. Appropriate food-based strategies should be targeted

towards the most vulnerable groups, usually women and children in poor households (Tontisirin et al., 2002).

Dietary modification and diversification also can best be undertaken through community-based approaches. Such an approach can be used to enhance awareness and understanding of micronutrient deficiency in the community, and help to empower the community to be more self-reliant towards addressing its nutritional problems (Tontisirin &Gillespie, 1999). In the case of β-carotene, which again in the absence of meat is the major dietary source of vitamin A in developing countries, it is important to consider factors which facilitate or prevent inhibition of its biological utilization. Positive clinical and biochemical responses to provitamin A carotenoids in foods have been observed in many studies conducted among populations with evidence of vitamin A deficiency (Tontisirin et al., 2002) and it is useful to remember that dark-green leafy vegetables, in addition to their being a major source of vitamin A, also provide other nutrients, including folic acid, vitamin C, Zn and phytonutrients (Tontisirin et al., 2002), and also vegetables are cost-effective and can substitute the animal food sources, which are expensive and beyond the purchasing power of the low income groups of population, especially in rural areas and should be explore as a source of β-carotene content (Ahamad et al., 2007), and green leafy vegetables constitute an indispensable constituent of the human diet in Africa in general. Apart from the variety 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 the daily diet (Ahamad et al., 2007).

2.3. Vegetables

Leaf vegetables are widely used in the human diet; they are low in calories and fat, but high in dietary fiber and minerals (Banerjee et al., 2012). Phytochemicals are plant-derived compounds, the plant`s way of protecting itself. In addition, they appear to have significant physiological effects on the human body. There are more than a thousand known phytochemicals. They are acting as antioxidants, stimulating enzymes, interfering with DNA replication, destroying bacteria, as well as they, seem to act to reduce the onset

of diseases such as cancer and heart diseases (Krishnaswamy, 1998) and manganese (Banerjee et al., 2012), a content of minerals, such as iron and calcium and very high in phytochemicals such as vitamin C, carotenoids, lutein and others (Duma et al., 2014). Among different vegetables, dark green leafy vegetables are a good source of phytochemicals (Raju et al., 2007).

2.3.1. Vegetable in Ethiopia

Ethiopia has always had a rural population with agriculture with dominating the economy and total life of the population. The agriculture of the country still has primitive roots and is subsistence farming in which more than 85% of the farm produce is consumed at home (Getahun, 1974), 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 16 lowlands (<1500 meters above sea level), Midlands (1500-2200 masl), and highlands (>2200 masl), respectively (Block et al., 2008). The development of 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 the 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. (Block et al., 2008).

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 percent (0.11%), which is insignificant as compared to food crops (Setegn, 2015).

Therefore Vegetables are a significant component of the Ethiopian diet, and traditional vegetable species are particularly important. These traditional vegetables have been relatively neglected, and their potential remains to be fulfilled. The enhancement of some traditional systems, in conjunction with modern scientific approaches, can contribute to the growth of the vegetable industry, thereby augmenting the national economy and improving people's health and standard of living. Increased attention needs to be focused nationally on traditional Ethiopian vegetables through research, conservation, and promotion of use (Asfaw, 1997).

2.3.2. Dark green leafy vegetable in Ethiopia

Studies showed that DGLVs are a good source of minerals and vitamins especially they figure as the most common rich sources of provitamin A, (Raju et al., 2007). Therefore they should be included in the diet to overcome various nutritional problems like vitamin A deficiency. Relatively easy to produce, and available practically all year round, inexpensive and accessible sources of provitamin A for most of the developing world (Rodriguez-Amaya, 1997) and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Kwenin et al., 2011). Dark-green leafy vegetables were found to increase serum retinol concentration in children of regions where the prevalence of vitamin A deficiency was high (Hussein, 1989; Devadas et al., 1980; Pereira, 1968).

In Ethiopia, dark green Leafy vegetables are consumed more during the rainy season when they grow in abundance and also when grains are in short supply. Leafy and other vegetables are also eaten more during Lent. Greater production of vegetables is needed to

cover consumption needs during such periods. Production during the off-season requires supplementary irrigation. Since some people tend to consider leafy vegetables very secondary as food sources, promotional methods should be integral parts of traditional vegetable enhancement schemes to forge wider public acceptance (Asfaw, 1997).

The frequent use of leafy vegetable foods in times of grain shortages in some localities may have led to the tendency to associate eating of leafy vegetables with famine, but their nutritional significance is being increasingly appreciated.

Vegetable production for home consumption had suffered in many places when rural families were resettled through the village program since they were unable to closely monitor and work on backyard plots. In urban and semi-urban settings, production and use of vegetables have grown in recent years, as have external demands (Asfaw, 1997). As shown in preliminary data collected from two regions Oromia and SNNPR, the most popular and repeatedly consumed dark green leafy vegetable is Kale.

2.4. KALE (*Brassica carinata*)

Ethiopia is the center of genetic diversity of *Brassica carinata,* and its cultivation is thought to have started there about 4000 years BC. Truly wild types are not known, but *Brassica carinata* often escapes from cultivation.

Brassica carinata A. Braun (n = 17), where n refers to the gametic or haploid number or the number of chromosomes in a gamete), commonly known as Abyssinian mustard, has been grown in and around Ethiopia for centuries and known they are hybrid between *Brassica nigra* and *Brassica oleracea,* (Carmody, 2017). The genus Brassica is one of 51 genera in the tribe *Brassiceae* belonging to the crucifer family and is the economically most important genus within this tribe, containing 37 different species (Gómez-Campo, 1980).

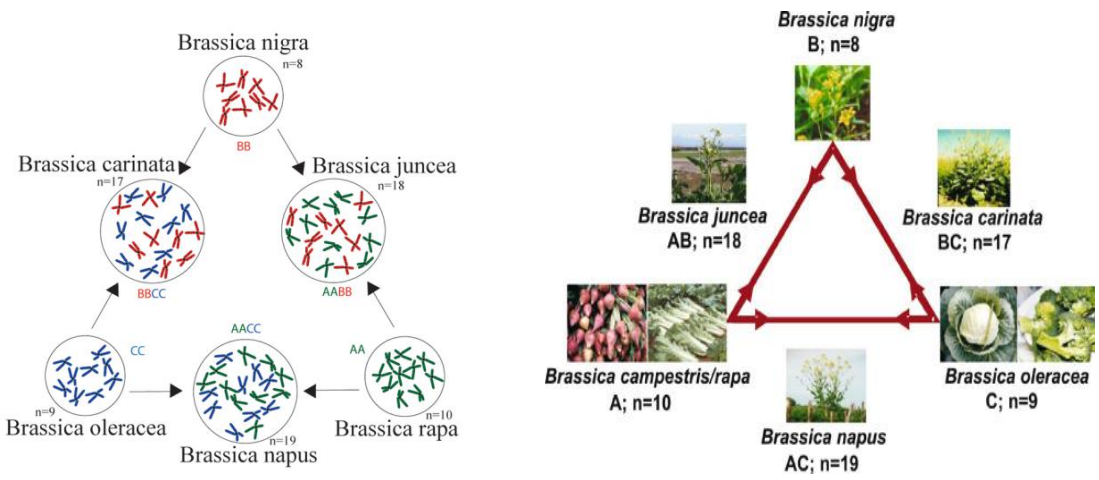


Figure 1: morphology and anatomy of the *Brassica* family

2.4.1. Morphology

Kale produces more leaves per plant, 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 gallous; taproot strong, leaves are alternate and usually simple. 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. Fresh market production of Ethiopian mustard leaves is usually under monoculture, the whole plants are harvested by uprooting 5 - 6 weeks after sowing in seedbeds (Adeniji & Aloyce, 2014) and the plant flowers earlier producing ripe seeds within 4 months of sowing.

Figure 2: Morphology and cultivation of Kale

2.4.2. Cultivation

Ecology: Ethiopian Kale is rather versatile and can be found in highland regions with a cool climate, but also in lowlands with relatively warm and dry conditions. It grows best in the dry season under irrigation. The crop is suited to a wide range of soils and especially the oil crop is often grown in marginal areas; the vegetable crop is mostly grown on more fertile soils. It is sensitive to salt and seeds may not germinate in soils with an above average salinity level, waterlogging is not tolerated (Asfaw, 1997).

Elevation: Being cold tolerant, Ethiopian Kale can be successfully grown at higher altitudes than most leafy vegetable crops, up to 2600 m (8500 ft.).

Rainfall: Ethiopian Kale has a long taproot and a more extensive root system than any other Brassicas family. Therefore, it only needs a moderate amount of rainfall, (600-1200 mm; 23-47 in) to produce a crop of leaves or seed, irrigation also improves the leaf crop in dry regions.

Soil types: Kale plant grows in almost any soil type except water-logged or saline soil. But loam soil recommends for better production if manure dug added into the soil protein content and amount of leaf production increases.

Temperature range: 15-20° C (59-68° F) the tiny seeds germinate rapidly in moist garden soil or in pots either in partial shade or full sun. Seed may be broadcast or planted 35-40 cm (14-16 in) apart in rows 50-60 cm (20-24 in) apart.

Harvesting and Seed Production: Leafs can be harvested from 35 days until 10 weeks, earlier being better for tenderness, re-growth, andf re-harvesting. Harvest the seed pods after 2 months when they begin to turn brown. Seed crops are harvested when the fruits turn brown. In frutescence are cut and placed on a tarpaulin or similar sheet, where they are allowed to dry without the risk of seed shattering. Complete drying of the pods on sheets spread in the shade but protected from birds. Threshing and winnowing should follow before storage. The seeds can be stored for several years in dry, dark conditions (Asfaw, 1997).

2.4.3. Diseases and pests

Ethiopian Kale is sensitive to turnip mosaic virus (TuMV) and especially the leaf crop is vulnerable. TuMV is transmitted by a range of aphids, of which the cabbage aphid *Brevicoryne brassicae* and the green peach aphid *Myzus persicae* are the most important. Oilseed types with bluish leaves have a thicker layer of leaf wax than green-leaved vegetable types and it has been noticed that leaf wax keeps aphids at bay to some extent. Leaf wax is also associated with the level of tolerance to Alternaria leaf spot. Ethiopian Kale is susceptible to black rot (*Xanthomonas campestris*), black spot, and to damping off and seedling root rot (*Rhizoctonia solani*). Cultivar 'Nanga' from Zambia has shown tolerance to black rot. Ethiopian Kale is tolerant of black leg disease *Leptosphaeria maculans* (asexual form: *Phoma lingam*) (Adeniji & Aloyce, 2014).

2.4.4. Production of Kale in Africa

Production of *Brassica carinata* for its seed is important only in Ethiopia; production in Canada and the Mediterranean region is still mainly experimental. Kale is mostly grown as a kitchen garden crop, although in Tanzania, Malawi, Zambia and to a lesser extent in Zimbabwe it is also grown as a market crop. Its use as a leaf crop appears to be declining because of higher yielding leaf cabbage (*Brassica oleracea*) and leaf mustard (*Brassica juncea*). No statistical data on its production is known. However, as a leafy vegetable, it is often grown in East and southern Africa, less so in West and Central Africa.

Figure 3: Distribution of Kale (*Brassica carinata*) in Africa

2.4.5. Production of Kale in Ethiopia

Ethiopian mustard is cultivated since ancient time and third in its production next to noug (*Guizotia abyssinica Casa*) and Linseed (*Linum ustatisumum* L.) in the highland areas of the country, grown as an oilseed crop in parts of Asia and Africa, Ethiopia is the main area of production (Alemayehu & Becker, 2002), was recently identified as a high-yielding, disease- and pest-tolerant crop for the highland areas of Ethiopia (Riley et al., 1983). Ethiopian farmers grow the plant as a leafy vegetable and harvest the seed for oil (Rakow, 2004). There are no known wild species of this crop type (Carmody, 2017).

In Ethiopia for Kale and pumpkin, there is no record of released variety from the national research system and also no seed imports, indicating that seed production of these vegetables is based on local varieties and farmers' indigenous knowledge in genetic resources conservation, selection, production and use (Ayana et al., 2014). However 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 & Aloyce, 2014).

2.4.6. Significance of Kale

In most parts of Africa, the primary use of *Brassica carinata* is as a cooked leafy vegetable, whereas in Ethiopia, the seed has major importance where it is called 'gomen zer'. Outside Africa, especially in western and southern Asia, it is occasionally grown as an oilseed crop or for mustard. The seeds are crushed and the oil is used for cooking and in the mustard industry. The oil has limitations for cooking because of the high contents of glucosinolates and erucic acid. In Ethiopia, it is used for oiling the baking plates of earthenware 'injera' stoves. It is also used for illumination. The seed is used in folk medicine to treat stomach-ache. The crop is occasionally used as fodder for livestock and the seeds to feed birds. The seed cake is used as high protein food for animals, there has been an interest in utilizing the oil, like other *Brassica* seed oils, as a biodiesel and for the preparation of special erucic acid derivatives (Gupta, 2011, Sanlier & Guler Saban, 2018, Jahangir et al., 2009).

2.4.7. Health benefit of Kale

Kale is rich in vitamin C, K, β-carotene and calcium, as well as cancer-fighting anti-oxidants (Adeniji & Aloyce, 2014). And they are also a good source of fiber (Jahangir et al., 2009) which helps to control blood sugar levels, maintain bowel health, lowers cholesterol levels. In developing world vitamin A deficiency remains a serious public health problem; therefore, dietary sources and adequacy of provitamins A continue to be a major concern (Rodriguez-Amaya, 1997). On the other hand, the focus in the developed world has shifted to the other health-promoting effects of carotenoids (Rodriguez-Amaya, 1997). Therefore as Kale is a member of green leafy vegetable which is a good source of provitamin A were different nutritional surveys from various countries consistently report β-carotene intake to be essential to meet vitamin A requirements (Sanlier & Guler Saban, 2018).

2.5. Carotenoid

Carotenoids are fat-soluble compounds that are associated with the lipidic fractions (de Quirós & Costa, 2006). There are many naturally occurring carotenoids more than 600 natural carotenoids are now known, including the enormous variety of carotenoids in algae, fungi, and bacteria (Rodriguez-Amaya, 2001). They are a group of natural pigments responsible for the yellow, orange or red color of many foods (Niizu & Rodriguez-Amaya, 2005), it is estimated that nature produces about 100 million tons of carotenoids annually (Rodriguez-Amaya, 1997).

2.5.1. Importance of carotenoids

The importance of carotenoids in foods goes beyond their role as natural pigments. Biological functions and actions have been increasingly attributed to these compounds (Abate-Pella et al., 2017). Besides the well-known provitamin A activity of some of these compounds, carotenoids are important for cardiovascular disease, and age-related disease they have also been associated with a lowered risk of developing degenerative diseases such as cancer, cataract, and macular degeneration. Some fruits and vegetables are rich sources of carotenoids, as well as other bioactive phytochemicals, and many vegetables

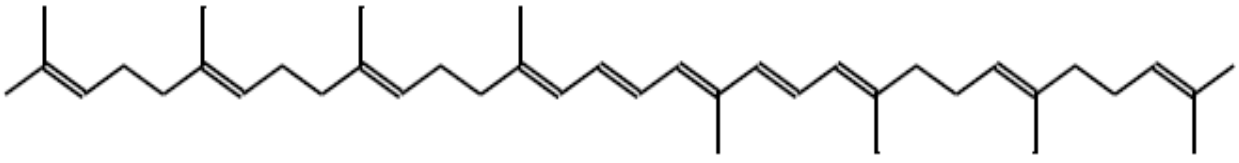
are widely available year-round therefore carotenoid can easily obtain (Abate-Pella et al., 2017; Niizu & Rodriguez-Amaya, 2005).

Additionally serves as a potent antioxidant, scavenging free radicals and quenching singlet oxygen. By this latter property, β-carotene is understood to reduce the risk of development of certain types of cancer (Veda et al., 2010). Thus, it is suggested that the major function of β-carotene in human nutrition is that of a provitamin A (Rodriguez-Amaya, 2015). A higher dietary intake of carotenoids was associated with a lower risk for age-related macular degeneration. Adjusting for other risk factors for AMD (Seddon et al., 1994) increasing the consumption of foods rich in certain carotenoids, in particular dark green, leafy vegetables may decrease the risk of developing advanced or exudative AMD, the most visually disabling form of macular degeneration among older people (Seddon et al., 1994).

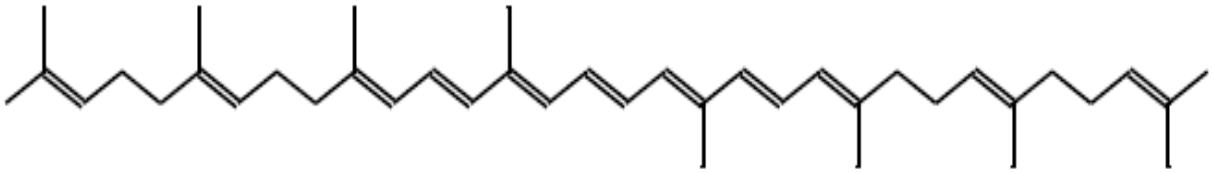
Provitamin A also has the advantage of being converted to vitamin A only when needed by the body; thus avoiding potential toxicity from an overdose of vitamin A (Rodriguez-Amaya, 1997). They are recognized as playing an important role in the prevention of human diseases and maintaining good health (Rao & Rao, 2007).

2.5.2. Common carotenoids in foods

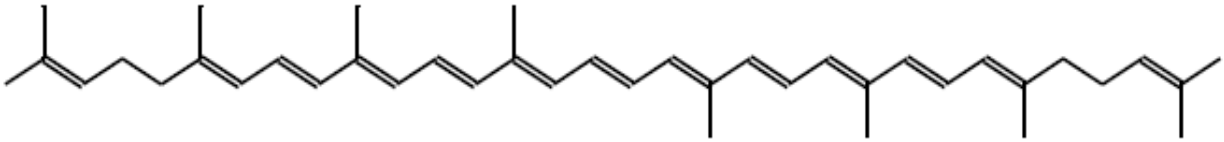
Carotenoids are widely distributed natural pigments and more than 600 naturally occurring carotenoids have been isolated and characterized, about 50 would be precursors of vitamin A based on structural considerations (Rodriguez-Amaya, 1997) among the 50 carotenoids with pro-vitamin A activity, the most common are α- and β-carotene (Carvalho et al., 2014).



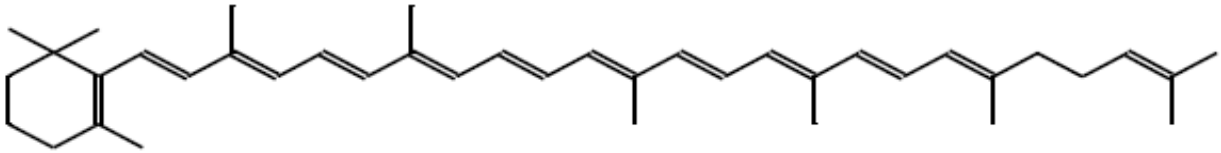
Phytofluene, acyclic, colorless



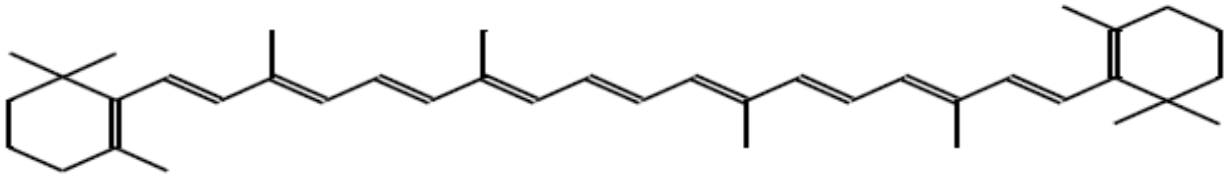
ζ-carotene, acyclic, light yellow



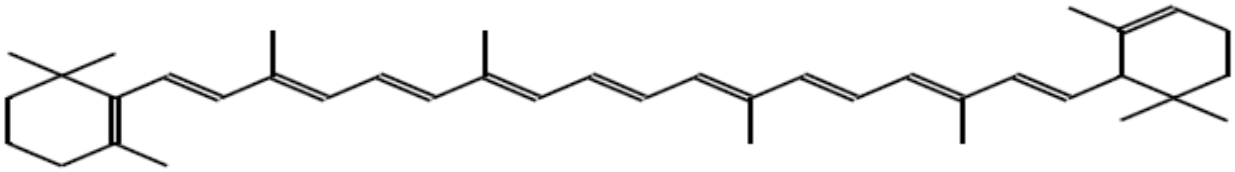
Lycopene, acyclic, red



γ-carotene, monocyclic, red-orange



α-carotene, bicyclic, yellow



β-carotene, bicyclic, orange

Figure 4: Structure and characteristics of common food carotenoids (Rodriguez-Amaya, 1997)

2.6. β-carotene

Among the common carotenoids with a provitamin, A activity β-carotene takes priority with conversion capacity to vitamin A. The bicyclic β-carotene is the most widespread of all food carotenoids, found in virtually all foods analyzed, as a minute or as the major

pigment (Rodriguez-Amaya & Kimura, 2004). β-carotene known the best-established function of carotenoids in terms of human health is the provitamin A activity (Rodriguez-Amaya, 2015).

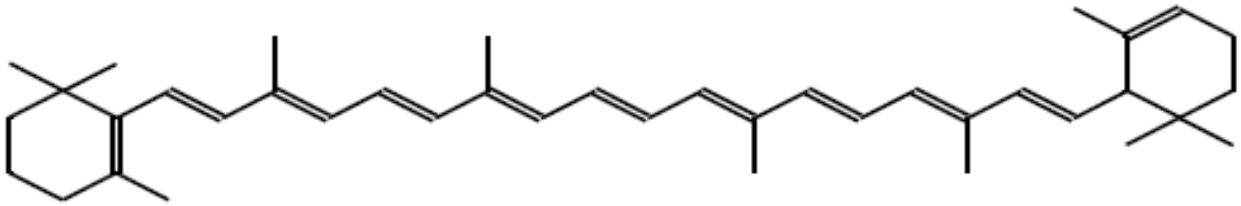


Figure 5: structure of β-carotene (Rodriguez-Amaya, 1997)

Thus, β-carotene is a potent provitamin A to which 100% activity is assigned, substituted β ring with an 11-carbon polyene chain is the minimum requirement for vitamin A activity. γ-Carotene, α-carotene, β-cryptoxanthin, and β-carotene-5, 6-epoxide, all of which have one unsubstituted ring, would have about half the bioactivity of β-carotene. β-carotene is converted in the gut to vitamin A (Sommer, 2001). Due to its unique structure and cleavage efficiency, β-carotene is the most efficient provitamin A carotenoid.

2.6.1. Factors affecting β-carotene content in food

There are many factors that affect carotenoid contents in food like, agroecology of environments like climatic condition or seasonal effects, agricultural and post-harvest handling like storage, processing, compositional differences of the raw material, as stage of maturity, cultivar, part of the Plant and way of utilized, can have a significant factors on carotenoid content of foods (de Sá & Rodriguez-Amaya, 2003) and also carotenoid sources varies with the vegetable, its ripeness, and the way it is prepared for dietary consumption (Sommer, 2001) such as amount, type, and physical form of the carotenoids in the diet; intake of fat fiber; protein and zinc status; existence of certain diseases; and parasite infestation (Rodriguez-Amaya, 1997).

There is evidence to suggest that vegetables produced in different climatic condition show different carotenoid concentration and composition (de Azevedo & Rodriguez-Amaya, 2005). And also post-harvest handling like storage conditions of food also accountable for the loss of color and biologic activity and the formation of volatile compounds that impart desirable or undesirable flavor in some foods and during storage carotenoids are susceptible to isomerization and oxidation (Rodriguez-Amaya, 1997). The as different study shows carotenoids are characteristically highly conjugated through double bonds, which lead to many isomers as well as susceptibility to oxidation and other chemical modifications (Abate-Pella et al., 2017). Among different factors as described above study shows that there is a significant loss of nutrients occur under processing which has a high effect on carotenoid contents of food and accountable for vitamin A deficiency for most of developing countries.

2.6.2. Effect of processing on β-carotene

Most vegetables are processed prior to consumption. Processing may be minimal or more extensive, involving procedures such as washing, peeling, cutting, blanching, an addition of processing chemicals, drying (dehydration), freezing and canning. All have a potential impact on carotenoid form and content (Thane & Reddy, 1997).

The leading factors for vitamin A deficiency is losing vitamin A and provitamin A from food due to processing, particularly home preparation cause carotenoid losses, sometimes to a greater extent. Because the effects of home preparation or processing on a food are dependent on many factors, sometimes with opposing consequences as well as their interactions, the findings of the various studies may appear at times inconsistent. The net effect will be due primarily to the prevailing factors.

β-carotene are highly susceptible to isomerization and oxidation during processing, the processing effect reason for the loss of color and biologic activity and the formation of volatile compounds that induce desirable or undesirable flavor in some foods. The occurrence of oxidation in food depends on the presence of oxygen, metals, enzymes, unsaturated lipids, pro-oxidants, or antioxidants; exposure to light; type and physical state of carotenoid present; severity of the treatment (destruction of the ultrastructure that

protects the carotenoids, increase of surface area, and duration and temperature of heat treatment): Heating promotes *trans-cis* isomerization (Rodriguez-Amaya, 1997).

2.6.2.1. Cooking effect

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 et al., 2013). Cooking is responsible for losses of vitamins and minerals in foods. However the bio availabilities 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).

Cooked vegetables would have variations in their carotenoid composition brought about by varying cooking conditions (e.g. time and temperature) (de Sá & Rodriguez-Amaya, 2003) while heat treatment involved in cooking is a necessary step in making the food palatable and in improving the digestibility of food components, the undesirable changes associated with cooking are reduction in nutrient content, which can be attributed to oxidation of chemicals in the food (Veda et al., 2010).

There are different kinds of cooking methods such as boiling, steaming, stir-frying, blanching etc. In which all affect 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 & Peacock, 2003; Lešková et al., 2006). Cooking losses of vitamins and minerals are depended upon the degree of heating, leaching into the cooking medium, the surface area exposed to water and oxygen, and cooking time (Lešková et al., 2006).

Methods, temperature, and duration of cooking may also affect 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 & Thornalley, 2007). However, Most of the data available on the provitamin A content of foods refer to the raw materials. It is evident, however, that data relating to the form in which the foods are consumed by the population are urgently needed and most green leafy vegetables are eaten as cooked therefore the influence of processing on provitamin A level has to be determined. This type of information and study on will help consumers to choose the processing conditions that favor pro vitamin A retention (de Sá & Rodriguez-Amaya, 2003).

3. MATERIALS AND METHODS

3.1. Study design for survey

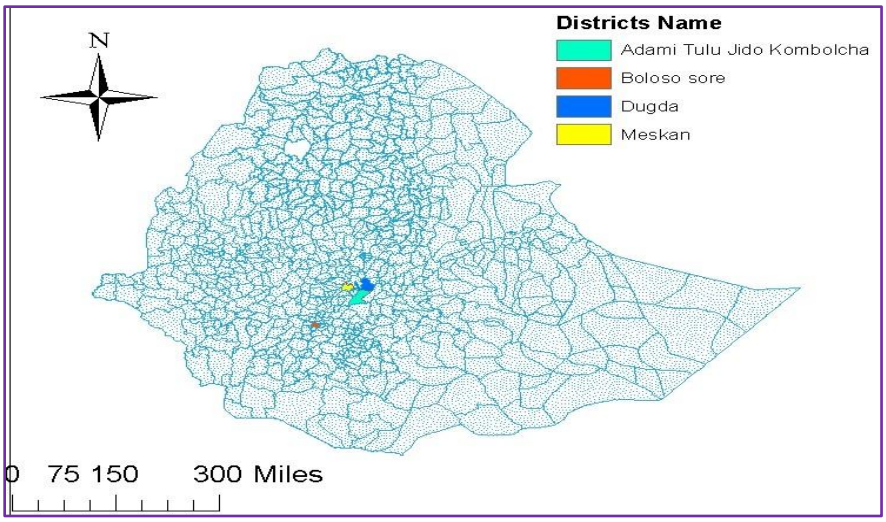


Figure 6: Selected study area for assessment

Purposive and structured questionnaires (Appendix-10) were used to get more information related to types of vegetables commonly used in the area, production, storage, marketing, knowledge, attitude and practice assessments regarding its benefit, cooking type and time of the process and other related question regarding vegetable from Ethiopia (Oromia and SNNPR).

From SNNNPR (Butagera and Wolita zone) was the target area. Meskan was one of selected Woreda from Butagera located at the base of the Zebidar massif in the Gurage zone, which has a latitude and longitude of $8^0$7'N $38^0$22'E and an elevation of 2131 masl; border on the south by the Silt zone, on the west by Muhor Na Aklil, on the north by the Oromia Region, on the northeast by Sodo, and on the southeast by Mareko. Boloso sore Woreda from Welayita zone has latitude and longitude of $7^0$4'N $37^0$42'E and an elevation of 1774 masl and 300 km from Addis Ababa bordered on the south by Sodo zurya and Demote Sore, on the west by Boloso Bombe on the north by Kembata Tembaro on the east by Damot Pulasa. From Oromia region (Zeway and Meki) wear the selected area, Adami Tulu is one of the woreds located in Zway 168 kilometers from Addis Ababa which has latitude and longitude $7^0$52'N $38^0$42'E respectively with an elevation of 1636

masl located in great rift valley bordered on south by Mirab Arsi zone on the west by SNNPR, on the north by Dugda Bora and on the east by Arsi zone. The last Woreda was Meki, the administrative center of Dugda woreda, located in Misraq Shewa zone it has altitude and longitude of $8^0$9'N $38^0$49'E with an elevation of 1636 masl. Based on the questioner administrated in different Woreda, can easily identify which vegetable is common and develop a flow chart on processing type.

Figure 7: Kale cooking practice in the rural parts of Ethiopia

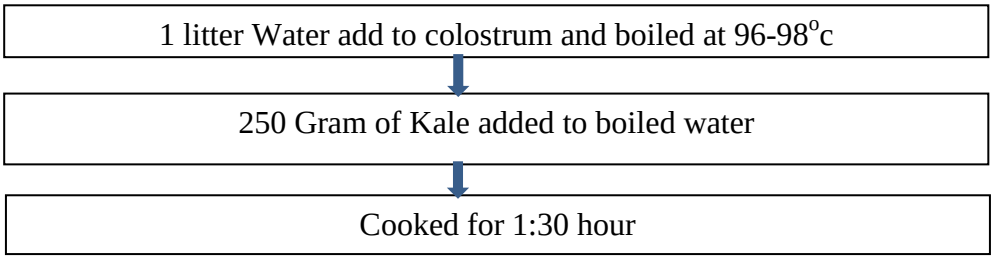
3.2. Experiment study area

The study was conducted at Addis Ababa, the capital city of Ethiopia and the experiments were carried out from November 2017 to July 2018 in Addis Ababa University Center for Food Science and Nutrition; Ethiopian food and drug administration; Ethiopian Public Health Institute and Bless Agri food lab.

3.3. Sample collection and preparation

About 5 kilo gram samples of Kale were collected from the local market of Addis Ababa, Ethiopia; then washed with tap water to remove all dust and insects particularly to aphids. The inedible parts were manually removed with the help of knife and hand then cut the leaf in to small pieces, thoroughly mixed and divided into three portions: - raw, process I and process II based on the cooking process of rural Ethiopia.

Process I



Process II

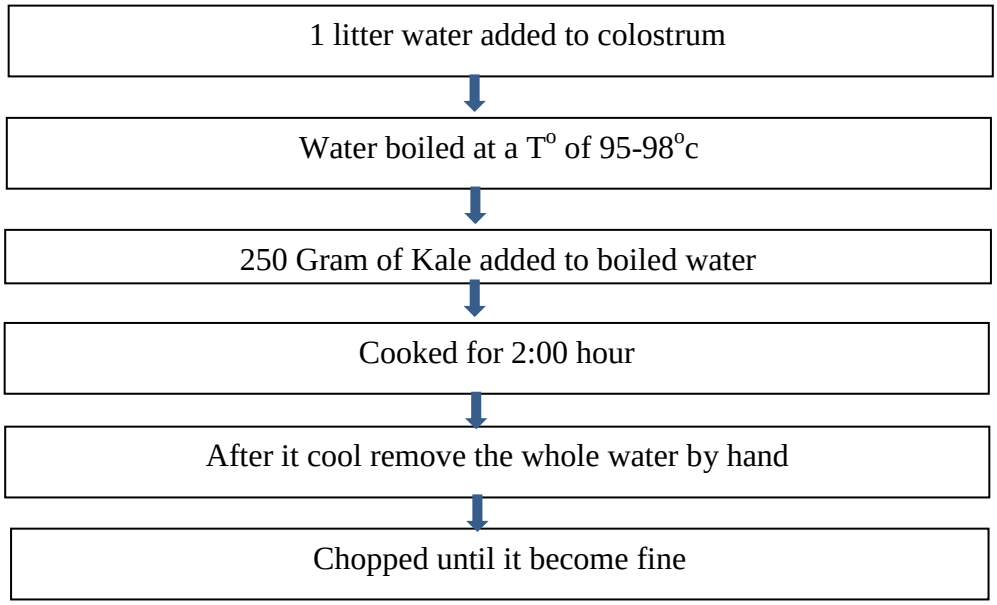


Figure 8: Traditional cooking flow chart

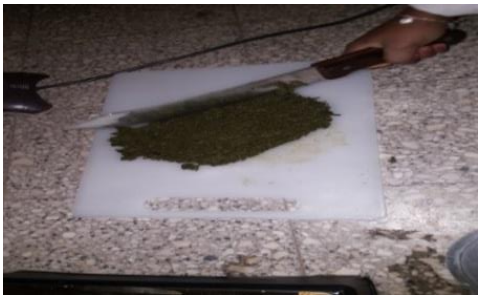
Process I

Cooked for 1:30 hours

Process II

Cooked for 2 hours

Remove the water



Chopped into small pieces

Figure 9: Sample preparation

3.4. Determination of proximate composition

3.4.1. Determination of moisture

The moisture content of Kale was analyzed using a drying oven (Model: Gallenkamp, OV 880, England) and moisture was determined according to AOAC (2000) using the official method 925.09. A clean dried and covered flat aluminum dishes were weighed and about 5gram of the dried samples were transferred into a previously cleaned, dried and weighed aluminum crucible (W2). The crucible with its content was put into a drying oven at 105°C for 5hr and cooled in a desiccator at room temperature for 30 minute. Then, the crucible with residue was re-weighed until a constant weight was obtained (W3) then the moisture content was estimated by:-

$$\text{Moisture (\%)} = \frac{(W2-W3)}{W1} * 100 \qquad (1)$$

Where W_1 = Weight of sample

W_2 = Final weight of crucible + Sample

W3 =Weight of crucible + Weight of sample after oven dried

3.4.2. Determination of ash

The ash content was determined by AOAC (2000) using the official method 923.03. The dish used for the analysis was washed and dried for an hour at 105^0c in an oven then the mass of the porcelain dish was measured using analytical balance (W1); About 2.5 gram of Kale powder was weighed into the porcelain dish (W2). The sample was gently heated over a hot plate until it charred at 120^oC on a hot plate for about 1 hour until the whole content becomes carbonized. Then the sample was placed in a furnace at 550^oC (Gallenkamp, model OV 880, England) for 5 hours until whitish color appears. The sample was removed from the furnace, placed and cooled in desiccators. Finally, the mass was weighed as (W3) then the total ash was calculated.

$$\text{Ash (\%)} = \frac{(W3-W2)}{W1} * 100 \qquad (2)$$

Where, W1= weight of sample

W2= weight of empty crucible

W3= weight of crucible and sample after ashing

3.4.3. Determination of crude protein

Crude protein was determined according to by the method of the Association of Official Analytical Chemists‟ AOAC (2000) using a Kjeldahl method using the official method 979.09. In a cleaned Tecator flask, 0.5g of the sample was weighed; 6mL of concentrated sulphuric acid (AR) was added and allowed to stand for 24hrs. After 24 hrs 3.5mL of H2O2 (30%) was added step by step. When the violent reaction stopped it was shacked and left in the rack. 3 g of accelerated reagent (a mixture of copper sulfate pentahydrate and anhydrous potassium sulfate) was added and left for 15 minutes. The mixture was digested in a digest stove (HYP-1008 eight holes) at 370^0C for 4hrs. After digestion it was cooled in the hood on the rack, 25mL of distilled water was added to dissolve the precipitate, 25 mL of 40% NaOH was added to the digested sample and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). 25mL of H_3B_O3 (saturated solution), 25mL of distilled water and 3 drops of methyl red were added in the 250mL conical flask and placed in the distiller (KDN-102F, nitrogen analyzer distillation device). In the distillation when about 150-200mL distillate was collected it was titrated with 0.1 N HCl and the amount of HCl was recorded. The amount of protein was calculated by using the following formula and the result is shown in Table 9.

$$\text{Crude protein \%} = \frac{V2-V1\times N\times 14.01\times 6.25}{10\ W} \qquad (3)$$

Where V1= Volume (ml) of hydrochloric acid solution required for the blank test

V2= Volume (ml) of hydrochloric acid solution required for the test sample

N= Normality of hydrochloric acid

W= Weight of the sample

14.01= Equivalent weight of nitrogen

6.25= Nitrogen to the protein conversion factor

3.4.4. Determination of crude fat

Crude fat of Kale was determined by Soxhelat extraction (Model: EV 16, SN: 4002824, Germany). The crude fat was extracted according to AOAC (2000) official method 4.5.01. The cleaned flask (cylinder) and boiling chips were dried in the drying oven at 1000C for 1h, cooled in the desiccators for 30minute and weighed. Two grams of sample was weighed in thimble containing fat-free cotton. The thimbles were placed in the thimble holders, 50mL of petroleum ether (boiling range of 40-60^{0}C) was poured in the flask, the thimble was immersed in the petroleum ether (in the flask) and heated at 80^{0}C in the fat determinator (SZC-C fat determinator) for 1hr, hanged the thimble and heated at the same temperature for 2hrs and then the solvent was recovered for 15 minute. The heater was switched off, the flask was dried in the drying oven at 900C for 30 minute, cooled in the desiccators for 15 minute and then weighed the flask with the extract. The amount of extractable fat was calculated by using the following formula and the result is shown in Table 9.

$$\%\text{crude fat}=\frac{(W3-W2)}{W1} *100 \qquad (4)$$

Where, W1= weight of the sample

W2= weight of extraction thimble

W3= weight of extraction thimble with the dried crude fat

3.4.5. Determination of crude fiber

Crude fiber analysis was conducted using the method of AOAC (2000) official method 962.09. About 1.5g sample was transferred into a 600 ml beaker and about 200 ml 1.25% sulfuric acid was added and boiled for 30 minutes. Recording took place by placing a watch glass over the mouth of the beaker. After 30 minutes heating by gently keeping the level constant with distilled water, 20 ml of 28% KOH was added and again boiled gently for further 30 minutes, and then the solution was filtered through sintered glass crucibles. Subsequently, washing was conducted with hot distilled water, 1% sulfuric acid, 1% NaOH solution and finally with acetone. Then, filtered and dried it in the electric oven (memmert 854 Schwabach, West Germany) at 92^{o}C for 4hrs. Furthermore, it was cooled

at room temperature for 2 hours in a desiccator and weighed, then transferred it to the crucible to muffle furnace (GALLENKAMP, Model FSL 340-0100, U.K.) for 30 minutes ashing at 550^0C. Finally, it was cooled again in a desiccator and reweighed. The crude fiber content was determined by using the formula and the result is shown in Table 9.

$$\text{Crude fiber (\%)} = \left(\frac{(W1-W2)}{W3}\right) * 100. \quad (5)$$

Where W1 = crucible weight after drying

W2 = crucible weight after ashing and W3 = weight of the sample

3.4.6. Determination of crude carbohydrate

The crude carbohydrate content was determined by the difference of the sum of the percentages of moisture, protein, ash and fat content from 100 with the exclusion of crude fiber.

$$\text{CHO(\%)} = 100 - (\%\text{Moisture} + \text{crude protein \%} + \%\text{ Ash} + \%\text{crude fat}) \quad (6)$$

3.5. Minerals analysis

3.5.1. Sample preparation

For mineral analysis 0.5g of raw, processed I and processed II Kale sample were taken then weighted in digestion vessel with added 5ml cold nitric acid, 3 ml of hydrogen peroxide; then close the vessel and digest the sample until the solution becomes colorless, after digestion it kept until it reaches to room temperature and transfer the digested clear solution to 25 ml volumetric flask and dilute to volume.

3.5.2. Quality control

Calibration standard was prepared from 1000mg/l of listed minerals standard solution which obtained from sigmal Aldrich. From 1000mg/l stoke solution by using dilution formula. 20mg/l from each were prepared and each calibration point, 1 mg/l, 2mg/l, 3 mg/l, 4 mg/l, and 5 mg/l calibration standard was prepared from 20mg/l listed minerals standard with 1 mg/l, 2 mg/l, 3 mg/l, 4 mg/l and 5 mg/l calibration points.

3.5.2.8. Determination of minerals

After standard preparation (Appendix 1) the digested raw Kale and processed Kale sample was determined by using flame atomic absorption spectroscopy. First atomize the hallo cathode lamps for 10 minute then adjust the alignment of the lamp turn on the flame and read the solution.

Table 2: Minerals calibration standard of Co, Ca, Mg, Mn, Zn, K, and Fe by Flame Atomic Absorption Spectroscopy

Calibration standard

Standard	Co	Ca	Mg	Mn	Zn	K	Fe
1	0.8 mg/l	1mg/l	0.2 mg/l	0.4 mg/l	0.2 mg/l	0.4 mg/l	1 mg/l
2	1.6 mg/l	2 mg/l	0.6 mg/l	0.8 mg/l	0.4 mg/l	0.8 mg/l	2 mg/l
3	2.4 mg/l	3 mg/l	1.2 mg/l	1.2 mg/l	0.6 mg/l	1.2 mg/l	3 mg/l
4	3.2 mg/l	4mg/l	1.6 mg/l	1.6 mg/l	0.8 mg/l	1.6 mg/l	4 mg/l
5	4.0 mg/l	5 mg/l	2.0 mg/l	2.0 mg/l	1.0 mg/l	2 mg/l	5 mg/l

3.6. Anti-nutritional factor

3.6.1. Determination of Oxalate

The oxalate content of Kale sample was determined according to AOAC 2005 which was determined by permanganate reduction using 0.1N $KMnO_4$ solutions. Weighted about 1 g of the sample in to 100 mL conical flasks then added 75ml of 3 mol/lH_2SO_4 and stir carefully intermittently with a magnetic stirrer for about 1 h then filter the slurry by whatman No.1 filter paper. Collected 25mL of filtrate sample then titrate the filtrate against hot (80^0 - 90^0) 0.1N $KMnO_4$ solution to the point when a faint pink color appeared that persisted at least 30s.

The concentration of oxalate in each sample obtained from the calculation:

1mL 0.1N permanganate = 0.0006303g oxalate

3.7. Vitamin C analysis

3.7.1 Reagents preparation

$9NH_2SO_4$: 25 ml of concentrated H_2SO_4 (sp.gr.1.84) was added to 50 ml of deionized water and cool, then followed by making the final volume to 100 ml. $85\%H_2SO_4$: were made by adding 90 ml of H_2SO_4 (sp.gr.1.84) to 10 ml deionized water. 5% meta phosphoric acid: was made by dissolved 5 gram of reagent grade HPO_3 into 80 ml of deionized water then made the final volume to 100 ml. 2% 2, 4-DNPH: were made by dissolving 2 gram of 2% 2, 4-DNPH in 100 ml of $9NH_2SO_4$ in volumetric flask and filter. 2%Thiourea: was made by dissolved 2 gram of Thiourea in 100 ml of 5% HPO_3. Saturated Bromine Solution: was made by in a hood added about 0.6-0.8 ml of bromine to 100ml of deionized water. 6% Trichloroacetic acid: were made by dissolve 6 gram of Trichloroacetic acid into 100 ml of deionized water. AA standard: made by weighing out 100 mg of AA in beaker 2-dissolve it with 5% metaphosphoric acid 3-make up the solution to 100ml with 5%metaphosphoric acids. Each ml of solution contains 20µg AA. To prepare 10, 20, 30, 40 & 50 µg take 0.5, 1, 1.5,2 & 2.5 ml in test tubes and dilute to 4 ml with 5% metaphosphoric acid.

3.7.2. Quality control

From 20ul Ascorbic acid 1ml of solution were taken and transfer to 50 ml volumetric flask and 1ml saturated bromine water solution was added and diluted to 50 ml with 5% metaphosphoric acid then aerated the solution in conical flask in alternate with added 0.5g thiourea to the solution to expel excess bromine from the clear solution then take 0.5ml in test tube and dilute to 4ml with 5% metaphosphoric acid, It has 10ul. Then five standard points were prepared 10, 20, 30, 40, and 50 with absorbance of 0.0165, 0.131, 0.2505, 0.3745, 0.499, and 0.623 respectively.

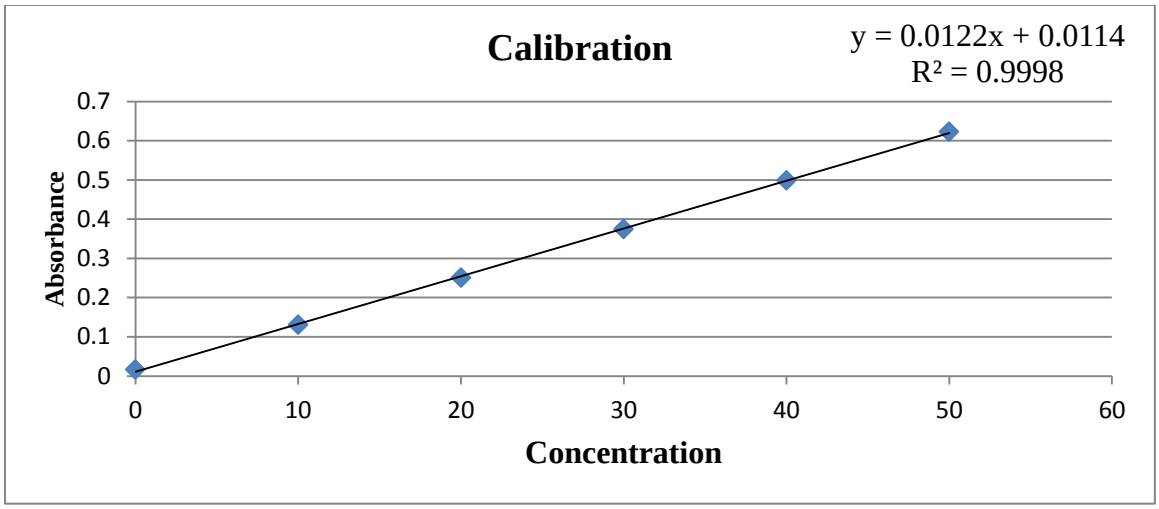


Figure 10: Calibration curve for vitamin C

3.7.3. Extraction and determination for vitamin C

Extract 5gram of Kale sample with 100ml of 6%TCA by mortar and pestle for 5 minute then remove the suspended solids by centrifuging or filtration. In a conical flask containing sample solution added 2 drops of saturated bromine solution was added with aeration then to 10ml aliquot, added 10ml of 2% thiourea then pipette 4ml from step 4 into each of 3 test tube then set one tube aside to serve as blank then to each of the remaining tubes add 1ml of 2, 4-DNPH then put all test tube in water bath at 37^0C For 3 hour and cool in an ice bath for approximately 5 minute. then Add slowly 5ml 85%H_2SO_4 while the tubes are in an ice bath then added 1ml of 2% DNPH to the blank and mixed all tubes followed by standing all tubes at room temperature for 30 minute, then read the absorbance of the standards, blank and test samples at 515 nm.

Figure 1: Standard and sample preparation

3.7.4. Calculation

$$\text{Mg AA/100g} = \left(\frac{(\mathbf{As-Ab})*\mathbf{10}}{\mathbf{A10\mu g\ Std-Ab}}\right) \quad \ldots\ldots (6)$$

Where: A_s Absorbance of samples

A_b Absorbance of blank

$A_{10\ \mu g\ Std}$. Absorbance of 10 µg AA standard

3.8. β- Carotene Analysis

3.8.1. β-carotene standard preparation

An analytical standard of β-carotene (β -carotene type II, synthetic, ≥ 95 %, HPLC) crystalline, Sigma-Aldrich, St. Louis, MO, 53103 USA) was used to calibrate and quantify the β-carotene.

The stock solution was prepared from10µg/ml by using HPLC grade n-hexane as a diluent; standards solutions were prepared in 100 ml volumetric flasks. From the stock solution (100ppm) β-carotene standard a concentration of (5, 10, 20, 40, 50, 80 and 100 ppm) were prepared for method validation. The prepared standards were transferred into vials and stored at 4^0C and protected from light to avoid deterioration of the β-carotene in the solution. All Chemicals used in the analysis of β-carotene were HPLC grade; acetone, petroleum ether, acetonitrile, methanol, ethyl acetate, triethylamine, and n-hexane.

3.8.3. Chromatography method validation

To evaluate the analytical performance of the instrument and validity of the method, first identification, precession, linearity, linearity cheek, LOD and LOQ, and work range were checked; and also quality control was done to identify whether there is poor methodology or not related to instrument and sample method and used to judge the quality, reliability, and consistency of analytical results and analytical procedure employed for a specific test for its intended use.

3.8.3.1. Identification

Identification of carotenoid was done based on their retention time of β-carotene standard at different time injecting at the same condition and its precision determined by the percent relative standard deviation %RSD.

3.8.3.2. Precision

The precision of a method is the extent to which the individual test results of multiple injections of a series of standards agree. The precision of the method was evaluated through the repeatability of the method by assaying 8 replicate injections of β-carotene standard at the same concentration (5, 10, 20,40,50,80 and100 ppm) during the same day under the same experimental conditions to obtain an acceptable % RSD.

3.8.3.3. Linearity

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analysts in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity determined by injecting a series of (5, 10, 20, 50, and 80 ppm) of β-carotene standards. The concentration range (5-80 ppm) and a regression equation were found by plotting the peak area (Y) versus the β-carotene concentration (X) expressed in ppm.

3.8.3.4. Linearity check and working range

According to the International Conference on Harmonization (ICH) guidelines, a minimum of five concentration levels, along with a certain specified range is recommended for the linearity checkup. The regression equation was found by plotting the β-carotene concentration in parts per million (ppm) (X) versus the corresponding peak area in (mv) (Y) for each concentration. The working range was obtained from the linearity study and depends on the intended application of the test method.

3.8.3.5. Limit of detection and limit of quantification

Limit of detection is the lowest concentration of analyte that the analytic process can reliably differentiate from background levels. The background level measured in the substrate blank plus 3 standard deviations of this baseline level. Limit of detection was determined by preparing serial dilution of 0.08 µg/ml, 0.05 µg/ml, 0.025 µg/ ml, 0.0125 µg/ml and 0.0025 µg/ml (ppm) of β-carotene standard from 5ppm to obtain the lowest amount of analyte greater than three times of noise level S/N > 3 and run each of them seven times. Therefore the signal (S) to noise (N) ratio were higher than 3 (S/N>3). In the same way, limit of quantification was determined by injecting 0.025 ppm of β-carotene, to obtain the lowest amount of analyte which can be reproducibly quantitated above the baseline noise, that gives S/N >10.

3.8.3.6. Accuracy and recovery

To simulate the actual analysis as closely as possible, the β-carotene standard were analyzed by injecting β-carotene standard. The recovery was used to determine the method accuracy. Accuracy is the degree of average measurement with an accepted reference. In this study, the accuracy of the technique was evaluated in terms of % recovery values between 70% and 120% were considered as an acceptable range. The recovery was performed by injecting a known amount of β-carotene standard and % of recovery was calculated as:-

%Recovery = (Obtained Concentration/Spiked Concentration) X 100%.

3.8.4. Sample β-carotene analysis

Extreme hydrophobicity, poor stability, and low concentration in biological samples make β-carotene difficult to analyze and difficult to develop analytical methods for aimed towards identification and quantification (Abate-Pella et al., 2017). However Several methods for carotenoid measurement have been published over the past decade, which all differ in isolation techniques and HPLC systems according to that this study depends on the method according to (de Sá & Rodriguez-Amaya, Rodriguez-Amaya and Kimura 2004) with slight modification.

3.8.4.1. Extraction

For this study 3 gram of representative Kale sample from (raw, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110 and 120 minute cooked) each was taken and put in the mortar, homogenize the sample with 50mL of cold acetone (acetone refrigerated for about 2 hours) for 1 minute and filter with suction through a sintered glass funnel then wash the mortar and residue with small amounts of acetone and receiving the washings in the funnel then repeat extraction and filtration (until the residue becomes colorless), then 0.1% BHT was added as antioxidant to solvent. The moisture content of the vegetable samples was estimated by drying 5gram of fresh vegetable leaves using moisture analyzer (ML-50, 04041-25, Japan).

Figure 2: Extraction of β-carotene from Kale leaves

3.8.4.2. Partition

The extraction was partitioned by placing about 40 mL of PE in a 500 mL separatory funnel then the acetone extract were slowly add in to the PE solution and distilled water (about 300-400 mL) by flow it along the walls of the funnel and allowed the two phases separate and discard the lower, aqueous phase by wash three to four times until completed removal of residual extraction solvent without discarding any of the upper phase, then collect the PE phase that contain carotenoids, then concentrate the extract in a rotary evaporator ($T \leq 35^{o}C$), dry under N2.

Figure 3: Partition the extract Kale with PE and removal of PE and acetone

3.8.4.3. Clean up

To remove color interference during HPLC analyses, the crude extract was cleaned up with open- column silica gel. Briefly, open glass column of 1.2mm diameter was cleaned, rinsed with n-hexane and dried. Then, a small piece of defatted cotton was put at the bottom to prevent silica gel loss. 15g of dried silica was dissolved in n-hexane. Then, the silica was packed into the open-column with continuous solvent addition to avoid bubble formation and cracking. The dried crude extract was reconstituted with small amount of n-hexane and loaded to the column. Then, the first 5mL of n-hexane was added into the column gradually. This resulted in the broader elution of orange pigment from the green extract. Then, to narrow the brown β-carotene pigment, a 4:1 combination of n-hexane to ethyl acetate was added gradually. The clear yellowish fraction was collected and dried under nitrogen. The fraction was kept at -20^{o}C until the HPLC analyses (Rodriguez-Amaya, 2001). Then, prior to injection into HPLC the extract was dissolved by 5 ml n-hexane then placed into sample vials.

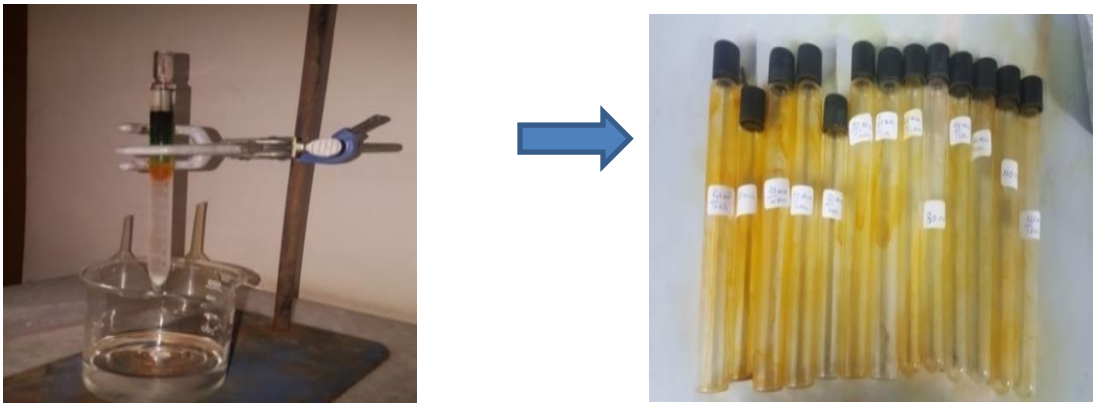


Figure 14: Clean up

3.8.4.4. HPLC analysis of β-carotene

β-carotene analysis was performed using Shimazu (model CTO-20AC S. NO. L20214605018 AE 220-240V~50-600VA made in Japan) consists of a binary pump, auto sampler, column, thermostat and chemistation software. Separated on the C18 monomeric column (Spherisorb ODS2), 3 µm, 4.6x150 mm.

The mobile phases used were a mixture of acetonitrile, methanol, ethyl acetate and triethylamine (0.05%) were added to acetonitrile, as recommended by Hart and Scott (1995) to improve carotenoid recovery from the column. Used isocratic method for better resolutions which elution program was set as follows 80:10:10, operated at a flow rate of 0.7mL/minute. The column temperature was 30^0C and the wavelength of UV/Visible was 450nm, the injection volume was 20µl the β-carotene content was calculated using the formula below

Figure 4: Standard, sample preparation and HPLC identification

3.8.4.5. Calculation

Calculate carotenoid concentration using the formula:

$$C_x\ (\mu g/g) = \frac{A_x X C_s\ (\mu g/mL) \text{x total volume of extract (mL)}}{A_s \text{ x sample weight (g)}}$$

CX = concentration of carotenoids of sample

Ax = peak area of carotenoids of sample

Cs = concentration of the standard

A s= peak area of the standard

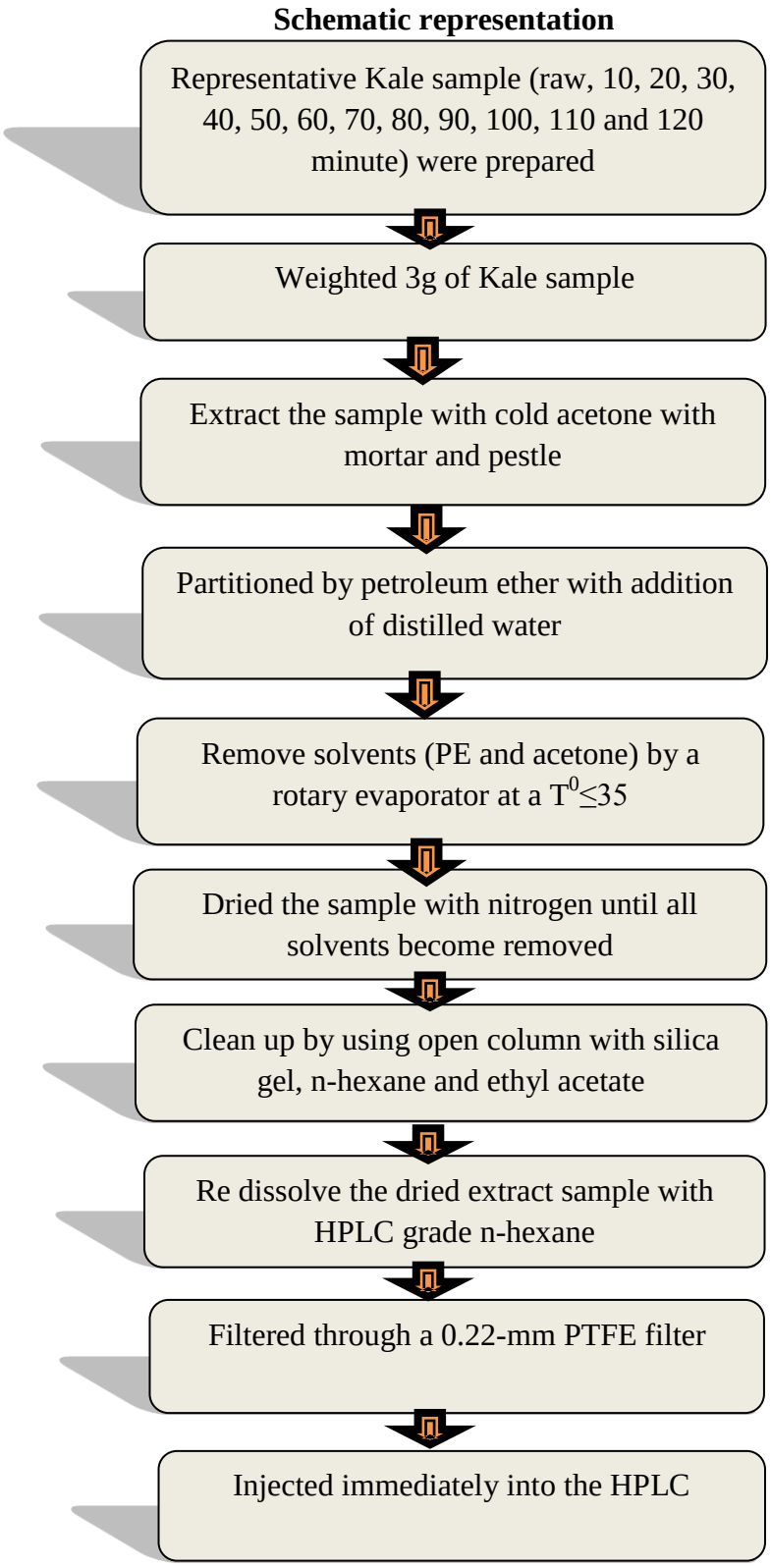


Figure 5: Schematic representation of sample extraction and identification

3.9. Sensory evaluation

Sensory attributes assessed the overall preference or acceptance of cooked Kale at different processing time from 20 panelists with nine-point hedonic scale sensory evaluation methods. Oriented about the test instructions to identify, name and classify a range of sample attributes (i.e. general appearance, taste, mouth feel and overall acceptability) and rate the samples for the intensity of each attribute.

3.10. Data analysis

Data analysis was performed by using SPSS version 20.0 and the required statistical analysis was calculated. The results were reported as mean ± SD and percentages. Least significant difference (LSD) was used for mean separation and $P\ values < 0.05$ were considered to be significant.

4. Result and Discussion

4.1. Survey report

Table 3: Assessment of green vegetables in selected districts of Oromia and SNNPR Regions of Ethiopia

	Variables	Response
Region	Oromia	45.3%
	SNNPR	45.3%
District	Zway	22.6%
	Meki	22.6%
	Bolososore	22.6%
	Butajira	22.6%
Sex of respondent	Male	11.3%
	Female	88.7%
Commonly consumed green leafy vegetable	Kale	84.9%
	Cabbage	9.4%
	Spinach	5.7%

As the result revealed above in Table 3, survey were collected from four villages of two regions: Oromia (Zway and Meki) and SNNPR (Bolososore and Burajira) of 48 households. From both regions among the household members 88.7% of the respondents were female and 11.3% male, this shows still in the rural parts of Ethiopian community female take the priority in most of the household activity. Since women are the main actors in the household for food and nutrition security.

In Oromia region the most common foods were maize and wheat, which are generally low in micronutrients and from vegetables like Kale, tomato, cabbage, onion and in SNNPR most root plants are common in daily bases like godere, sweet potato, potato, ‘enset’ in the form of ‘kocho’ and ‘bula’ and vegetables like Kale, cabbage, tomato, onion, spinach. Among the commonly consumed dark green leafy vegetable based on the assessment 84.9% household consumed Kale in both region.

Table 4: Assessment on green leafy vegetables production, storage and marketing

Variables		Response
Highly engaged with the vegetable gardening activity	Male	58.5%
	Employs	9.4%
	Females	32.1%
Are green leafy vegetable available yearly round	Yes	35.8%
	No	54.7%
High season of production	Winter	90.6%
	Summer	9.4%
What do you use to increase the yield of green leafy vegetable	Manure	74%
	Animal Dung	26%
what is the source of water for gardening of green leafy vegetables	rain water	47.2%
	river water	15.1%
	tap water	28.3%
Major challenges that negatively affect the production	Heavy rain	24.2%
	Pathogen	3.8%
	Insects/pest	72%
Vegetable transport from farm to market	Animal	87.4%
	Vehicle	12.6%
Time of day do you get quality and cheap vegetable in the market	Morning	90.6%
	Evening	9.4%

In Ethiopian community female take the priority in most of the household activity however, 58.5% of the household males were highly engaged with vegetable gardening activity and 32.1% were females from the household and the other 9.4% were.

35.8% of household respond that green leafy vegetable available year round; most of the members were from SNNPR and 54.7% of the household responds green leafy vegetable is not available year-round and about 90.6% household response vegetable grow in abundance in rainy season and also when grains are in short supply. To increase the yield of green leafy vegetable 74% of the household used manure and 26% used animal dung. In both regions to increase the yield of Kale production, they don't use artificial fertilizer which is much appreciated and needs to encourage for the future to reduce noncommunicable diseases and other risk factors. 72% of household respond that insects were the major challenges that negatively affect the production of Kale particularly to aphids and 24.2% responded that heavy rain has a high impact on the yield especially at the time of harvesting period and 3.8% respond pathogens wear a major challenge.

Therefore plant protection lesson need to be a concern through nutrition-specific intervention and Agriculture sectors.

87.4% of household and vegetable merchants respond that vegetables were transported from farm to market by animal and 12.6% by vehicle those respondents were from Wolita Woreda while the market was also very suitable for vehicles than others Woreda and the freshness of Kale that transported by animals were not good relatively to vehicle. 90.6% of the community obtained quality and cheap vegetable from the market in the morning and 9.4% obtained at evening.

Table 5: Knowledge survey response

Variable		Response
Have you ever learned about the right way of cooking	Yes	27.0%
	No	73.5%
How do you know cooking is enough	Change color	66.0%
	Ending of large amount of fire and time	17.0%
	Become very soft	3.8%
How to maintain the quality of vegetable	Put in a plastic bag	71.7%
	Immediately cook	18.9%

Based on the above Table 5, households have different level of awareness in related to vegetable cooking and eating pattern. As reported about the right way of cooking 27.0% of the household learned about the right way of cooking through education of different governmental bodies and non-governmental bodies like NGO. The other 73.5% of household have not been ever learned about the right way of cooking and only obtained knowledge from conventional methods.

Assessment for way of knowing whether cooking is enough or not, 66.0% of the household respond that cooking is enough when the color of Kale change its color from green to very light color/ complete removal of green color and the other 17.0% respond when ending of large amount fire and time, 3.8% of household respond when the leaf become very soft.

The consumption pattern is different among households within different regions and kebelas. This consumption pattern is highly variable and depends on factors such as

poverty status (Poor households use Kale more than their wealthier counterparts), degree of urbanization, ethnicity (ethnicity was shown to strongly influence households way of cooking and eating pattern and it is also a way of certifying a women cooking ability particularly in Gurage Zone where Kale cooked until it loses its color and become very soft and consumed after cooing and recooked which is known by 'ye,gomen kitfo'), distance to fresh produce markets and season of the year also other factor. Therefore traditional cooking of vegetable is more of based on knowledge of the society have.

Table 6: Attitude survey response

Variable		Response
Do you believe that there are certain foods that make people healthy and not healthy	yes there are foods that makes healthy and unhealthy	9.4%
	food is food can't be categorizes	50.9%
	yes there are foods that makes healthy and unhealthy	39.6%
Do you think eating vegetable will make a person healthy why	Unhealthy it is poor food free from fat	34.0%
	It is food why not healthy	11.3%
	Have no idea	13.2%
	Yes it is healthy	41.5%
Quality parameter when buying green leafy vegetable	Fresh	49%
	Clean	17.0%
	Free from diseases	17.0%
	Green and young leaves	17%

Based on the assessment the community knowledge was different depend on eating vegetable whether it make healthy or not, 34.0% respond vegetables are unhealthy it is poor food which is free from fat; 11.3% respond it is a food why not healthy; 41.5% respond yes it is healthy and the other 13.2% have no idea and further investigation and nutrition education needs to work on that.

Quality parameter during buying, 17.0% of household were responded when the leaf is free from diseases that means that is quality, the other 49.1% were its freshness; 17.0% of household respond its cleanness, and 7.5% of the household preferred when the leaf is green and young. 71.7% of the household maintain the quality by putting the Kale in plastic bag and the other 18.9% cooked the vegetable immediately after buying or harvesting. Once its prepared 79.2% of household prepare and consumed Kale for only

one day, the others 11.3% household consume for 2 days after prepared and 9.5% household prepared and consume immediately after the cook.

Table 7: Practice survey response

Variable		Response
How often do you buy vegetable	Every day	32.1%
	4-5 out of 7 days	62.8%
	Twice in a week	5.7%
The processing method of vegetable-based meal	Boiling	66.0%
	Boiling then remove water and chopped then recooked	26.5%
	Frying	7.5%
Will you consume it alone or with other food items	Alone	58.5%
	With other	32.1%
After a while will it served after reheating	Yes	62.3%
	No	28.3%
For how long reheated	5 minute	15.1%
	15 minute	49.1%
	10 minute	26.4%
Source of energy for cooking green leafy vegetable	Wood fire	43.4%
	Charcoal	47.2%
Once prepared for how long it can be consumed	1 day	79.2%
	2 days	11.3%
	Half day	9.5%

Kale were common vegetable with the frequency of buying 4 - 6 out of 7 days in the rural parts of Ethiopia particularly in the above-mentioned regions and Woreda. The common method of cooking for Kale in the household were boiling which accounted by 66.0% of the household and 26.5% were use boiling then remove the liquid then recooked after chopped, the later processing may accountable for loss of most valuable nutrients during extracting of liquids and the time it takes to cook. 7.5% of households were cooked vegetable by frying with oil this may enhance the bioavailability of nutrients like β-carotene and the oil may bind other nutrients from loss. Kale dishes could be prepared alone or with other ingredients like meat, potato and Kale alone made from a combination of different species to enhance the taste; major are salt oil, onion, and peeper. Edible part of Kale was only leafs in 77.4% of the household particularly in

Butajira, Meskane Woreda and Wolita, Areka Woreda to make "*ye gomen kitfo"* this mostly serve as a side dish with "*kitfo*" and 13.2% were used both leafs and stem especially this intend to cook "*gomen besiga"*.

Total cooking time was recorded in 66.0% of household cook Kale for about 2 hours and 11.3% of household cook Kale for about 1:30 hour and 11.3% of the household were an about1 hours and the rest record for 40-45 minute1.9%. In the rural parts, wood fire and charcoal were common sources of energy for cooking in both regions with recorded temperature 96^{o}c-$98\ ^{o}$c.

Other significant factor for the nutrient instability in Kale were reheating, 62.3% of household; 49.1% household recooked for about 15minute and 26.4% responded recooked for 10 minute and 15.1% for 5 minute.

Table 8: Observation assessment

	Variable	Response
Name of green leafy vegetable	Kale	90.6%
Edible part	Leaves	77.4%
	Leaves and stem	13.2%
Cooking utensils used	Metal colostrum	90.6%
	Clay colostrum	9.4%
Energy source used	Wood fire	39.6%
	Charcoal	50.9%
Cooking total time	60 minute	11.3%
	90 minute	11.3%
	120 minute	66.0%
	40-45 minute	1.9%
Cooking temperature	95^{0}c	3.8%
	96^{0}c	39.6%
	97^{0}c	28.3%
	98^{0}c	18.9%

Observation assessments were taken when the household member cooked by them self. 90.6% household start cooking by Kale and 77.4% use only the leafs part and drop the stem, this type of process was more of process II type and 13.2% of households use the leafs in coupled with stem, this type of process was more of process I. In the rural part of Ethiopia 90.6% household use metal colostrum for cooking, only in Meki Wereda 9.4%

household use clay colostrum for cooking. Source of energy for cooking were charcoal which used by 50.9% household and 39.6% used wood fire. Cooking of total time was ranged from 40-120 minute. Based on the assessment flow chart of processing type and time of traditional cooking were develop for further laboratory analysis and reported in alternative section.

4.2. Proximate composition

Table 9: Proximate composition of raw and processed Kale

Tested parameters	Sample analyzed		
	Raw	Process I	Process II
Moisture (%)	11.57 ± 0.00^{a}	14.77 ± 0.06^{c}	12.78 ± 0.03^{b}
Protein %	30.03 ± 0.06^{c}	29.87 ± 0.06^{b}	27.46 ± 0.01^{a}
Crude fat in (%)	3.10 ± 0.10^{a}	3.03 ± 0.05^{a}	3.07 ± 0.05^{a}
Ash %	16.23 ± 0.02^{c}	13.73 ± 0.01^{b}	12.33 ± 0.15^{a}
Fiber %	9.16 ± 0.04^{c}	7.33 ± 0.02^{b}	7.04 ± 0.03^{a}
CHO%	55.301	52.327	56.327

Data are expressed means ± SD
Means in the same row with different letter superscripts indicate significant differences ($p<0.05$)

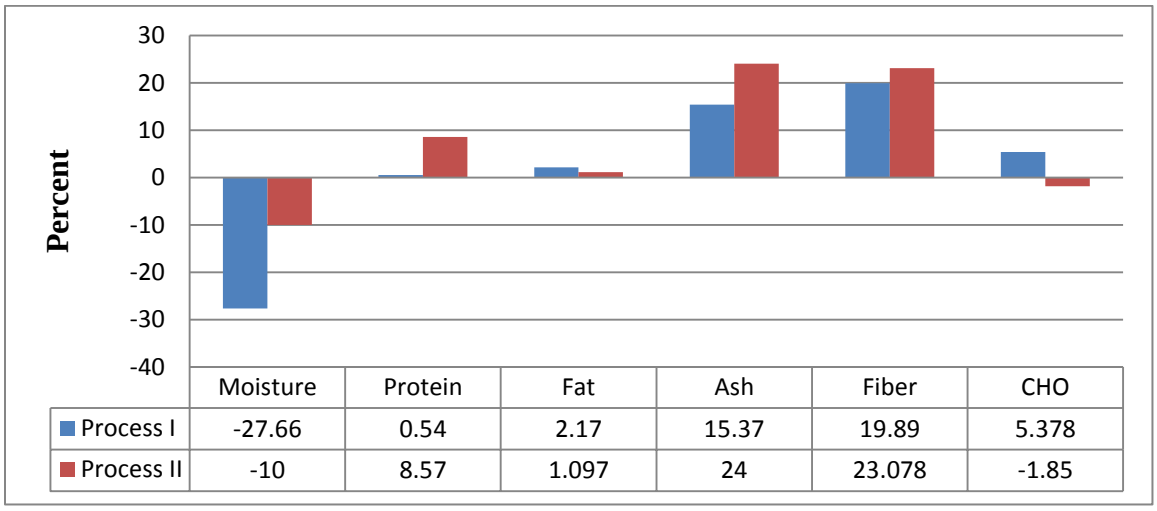


Figure 17: Percent loss in proximate composition of processed Kale

There is no efficient information on the nutritional composition of *Brassica carinata* leafs. However, it is proven that Kale contains a high amount of vitamin, mineral, and fiber as well as useful phytochemicals. In addition, Kale contains well-

known antioxidants such as vitamins C and E, carotenoids and antioxidant, enzymes such as catalase, superoxide dismutase (SOD) and peroxidase (Jahangir et al., 2009).

Current researches showed that the protein content of Kale was high as compared to other dark green leafy vegetables. As presented in Table 9, protein was ranged between 27.47% and 30.03%. According to other studies the protein content of *Brassica carinata* are high, 25–45% and comparable to that of pulses (Gupta, 2011; PROTA, 2018). Vegetables are a good source of protein; the protein content of minute, Spinach, Cauliflower, and Amaranth was ranged from 26.2% and 30.9% (Singh et al., 2001).

While heat treatment involved in cooking is a necessary step in making the food palatable and in improving the digestibility of food components like protein, the undesirable changes associated with cooking are a reduction in nutrient content, therefore along high processing protein can be attributed to reducing (Veda et al., 2010).

The fat content of raw Kale and processed Kale were 3%, there was no difference between raw and processed. The seeds are rich in oil, containing 25–47% depending on cultivar and growing conditions; however, according to Jahangir investigation Kale was contained low fat content (Jahangir et al., 2009 ; Sanlier & Guler Saban, 2018) highly deviate to the current study.

Brassica carinata were found a good source of fiber and recorded between 7.0% -9.1%. Dietary fiber content was high in raw Kale which shows there is a significant difference $(p<0.05)$ from processed I and processed II. According to studies fiber content of Kale was high (Jahangir et al., 2009). Carbohydrate content of Kale also very high which obtained by difference but could not found other studies which comparable with this result.

4.3. Mineral composition

Table 10: Mineral composition of raw and processed Kale

Samples analyzed (ppb)	Raw	Process I	Process II
Iron	206.70 ± 0.57^{b}	206.28 ± 0.50^{b}	200.20 ± 0.35^{a}
Calcium	22085.20 ± 0.20^{c}	21276.76 ± 0.68^{b}	21274.50 ± 0.50^{a}
Potassium	19855.45 ± 0.05^{c}	19175.33 ± 0.57^{b}	19085.00 ± 0.50^{a}
Copper	8.93 ± 0.06^{c}	5.58 ± 0.13^{b}	4.59 ± 0.09^{a}
Zinc	40.30 ± 0.26^{c}	33.64 ± 0.12^{b}	26.40 ± 0.37^{a}
Magnesium	4632.80 ± 0.72^{c}	4160.38 ± 0.34^{b}	4120.95 ± 0.93^{a}
Manganese	41.61 ± 0.53^{b}	39.26 ± 0.37^{a}	39.09 ± 0.15^{a}

Data are expressed means ± SD
Means in the same row with different letter superscripts indicate significant differences ($p<0.05$).

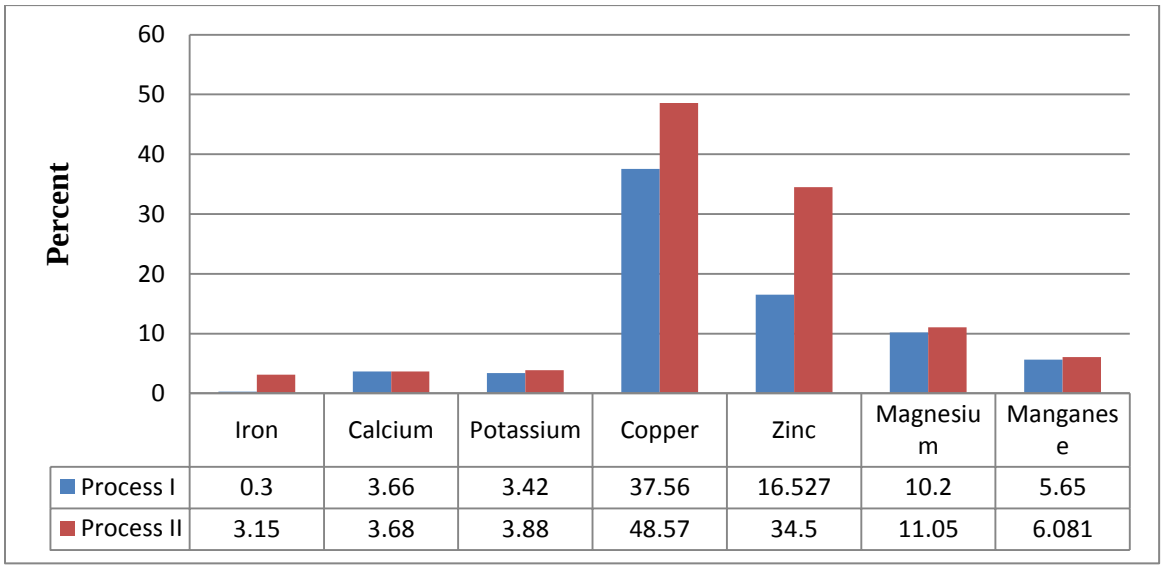


Figure 18. Percent loss in Mineral content of processed Kale

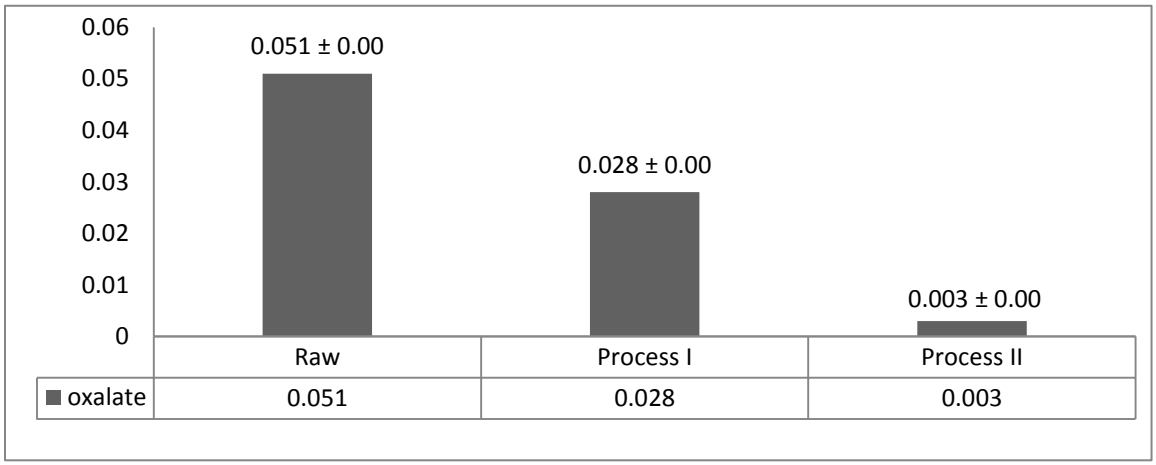
Among green leafy vegetables, Kale is an important mineral source that accumulates high levels of P, S, Cl, Ca, Fe, Sr and K (Sanlier & Guler Saban, 2018, Jahangir et al., 2009). The mineral compositions of raw and traditionally cooked leafy vegetables presented in Table 10, showed that the mineral content of raw and traditionally cooked leafy vegetable was reduced but decreased at a very decreasing rate.

Minerals are inorganic substances, present in all body tissues 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, Hunt 1996).

The result for mineral analysis of Kale suggests consumption of an efficient amount of Kale with minimal time cooking recommended to meet the mean recommended daily allowance for minerals.

4.4. Oxalate determination



mean ± standard deviation of triplicate determinations

Figure 19: Oxalate content of raw and processed Kale 0.001

As shown in Figure 19, the amount of oxalate was highly decreased as heat treatment. Oxalates are generally anti-nutritional factors and negatively affect the nutritional value of Kale by impairing protein digestibility, vitamin and mineral availability to the body. Based on study oxalate in raw Kale was 0.051gram, after processing for about 1 hour and 30 minutes showed a significant reduction along with processing and 0.028gram oxalates were obtained from processed Kale for about 2 hours and recorded 0.003gram which is not a risk for the inability of nutrients to take by the body. Therefore, decreasing of oxalate content along heat increment were good aspects of processing for this case.

4.5. Vitamin C

Table 11: Vitamin C content in raw and processed Kale

	Raw	Process I	Process II
Vitamin C	31.66 ± 0.43^{c}	14.28 ± 0.06^{b}	9.96 ± 0.12^{a}

All the values are the mean ± standard deviation

Means within the same row with different letter superscripts indicate significant differences ($p < 0.05$)

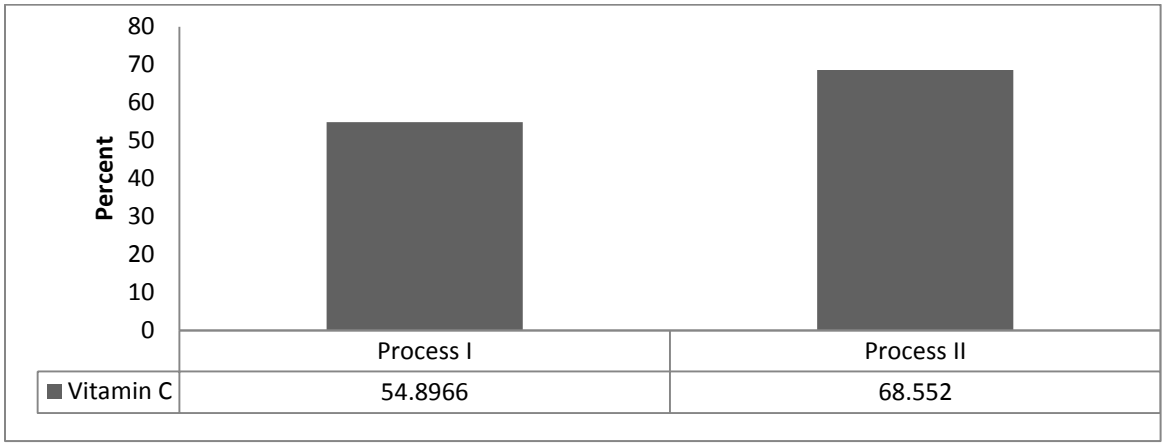


Figure 20: Percent loss of vitamin C in processed Kale

Vitamin C content of raw and traditionally cooked leafy vegetable was presented in Table 11, it indicate vitamin C content of the raw and traditionally cooked kale significantly different ($p< 0.05$) from each other. The result illustrate that a high percentage of vitamin C 68.5% were lost during process II and 54.89% of vitamin C was lost during process I with respect to raw Kale.

Vitamin C was greatly affected by processing and this result also paralleled with other findings, decrease in ascorbic acid by 19% in cooked amaranth, 61% in dried Vernonia amygdalina and by almost 100% in dried adonsonia digitata (Redhead, 1990). Also loss as a result of cooking was justified since vitamin C is water-soluble and heat labile, Olayiwola and Oyeleke explained as time of boiling increases vegetables are subjected to denaturation and tend to bring vitamin C level to zero (Olayiwola & Adeoye, 2012) this results also n accordance with what was obtained by (Davey et al., 2000).

Brassicas own a broad array of health-promoting compounds, emphasized (Domínguez-Perles 2014) according to different results in *Brassica oleracea* 31.0 mg/100g of vitamin C were recorded (McGuire, 2011), in spinach 36.8mg/100g (Singh et al., 2001) recorded. But the result was not comparable with the finding of (Sanlier & Guler Saban, 2018) recorded in Kale which was 120mg/100g.

As FAO and WHO report RNI (mg/day) for infants and children 0–6 months 25 mg/day, 7–12 months 30 mg/day, 1–3 years 30 4–6 years 30 mg/day , 7–9 years 35 mg/day adolescents 10–18 years 40 mg/day Adults 19–65 years 45 mg/day 65+ years 45 pregnant women 55 mg/day and for lactating women 70 mg/day (Joint & Organization, 2005) therefore Kale with minimal cooking time can satisfy vitamin C requirement for most of age group.

Vitamin C has been pointed out as an essential nutrient with an active role in the maintenance of body functions, displaying a wide range of therapeutic properties such as antioxidant, anti-carcinogenic, co-factor in the collagen synthesis, and promoter of iron absorption (Arrigoni & De Tullio, 2002).

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 (Naidu, 2003), Since our bodies cannot produce or store vitamin C, an adequate daily intake of this nutrient is essential for optimum health. 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 & Brannock, 1998). Therefore with regard to the drastic decrease in vitamin C during cooking, keeping vitamin C from loss during processing is very essential.

4.6. β-carotene chromatographic method validation

Chromatographic method validation has been done by checking different parameters such as identification, accuracy, recovery, linearity, working range, LOD and LOQ that set to measure the analytical performance of the instrument and to validate the method used to analyze β-carotene on the commonly processing type of Kale from selected rural part of Ethiopia.

4.6.1. Identification

Identification of β-carotene from the test sample was done according to their retention time on the HPLC chromatogram which is obtained after running 100ppm standard on different concentration (5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm, and 100ppm). According to the result, β-carotene retention time was 9.09; the precision of the retention time measured by using percent relative standard deviation which is 0.19 % according to FDA percent relative standard below 2% is acceptable (FDA, 2002).

Table 12: β-carotene standard identification

β-carotene (minute) in (5,10,20,40,50,80 and 100ppm) concentration	Mean	N	Standard Deviation	% RSD
	9.09	8	0.018	0.19

N= number of replications, RSD= Relative Standard Deviation

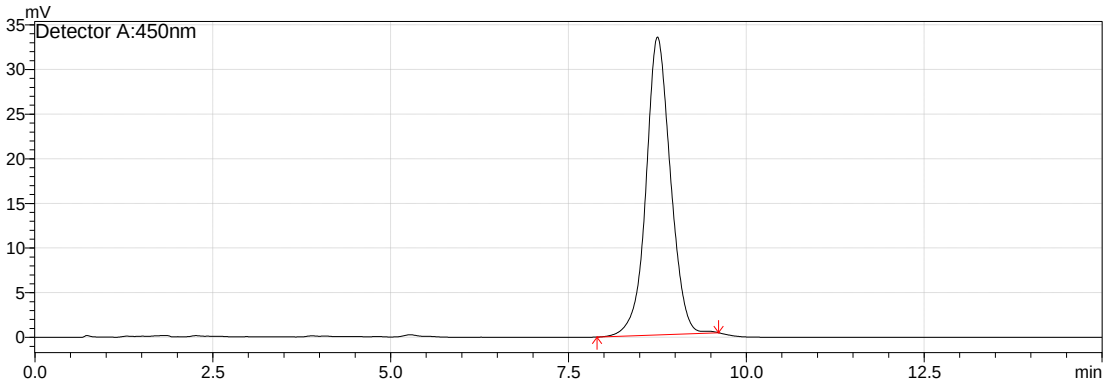


Figure 6: β-carotene standard identification chromatogram

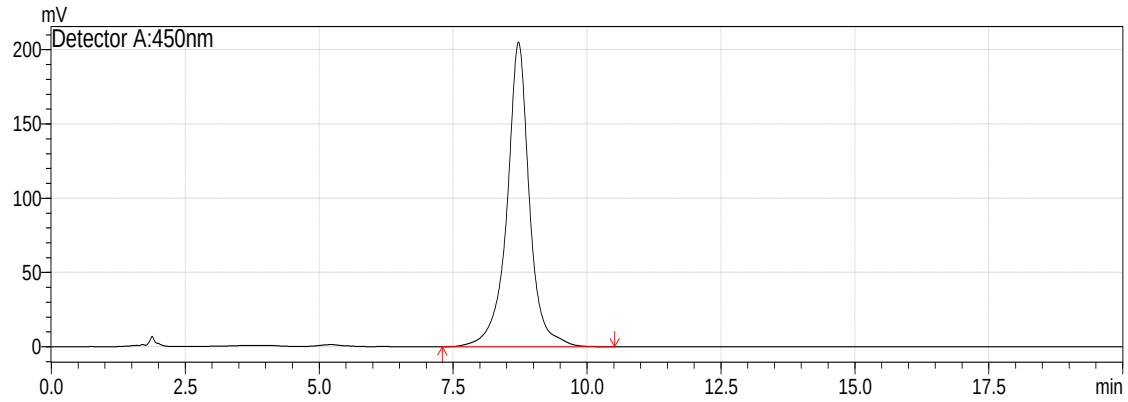


Figure 7: β-carotene identification of raw Kale

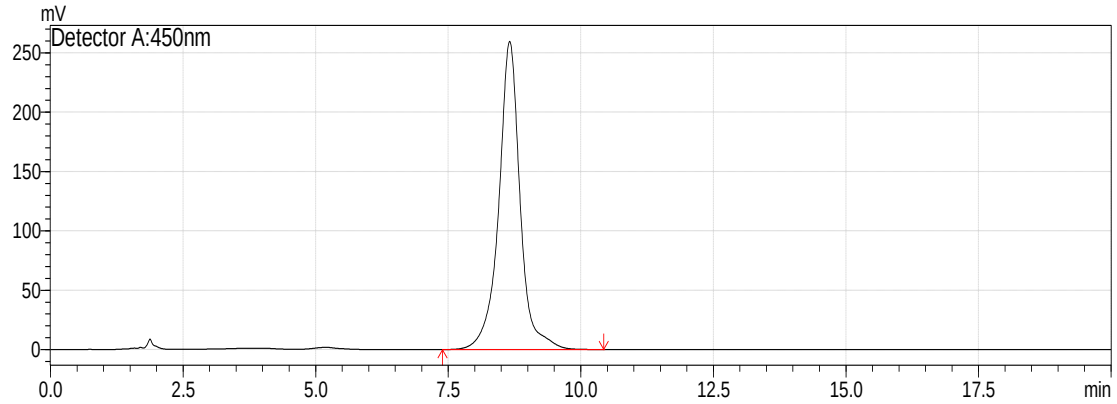


Figure 8: β-carotene identification of processed Kale

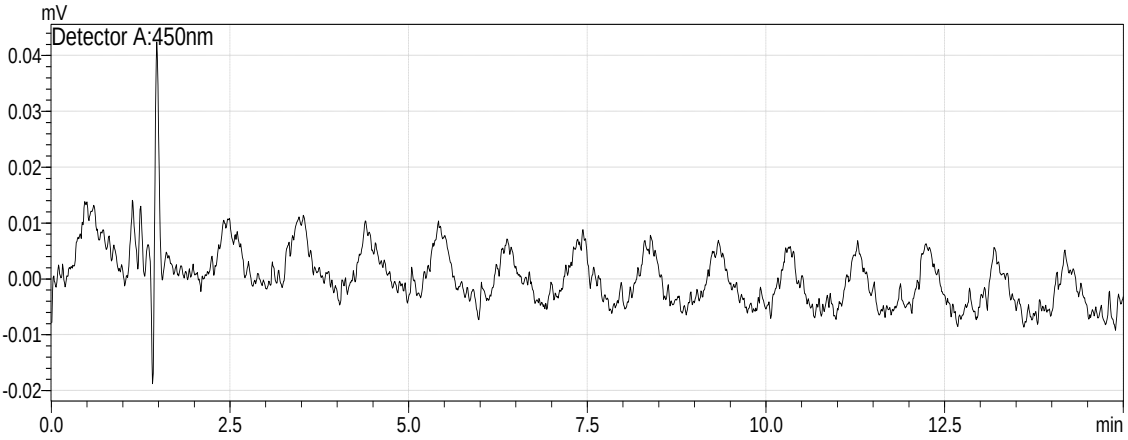


Figure 9: Chromatogram of mobile phase

Blank chromatogram indicated, there was no interference of mobile phase

4.6.2. Precision

The repeatability of the analytical method was tested by injecting 7 replicates of 5ppm, 10ppm, 20ppm, 40ppm, 50ppm, 80ppm and 100ppm of the β-carotene standard under the same analytical condition within the same day. Based on FDA standard the acceptable level of percent relative standard deviation for precision is ≤ 2. The result obtained after the assay is described in Table 13, therefore percent relative standard was less than 2 which is acceptable.

Table 13: Repeated injection of different standard β-carotene concentrations to check the precision of the method

	β-carotene concentration						
	5ppm	10ppm	20ppm	40ppm	50ppm	80ppm	100ppm
Peak Area	444848	1026147	1821854	3003999	4526516	6852071	7889030
	444062	1025374	1823599	2999233	4546285	6850527	7882525
	447008	1027291	1824384	2997094	4563495	6855641	7890406
	448243	1029663	1826946	2995487	4577869	6861285	7876587
	447564	1031168	1827463	2994531	4594261	6867161	7869765
	448746	1035209	1831823	2993971	4610502	6873452	7860232
	448341	1038440	1835939	2993182	4629732	6879870	7862357
Mean	446973	1030470	1827429	2996785	4578380	6862858	7875843
S D	1824.27	4862.80	4949.71	3781.43	36200.47	11129.01	12204.4
% RSD	0.41	0.47	0.27	0.13	0.79	0.16	0.15

SD= Standard Deviation RSD= Relative Standard Deviation

4.6.3. Limit of detection and limit of quantification

Among the concentration of 0.08, 0.05, 0.025 and 0.0125 ppm of β-carotene standard, the ability of the instrument to detect the smallest change was 0.0125 ppm with a signal to noise ratio 8.375 which is greater than 3. The limit of quantification of the instrument was 0.025 with a signal to noise ratio 17.6 which is greater than 10 and accepted according to (FDA, 2002) standard.

Table 14: Limit of detection and limit of quantification

β-carotene	LOD (ppm)	Signal to noise ratio (S/N)	LOQ (ppm)	Signal to noise ratio (S/N)
	0.0125	8.375	0.025	17.6

LOD= Limit of Detection LOQ=Limit of Quantification S/N=Signal to Noise ratio

The ability of the instrument to detect the smallest change on the analyte was 0.0125 ppm with a signal to noise ratio of 8.375 which is greater than 3, were acceptable for the limit of detection of the instrument.

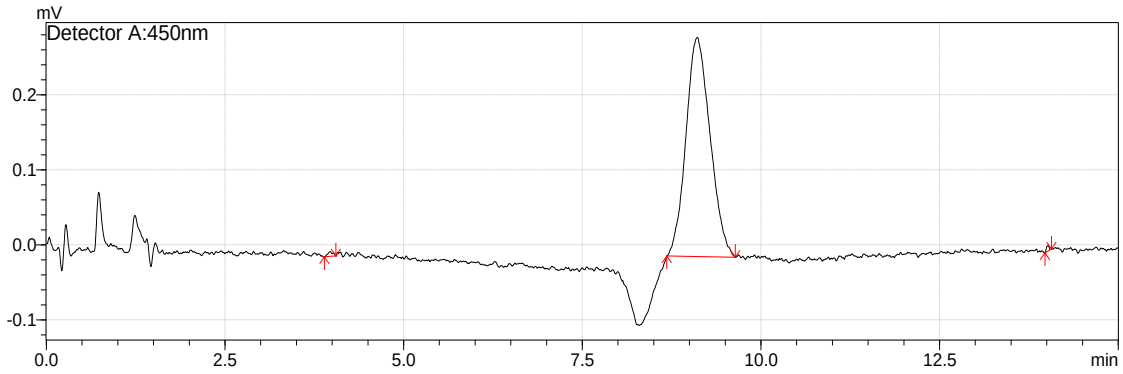


Figure 10: Chromatogram of Limit of detection

The limit of quantification of the instrument was 0.025 with a signal to noise ratio 17.6. The acceptable level of the signal to noise ratio for the limit of quantification is greater than 10.

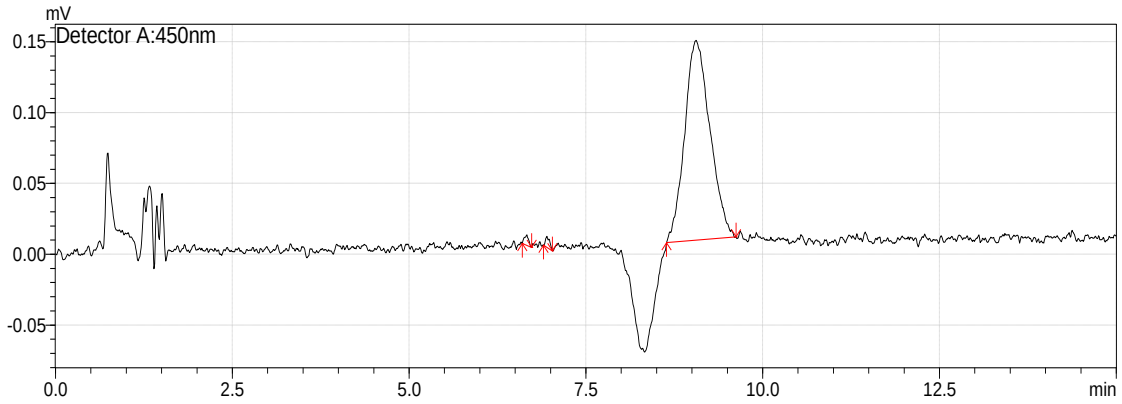


Figure 26: Chromatogram of Limit of quantification

The inability of the instrument to detect the smallest change of the analyte, the result (0.0025) was less than 3 and it is in acceptable by FDA standard.

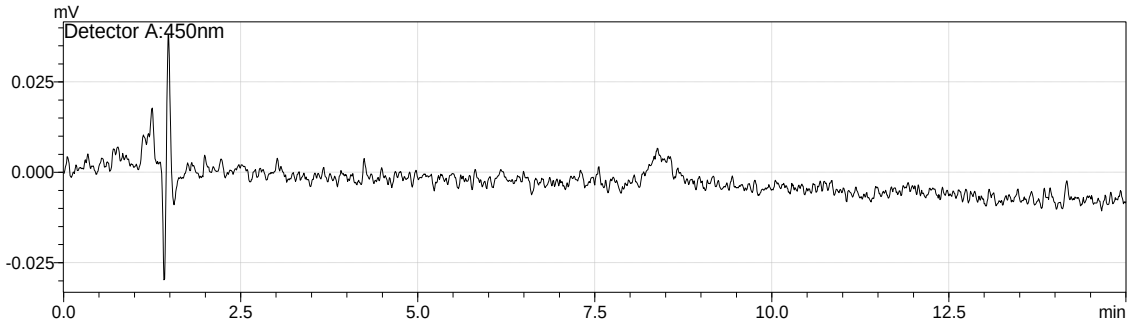


Figure 27: Not detected chromatogram

4.6.4. Linearity

The coefficient of determination (r2) is the main criteria that FDA uses to check the acceptability of linearity data, which obtained from y-intercept of the linear regression line for the peak area versus concentration plot. As the data described below in the Table 15, and Figure 30, the coefficient of determination lie between 0.9945-0.9999, which indicates the presence of a strong relationship between the concentration of the analyte and peak area. The coefficient of determination > 0.998 is generally acceptable as FDA standard.

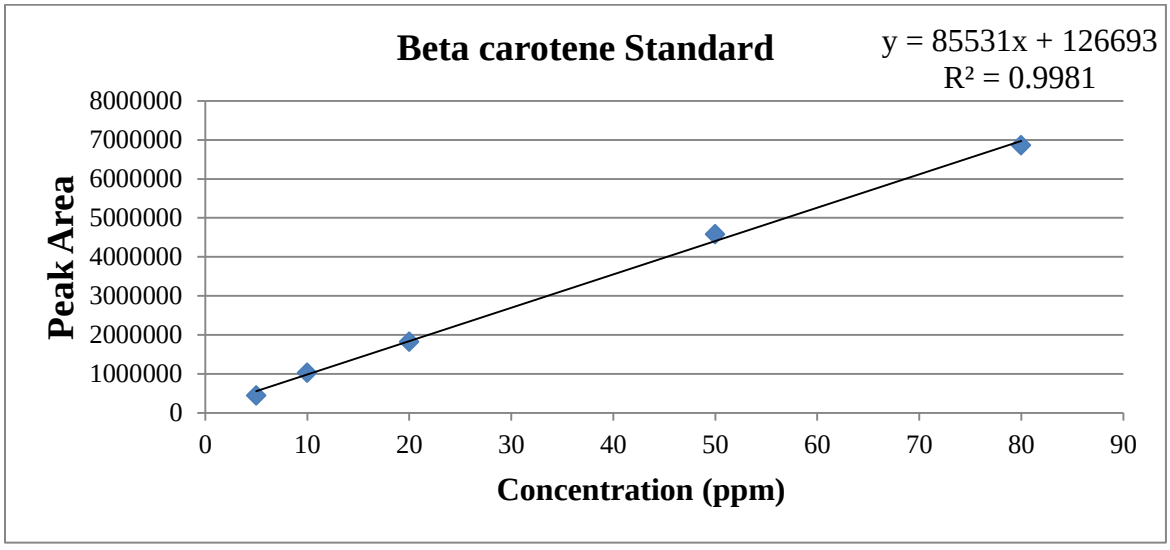


Figure 28: Calibration curve for β-carotene

4.6.5. Linearity check

The linearity of the analysis was evaluated by injecting five series of β-carotene standards (5, 10, 20, 50, and 80 ppm) to indicate the presence of a direct linear relationship between the concentration of the analyte and the peak area on the chromatogram with 7 runs.

Table 15: Linearity Check

	Number of runs (N)	Calibration curve equation	R2
β-carotene	7	Y=85531X+126693	0.9981

4.6.6. Work range

The working range was obtained from the linearity study and depends on the intended application of the test method. As the results described in Table 16, obtained during the linearity studies was used to assess the range of the assay method and shows sample lay between the described working ranges.

Table 16: Work range

β-carotene standard concentration	Concentration
5ppm	3.74
10ppm	10.56
20ppm	19.80
50ppm	52.04
80ppm	78.75

4.6.7. Accuracy and recovery

According to FDA, (2002) mean percent recovery was lay within the range of 70-120 which was acceptable. Table 16 showed that the method was accurate within the desired recovery range that set by FDA and the percent relative standard deviation was less than 1 (FDA, 2002).

Table 17: Accuracy and recovery check of different concentrations of β-carotene injected into HPLC

	β-carotene Spiking Concentration					β-carotene %Recovery				
	5ppm	10ppm	20ppm	50ppm	80ppm	5ppm	10ppm	20ppm	50ppm	80ppm
	3.74	10.51	19.81	51.44	78.63	74.80	105.10	99.05	102.80	98.28
	3.71	10.50	19.83	51.67	78.61	74.20	105.00	99.15	103.30	98.26
	3.74	10.52	19.84	51.87	78.67	74.90	105.20	99.2	103.74	98.33
	3.75	10.55	19.87	52.04	78.73	75.10	105.50	99.35	104.08	98.41
	3.75	10.57	19.88	52.23	78.80	75.00	105.70	99.4	104.46	98.50
	3.76	10.62	19.93	52.42	78.88	75.30	106.20	99.65	104.84	98.60
	3.76	10.65	19.98	52.64	78.95	75.20	106.50	99.9	105.28	98.68
Mean ± SD	3.74 ± 0.01	10.56 ± 0.05	19.87 ± 0.05	52.04 ± 0.42	78.75 ± 0.12	74.90 ±0.36	105.60 ± 0.57	99.38 ± 0.29	104.00 ± 0.86	98.44 ± 0.16
% RSD	0.26	0.53	0.29	0.8	0.16	0.48	0.54	0.30	0.82	0.16

Once the method was validated the β-carotene content of Kale was determined

4.7. β-carotene composition

Raw and processed Kale samples were analyzed for β-carotene content by using HPLC. Each sample analyzed in triplicate and the results reported in μg/g as shown in Table 18.

Table 18: β-carotene content in raw and cooked Kale

Cooking time	β –carotene content μg/100g
Raw	3582.96 ± 0.99
10 minute	3008.73 ± 13.72
20 minute	3519.25 ± 54.29
30 minute	3485.54 ± 4.09
40 minute	2582.34 ± 4.82
50 minute	2582.32 ± 4.82
60 minute	1909.61 ± 5.34
70 minute	1786.84 ± 2.06
80 minute	1475.79 ± 0.56
90 minute	1463.28 ± 4.91
100 minute	1407.02 ± 4.67
1:10 minute	1314.39 ± 3.20
1:20 minute	1204.27 ± 1.78

Data are expressed means ± SD

Based on the present investigation β-carotene content in raw Kale was very high 3582.96 ± 0.99, but at 10 minutes cooking the content of β-carotene decreased to 3008.73 ± 13.72, however at 20 minute and 30 minute β-carotene increased 3519.25 ± 54.29 and 3485.54 ± 4.09 respectively. When processing time increased until 120 minutes the content of β-carotene decreased at increasing rate and showed there is a significant difference (*p<0.005*) between differently timed cooked kale.

Cooking leafy vegetable increases the bioavailability of α- and β-carotene (Howard, et al., 1999; Khachik et al., 1992). Carotenoids are bounded by protein, thus heat treatment helps to release bound carotenoids and enables them to be readily extracted (Howard et al., 1999). This could be attributed to the increased extractability on cooking due to destruction of enzymes which otherwise could cause carotene degradation (Kala & Prakash, 2004) as the time increased cooking affect the stability and retention of carotenoids by the method and severity of processing, (Gupta, 2011, Uusiku et al., 2010), while heat treatment involved in cooking is a necessary step in making the food palatable and in improving the digestibility of food components, however the undesirable changes associated with cooking are reduction in nutrient content (Veda et al., 2010) therefore cooking have a significant effect on Kale carotenoid content to its retention and stability.

4.7.1. Retention

Retention was calculated using apparent retention. Apparent retention is defined as the ratio of the nutrient content in the cooked food to the nutrient content in the raw food (Murphy et al., 1975).

Table: 19. Retention β–Carotene Content on different cooking time

cooking time (minute)	β-carotene content (µg/g)	Retention of β-carotene%	(%)in loss
10	30.08	83.97	16.03
20	35.19	98.22	1.78
30	34.85	97.28	2.72
40	25.82	72.07	27.9
50	25.82	72.07	27.9
60	19.09	53.29	46.71
70	17.86	49.87	49.43

80	14.76	41.19	58.81
90	14.63	40.84	59.16
100	14.07	39.27	60.74
110	13.14	36.68	63.31
120	12.04	33.61	66.38

Generally preparations of vegetables at home are based on taste preference and convenience rather than retention of nutrients and health-promoting compounds (Igwemmar et al., 2013). As shown in the Table 19, retention of β–Carotene were higher in 20 and 30 minute 98.22% and 97.28 % of respectively then the retention become decreased along severity of process.

As shown in the Table 19, high percent of loss recorded in 120 minute cooked Kale 66.38% followed by 110 minute 63.31%, 90 minute 59.16%, 80 minute 58.81%, 70 minute 49.43%, 60 minute 46.71% and relatively lower loss recorded in 50 minute 27.9%, 40 minute 27.9% and 10 minute 16.03% and the lowest carotenoid percent lose recorded in 20 minute cooked Kale 1.78% followed by 30 minute cooked Kale 2.72%. As shown in the chart depending on cooking time (longer cooking periods lowering retention).

4.8. Sensory evaluation

As reported in Table 20, there is a significant difference ($p<0.05$) within different time cooked kale along sensory attributes (appearance, test, mouthfeel, and overall acceptability). Panels were oriented about the test instructions to identify, name and classify a range of sample attributes and rate the samples for the intensity of each attribute.

Table 20: Sensory attributes in Kale samples at different cooking time

Cooked Kale	Appearance	Test	Mouth feel	Overall acceptability
10 minute	7.70 ± 0.66^{cd}	5.20 ± 1.82^{a}	5.65 ± 1.63^{a}	5.15 ± 2.13^{a}
20 minute	7.00 ± 1.25^{bc}	5.20 ± 1.5^{a}	5.15 ± 1.75^{a}	4.75 ± 2.14^{a}
30 minute	7.85 ± 0.36^{d}	6.70 ± 1.17^{b}	$7.25 \pm .0.85^{b}$	7.35 ± 0.812^{b}
40 minute	6.30 ± 1.52^{b}	5.30 ± 2.00^{a}	5.40 ± 1.63^{a}	5.40 ± 2.56^{a}

90 minute	5.05 ± 1.7[a]	5.45 ± 2.00[a]	4.70 ± 1.52[a]	5.20 ± 2.48[a]

Data are expressed means ± SD

Means in the same column with different letter superscripts indicate significant differences ($p<0.05$)

As the Table showed the highest appearance which consisted the size, shape, and color was scored in 10 minute 7.70 ± 0.65 and 30 minute 7.85 ± 0.36 followed by 20, 40 and 90 minute cooked Kale. According to different studies colorful vegetables take more attention and attract to eat, this result was taken under comparison of the finding of other studies that used a comparison between conventional and microwave methods; showed that in case of amaranth and shepu, color of microwave cooked greens was preferred more than conventional which responsible for loses of color (Kala & Prakash, 2004).

In Table 20, differently cooked Kale showed a significant difference in the taste scores and the highest test parameter counted by 30- minute cooking 6.7 ± 1.17. Mouth feel of differently cooked Kale with great significant 7.25 ± 0.85 recorded at 30-minute cooking. Overall acceptability among differently cooked Kale was highly recorded at 30 minute higher ratings 7.35 ± 0.81. Therefore, in terms of appearance, test, mouthfeel, and overall acceptability the highest score was counted by 30- minute cooking.

5. Conclusion and Recommendations

Kale is the most common dark green leafy vegetable consumed widely in Ethiopia. It is a rich source of β-carotene with the ability of tackling the burden of vitamin A deficiency where vitamin A deficiency contributes to high morbidity and mortality rates in developing countries and remains a serious public health problem. However this important natural compound lost during processing. In the rural parts of Ethiopia Kale was highly affected by traditional cooking where Kale eaten by the society almost in an overcooked form and extract and remove the liquid part which may contain the essential minerals and vitamins.

- Therefore, it is recommended to consume cooked leafy vegetables before extraction of the liquid.
- It is advisable to cook Kale at 20-30 minutes to retain its nutrients.
- It is recommended to consume adequate amount of cooked Kale to meet recommended daily allowance of vitamins particularly β-carotene and vitamin c.
- All stakeholders who work on combating VAD should take their part to raise awareness of public way of cooking for the retention vitamin.

6. References

Abate-Pella, D., Freund, D. M., Slovin, J. P., Hegeman, A. D., & Cohen, J. D. (2017). An improved method for fast and selective separation of carotenoids by LC-MS. *Journal of Chromatography B.*

Adeniji, O., & Aloyce, A. (2014). Participatory identification of agronomic and leaf quality traits in Ethiopian mustard (Brassica carinata A. Braun) genotypes in Tanzania. *Agr. Biol. JN Am, 5*, 245-251.

Adongo, S., Murungi, J., & Wanjau, R. (2012). Determination of levels of selected essential elements in the medicinal plants used by Chuka Community, Meru-Kenya using AAS. *International Journal of Physical and Social Sciences, 2*(5), 2249-5894.

Ahamad, M. N., Saleemullah, M., Shah, H. U., Khalil, I. A., & Saljoqi, A. (2007). Determinuation of beta carotene content in fresh vegetables using high performance liquid chromatography. *Sarhad Journal of Agriculture, 23*(3), 767.

Alemayehu, N., & Becker, H. (2002). Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (Brassica carinata A. Braun). *Genetic Resources and Crop Evolution, 49*(6), 573-582.

Allen, L. H., De Benoist, B., Dary, O., Hurrell, R., & Organization, W. H. (2006). Guidelines on food fortification with micronutrients.

Arrigoni, O., & De Tullio, M. C. (2002). Ascorbic acid: much more than just an antioxidant. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1569*(1-3), 1-9.

Asfaw, Z. (1997). *Conservation and use of traditional vegetables in Ethiopia.* Paper presented at the Traditional African Vegetables: Proceedings of the IPGRI International Workshop on Genetic Resources of Traditional Vegetables in

Africa. Conservation and Use. ICRAF-HQ, Nairobi. Institute of Plant Genetic and Crop Plant Research, Rome.

Ayana, A., Afari-Sefa, V., Emana, B., Dinssa, F. F., Balemi, T., & Temesgen, M. (2014). Analysis of vegetable seed systems and implications for vegetable development in the Humid Tropics of Ethiopia. *International Journal of Agriculture and Forestry, 4*(4), 325-337.

Ayele, Z., & Peacock, C. (2003). Improving access to and consumption of animal source foods in rural households: the experiences of a women-focused goat development program in the highlands of Ethiopia. *The Journal of nutrition, 133*(11), 3981S-3986S.

Balcha, H. M. (2001). Experience of World Vision Ethiopia Micronutrient Program in Promoting the Production of Vitamin A—Rich Foods. *Food and nutrition bulletin, 22*(4), 366-369.

Bao, B., & Chang, K. (1994). Carrot pulp chemical composition, color, and water-holding capacity as affected by blanching. *Journal of food science, 59*(6), 1159-1161.

Bartholomew, B., & Ogden, L. (1990). Effect of emulsifiers and fortification methods on light stability of vitamin A in milk. *Journal of dairy science, 73*(6), 1485-1488.

Black, R. (2003). Micronutrient deficiency: an underlying cause of morbidity and mortality: SciELO Public Health.

Block, P. J., Strzepek, K., Rosegrant, M. W., & Diao, X. (2008). Impacts of considering climate variability on investment decisions in Ethiopia. *Agricultural Economics, 39*(2), 171-181.

Cadet, J. L., & Brannock, C. (1998). Invited Review Free radicals and the pathobiology of brain dopamine systems. *Neurochemistry international, 32*(2), 117-131.

Carmody, S. M. (2017). *Light Leaf Spot and White Leaf Spot of Brassicaceae in Washington State.* Washington State University.

Carvalho, L. M. J. d., Smiderle, L. d. A. S. M., Carvalho, J. L. V. d., Cardoso, F. d. S. N., & Koblitz, M. G. B. (2014). Assessment of carotenoids in pumpkins after different home cooking conditions. *Food Science and Technology (Campinas), 34*(2), 365-370.

Davey, M. W., Montagu, M. v., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., . . . Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability, and effects of processing. *Journal of the Science of Food and Agriculture, 80*(7), 825-860.

Dawit, N. T. (2014). *Assessment Of Trade Standards On Ethiopia's Fresh Fruit Export Volume.* Mekelle University.

de Azevedo, C. H., & Rodriguez-Amaya, D. B. (2005). Carotenoid composition of Kale as influenced by maturity, season and minimal processing. *Journal of the Science of Food and Agriculture, 85*(4), 591-597.

Demissie, T., Ali, A., & Zerfu, D. (2009). Availability and consumption of fruits and vegetables in nine regions of Ethiopia with special emphasis to vitamin A deficiency. *Ethiopian Journal of Health Development, 23*(3), 216–222.

de Quirós, A. R.-B., & Costa, H. S. (2006). Analysis of carotenoids in vegetable and plasma samples: A review. *Journal of Food Composition and Analysis, 19*(2), 97-111.

de Sá, M. C., & Rodriguez-Amaya, D. B. (2003). Carotenoid composition of cooked green vegetables from restaurants. *Food Chemistry, 83*(4), 595-600.

de Sá, M. C., & Rodriguez-Amaya, D. B. (2004). Optimization of HPLC quantification of carotenoids in cooked green vegetables—Comparison of analytical and calculated data. *Journal of Food Composition and Analysis, 17*(1), 37-51.

Devadas, R., Saroja, S., & Murthy, N. (1980). Availability of beta-carotene from papaya fruit and amaranth in preschool children. *Indian journal of nutrition and dietetics, 17*(2), 41-44.

Domínguez-Perles*, R., Mena*, P., Garcia-Viguera, C., & Moreno, D. (2014). Brassica foods as a dietary source of vitamin C: A review. *Critical reviews in food science and nutrition, 54*(8), 1076-1091.

Duma, M., Alsina, I., Zeipina, S., Lepse, L., & Dubova, L. (2014). *Leaf vegetables as source of phytochemicals.* Paper presented at the 9th Baltic Conference on Food Science and Technology "Food for Consumer Well-Being".

FDA, Analytical Procedure. (2000). Methods validation: chemistry, manufacturing and controls documentation, availability. *Federal Register (Notices), 65*(169), 52776-52777.

Frassinetti, S., Bronzetti, G. L., Caltavuturo, L., Cini, M., & Della Croce, C. (2006). The role of zinc in life: a review. *Journal of environmental pathology, toxicology and oncology, 25*(3).

Gebremedhin, S., & Enquselassie, F. (2011). Correlates of anemia among women of reproductive age in Ethiopia: Evidence from Ethiopian DHS 2005. *Ethiopian Journal of Health Development, 25*(1), 22-30.

Getahun, A. (1974). The role of wild plants in the native diet in Ethiopia. *Agro-ecosystems, 1*, 45-56.

Gómez-Campo, C. (1980). Morphology and morpho-taxonomy of the tribe Brassiceae. *Morphology and morpho-taxonomy of the tribe Brassiceae.*, 3-31.

Gupta, S., Lakshmi, A. J., Manjunath, M., & Prakash, J. (2005). Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. *LWT-Food Science and Technology, 38*(4), 339-345.

Gupta, U. (2011). *What's new about crop plants: novel discoveries of the 21st century*: CRC Press.

Harrison, E. H. (2012). Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids, 1821*(1), 70-77.

He, F. J., & MacGregor, G. A. (2008). Beneficial effects of potassium on human health. *Physiologia plantarum, 133*(4), 725-735.

Howard, L., Wong, A., Perry, A., & Klein, B. (1999). β-Carotene and ascorbic acid retention in fresh and processed vegetables. *Journal of food science, 64*(5), 929-936.

Hussein, L., & el-Tohamy, M. (1989). Effect of supplementation with vitamin A or plant carotenes on plasma retinol levels among young Egyptian males. *International journal for vitamin and nutrition research. Internationale Zeitschrift fur Vitamin- und Ernahrungs forschung. Journal international de vitaminologie et de nutrition, 59*(2), 229-233.

Igwemmar, N., Kolawole, S., & Imran, I. (2013). Effect of heating on vitamin C content of some selected vegetables. *International Journal of scientific & technology research, 2*(11), 209-212.

Jahangir, M., Kim, H. K., Choi, Y. H., & Verpoorte, R. (2009). Health-affecting compounds in Brassicaceae. *Comprehensive Reviews in Food Science and Food Safety, 8*(2), 31-43.

Joint, F., & Organization, W. H. (2005). Vitamin and mineral requirements in human nutrition.

Kala, A., & Prakash, J. (2004). Nutrient composition and sensory profile of differently cooked green leafy vegetables. *International Journal of Food Properties, 7*(3), 659-669.

Kapil, U., & Bhavna, A. (2002). Adverse effects of poor micronutrient status during childhood and adolescence. *Nutrition reviews, 60*(suppl_5), S84-S90.

Kebede et al., (2013). Ethiopia National Food Consumption Survey. *Ethiopian Public Health Institute, 3*, 54–67.

Khachik, F., Goli, M. B., Beecher, G. R., Holden, J., Lusby, W. R., Tenorio, M. D., & Barrera, M. R. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry, 40*(3), 390-398.

Khan, N. C. (2006). *The role of plant food sources in controlling vitamin A deficiency in Vietnam*.

Kwenin, W., Wolli, M., & Dzomeku, B. (2011). Assessing the nutritional value of some African indigenous green leafy vegetables in Ghana. *Journal of Animal and Plant Sciences, 10*(2), 1300-1305.

Lešková, E., Kubíková, J., Kováčiková, E., Košická, M., Porubská, J., & Holčíková, K. (2006). Vitamin losses: Retention during heat treatment and continual changes expressed by mathematical models. *Journal of Food Composition and Analysis, 19*(4), 252-276.

Mason, J. B., Lotfi, M., Dalmiya, N., Sethuraman, K., & Deitchler, M. (2001). The micronutrient report. Current progress and trends in the control of vitamin A iodine and iron deficiencies.

McGraw, K. J., Hill, G. E., Navara, K. J., & Parker, R. S. (2004). Differential accumulation and pigramenting ability of dietary carotenoids in colorful finches. *Physiological and Biochemical Zoology, 77*(3), 484-491.

McGuire, S. (2011). US department of agriculture and US department of health and human services, dietary guidelines for americans, 2010. Washington, DC: US government printing office, January 2011: Oxford University Press.

Mena, I. (1974). The role of manganese in human disease. *Annals of Clinical & Laboratory Science, 4*(6), 487-491.

Mithen, R. F., Dekker, M., Verkerk, R., Rabot, S., & Johnson, I. T. (2000). The nutritional significance, biosynthesis, and bioavailability of glucosinolates in human foods. *Journal of the Science of Food and Agriculture, 80*(7), 967-984.

Mornet, E., Stura, E., Lia-Baldini, A.-S., Stigbrand, T., Ménez, A., & Du, L. (2001). Structural evidences for a structural role of human non-specific alkaline phosphatase in bone mineralization. *Journal of Biological Chemistry*.

Mosha, T., Pace, R., Adeyeye, S., Laswai, H., & Mtebe, K. (1997). Effect of traditional processing practices on the content of total carotenoid, β-carotene, α-carotene and vitamin A activity of selected Tanzanian vegetables. *Plant Foods for Human Nutrition, 50*(3), 189-201.

Naidu, K. A. (2003). Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal, 2*(1), 7.

Niizu, P. Y., & Rodriguez-Amaya, D. B. (2005). New data on the carotenoid composition of raw salad vegetables. *Journal of Food Composition and Analysis, 18*(8), 739-749.

Olayiwola, O. A., & Adeoye, G. O. O. M. D. (2012). Mineral composition and effect of boiling time on vitamin C in extract of fresh and dried Nigerian vegetables with and without addition of potash: Iree, Nigeria as a case study. *Studies, 6*.

Olivares, M., & Uauy, R. (1996). Copper as an essential nutrient. *The American journal of clinical nutrition, 63*(5), 791S-796S.

Organization, W. H. (2003). *Diet, nutrition, and the prevention of chronic diseases: report of a joint WHO/FAO expert consultation* (Vol. 916): World Health Organization.

Organization, W. H. (2009). Global prevalence of vitamin A deficiency in populations at risk 1995-2005: WHO global database on vitamin A deficiency.

Pereira, S. (1968). A. BEGUM. *Studies in the prevention of vitamin A deficiency. Indian J. Med. Res, 56*, 362.

Power, M. L., Heaney, R. P., Kalkwarf, H. J., Pitkin, R. M., Repke, J. T., Tsang, R. C., & Schulkin, J. (1999). The role of calcium in health and disease. *American journal of obstetrics and gynecology, 181*(6), 1560-1569.

Qureshi, G. A., Memon, S. A., Memon, A. B., Ghouri, R. A., Memon, J. M., & Parvez, S. H. (2005). The emerging role of iron, zinc, copper, magnesium and selenium and oxidative stress in health and diseases. *Biogenic amines, 19*(2), 147.

Raju, M., Varakumar, S., Lakshminarayana, R., Krishnakantha, T. P., & Baskaran, V. (2007). Carotenoid composition and vitamin A activity of medicinally important green leafy vegetables. *Food Chemistry, 101*(4), 1598-1605.

Rakow, G. (2004). Species origin and economic importance of Brassica *Brassica* (pp. 3-11): Springer.

Rao, A. V., & Rao, L. G. (2007). Carotenoids and human health. *Pharmacological research, 55*(3), 207-216.

Rao, P. (2018). Atomic Absorption Spectrophotometer (AAS) analysis for evaluation of variation in mineral content in different varieties of Trigonella foenumgraecum L. *Legume Research: An International Journal, 41*(1).

Redhead, J. (1990). Utilization of tropical foods: fruits and leaves. *FAO Food and Nutrition Paper, 47*(7).

Riley, K., Tadesse, N., Alemaw, G., & Belayneh, H. (1983). Response of three oilseed Brassica species to different planting dates and seed rates in highland Ethiopia.

Rodriguez-Amaya, D. (2015). *Food carotenoids: Chemistry, biology, and technology*: John Wiley & Sons.

Rodriguez-Amaya, D. B. (1997). *Carotenoids and food preparation: the retention of provitamin A carotenoids in prepared, processed and stored foods*: John Snow Incorporated/OMNI Project Arlington, VA.

Rodriguez-Amaya, D. B. (2001). *A guide to carotenoid analysis in foods*: ILSI press Washington, DC.

Rodriguez-Amaya, D. B., & Kimura, M. (2004). *HarvestPlus handbook for carotenoid analysis* (Vol. 2): International Food Policy Research Institute (IFPRI) Washington.

SanJoaquin, M. A., & Molyneux, M. E. (2009). Malaria and vitamin A deficiency in African children: a vicious circle? *Malaria journal, 8*(1), 134.

Sanlier, N., & Guler Saban, M. (2018). The Benefits of Brassica Vegetables on Human Health. *J Human Health Res, 1*, 104.

SCn, U. (2004). Fifth report on the world nutrition situation: Nutrition for improved development outcomes. *UN SCN, Geneva*, 22-27.

Seddon, J. M., Ajani, U. A., Sperduto, R. D., Hiller, R., Blair, N., Burton, T. C., . . . Miller, D. T. (1994). Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. *Jama, 272*(18), 1413-1420.

Setegn, D. (2015). *Analysis of vegetable market chain in Dugda Woreda, east Shoa Zone, Oromia Region, Ethiopia.* Addis Ababa University.

Singh, G., Kawatra, A., & Sehgal, S. (2001). Nutritional composition of selected green leafy vegetables, herbs, and carrots. *Plant Foods for Human Nutrition, 56*(4), 359-364.

Sommer, A. (2001). *Vitamin A deficiency*: Wiley Online Library.

Song, L., & Thornalley, P. J. (2007). Effect of storage, processing and cooking on glucosinolate content of Brassica vegetables. *Food and Chemical Toxicology, 45*(2), 216-224.

Thane, C., & Reddy, S. (1997). Processing of fruit and vegetables: effect on carotenoids. *Nutrition & Food Science, 97*(2), 58-65.

Thompson, B. (2011). 15 Combating Iron Deficiency: Food-based Approaches. *Combating micronutrient deficiencies: food-based approaches*, 268.

Tomkins, A. (2000). Malnutrition, morbidity, and mortality in children and their mothers. *Proceedings of the Nutrition Society, 59*(1), 135-146.

Tontisirin, K., Nantel, G., & Bhattacharjee, L. (2002). Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Proceedings of the Nutrition Society, 61*(2), 243-250.

Underwood, B. A. (2004). Vitamin A deficiency disorders: international efforts to control a preventable "pox". *The Journal of nutrition, 134*(1), 231S-236S.

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis, 23*(6), 499-509.

van der Beek, E. J. (1991). Vitamin supplementation and physical exercise performance. *Journal of sports sciences, 9*(S1), 77-89.

Veda, S., Platel, K., & Srinivasan, K. (2010). Enhanced bioaccessibility of β-carotene from yellow-orange vegetables and green leafy vegetables by domestic heat processing. *International journal of food science & technology, 45*(10), 2201-2207.

West Jr, K. P. (2003). Vitamin A deficiency disorders in children and women. *Food and nutrition bulletin, 24*(4_suppl2), S78-S90.

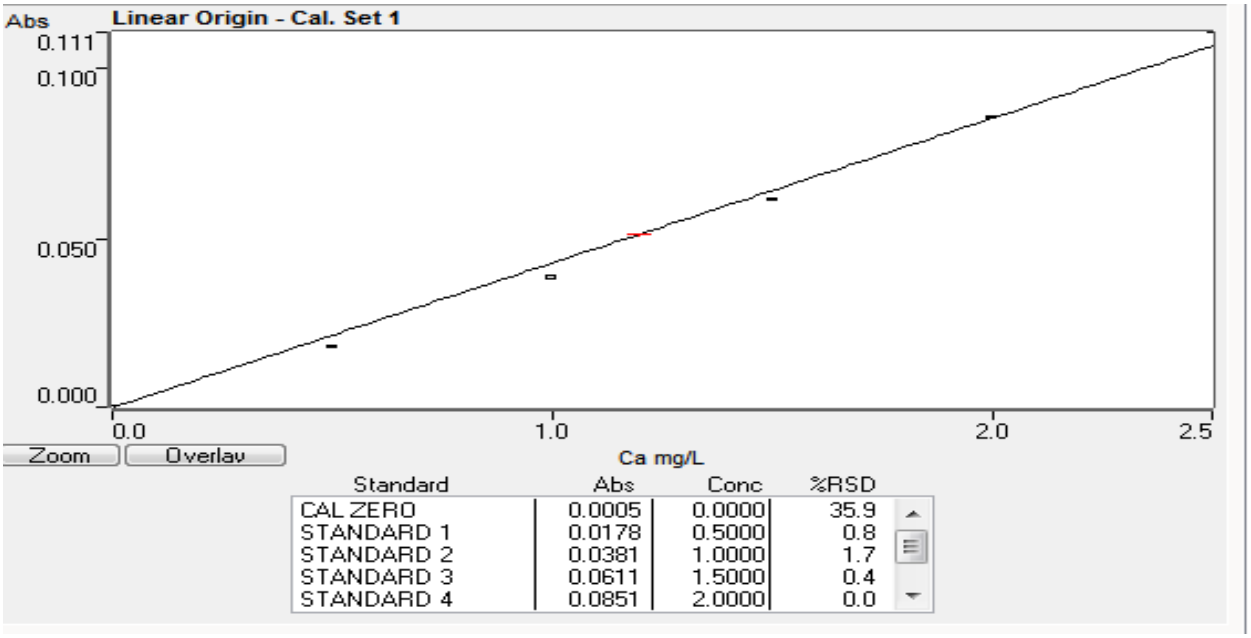
Whitney, E., & Rolfes, S. (2008). Planning a healthy diet. *Understanding Nutrition, 11th ed.; Thomson Wadsworth: Belmont, CA, USA*, 37-63.

Zerfu et al., (2016). Ethiopian national micronutrient survey report. *Ministry of Health*, 1–113.

Zewditu Getahun, Kelbessa Urga, Timotewos Ganebo, A. N. (2010). Review of the status of malnutrition and trends in Ethiopia. *Ethiopian Journal of Health Development*, *24* (SPEC. ISSUE 1), 105–109.

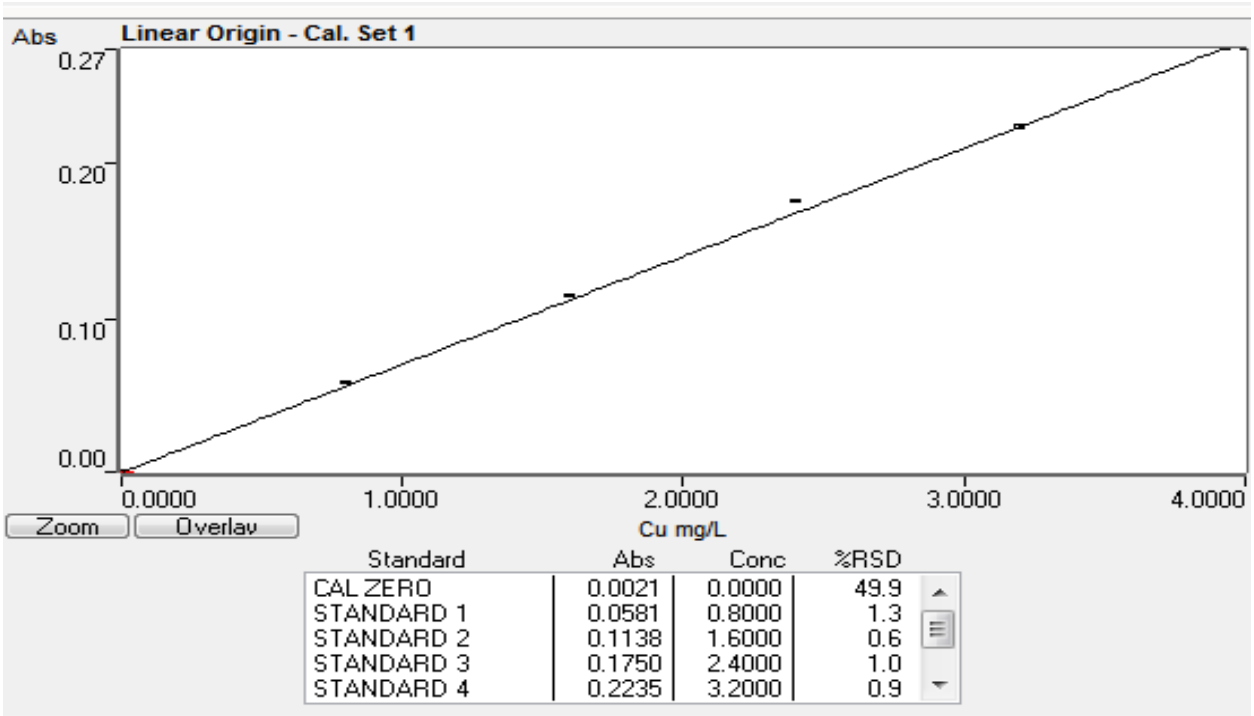
Appendix

Appendix 1: Calibration curve for calcium (Ca)



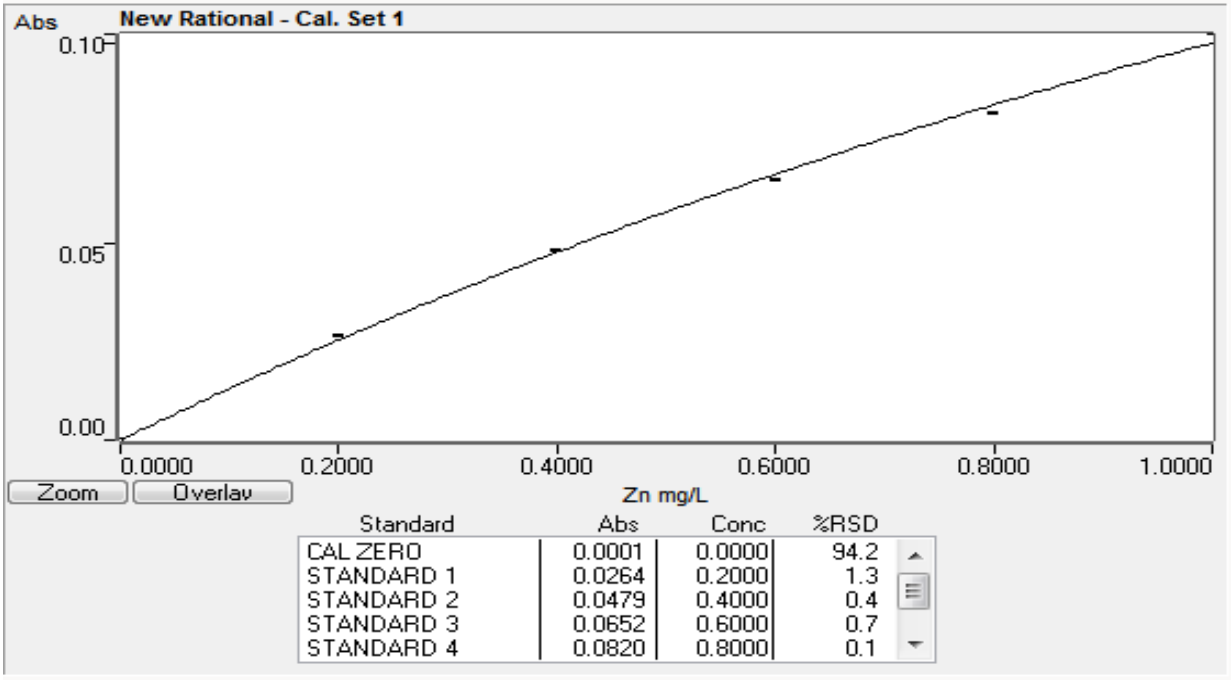
Standard	Abs	Conc	%RSD
CAL ZERO	0.0005	0.0000	35.9
STANDARD 1	0.0178	0.5000	0.8
STANDARD 2	0.0381	1.0000	1.7
STANDARD 3	0.0611	1.5000	0.4
STANDARD 4	0.0851	2.0000	0.0

Appendix 2: Calibration curve for copper (Cn)



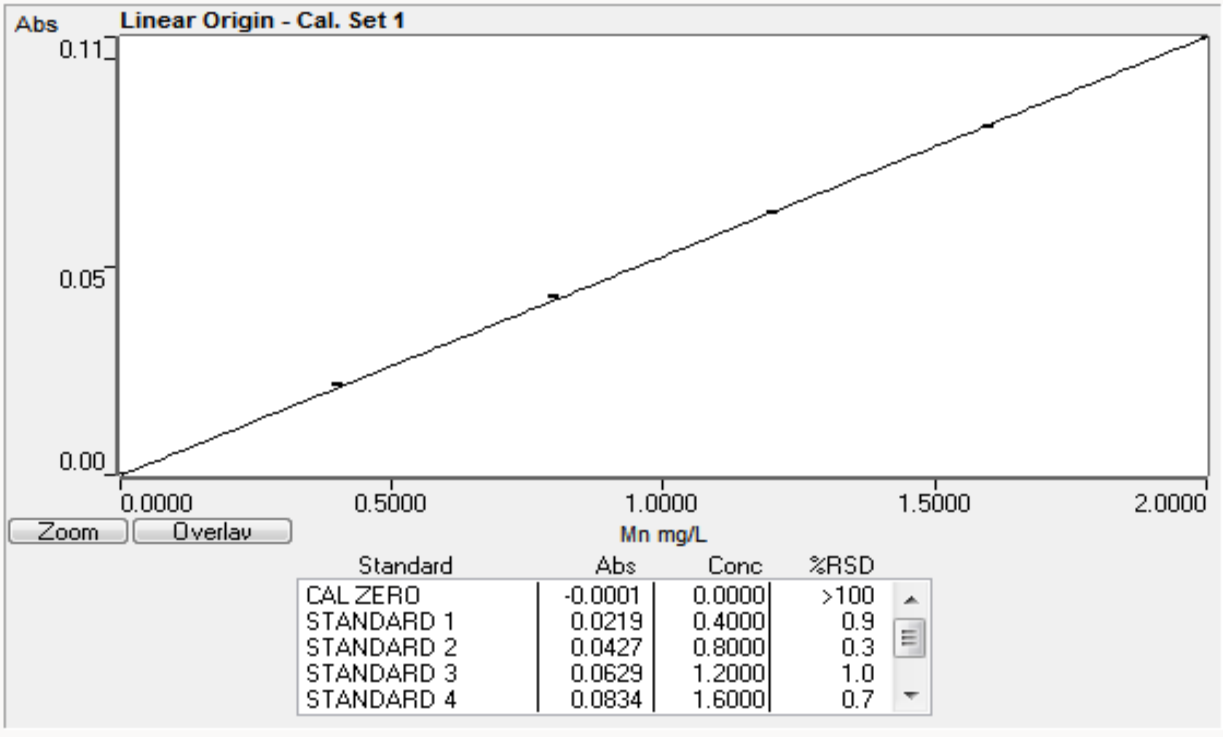
Standard	Abs	Conc	%RSD
CAL ZERO	0.0021	0.0000	49.9
STANDARD 1	0.0581	0.8000	1.3
STANDARD 2	0.1138	1.6000	0.6
STANDARD 3	0.1750	2.4000	1.0
STANDARD 4	0.2235	3.2000	0.9

Appendix 3: Calibration curve for zinc (Zn)



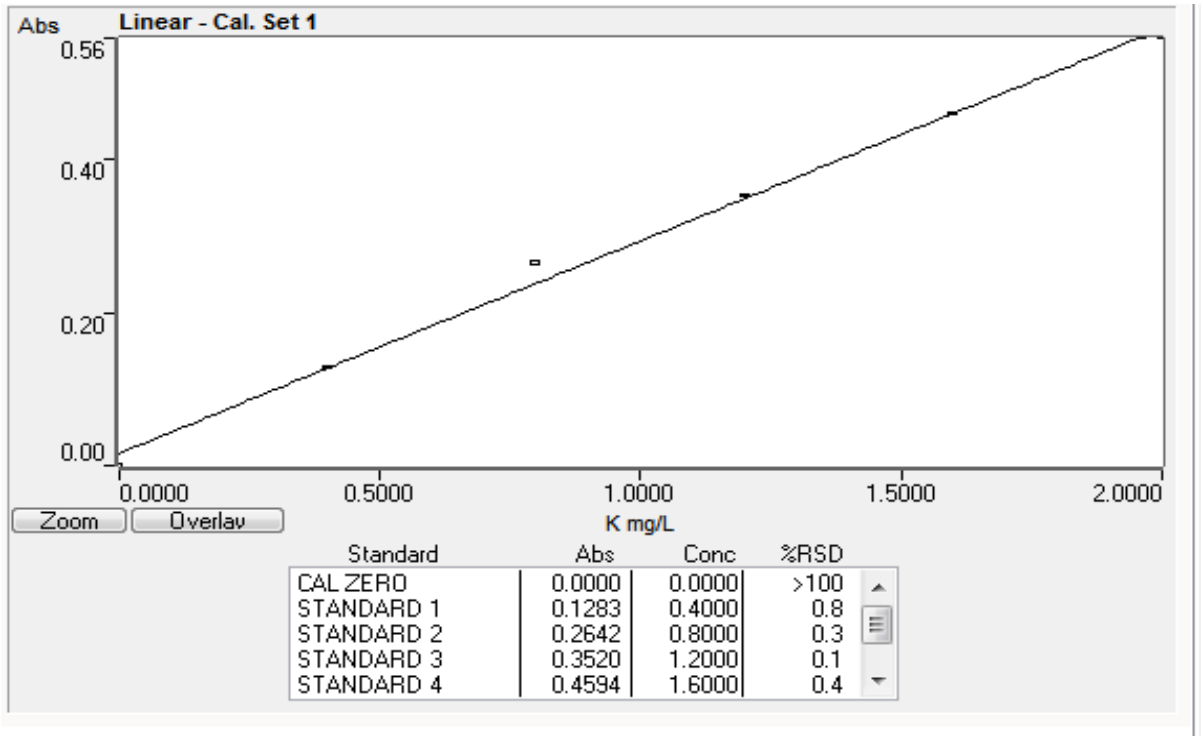
Standard	Abs	Conc	%RSD
CAL ZERO	0.0001	0.0000	94.2
STANDARD 1	0.0264	0.2000	1.3
STANDARD 2	0.0479	0.4000	0.4
STANDARD 3	0.0652	0.6000	0.7
STANDARD 4	0.0820	0.8000	0.1

Appendix 4: Calibration curve for manganese (Mn)



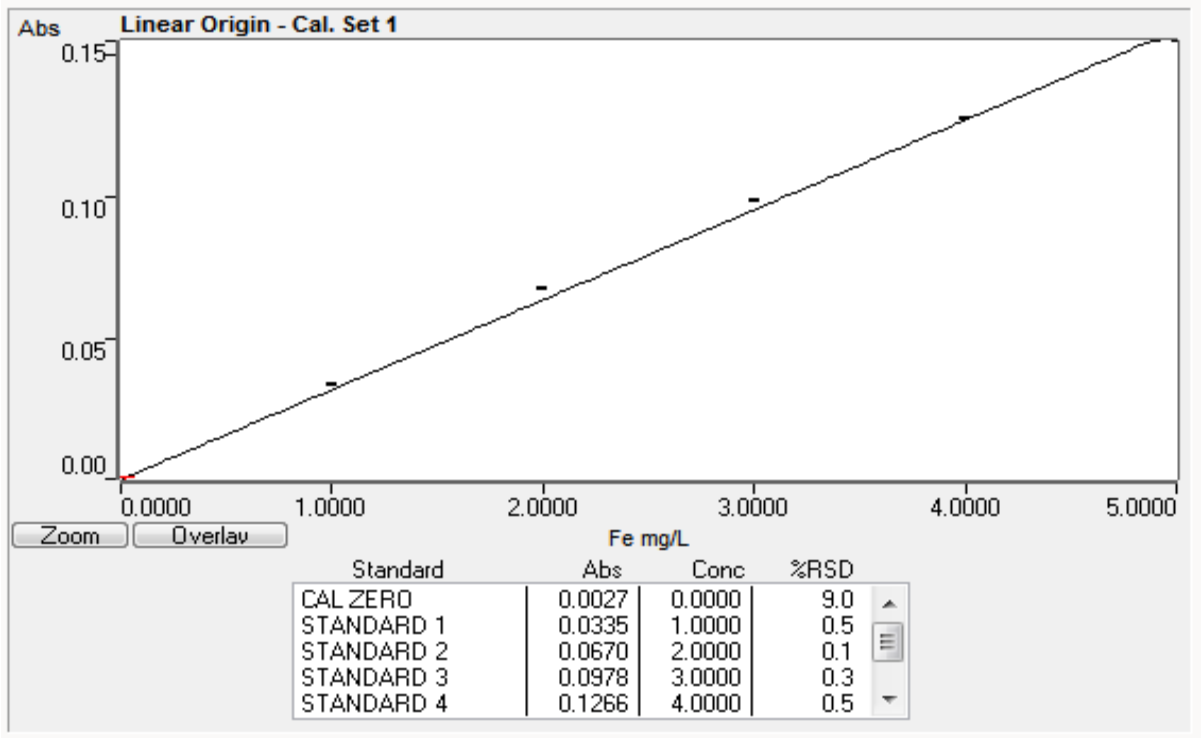
Standard	Abs	Conc	%RSD
CAL ZERO	-0.0001	0.0000	>100
STANDARD 1	0.0219	0.4000	0.9
STANDARD 2	0.0427	0.8000	0.3
STANDARD 3	0.0629	1.2000	1.0
STANDARD 4	0.0834	1.6000	0.7

Appendix 5: Calibration curve for potassium (K)



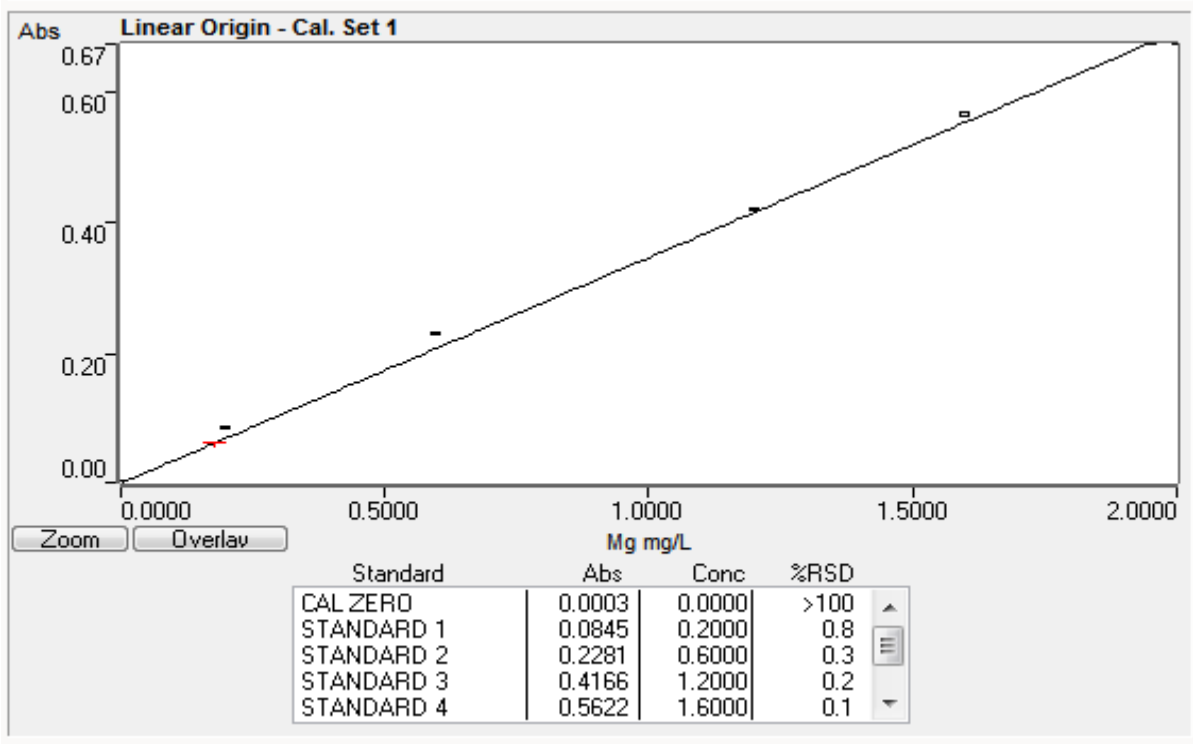
Standard	Abs	Conc	%RSD
CAL ZERO	0.0000	0.0000	>100
STANDARD 1	0.1283	0.4000	0.8
STANDARD 2	0.2642	0.8000	0.3
STANDARD 3	0.3520	1.2000	0.1
STANDARD 4	0.4594	1.6000	0.4

Appendix 6: Calibration curve for iron (Fe)



Standard	Abs	Conc	%RSD
CAL ZERO	0.0027	0.0000	9.0
STANDARD 1	0.0335	1.0000	0.5
STANDARD 2	0.0670	2.0000	0.1
STANDARD 3	0.0978	3.0000	0.3
STANDARD 4	0.1266	4.0000	0.5

Appendix 7: Calibration curve for magnesium (Mg)



Standard	Abs	Conc	%RSD
CAL ZERO	0.0003	0.0000	>100
STANDARD 1	0.0845	0.2000	0.8
STANDARD 2	0.2281	0.6000	0.3
STANDARD 3	0.4166	1.2000	0.2
STANDARD 4	0.5622	1.6000	0.1

Appendix 8: HPLC- β-carotene standard result

<Detector A> Summery (concentration)

Title	Sample Name	B-C
5ppm.1cd	5ppm	444848
5ppm.1cd	5ppm	444062
5ppm.1cd	5ppm	447008
5ppm.1cd	5ppm	448243
5ppm.1cd	5ppm	447564
5ppm.1cd	5ppm	448746
5ppm.1cd	5ppm	448341
10ppm.1cd	10ppm	1026147
10ppm.1cd	10ppm	1025374
10ppm.1cd	10ppm	1027291
10ppm.1cd	10ppm	1029663
10ppm.1cd	10ppm	1031168
10ppm.1cd	10ppm	1035209
10ppm.1cd	10ppm	1038440
20ppm.1cd	20ppm	1821854
20ppm.1cd	20ppm	1823599
20ppm.1cd	20ppm	1824384
20ppm.1cd	20ppm	1826946
20ppm.1cd	20ppm	1827463
20ppm.1cd	20ppm	1831823
20ppm.1cd	20ppm	1835939
40ppm.1cd	40ppm	3003999
40ppm.1cd	40ppm	2999233
40ppm.1cd	40ppm	2997094
40ppm.1cd	40ppm	2995487
40ppm.1cd	40ppm	2994531
40ppm.1cd	40ppm	2993371
40ppm.1cd	40ppm	2993182
50ppm.1cd	50ppm	4526516
50ppm.1cd	50ppm	4546285
50ppm.1cd	50ppm	4563495
50ppm.1cd	50ppm	4577869
50ppm.1cd	50ppm	4594261
50ppm.1cd	50ppm	4610502
50ppm.1cd	50ppm	4629732
80ppm.1cd	80ppm	6852071
80ppm.1cd	80ppm	6850527
80ppm.1cd	80ppm	6855641
80ppm.1cd	80ppm	6861285
80ppm.1cd	80ppm	6867161
80ppm.1cd	80ppm	6873452
80ppm.1cd	80ppm	6879870
100ppm.1cd	100ppm	7889030
100ppm.1cd	100ppm	7882525
100ppm.1cd	100ppm	7890406
100ppm.1cd	100ppm	7876587
100ppm.1cd	100ppm	7869765
100ppm.1cd	100ppm	7860232
100ppm.1cd	100ppm	7862357
Blank-1.lcd	Blank-1	0
Blank-2.lcd	Blank-2	0
Blank-3.lcd	Blank-3	0

Appendix 9: HPLC- Kale- β-carotene result

<Detector A> Summery (concentration)

Title	Sample Name	B-C
Raw-1.1cd	Raw-1	5641304
Raw-2.1cd	Raw-2	5642908
Raw- 3.1cd	Raw-3	5644373
10-1.1cd	10-1	4734434
10-2.1cd	10-2	4770247
10-3.1cd	10-3	4771760
20-1.1cd	20-1	5641300
20-2.1cd	20-2	5496968
20-3.1cd	20-3	5496062
30-1.1cd	30-1	5485642
30-2.1cd	30-2	5496967
30-3.1cd	30-2	5496060
40-1.1cd	40-1	4093893
40-2.1cd	40-2	4105419
40-3.1cd	40-3	4107757
50-1.1cd	50-1	4093855
50-2.1cd	50-2	4105381
50-3.1cd	50-3	4107719
60-1.1cd	60-1	3057857
60-2.1cd	60-1	3067933
60-3.1cd	60-1	3074159
70-1.1cd	70-1	2874371
70-2.1cd	70-2	2877809
70-3.1cd	70-3	2880708
80-1.1cd	80-1	2398233
80-2.1cd	80-2	2399743
80-3.1cd	80-3	2398274
90-1.1cd	90-1	2372322
90-2.1cd	90-2	2387397
90-3.1cd	90-3	2378755
100-1.1cd	100-1	2285095
100-2.1cd	100-2	2294282
100-3.1cd	100-3	2299268
110-1.1cd	101-1	2144605
110-2.1cd	101-2	2152597
110-3.1cd	101-3	2153594
120-1.1cd	120-1	1978718
120-2.1cd	120-2	1979648
120-3.1cd	120-3	1983865
Blank-1.lcd	Blank-1	0
Blank-2.lcd	Blank-2	0
Blank-3.lcd	Blank-3	0

Appendix 10: Assessment on processing methods of green vegetables in selected districts of Oromia and SNNPR regions of Ethiopia

Section 1. Survey basic information

1.1.Questionnaire No.: _____

1.2.Date of Interview: dd_____ /mm _____/yy_____

Staring at (time):_____ Ended at (time):_____

1.3.Region:_____

1.4.District:_____

1.5.Kebele:_____

1.6.House no:_____

1.7.Family size of the household:_____

No children: _____Boys:_____ Girls: _____ School age children: _____

1.8.Location of the district: 1. Urban/ 2. Rural

1.9.Name of the interviewer:_____

1.10. Name of the Principal Investigator (PI):_____

1.11. Gender of respondent: 1. Male/ 2. Female

1.12. Age of respondent:_____

1.13. Role of respondent in the house (house wife, employee, etc):_____

1.14. Language of the version of the questionnaire:_____

Section 2. Survey on Commonly Consumed Foods

2.1. What are the commonly consumed food items in the village?

_____

2.2. How often do you eat vegetables ?

1.On daily basis 2. Weekly basis 3. Monthly basis

4.Others (specify)_____

2.3. What are the commonly consumed green leafy vegetables in the village?

1. Kale 2. Cabbage 3. Lettuce 4. Carrot 5. Broccoli

6. Others_____

2.4. How often do you consume green leafy vegetables

1.On daily basis 2. Weekly basis 3. Monthly basis

4. Others (specify)_____

2.5. Once the vegetable is prepared for how long it can be consumed?

1. For one day 2. For two days

3. For three days 4. Others (specify)_____

Section 3. Survey on Green Leafy Vegetables Production

3.2 Are green leafy vegetables available yearly round? 1. Yes 2. No

3.3. If No, in which month (season) is the highest production of green leafy vegetables?

_____

_____

3.4. Who is highly engaged with the vegetable gardening activities?

1. Male adults 2. Employees

3. Female adults 4. Others (specify) _____

3.5. What is the source of water for gardening of green leafy vegetables?

1. Rain water 2.Tap water

3. River water 4. Others (specify)_____

3.6. What do you use to increase yield of green leafy vegetables?

1. Artificial fertilizers 2. Manure

3. Animal dungs 4. Pesticides/insecticides

5. Others (specify) _____

3.7. What are the major challenges that negatively affect the production of green leafy vegetables?

1. Heavy rain 2. Pathogens

3. Solar dehydration 4. Insects/pests

5. Others (specify) _____

Section 4. Survey on Marketing of Green Leafy Vegetables

4.1. Are green leafy vegetables available in the market year round? 1. Yes 2. No

4.2. If yes, what time of the year are green leafy vegetables abundantly available in the market?

_____

4.3. What are the common green leafy vegetables available in the market?

1. Kale 2. Cabbage 3. Lettuce 4. Carrot 5. Broccoli

6. Others_____

4.4. How are the vegetables transported from farm to the markets?

1. Cold chain (refrigeration) 2. Merchants carry them using basket

3. Vehicles 4. Animals

5. Others (specify)_____

4.5. At what time of the day do you get quality and cheap vegetables in the market?

1. Early morning 2. At Noon

3. Just before noon 4. After noon 5.Round evening time

4.6. What are the quality parameters that you consider when buying green leafy vegetables?

_____

Section 5. Survey on Storage of Green Leafy Vegetables

5.1. Do you have cooling and bulking facilities to handle green leafy vegetables?

1. Yes 2. No

5.2. If No, how do you maintain the quality of the vegetables after harvest or purchase until it is processed?

_____

Section 6. Survey on Hygiene and Sanitation Conditions

6.1. Who is responsible for keeping cooking utensils / food items/ clean and hygienic among the family members?

1. Husband 2. Wife

3. Children 4. Others (specify)_____

Section 7. Survey on Knowledge, Attitude and Practice of Green Leafy Vegetables

a) Knowledge

7.1. Do you think eating vegetables will make you healthy? Why?

_____

7.2. What are the common methods of cooking vegetables in the household?

1. Boiling 2.Blanching

3. Frying 4.Others (specify)_____

7.3. Among the methods mentioned above, which are unhealthy methods of cooking food? Why?

1. Boiling 2. Blanching

3. Frying 4. Others (specify) _____

7.4. Accordingly, what foods do you eat less often? What foods do you eat more often?

_____

7.5. Have you ever learned about the right way to cook/to eat and how frequently to eat?

1. Yes 2. No

7.6. If Yes, where did you learn about these information

1. School 2. Friends 3. Radio 4. Newspaper 5. Agriculture officers

6. Parents 7. TV 8. Magazines 9. Health officers

10. Others (specify)_____

b) Attitude

7.7. Do you believe that there are certain foods that make people healthy and not healthy? Why?_____

c) Practices

7.8. Do you produce any of your own food items? What do you produce?

_____

7.9. What foods items do you buy?

_____

7.10. Who is responsible for buying any food items for the household?

_____

7.11. Who is responsible for preparing your food items?

_____

7.12. Who makes the decision as to what food item to be bought and prepared?

_____

7.13. How often do you buy vegetables?

_____

7.14. Do you eat vegetables daily? 1. Yes 2. No

7.15. Which of the following vegetables do you eat frequently?

Rank vegetables based on frequency of consumption (1, 2, 3,….)

Cabbage:___	Tomatoes:_____	Lettuce:____
Round cabbage:____	Cucumber:_____	Cauliflower:_____
Long beans:_____	Green pepper:____	Onion:_____
Short beans:_____	Carrots:____	Garlic:____
Eggplant:_____	Kale:_____	Broccoli:____

Others(specify)_____

Section 8. Survey on Processing of Green Leafy Vegetables

8.1. What utensils do you use for cooking green leafy vegetables commonly?

1. Metal Casserole 2. Clay casserole

3. Pan 4. Others (specify) _____

8.2. What is the source of water used for cooking?

1. Tap water 2.Underground water

3. River water 4. Others (specify)_____

8.3. What is the source of energy for cooking green leafy vegetables?

1. Wood fire 2.Charcol

3. Animal dung 4. Biogas

5. Others (specify) _____

8.4. What is the amount of each recipe to make a vegetable-based meal?

Ingredient (Recipe)	Amount (gram, Lit or specify if different)
Onion	
Oil	
Green leafy vegetable (_____)	
Water	
Other ingredients	

8.5. What is commonly applied processing method of green leafy vegetables? (In detail) (Processing Flow Diagram)

_____

8.6. How do you know the cooking is enough/done?

_____

8.7. Once cooked, will it be served directly? 1. Yes 2. No

8.8. If 'Yes', will you consume it alone or with other food items? 1. Alone 2. With other food items

If you consume it with other food items, which food items?

_____

8.9. If the answer for question 8.7 is No, how it will be served?

_____

8.10. If you consume the cooked vegetable after a while, will it be served after re-heating? 1. Yes 2. No

8.11. If Yes, for how long will it be re-heated?

1. For 5 minute 2. For 15 minute
3. For 10 minute 4. Others (specify) _____

8.12. How often do you prepare/cook green vegetable based foods?

1. Once in a day 2. Once in two days
3. Twice in a day 4. Others (specify) _____

Section 9. Survey from Agriculture Office in Each Kebele

9.1. Commonly what is the time lapse between harvest/purchase of green leafy vegetables and cooking/processing?

_____

9.2. How much is the annual vegetable production in the:

a) District: _____

b) Household: _____

9.3. How much is the annual green leafy vegetable production in the:

a) District: _____

b) Household: _____

9.4. What time of the year are green leafy vegetables abundantly available and shortage in production, supply and demand?

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Months of high production												
Months of high demand												
Months of high supply												
Months of high shortage												

9.5. What are the common green leafy vegetables available in the market?

_____

9.6. Which NGO's work in the village?

_____

9.7. On which issues, these NGOs focus to work on?

_____

9.8. Is there any program in the district working on nutrition? 1. Yes 2. No

9.9. If Yes, on what nutrition related problems the program target to work on? For how long?

_____

9.10. Was there any nutritional intervention in the district (vitamin/mineral supplementation, fortification etc)? 1. Yes 2. No

9.11. Does home gardening activities helped you to increase your vegetable consumption?

1. Yes 2. No

9.12. Is there any food borne disease outbreak recently in the village? 1. Yes 2. No

9.13. If yes, what was the cause?

_____

9.14. What other communicable and non-communicable diseases are common in the village?

_____

Section 10. Interviewer's Observation guide

10.1. The sanitation of cooking materials

_____

10.2. The sanitation of water source which is used for gardening and cooking

_____

10.3. The sanitation of gardening

_____

10.4. Over all condition of the vegetable market of the region

_____

Section 11. Closure

11.1. Give them a chance to ask any questions

_____

11.2. Are there any particular nutritional information you want?

_____

Survey on Green Leafy Vegetables Processing

Data Collection Form

Name of data collector_____

Date of interview:dd_____/mm_____/yy_____

Time started: _____ Ended:_____

House hold number: _____ Name of respondent: _____

Region:_____District:_____ Kebele:_____

1. Green leafy vegetable (Name:_____,

   Edible part:_____)

   Cooking utensils used _____

   Energy source used: 1. Wood fire 2. Animal dunk 3. Biogas. 4. Charcoal 5. Others (specify):_____

   Cooking temperature: _____^{o}C

   Cooking started at:_____; Ended at:_____,

   Total time:_____

Ingredients	Amount used
Green leafy vegetable (name:_____)	
Onion	
Oil	
Water	
Other Ingredients	

Processing Steps observed while cooking (in detail)

_____

Appendix 11: Sensory evaluation

Nine-point Hedonic scale for cooked Kale

Dear participants, you are invited to participate in a study entitled "Commonly used processing methods on Kale (*Brassica Carinata*) in rural parts of Ethiopia; Effect on proximate, mineral and beta-carotene composition and optimization of the process for the nutrient retention". The overall objective of this study is to assess the overall preference of cooked Kale at different processing way with a nine-point hedonic scale sensory evaluation methods. You will be oriented about the test instructions to identify, name and classify a range of sample attributes (i.e. **general appearance, test, mouthfeel, and overall acceptability**). You will be asked to rank them in level of score given in the table. If you have prior experience of any allergic reactions to vegetables you should not participate in this sensory evaluation.

Please, rinse your mouth with water between samples, and wait for 30 seconds before you taste the next sample.

You will get 5 processed Kale samples cooked for (10 minute, 20 minute, 30 minute, 40 minute, and 90 minute). Please read the instructions carefully and answer the questions. Keep in minutes that you are asked to answer the questions as a representative of the consuming population; it is your personal opinion of liking and preference that is of interest. Since we want your personal opinion, please do not talk to the other participants during the test. Along with each question, there is room for comments. Use this room to try to explain the reason to your choice as detailed as possible.

Dear participants, as a representative of the consuming population, quantify the degree of liking or disliking the products one by one separately. Please taste each of the coded samples in the set and rank them in the level of score given in the table which sequence presented from **top to bottom**. Use the appropriate scale to show your attitude by checking at the point that best describe your feeling about the sample. Please give a reason for this attitude. Remember you are the only one who can tell what you like. An honest expression of your personal feeling will help us. Please put a"√" sign in the box that best describes your overall opinion of the sample.

Don't forget to rinse your mouth with water in between the samples.

Sample Code 111

score	Sensory perception	Sensory quality attributes			
		Appearance	Taste	Mouthfeel	Overall acceptability
9	Like extremely				
8	Like very much				
7	Like moderately				
6	Like slightly				
5	Neither like nor dislike				
4	Dislike slightly				
3	Dislike moderately				
2	Dislike very much				
1	Dislike extremely				

Sensory evaluation