NUTRIENTS AND ANTINUTRITIONAL FACTORS AT DIFFERENT MATURITY STAGES OF SELECTED INDIGENOUS AFRICAN GREEN LEAFY VEGETABLES

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ABSTRACT

A study was conducted to determine the effect of maturity stage on selected nutrients and anti-nutritional factors in selected indigenous African leafy vegetables namely Amaranths (Madiira Ex zim, and Madiira AM 38), African nightshade (Nduruma BG 16 and Olevolosi SS 49) and Spider plant. Vegetables were planted on plots and harvested at three maturity stages (21, 28 and 35 days). Chemical analyses were done to determine levels of vitamin C, iron and zinc and anti-antinutional factors (oxalate, phytate and nitrate).Vitamin C increased significantly ($p<0.05$) with maturity in all vegetables except African nightshade Nduruma BG 16. Vitamin C content was highest (162.7 ± 1.2 mg/100g) in Spider plant and lowest (29.0 ± 1.5 mg/100g) in African nightshade Olevolosi SS 49. Iron content increased significantly ($p<0.05$) at all maturity stages. Amaranths Madiira Ex zim had the highest iron concentration (999.0 ± 3.7 mg/100g) while African nightshade Olevolosi SS 49 had the lowest value (231.1 ± 1.5 mg/100g). Zinc content decreased with plant age although not significantly ($p>0.05$) between each stage. Zinc was highest (76.9 ± 1.0 mg/100g) in Spider plant and lowest (44.8 ± 0.7 mg/100g) in amaranths Madiira Ex zim. Oxalic acid concentration increased with plant age. African nightshade Nduruma BG 16 had lowest oxalic acid concentrations (28.7 ± 0.0 mg/100g) while African nightshade Olevolosi SS 49 had highest value (60.9 ± 0.9 mg/100g). Phytic acid increased significantly between stages of maturity in all varieties except in Spider plant. Highest phytic acid value was in amaranths Madiira AM 38 (0.9 ± 0.0 mg/100g) while the lowest value (0.01 ± 0.0 mg/100g) was in Spider plant Nitrate content decreased with plant age in all samples. Highest nitrate content was 86.1 ± 1.1 mg/100g in Olevolosi SS 49 whereas lowest value was 45.8 ± 0.6mg/100g in amaranths Madiira AM 38. The study concludes that nutrients and anti-nutritional factors vary with plant maturity and therefore the vegetables need to be harvested at 28 days.

DECLARATION

I, Teddy Mamboleo, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

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DEDICATION

This dissertation is dedicated to my parents Mr. Frederick and Mrs. Irmina Mamboleo, my beloved husband Camil Shayo, my lovely sons Baraka and Ibrahim and daughters Sekonsia and Sarah.

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LIST OF ABBREVIATIONS

ADI	Acceptable Daily Intake
AMA 38	Amaranths Madiira I AM 38
AMZ	Amaranths Madiira II Ex Zim
ANN 16	African nightshade Nduruma BG 16
ANO 49	African nightshade Olevolossi SS 49
AOAC	Association of Official Analytical Chemists
AVRDC	African Vegetable Research Development Centre
Cm	Centimeter
DM	Dry Matter
FAO	Food and Agriculture Organization
FNBIOM	Food and Nutrition Board of Institute of Medicine
g	Gramme
GLV	Green Leafy Vegetables
IFPRI	International Food Policy Research Institute
NADPH	Nicotinamide Adenine Di-Phosphate
SPC	Spider plant *Cleome gynandra*
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Vegetables are generally succulent parts of plants grown in gardens and consumed as a side dish with starchy staples (Nangula *et al*., 2010). Several vegetable species exist in the world. Green Leafy Vegetables (GLVs) are a rich source of vitamins and other components that contribute to antioxidant activity in the diet. They are also a great source of minerals such as zinc and iron (Weinberger and Msuya, 2004). In recent study by Moyo *et al.* (2013), it has been reported that GLVs contain non-nutrient bioactive phytochemicals that have been linked to protection against cardiovascular and other degenerative diseases.

African indigenous GLVs are reported to play a very important role in income generation and subsistence (Schippers, 2000). They are important commodities for poor households because their prices are relatively affordable when compared to other food items. In Tropical Africa where the daily diet is dominated by starchy staples, indigenous GLVs are the cheapest and most readily available sources of important minerals and vitamins (Dzomeku *et al.*, 2011). In addition, indigenous vegetables have the added advantage of possessing other desirable traits such as aroma and flavor which make them quite acceptable in the local communities. These vegetables are often easier to grow, resistant to pests and diseases and do not require intensive management (Moyo *et al.,* 2013). When available, indigenous vegetables are preferred over exotic varieties.

On the other hand, GLVs contain dietary components that compromise digestion and absorption of vital nutrients (Nangula *et al*., 2010). These components occur in combinations and may act synergistically or may have contraindicating effects with each

other. Understanding the role of these components is crucial for managing micronutrient deficiencies and chronic diseases of lifestyle.

Micronutrient malnutrition is highly prevalent in the Tropics where per capita vegetable consumption is far less than minimum recommended 73kg per person per year (Engle *et al.,* 2003). In Sub Sahara Africa, per capita vegetable supplies are only 43% of what is needed leading to widespread micronutrient deficiencies (Ruel *et. al.*, 2005). A study by Ezzati *et al*. (2002) estimated that insufficient consumption of vegetables causes 2.7 million deaths annually worldwide and belongs to the top ten risk factors contributing to mortality. Increased production and consumption of green leafy vegetables is among the best food based strategy for alleviating micronutrient deficiencies especially in resource poor communities (Ismail and Fun, 2003; Aphane *et al.,* 2002).

1.2 Problem Statement and Justification

Green leafy vegetables are harvested by rural communities from crop fields at different stages of plant growth (Modi *et al*., 2006). For most of GLVs there is a preferred stage of plant development when flavor and palatability are favorable for human consumption. Food insecurity pressures (such as lack of food or vegetables) and other factors associated with human preference (example, age, gender and culture) may also influence the stage of plant development when leafy vegetables are harvested and consumed (Mathenge, 1997). Moreover, studies have indicated that levels of nutrients and toxic substances in vegetables are influenced by stages of plant development (Khader and Rama 1998), Modi *et al*., 2006). Unlike non indigenous vegetables, information about the stage of plant development to define harvest maturity for indigenous GLVs is scarce.

Since consumption of GLV is recommended as a strategy of preventing and alleviating minerals and vitamin deficiencies in both rural and urban societies, it is important that vegetables are harvested and consumed when the levels of micronutrients is at maximum; and when the quantities of anti-nutritional factors is at the minimum. However there is limited information about the stage of plant development at which minerals and vitamins are at maximum. It is in this view that the research is designed to investigate the effect of plant age at harvest on the levels of some micronutrients and anti-nutritional factors in the selected vegetables so that the nutritional potential of the selected vegetables can be fully harnessed.

1.3 Objectives

1.3.1 Main objectives

The main objective of this study was to investigate the contents of nutrients and anti-nutritional factors at different maturity stages of selected indigenous African green leafy vegetables namely Amaranths (Madiira 1 EX Zim and Madiira II AM 38), African nightshade (Nduruma BG 16 and Olevolosi SS49) and Spider plant.

1.3.2 Specific objectives

Three specific objectives were intended in this study:

i. To determine the levels of vitamin C in the vegetables at 21, 28 and 35 days age of the plants.
ii. To determine the levels of minerals (iron and zinc) in the vegetables at 21, 28 and 35 days age of the plants.
iii. To determine the levels of anti-nutritional factors (phytic acid, nitrate and oxalic acid) in the vegetables at 21, 28 and 35 days age of the plants.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Green Leafy Vegetables

Indigenous green leafy vegetables are native or introduced to particular region from another geographical area but have been used over a long period of time (Engle *et al*., 2003). Indigenous vegetables have a potential for introduction or greater use as cash crops in peri-urban systems, as vegetables for daily sustenance in home gardens and as a means of diversifying production systems and diets (Jansen *et al*., 2007).

African indigenous GLVs have an advantage of possessing desirable agronomic and organoleptic traits. They are often easier to grow, resistant to pests and diseases, and are quite acceptable to local tastes (Onyango and Ekisa, 2007). They contribute significantly to household food security and add a variety to cereal staple diets. Traditionally these vegetables are cooked and eaten as a relish together with a starchy staple food (Vainio-Mattila, 2000). Leafy vegetable dishes may be prepared from a single plant species or from a combination of different species.

Preference of indigenous GLVs species differs depending on gender and age of consumers as well as their cultural background and geographical location (Jansen *et al*., 2004). A study by Onyango and Ekisa (2007) indicated that women have higher preference to sour and bitter tasted vegetables compared to men. In addition, they found out that fear to bitterness or sourness of the vegetables decrease with age. African indigenous green leafy vegetables are preferred over exotic vegetables and studies have indicated that they contain micronutrient levels as high as or even higher than those found in exotic leafy vegetables (Weinberger and Msuya, 2004). Cooking and processing methods may affect

the nutritional value as well as consumer acceptance of GLVs (Mepba *et al.,* 2007; Gimba *et al.,* 2012).

2.2 Description of Vegetables Used in This Study

2.2.1 Amaranths cruentus

Amaranth belongs to the *Amaranthaceae* family and has got many species some of which are cultivated and others are wild (Schippers, 2000). Amaranths are popular leafy vegetables cultivated in many parts of Africa, America and India although they originated from South India (Plate 1).

In most varieties, both the leaves and grains are edible and play a vital role in combating micronutrient malnutrition. Amaranths leaves are rich in iron, vitamin A and C. High oxalate and nitrate contents are reported from leaves of various species (Amanabo *et al*., 2011).

Amaranths grow well in both tropical and sub-tropical regions and in well drained loamy soils rich in organic matter. Cultivated leafy amaranth varieties and cultivars differ in size, shape and colour of leaves and stem. Some varieties of amaranths can be produced by either direct sowing the seeds or by transplanting. For good yield amaranths require frequent irrigation. Amaranths leaves are mature for harvest between 30 and 60 days after sowing depending on variety and method of harvesting (pulling the whole plant or periodic picking). The height of mature plants varies between 0.3 m and 2 m, depending on the species, growth habit and environment (Schippers, 2000; Vorster, 2005). The demand for amaranths as vegetable has increased, especially in the urban centers where people are not involved in primary production (Schippers, 2000).

Plate 1: Amaranths (*Amaranths cruentus*)

2.2.2 African nightshade (*Solanum villosum*)

Solanum genus consists of a wide ranging group of plants commonly called "nightshade" (Plate 2), which includes edible, medicinal and poisonous species (Jansen *et al*., 2004). The broad-leafed *Solanum villosum* (African nightshade) is widely cultivated in sub-Saharan Africa on smaller plots and home gardens. African nightshade is also gaining popularity near cities, for city dwellers who crave a taste of home. Young shoots and leaves are blanched, boiled or stir-fried, cooked with other vegetables or added to soups. The vegetable can be very bitter and milk or salt is often added to reduce bitterness. Unlike some other nightshades, the fruit of the African nightshade is not eaten (AVRDC, 2004).

The vegetable is an excellent source of minerals (iron, calcium, iodine, magnesium, and zinc) and vitamins A and C (Kamga *et al*., 2013). The high nutritional value makes African

nightshade especially important for poor people, as well as helping people suffering from HIV/AIDS get better nutrition.

The African nightshade is naturally common in both lowland and highland areas in West, Central, and East Africa. It can grow in a wide range of soils, but it does better in nutrient-rich soil with high levels of organic matter (AVRDC, 2004).

Plate 2: African nightshade (*Solanum villosum*)

2.2.3 Spider plant (*Cleome gynandra)*

Cleome gynandra also known as spider plant or cat's whiskers (Plate 3) is a wild green leafy vegetable that grows all over tropical Africa, Asia, and the Americas. The African native most likely originated in Eastern Africa, Ethiopia and Somalia. It is not formally

cultivated, but there is some limited cultivation of commercial leafy varieties in several countries in East and Southern Africa (Madisa *et al*., 1997). Spider plant is known to contain high levels of beta-carotene, vitamin C, and moderate levels of calcium, magnesium, and iron (Lyimo *et al.*, 2003). Spider plant has a number of medicinal uses such as to alleviate migraine, vomiting, diphtheria, headache, pneumonia, septic ears, and stomach ailments. The plant is also used as eyewash and fed to boys after circumcision (Schippers, 2000; Kokwaro, 1993).

The spider plant is a hardy native, and can withstand high daytime temperatures, intense sunlight, and drought. It thrives in sandy and degraded soils, although it does not do well in water logged or heavy clay soils. It is a fast growing plant and in the right conditions can be harvested in as few as three weeks after planting making it important for food security for rural populations (Jansen *et al*., 2007).

Plate 3: Spider plant (*Cleome gynandra*)

2.3 Quality Attributes of Green Leafy Vegetables

Quality is the total of all characteristics that impart value to the commodity for the consumer. Consumers consider good quality green leafy vegetables to be those which look good (bright deep green), are firm and have good flavor and nutritive value. At first consumers buy vegetables based on appearance and texture but repeated purchase would in addition base on flavor. The quality attributes of green leafy vegetables are affected by pre and post-harvest factors (Lee and Kader, 2000).

2.4 Role of Selected Micronutrients in Health

GLVs are reported to contribute significantly to the dietary vitamin and mineral intakes of human beings populations (Okeno *et al.,* 2003). Several of these indigenous green leafy vegetables continue to be used for prophylactic and therapeutic purposes. African indigenous GLVs are reported to contribute significantly to the dietary vitamin and mineral intakes of human beings populations (Mulokozi *et al*., 2004).

2.4.1 Vitamin C

Vitamin C refers to a number of vitamins that have vitamin C activity in animals, including ascorbic acid and its salts, and some oxidized forms of the molecule like dehydroascorbic acid (Walingo, 2005). Green leafy vegetables are good sources of vitamin C (Amanabo *et al*. 2011; Lee and Kader, 2000) which is an essential nutrient for humans and other animal species.

Vitamin C has numerous metabolic functions that are largely dependent on its reducing properties (Sen *et al.,* 2014). It is required for the maintenance of healthy skin, gums and blood vessels. It is also known to have many biological functions in collagen formation, absorption of inorganic iron, reduction of plasma cholesterol level, inhibition of

nitrosoamine formation, enhancement of the immune system, and reaction with singlet oxygen and other free radicals (Amanabo *et al*., 2011). Vitamin C, as an antioxidant, reportedly reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Nyonje *et al*., 2014).

Vitamin C deficiency leads to scurvy, a disease characterized by weakness, small hemorrhages throughout the body that cause gums and skin to bleed, and loosening of the teeth. Vitamin C is water soluble, with dietary excesses not absorbed, and excesses in the blood rapidly excreted in the urine. It exhibits remarkably low toxicity. Vitamin C cannot be synthesized in the body cells, nor does the body store it. It is therefore important to include plenty of vitamin C containing foods in daily diet (Garg *et al*., 1992).

2.4.2 Iron

Some African indigenous GLV are excellent sources of iron. Iron levels in vegetables is influenced by factors such as soil type, soil p^H, water availability to the plant, climatic conditions, plant variety, plant age and the use of fertilizers (Khader and Rama, 1998).

Iron is a mineral found in every cell of the body. The human body needs iron to make the oxygen-carrying proteins hemoglobin and myoglobin. Hemoglobin is found in red blood cells and myoglobin is found in muscles. Iron also makes up part of many proteins in the body. Iron is considered an essential mineral because it is needed to make part of blood cells.

Africa has the highest proportion of individuals affected by anemia with an increase in prevalence from 48% in 1993 to 68% in 2005 (De Benoist *et al*., 2008). Women especially

during pregnancy and post-partum, low birth weight babies and malnourished children are more at risk of developing anemia.

2.4.3 Zinc

Zinc is an essential component of many enzymes and it affects multiple aspects of the immune system (Shankar and Prasad, 1998). Zinc is crucial for normal development and function of cells mediating innate immunity cells. The ability of zinc to function as an anti-oxidant and stabilize membranes suggests that it has a role in the prevention of free radical-induced injury during inflammatory processes. Zinc deficiency adversely affects the growth and function of immune cells. Its deficiency is also associated with impaired gastrointestinal and immune system (Roth *et al.,* 2008). An estimated 6.8% of deaths in children less than 5 years of age in 2004 were due to zinc deficiency in sub-Sahara Africa where iron deficiency has been reported. This is because iron and zinc have similar distribution in the food supply and some dietary components affect the absorption of both iron and zinc (Lonnerdal, 2000).

2.5 Anti-nutritional Factors in Vegetables

Anti-nutritional factors are substances that are present in food which interfere with metabolic processes such as growth and bioavailability of nutrients after being consumed (Agbaire, 2012). Example of anti-nutritional factors in food systems includes phytic acid, oxalic acid and nitrates. Phytic acid and oxalic acid have the ability to form chealates with di-and tri-valent metallic ions such as Cd, Mg, Zn and Fe to form poorly soluble compounds that are not readily absorbed from the gastrointestinal tract thus decreasing their bioavailability (Nangula *et al*., 2010). Ladeji *et al*. (2004) reported that oxalic acid cause irritation and swelling in the mouth and throat. He further noted phytic acid to inhibit the functions of some digestive enzymes.

2.5.1 Oxalic acid

Oxalic acid is present in many African indigenous GLVs. Depending on species, oxalic acid can occur as soluble salt of potassium and sodium and insoluble salt of calcium, magnesium and iron or as a combination of the two forms (Ladeji *et al.,* 2004). Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as rickets and osteomalacia. Oxalic acid may also crystallize with calcium in the renal vasculature and infiltrates vessel walls causing renal tubular obstruction, vascular necrosis and hemorrhage which leads to anuria, uraemia and electrolyte imbalances. The risk factors involved in stone formation are low volume of urine, increased urinary excretion of oxalic acid, persistently low or high urine pH and a low concentration of urinary inhibitors (Agbaire and Emoyan, 2012). Addition of a source of calcium to vegetables containing high levels of soluble oxalate has shown to reduce intestinal available oxalic acid content in such foods (Radek and Savage, 2008).

Processing methods for example soaking, blanching and cooking can significantly help to reduce oxalic acid in vegetables (Virginia *et al.,* 2012). During soaking, oxalic acid may be removed by leaching in water. Blanching and cooking leafy vegetables greatly reduces their oxalate content. This is because the concentrations of anti-nutritional factors are highest in the superficial layer of vegetables and blanching and cooking ruptures this layer.

2.5.2 Phytic acid

Phytic acid is the major phosphorus storage compound in African leafy vegetables (Schlemmer *et al*., 2009). Although phytic acid is an antioxidant, it has been shown to inhibit absorption of minerals. Phytic acid chelates multivalent metal ions such as calcium, iron and zinc, thus it is a strong inhibitor of iron mediated free radical generation. The

disadvantage of this is that the diet high in phytic acid content reduces bioavailability of zinc and iron. A number of studies indicate that green leafy vegetables contain various amounts of phytic acid (Agbaire and Emoyan, 2011; Nkafamiya *et al*., 2010).

While some studies (Imaobong *et al.*, 2013; Yadav and Sehgal, 2002) reveal that domestic thermal processing methods can significantly reduce phytic acid content in vegetables, other studies (Embaby, 2010) report that phytic acid content increases or remains unchanged when heat processed.

2.5.3 Nitrates

Nitrates are fairly stable nitrogen compounds that can be degraded into nitrites. Dietary nitrate is metabolized to nitrite by bacterial flora on the posterior surface of the tongue leading to increased salivary nitrite concentrations. Nitrites are unstable and can combine readily with other compounds in the digestive tract to form carcinogenic nitrosamines. The amount of nitrate in plants is determined mainly from its genetically based metabolism, the age of the plant, and the amount of available nitrate in the soil (Huarte-Mendicoa *et al.,* 1997). Leafy green vegetables and some root crops contain the highest concentrations of nitrates (Gupta *et al*., 2005). Among commonly eaten vegetables, beetroot, celery, lettuce, spinach, and radishes have high quantities of nitrate. About 87% of the total nitrate concentration in a normal diet is believed to be a direct result of vegetable intake.

Nitrates form part of the essential chemistry of soils and plants. Thus plant roots are able to absorb nitrate directly from the soil. Nitrate contamination in vegetables occurs when crops absorb more than they require for their sustainable growth. Nitrate content of vegetables may range from 1 to 10,000 mg/kg (Ximenes *et al*., 2000). The main concern for the public health is the link between nitrates and stomach cancer due to the fact that

nitrates may lead to formation of carcinogenic nitrosamines. The elevation of gastric pH > 5.5 leads to bacterial growth followed by rapid conversion of nitrate to nitrite which is a precursor in the formation of nitrosamines.

2.6 Factors Affecting Nutritional Quality of Vegetables

The major factors affecting the nutrient content of vegetables can be grouped as pre-harvest, harvest and postharvest factors.

2.6.1 Pre-harvest factors

2.6.1.1 Soil factors

Soils vary greatly in their proportions of sand, silt, clay and organic matter which can significantly affect such properties as pH, water holding capacity, porosity, cation exchange capacity, and mineral composition. Some crops require a specific range of soil pH for optimum growth. The relation between soil pH and macro- and micronutrient solubility determines the availability of soil nutrients; in turn, growth and yield of crops and their ultimate nutrient contents are affected. Soil structure is essential for water and nutrient movement, penetration, and retention. Large spaces between aggregates allow soil water and nutrients dissolved therein to move more freely, resulting in leaching losses. Small or no spaces between aggregates, especially due to compaction, prevent water from moving through the soil profile, resulting in runoff (Bellows, 2001).

Maintaining good, long-term soil health and quality remains a primary goal of organic production systems. Achieving this goal will ultimately benefit the postharvest quality of vegetables grown on the farm, as the availability of the optimal levels of plant nutrients throughout the growing season will allow for optimal quality of the vegetables throughout

the packing and distribution processes. Deficiencies or overabundances of certain plant nutrients can affect positively or negatively the nutritional quality of vegetables.

2.6.1.2 Climate

Light intensity, temperature and rainfall interact to affect the nutrient content of plants, and each varies considerably depending on the season and specific growing conditions (Howard *et al.,* 1999). Increases in light intensity can significantly affect the ascorbic acid (vitamin C) content of vegetables. In general, the lower the light intensity the lower the ascorbic acid concentration is in plant tissues. Moreover, temperature influences uptake and metabolism of mineral nutrients by plants because transpiration increases with temperature. Rainfall affects the water supply to the plants which may affect the composition of the harvested part and its susceptibility to mechanical damage during subsequent harvesting and handling operations. For example, a study by Xuedong *et al*. (2012) revealed that excess soil water diluted the nitrate in the soil solution and restricted crop growth and caused loss of nitrate by denitrification.

2.6.1.3 Agronomic practices

Agronomic practices such as mulching, irrigation and fertilization have effect on the nutritional quality of plants as they influence the water and nutrient supply to the plant (Lee and Kader, 2000). The study conducted by Antonio *et al* (2007) revealed that vitamin C content of sweet pepper fruits grown organically was higher (100.13 ± 0.18mg/100g) compared to that of the peppers grown conventionally (77.96 ± 0.12mg/100g). The effect of fertilizers on mineral content of plants is very significant. Lee and Kader (2000) reported that increasing the amount of nitrogen fertilizer from 80 to 120 kg/ha decreased the vitamin C content by 7% in cauliflower. Nitrogen fertilizers are also known to increase plant foliage and thus may reduce the light intensity and accumulation of ascorbic acid in

shaded parts. Furthermore, excess use of nitrogen fertilizers increases the concentration of nitrite therefore it may have a double negative effect on the quality of plant foods. On the contrary, increased potassium fertilization increased vitamin C content in vegetables.

Adequate soil moisture during the pre-harvest period is essential for the maintenance of post-harvest quality. Water stress during the growing season can affect the size of the harvested plant organ, and lead to soft or dehydrated fruit that is more prone to damage and decay during storage. On the other hand, vegetables experiencing an excess of water during the growing season can show a dilution of soluble solids and acids, affecting flavor and nutritional quality (Shewfelt and Prussia, 1993). Excess moisture on the harvested vegetable can also increase the incidence of post-harvest diseases. To minimize the amount of water on the harvested vegetable brought into storage, it may be beneficial to choose surface or subsurface irrigation rather than overhead irrigation. Vegetables harvested in the early morning, during rainy periods, and from poorly ventilated areas can also experience increased post-harvest decay.

2.6.1.4 Vegetable variety and cultivars

Various crops differ in their nutrient requirements. For example, nitrogen-fixing legumes do not require nitrogen fertilizer while cereals, some vegetable and fruit crops do. Each crop has its own specific macro- and micronutrient requirements for optimum growth and yield. In addition, the nutrient requirements and final nutrient contents of a particular crop can also vary depending on the cultivar (Singh *et al.,* 2001). For example, vitamin C contents were found to vary between 64 to 168mg/100g in five cultivars of fresh pepper (Lee *et al*., 1995).

2.6.2 Harvest factors

2.6.2.1 Maturity stage at harvest

Mature stage of fruits and vegetables is the stage of fully development of tissue of the crop at which it will ripen normally. Harvest maturity is a stage of plant development at which quality attributes preferred for consumption (volume, flavor, appearance, chemical composition and adequate shelf life) are at maximum. Age at harvest is known to affect chemical composition of leafy vegetables. Study by Modi (2007) indicate that boiled amaranths that were harvested 60 days after sowing displayed higher antioxidant activity compared with those harvested at either 20 or 40 days after sowing. Therefore the stage of plant development should be considered for optimum antioxidant activity.

2.6.2.2 Harvesting method

The method of harvest can determine the extent of variability in maturity and physical injuries, and consequently influence nutritional composition of fruits and vegetables. Mechanical injuries such as bruising, surface abrasions, and cuts can result in accelerated loss of vitamin C (Lee and Kader, 2000). The incidence and severity of such injuries are influenced by the method of harvest and handling operations. Proper management to minimize physical damage on vegetables is a must whether harvesting is done by hand or by machine.

2.6.3 Post harvest factors

Fresh fruits and vegetables as living tissues are subject to continual changes after harvest. Such changes cannot be stopped but can be controlled within certain limits by using various post-harvest processes.

2.6.3.1 Preparation procedures applied to the vegetables

Various procedures applied to vegetables during preparation for consumption or further processing affect their nutritional composition. These include cleaning, sorting to remove

defects, treatment with fungicides to control decay, heat treatments and irradiation. Excessive trimming of leafy vegetables results in loss of outer green leaves which contain more vitamins than inner leaves. Trimming of outer leaves and of the core and associated inner leaves of Chinese cabbage had a greater effect on reduction of vitamin C content than untrimmed leaves (Klieber and Franklin, 2000).

2.6.3.2 Processing procedures

In general, the nutrient levels in freshly harvested vegetables are higher compared to those held in storage for some time. Postharvest processing procedures such as cooking, drying, freezing, canning, packaging markedly help in increasing shelf life but can affect positively or negatively the nutritional quality of green leafy vegetables (Lee and Kader, 2000). For example, the study by Souzan and Abd-El-Aal (2007) revealed that there was some moisture loss in fresh green leafy vegetables under refrigerated storage for eight days compared to zero day-refrigerated vegetables.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out at Sokoine University of Agriculture (SUA) whereby vegetables were planted and managed at Horticulture Department and assessed for their chemical composition at laboratories of the Departments of Food Science and Technology and Soil Science.

3.2 Materials and Their Sources

3.2.1 Vegetables

Five varieties of African indigenous Green Leafy Vegetables namely Amaranths Madiira EX Zim (AMZ), Amaranths Madiira AM 38 (AMA 38), African nightshade Nduruma BG 16 (ANN 16), African nightshade Olevolosi SS 49 (ANO 49) and Spider plant (SPC) were used in the study. Seeds of these vegetables were obtained from The World Vegetable Centre (AVRDC) – Tengeru, Arusha- Tanzania and from the Horticulture Unit of Sokoine University of Agriculture (SUA), Morogoro.

3.2.2 Chemicals and reagents

The chemicals and reagents used were obtained from Sokoine University of Agriculture and were of analytical grades and were obtained from the university laboratories. Details are shown in section 3.3.5.

3.3 Methods

3.3.1 Research design

Completely Randomized Block Design (CRBD) was used in the study to determine and

compare the effects of principal factors (variety and stage of maturity) on the micronutrients and anti-nutritional factors of leafy vegetables. The design of plots by using CRBD is shown in Fig. 1.

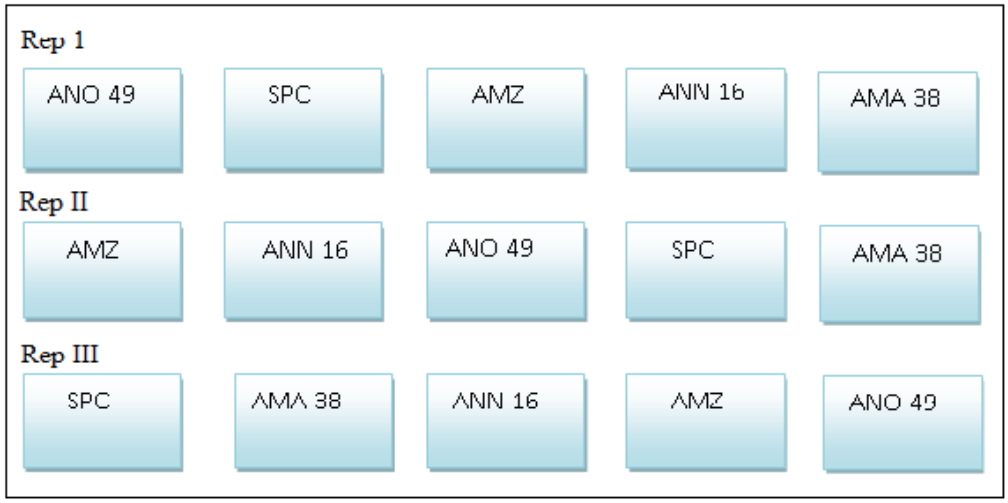


Figure 1: Layout of the experimental plot

Key: ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant, AMZ= Amaranths (Madiira Ex Zim) ANN 16=African nightshade (Nduruma BG 16), and AMA 38 =Amaranths (Madiira AM 38)

3.3.2 Planting and management

The seeds were sown at the experimental plots as shown on Fig. 1. The land was cleared, ploughed, harrowed and fertilized using poultry manure followed by seed sowing on the plot in three replicates. The area was divided into three rows for the three replications and each row was divided into five beds, one for each vegetable variety. The seeds were sown in 30 cm inter and intra spacing. The density of the plants was 45 plants per bed. The vegetables were watered twice daily (mornings and evenings) and weeding was done weekly.

3.3.3 Harvesting and collection of samples

The three maturity stages were 21 days, 28 days and 35 days from sowing the seeds. At each stage, about 500 to 600 g of the edible parts (leaves and young stems) were harvested by uprooting the whole plant and picking the edible leaves and shoots. The picked leaves were placed in dark colored polythene bags and transported to the laboratory for chemical analyses.

3.3.4 Sample preparation for chemical analyses

The edible portions of the samples were washed with running tap water, drained to remove excess water, cut into small pieces (about 2 mm) using domestic sharp knife and cutting board and homogenized. Two grams of each fresh sample were taken for vitamin C analysis while the remaining samples were oven dried at 60^{o}C for 24 hours. The dry samples were removed from the oven and immediately ground into a fine powder using motor and pestle. The powdered samples were placed in transparent polythene bags, labeled and stored ready for mineral analyses.

3.3.5 Chemical analyses

3.3.5.1 Vitamin C determination

The vitamin C concentration was determined using procedure outlined by Kumar *et al*. (2013) where 10 g of each of the sample was accurately weighed and ground using mortar and pestle with an additional of 20 ml of metaphosphoric acid and acetic acid. The mixture was further ground and strained through Whatman filter paper no. 1 and the extract was made up to 100 ml with the metaphosphoric-acetic acid mixture. Five ml of the metaphosphoric acid-acetic acid solution was pipetted into three of the 50 ml Erlenmeyer flask followed by 2 ml of the samples extract. The samples were titrated separately with the indophenol dye solution until a light rose pink persisted for 5 s. The amount of dye

used in the titration was determined and used in the calculation of vitamin C content. The L-dihydroascorbic acid was calculated using the following formula (Equation 1):

$$R = \frac{C\,x\,(V_1 - B)\,x\,V_2\,X\,100}{S_a\,x\,V_3} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots. (1)$$

Where, R was the concentration of ascorbic acid in mg/100g of the sample, C was the concentration of 2, 6–dichlorophenolindophenol dye, V_1 was the volume of DCIP used for the sample, B was the volume of DCIP used for the blank, V_2 was the total extraction volume, S_a was the sample weight taken and V_3 was the sample extract analysed.

3.3.5.2 Iron and zinc analyses

Iron and Zinc contents were determined by following a procedure as described by Eslami *et al.* (2007). Ten grammes of sample were incinerated into ash. The obtained ash was dissolved in 6M HCL acid and filtered using Whatman filter paper no.1 to obtain clear solution. The clear solution was then subjected to Atomic Absorption Spectrophotometer using the hollow cathode lamps set at 248.3 nm and 213.9 nm to determine iron and zinc contents respectively.

3.3.5.3 Determination of anti-nutritional factors

i. Oxalic acid content

The titration method as described by Baker (1952) was followed. Two grammes of powdered sample were heated in 50 ml distilled water and 0.3M HCl was added to the sample and left to cool. The cold filtrate was treated with 3 drops of methyl red indicator and NH_4OH solution before being heated to 100^0C. The mixture was left to cool where the filtrate was heated further before addition of 10 cm^3 of 10% $CaCl_2$ solution and allowed to stand overnight. The mixture was filtered by using Whatman paper no I and the precipitate

formed was washed to remove traces of Ca^{2+} before dissolving in H_2SO_4 solution. The solution formed was boiled by heating before warm titrating with 0.05 M $KMnO_4$ solution until a faint pink colour persisted for at least 30 seconds. The oxalate content was then calculated by taking 1ml of 0.05 m $KMnO_4$ as equivalent to 2.2 mg oxalate using the formula below (equation 2):

$$O = \frac{T_s \times M_d \times M_o \times 100}{W_s} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

Where, O was oxalate concentration in mg/100g, T_s was the volume of potassium permanganate used for sample, M_d was the number of moles of potassium permanganate reacted, M_o was the number of moles of Oxalate reacted and W_s was the sample weight.

ii. Phytic acid content

Phytic acid contents of the vegetable samples were determined by method as described by Wheeler and Ferrel (1971). About 0.2g of each powdered sample was weighed into a 125 ml Erlenmeyer flask and 50 ml 3% TCA for 30 minutes with occasional swirling by hand for 45 minutes to extract phytic acid. The suspension was centrifuged by Braid and Tallock Auto Bench Centrifuge Mark IV, UK at 3000 rpm for 10 minutes and 10 ml aliquot of the supernatant was transferred to a 50 ml conical flask. Four milliliters of $FeCl_3$ solution was added to the aliquot by lowering rapidly from the pipette and the content was heated in a boiling water bath for 45 minutes. After 30 minutes, two drops of 3% Sodium sulphate were added in 3% TCA extract and continued to be heated. The supernatant was centrifuged for 15 minutes and decanted. The precipitate was washed twice by dispensing well in 20-25 ml of 3% TCA, heated in boiling water bath for 10 minutes and centrifuged. Washing with water was repeated. The precipitate was dispersed in 27 ml of water and 3 ml of 1.5N NaOH with mixing. The volume was brought to approximately 30 ml with

water and heated in boiling water bath for 30 minutes. The precipitate was filtered through a moderately retentive paper Whatman No.2. The precipitate was washed with 70 ml hot water and the filtrate was discarded. The precipitate was dissolved from the paper with 40ml 3.2N HNO_3 into a 100ml volumetric flask. The filter paper was washed with several portions of water and the washings were collected in the same flask taking care not to exceed the 100 ml volume. The flask was cooled at room temperature and diluted to volume with water. A 5 ml aliquot was transferred to another 100 ml volumetric flask and diluted to approximately 70 ml. Twenty milliliters of 1.5M KSCN was added and diluted to volume and color was read immediately within 1min at 480 nm. A reagent blank was run with each set of samples. Phytic acid content in sample was calculated using the formula shown on Equation 3:

$$\text{Phytate content in } \frac{\text{mg}}{100\text{g}} \text{ sample } = \frac{C \; x \; E \; x \; 100}{S \; x \; Av} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

Where C was phytate acid concentration from standard graph, E was total extraction volume, S was the analytical sample taken and Av was Analytical volume.

iii. Nitrate content

Determination of nitrate contents was done by using spectrophotometric method as outlined by Gaya and Alimi (2006) where 10g of each sample was taken into a 250cm^3 beaker and 2.5ml of 4% NaOH was added. The content of the beaker was warmed at 800^{0}C for 25minutes with occasional shaking. The resulting solution was filtered through a fluted filter paper into 100 cm^3 volumetric flask and made up to the mark. An aliquot of 4 cm^3 was taken into a test tube cooled in ice. One cm^3 of 5% Ag_2SO_4 solution was added followed by subsequent addition of 7 cm^3 of 98% H_2SO_4 and 0.1 cm^3 of 5% phenol

solution. The solution was allowed to stand for 20 minutes while shaking occasionally. The resulting mixture was extracted in 50 cm^3 separating funnel by adding toluene and shaking for 5 to 10 minutes. The lower aqueous layer was discarded. The organic phase was washed twice with 10ml of distilled water by shaking for 2 minutes and each time discarding the aqueous phase. The organic phase was extracted again by shaking for 1 minute with 10 cm^3 of 10% Na_2CO_3 solution and collected in a test tube. Absorbance was read at 407 nm. Since 4cm^3 of the 100cm^3 filtrate was used for analysis. The amount of nitrate (mg/g) in the vegetable sample was calculated by the following formula depicted in Equation 4:

$$\text{Nitrate} = \frac{C \ x\, 100}{W\ x\ 4} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

Where C = Concentration of nitrate in the sample as from calibration graph (mg/cm^{-3}) and W = Weight of the sample used (g)

3.3.6 Statistical data analysis

The data obtained were analysed using SPSS for windows (version 15.0, SPSS, Chicago, USA) using one way analysis of variance (ANOVA) to determine the significant difference between the main factors (Variety and maturity stage) at 5% level of significance. The mathematical expression is shown in Equation 5.

$$yij = \mu i + \tau i + \varepsilon ij \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

i=1, 2,..., t, j=1,2,...,ni

Where μ is the overall mean concentration of parameters, i is the concentration between maturity stages, j is the concentration between varieties, and εij is the random error effect due to treatment. Mean values were separated by using Turkey's Honest Significant Difference at $p<0.05$. Data were expressed as Mean ± SD and presented in tabular and graphical forms.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Effect of Maturity Stage and Variety on Vitamin C Content of the Vegetables

4.1.1 Effect of maturity stage

Fig. 2 shows a trend of vitamin C contents (mg/100g Dry Matter) of different vegetables at different stages of maturity. Vitamin C contents increased from maturity stage I to maturity stage II in all samples except ANN16. The vitamin increased much in SPC while in sample AMA 38 it increased only a small amount. From maturity stage II to stage III the concentration of the vitamin increased in all samples. The increase at this stage was higher in all samples as compared to the former stage (Fig. 2).

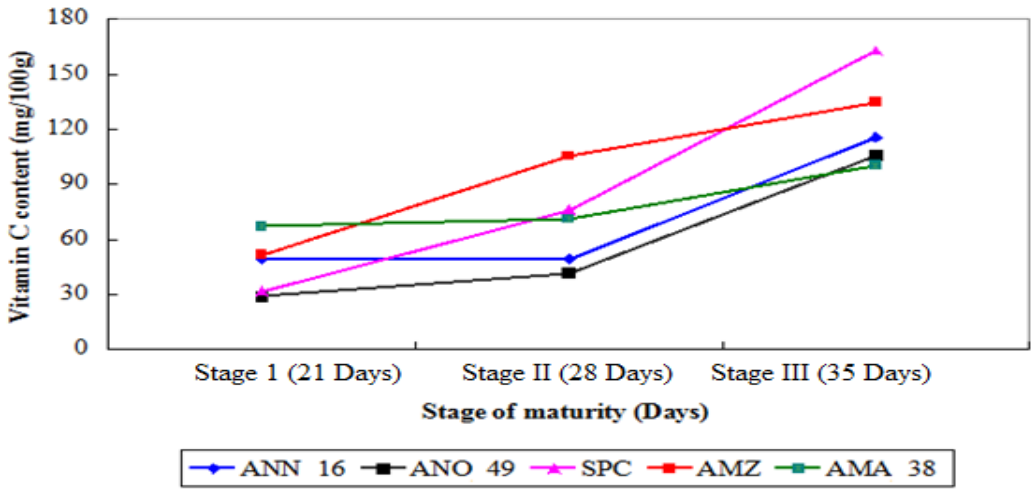


Figure 2: **Vitamin C contents (mg/100 g DM) in different vegetables at different stages of maturity: ANN 16= African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)**

The effect of maturity stage on vitamin C of each vegetable variety was significant ($p< 0.05$) as shown in Table 1. The results show that for ANN16 the mean quantity of vitamin C during the first and the second stage were statistically equal ($p > 0.05$). However vitamin C content of ANN16 during the third stage was higher than that of the first and second stages and the difference was statistically significant at 95% level of confidence ($p < 0.05$). In addition, the results indicated that vitamin C content of the remaining varieties increased with the stage of maturity and the quantity at subsequent stages were significantly ($p < 0.05$) different from the preceding ones.

Sample AMA 38, AMZ and SPC had highest scores of vitamin C content (67.1 ± 2.0 mg/100g, 105.5 ± 1.0 mg/ 100g and 162.7 ± 1.2 mg/100g) at maturity stages I, II and III respectively. The lowest Vitamin C scores were observed in sample ANO 49 (29.0 ± 1.5 mg/100g and 41.5 ± 1.2 mg/100g) at maturity stage I and II respectively and in AMA 38 (134.6 ± 3.5 mg/100g) at maturity stage III (Table 1).

Table 1: Vitamin C contents (mg/100 g DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	49.2 ± 0.0^{b}	49.2 ± 0.0^{b}	115.5 ± 0.5^{a}
ANO 49	29.0 ± 1.5^{c}	41.5 ± 1.2^{b}	105.7 ± 1.6^{a}
SPC	31.8 ± 1.8^{c}	76.0 ± 2.0^{b}	162.7 ± 1.2^{a}
AMZ	51.6 ± 1.2^{c}	105.5 ± 1.0^{b}	134.6 ± 3.5^{a}
AMA 38	67.1 ± 2.0^{c}	71.2 ± 1.1^{b}	100.4 ± 0.6^{a}

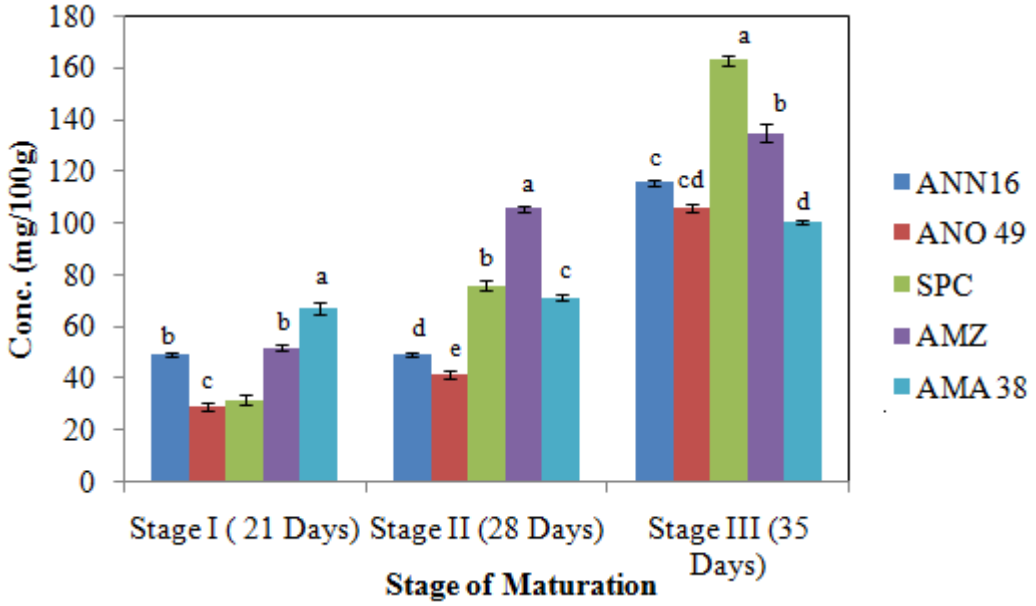
Data presented as arithmetic means ± SD (n = 3).

Means within the vegetable variety in row with different superscript letters are significantly different at $p<0.05$.

These results are in agreement with the work of Amanabo *et al*. (2011) who found that, the vitamin C content increased from market maturity (94.60±5.60 mg/100g) to reproductive stage (160.50 ± 7.10 mg/100g) in *Amaranthus cruentus*. Antonio *et al* (2007) found that vitamin C content of sweet pepper grown organically increased with maturity (5.08 ± 0.04 mg/100g, 100.13 ± 0.18 mg/100g and 148.85 ± 0.37 mg/100g fresh weight) in immature green, mature green and red ripe stages respectively. Howard *et al*. (1999) also reported about 30% higher value of total vitamin C in ripe pepper than that of unripe pepper. Although vitamin C occurs in all plant tissues, usually being higher in photosynthetic cells and meristems and some fruits, its concentration is highest in mature leaves with fully developed chloroplasts (Taqi *et al.,* 2011). This suggests that harvesting green leafy vegetables at advanced age potentially provides the greatest concentrations of vitamin C.

4.1.2 Effect of variety on vitamin C content

Fig. 3 shows the effect of variety on vitamin C contents in different vegetables within each stage of maturity. There were significant ($p < 0.05$) differences in vitamin C content between the varieties. The results show that at maturity stages I and II, varieties ANO 49 and SPC had statistically ($p > 0.05$) same concentrations of vitamin C. Likewise there was no significant ($p > 0.05$) difference in vitamin C contents of varieties ANN 16 and AMZ but the two varieties had significantly higher vitamin C contents than varieties ANO 49 and SPC. At maturity stage I variety AMA_38 had the highest vitamin C concentration (67.1 ± 2.0 mg/100g) while variety ANO 49 (29.0 ± 1.5mg/100g) had lowest concentration of the vitamin. At maturity stages II and III, vitamin C contents of each variety were significantly ($p < 0.05$) different. At stage II, AMZ had the highest vitamin C content (105.5 ± 1.0 mg/100g) while ANO 49 had the lowest content (41.5 ± 1.2 mg/100g). The highest values of vitamin C at maturity stage III were in SPC (162.7 ± 1.8 mg/ 100 g) while AMA 38 scored least values of the vitamin at this stage (Fig. 3 and Appendix 1).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 3: Vitamin C content (mg/100 DM) in different vegetable varieties within the stages of maturity; ANN 16 =African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

Such a wide variation of vitamin C contents among different varieties of fruits and vegetables was also reported by Ogunlesi *et al*. (2010) and Lee and Kader (2000). Lee *et al*. (1995) reported a range from 64 to 168 mg/100g of vitamin C in five fresh pepper cultivars.

Other factors affecting vitamin C concentration in vegetables apart from maturity and variety include agronomic practices, postharvest handling, conditions (time lapse between harvest to consumption and extent of physical damage), storage and processing methods

such as blanching, cooking, drying, condition and canning. The processing methods lead to significant losses of vitamin C (Lee and Kader, 2000).

4.2 Effect of Maturity Stage and Variety on Mineral Contents of the Vegetables

4.2.1 Iron content

4.2.1.1 Effect of maturity stage on iron content

Fig. 4 presents the effect of maturity stage on iron content in the five vegetable varieties. The results show that there was progressive increase of iron concentration from stage I to stage II and from stage II to stage III in all varieties. The increase from maturity stage I to stage II was slightly lower as compared to that of stage II to stage III (Fig. 4).

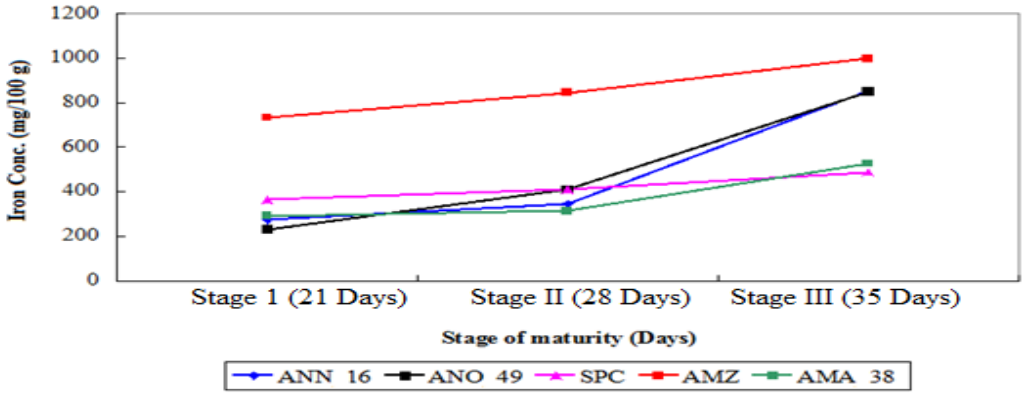


Figure 4: Iron contents (mg/100 g DM) in different vegetables at different stages of maturity.ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

Table 2 presents the results of ANOVA for comparison of mean quantity of iron at different maturity stages for each variety. Overall results suggest that iron content increased significantly ($p < 0.05$) from stage I to stage III of maturation in all five

varieties. Sample AMZ scored the highest iron concentrations at all three maturity stages (733.2 ± 0.3 mg/100g, 845.9 ± 0.6 mg/100g and 999.0 ± 3.7 mg/100g) at maturity stages I, II and III respectively while samples ANO 49, AMA 38 and SPC had the lowest iron concentrations (231.1 ± 1.5mg/100g, 313.3 ± 0.8 and 485.7 ± 3.3) at maturity stages I, II and III respectively (Table 2).

Table 2: Iron contents (mg/100 g DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	273.5 ± 0.8^{c}	345.5 ± 0.5^{b}	$853. \pm 0.8^{a}$
ANO 49	231.1 ± 1.5^{c}	411.4 ± 0.4^{b}	851.0 ± 0.6^{a}
SPC	363.3 ± 3.3^{c}	409.2 ± 3.6^{b}	485.7 ± 3.3^{a}
AMZ	733.2 ± 0.3^{c}	845.9 ± 0.6^{b}	999.0 ± 3.7^{a}
AMA 38	291.8 ± 0.6^{c}	313.3 ± 0.8^{b}	526.0 ± 1.3^{a}

Data presented as arithmetic means ± SD (n = 3).

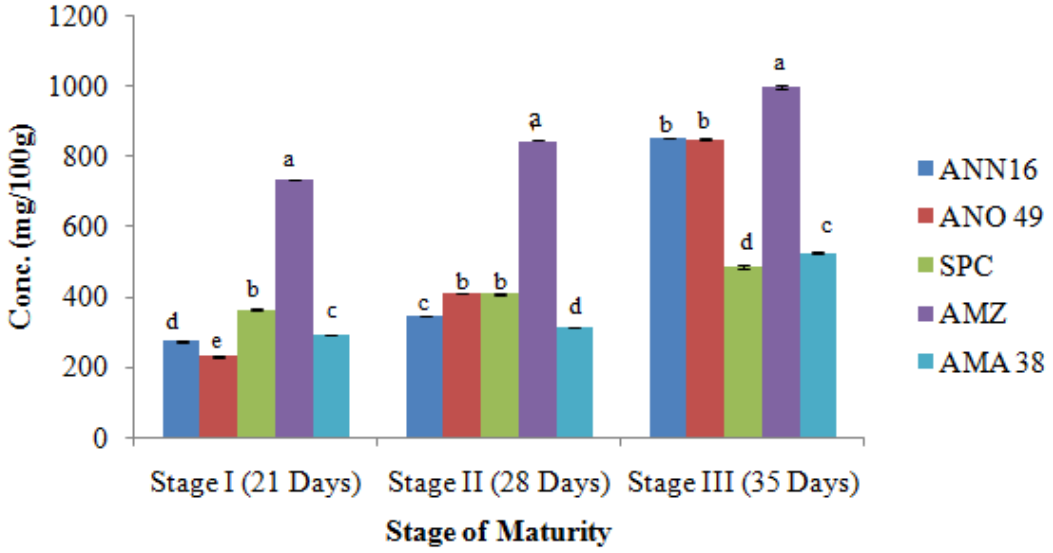
Means within the vegetable variety row with different superscript letters are significantly different at $p < 0.05$.

These findings were similar to those of Flyman and Afolayan (2007), who reported increase in iron content at each stage in *Vigna unguiculata* as the plant matured from 21 to 57 days after sowing the seeds. Modi (2007) found that iron concentration in *Amaranthus cruetus* increased significantly ($p < 0.05$) from40 ± 2.5 mg/100g at 20 Days after sowing to 58 ± 2.1 mg/100g at 40 days after sowing. However, the results differ from the findings of Khader and Rama (1998) who reported decrease in iron concentration in green leafy vegetables from 30 to 45 days. The likely reason for the decrease of iron may have been due to possible translocation of some of its content to the developing fruits and a decline

in the content and activity of chlorophyll and associated light absorbing pigments following senescence induced by fruit formation and maturation (Noggle and Fritz, 2006). The increasing trend of iron suggests that the mineral may be an indissociable ion and accumulates as age increases.

4.2.1.2 Effect of variety on iron content

The variations in iron concentrations among the vegetable varieties within each stage of maturity are presented in Fig. 5. At maturity stage I, iron contents of all varieties were statistically different ($p<0.05$) from each other. Likewise, at stage II, varieties ANN 16 AMZ and AMA 38 had significant difference in iron contents while varieties ANO 49 and SPC had no significantly different iron contents. At maturity stage III, ANN16 and ANO 49 had statistically same iron contents. At the same stage, varieties SPC and AMA 38 had statistically different iron contents. Highest iron contents within each maturity stage were 733.2 ± 0.3mg/100g, 845.9 ± 0.6 mg/100g and 999.0 ± 3.7mg/100g in AMZ at maturity stages I, II and III respectively while lowest iron contents were in ANO 49 (231.1 ± 1.5 mg/100g), AMA (38 313.3 ± 0.8 mg/100g) and SPC (485.7 ± 3.3 mg/100g) at maturity stages I, II and III respectively (Fig. 5 and Appendix 2).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 5: Iron content (mg/100g DM) in different vegetable varieties within the stages of maturity; ANN16=African nightshade (Nduruma BG 16), ANO49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (*Cleome gynandra*), AMZ= Amaranths AMZ, and AMA38 =Amaranths (Madiira AM 38)

The different iron contents among different varieties within same maturity stages were also reported by other researchers. Atta *et al.* (2010) reported different iron contents among three ecotypes of roselle leaves within same stages of maturity. The varietal differences in iron content may be due to the different rooting systems (root surface area) of the vegetables which determine plant's efficiency to take up mineral elements from the soil (Baligar *et al.*, 2001).

4.2.2 Zinc content

4.2.2.1 Effect of maturity stage on zinc content

The zinc contents of the vegetables at stages I, II and III of maturation are shown in Figure 6. Results show that zinc contents decreased continuously with maturity in all five

varieties, although the decrease was not much between the stages. Furthermore, variety SPC had highest concentration of zinc at all maturity stages while variety AMZ had the lowest zinc contents at all stages (Fig. 6).

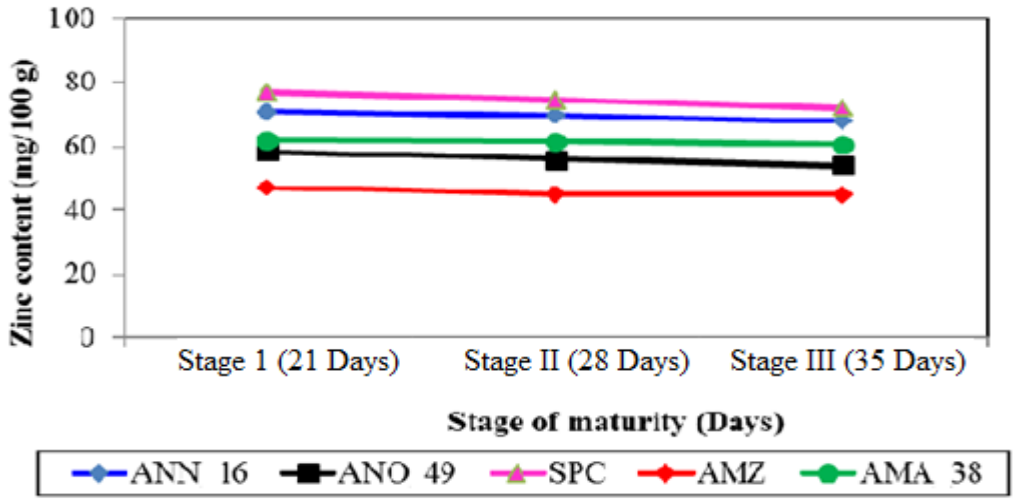


Figure 6: Zinc content (mg/100g DM) in different vegetables at different stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

Further results of ANOVA for comparison of mean content of zinc for each variety across different stages of maturity are shown in Table 3. Overall results showed that, zinc content decreased with plant age. The highest zinc contents were 76.9 ± 1.0 mg/100g, 74.5 ± 3.4 mg/100g and 72.2 ± 1.4 mg/100gin sample SPC at maturity stages I, II and III respectively. The lowest zinc contents were 47.0 ± 0.5 mg/100, 45.0 ± 0.6 mg/100g and 44.8 ± 0.7mg/100g in sample AMZ at maturity stages I, II and III respectively. The results imply that the decrease was not significant ($p < 0.05$). These results were consistent for all five varieties of vegetables under investigation (Table3).

Table 3: Zinc contents (mg/100 g DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	71.0 ± 1.1^{a}	69.7 ± 0.9^{a}	68.00 ± 1.2^{a}
ANO 49	58.4 ± 0.9^{a}	56.2 ± 1.1^{a}	54.2 ± 1.5^{a}
SPC	76.9 ± 1.0^{a}	74.5 ± 3.4^{a}	72.2 ± 1.4^{a}
AMZ	47.0 ± 0.5^{a}	45.0 ± 0.6^{a}	44.8 ± 0.7^{a}
AMA 38	62.1 ± 0.9^{a}	61.7 ± 0.9^{a}	60.8 ± 0.5^{a}

Data presented as arithmetic means ± SD (n = 3)

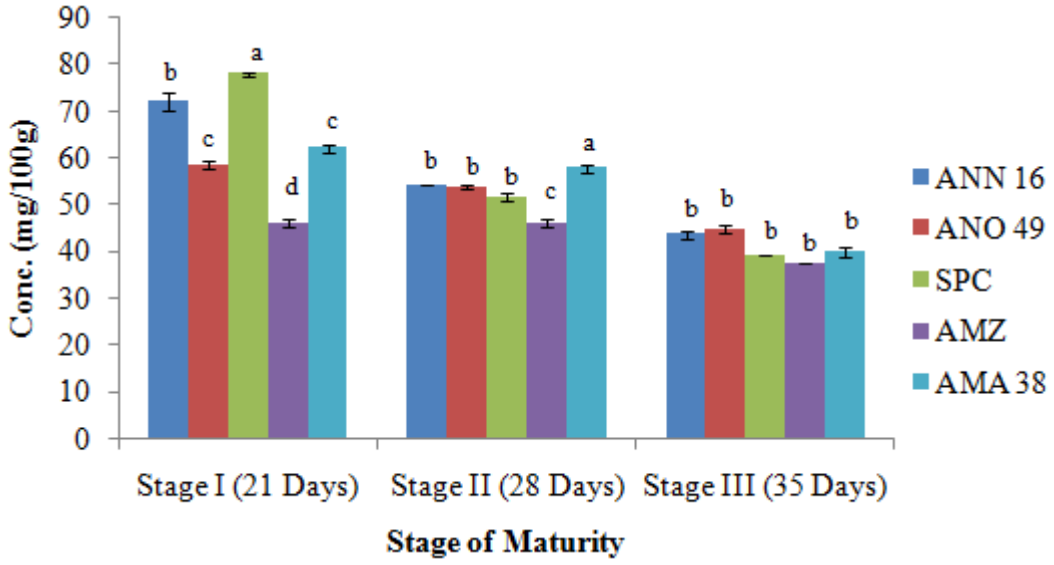
Means within the vegetable variety in row with different superscript letters are significantly different at $p<0.05$.

The decreasing zinc contents with increasing maturity stages were also reported by Khader and Rama (1998) who observed that zinc content was higher at 15 days and decreased continuously from 15 to 45 days of plant age in Amaranths and Spineces spicies. Flayman and Afolayan (2012) found that zinc content was higher at 21 days (389.08 ± 5.08 mg/100Kg) and decreased continuously to 161.53 ± 3.59 mg/100Kg at 28 days and decreased further to 85. 83 ± 1.30 mg/100Kg at 35 days after sowing. Amanabo *et al.* (2011) observed that zinc content in *Amaranthus cruentus* decreased from 0.08 ± 0.01mg/100g dry weight at market maturity (vegetative) stage to 0.05 ± 0.01 mg/100g dry weight at heading (reproductive) stage. The decreasing zinc content may be attributed to diversion of this mineral towards flower development. During fruit initiation and development, some metabolites for cellular synthesis and growth substances are translocated from the leaves, stems, and roots to the developing fruits (Khader and Rama, 1998). Moreover, Lanyasunya *et al*. (2007) observed that the rapid uptake of mineral by plants during early growth and the gradual dilution that occurs as plant matures would have been responsible for the decrease in some of the mineral content during fruiting.

4.2.2.2 Effects of variety on zinc content

The varietal differences in zinc contents at each stage of maturity are presented in Fig. 7. At maturity stage I variety SPC had significantly ($p<0.05$) highest zinc content followed by ANN 16 while variety AMZ had lowest zinc content. At stage II of maturity AMA 38 had highest zinc content while variety AMZ had lowest zinc levels. At the same stage, varieties ANN 16 and ANO 49 had very little difference in their zinc contents. At maturity stages II and III there was no great difference in zinc contents of all varieties as compared to the concentrations of stage I (Fig. 7).

Results from ANOVA revealed that at maturity stage I varieties ANO 49 and AMA 38 had statistically same zinc contents while other three varieties (ANN 16, SPC and AMZ) had significantly different ($p< 0.05$) zinc contents. At maturity stage II, the zinc contents of varieties ANN 16, ANO 49 and SPC were statistically not different. At maturity stage III, zinc contents of all five varieties were statistically the same (Fig. 7). Varieties SPC, AMA 38 and ANO 49 had highest zinc contents at the three maturity stages (77.9 ± 0.4mg/100g, 54.0 ± 0.0 mg/100g and 44.7 ± 0.0 mg/100g at maturity stages I, II and III respectively) while lowest values were 46.0 ± 0.9 mg/100g, 46.0 ± 0.8 mg/100g and 37.3 ± 0.0 mg/100g at maturity stages in I, II and III respectively in variety AMZ (Fig. 7 and Appendix 3).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 7: Zinc content (mg/100g DM) in different vegetable varieties within the stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

The statistically same zinc contents among different varieties suggest that the plants have same root system and hence equal efficiency in exploiting and taking up nutrients from the soil (Welch and Graham, 2004).

Other factors which influence concentration of trace minerals by vegetables include the absorption area of the roots/leaves (Sadeghzadeh, 2013). The larger the absorption area the higher is the effective uptake. Increased biomass production increases the uptake of elements.

4.3 Effect of Maturity Stage and Variety on Anti-nutritional Factors of the Vegetables

4.3.1 Oxalic acid content

4.3.1.1 Effect of maturity stage on oxalic acid content

Variation of oxalic acid concentration in the vegetable samples at the different maturity stages are presented on Fig. 8. The concentration of oxalic acid increased from maturity stage I to stage II in all varieties. Oxalic acid concentration continued to increase to stage III of maturity in ANN 16, ANO 49 and AMZ. In varieties SPC and AMA 38 oxalic acid contents decreased from stage II to stage III (Fig. 8).

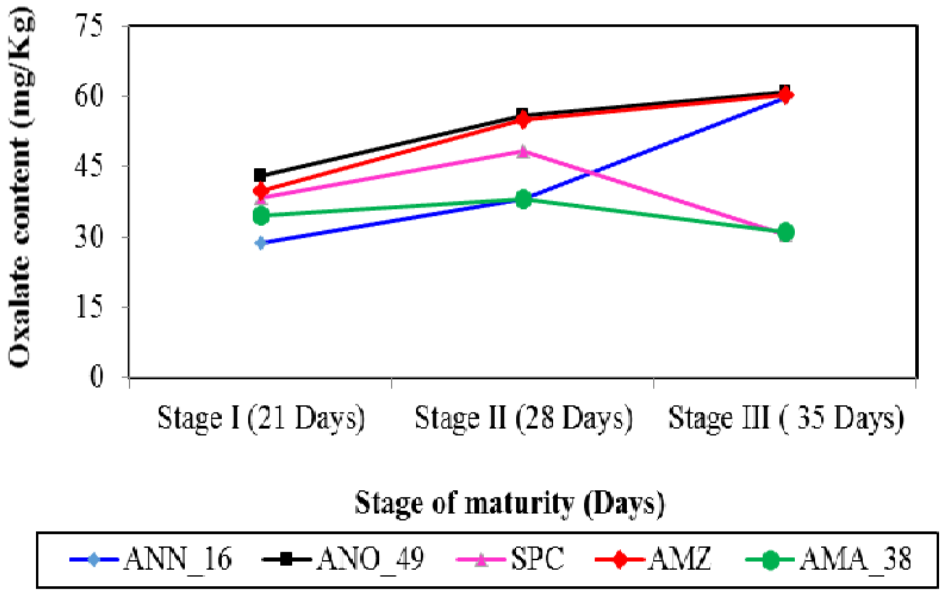


Figure 8: Oxalic acid content (mg/100g DM) in different vegetables at different stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM)

Table 4 shows the results of ANOVA for comparison of mean concentration of oxalate in individual varieties of vegetables at different stages of maturity. The results showed an increase of oxalic acid concentration with plant age from maturity stage I to stage III in ANN 16 (from 28.7 mg/100g to 59.6 mg/100g), in ANO 49 (from 42.9 mg/100g to 60.3mg/100g) and in AMZ (from 39.9 mg/100g to 60.3 mg/100g) respectively. In SPC and AMA 38, the concentration of oxalic acid increased from maturity stage I to stage II (from 38.4 ± 1.7 mg/100g to 48.3 ± 1.5 mg/100 g) in SPC and from 34.6 ± 0.6mg/100g to 38.1 ± 0.3 mg/100g in AMZ) respectively but decreased at maturity stage III (30.5 ± 0.5 mg/100g in SPC and 31.1 ± 1.2 mg/100g in AMA38). When the mean values were separated by using Tukey's Honest Test it was revealed that oxalate concentration in ANN 16 at maturity stages I and II was statistically equal ($p < 0.05$) while at stage III was statistically different from the preceding stages ($p < 0.05$). In addition, ANO 49SPC and AMZ indicated insignificant difference of oxalate concentration at maturity stages I, II and III ($p < 0.05$).Results further show that variety AMA 38hadno significant difference in oxalic acid concentration at maturity stages I, III ($p < 0.05$); but a significant different ($p < 0.05$) concentration at maturity stage II (Table 4).

Table 4: Oxalic acid content (mg/100 DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	Stage II	Stage III
ANN 16	28.7 ± 0.0^{c}	38.2 ± 1.5^{c}	59.6 ± 0.3^{a}
ANO 49	42.9 ± 0.2^{a}	55.9 ± 1.2^{a}	60.9 ± 0.9^{a}
SPC	38.4 ± 1.7^{b}	48.3 ± 1.5^{b}	30.5 ± 0.5^{b}
AMZ	39.9 ± 0.8^{a}	54.9 ± 0.5^{a}	60.3 ± 0.5^{a}
AMA 38	34.6 ± 0.6^{b}	38.1 ± 0.3^{c}	31.1 ± 1.2^{b}

Data presented as arithmetic means ± SD (n = 3).

Means within the vegetable variety in row with different superscript letters are significantly different at $p < 0.05$.

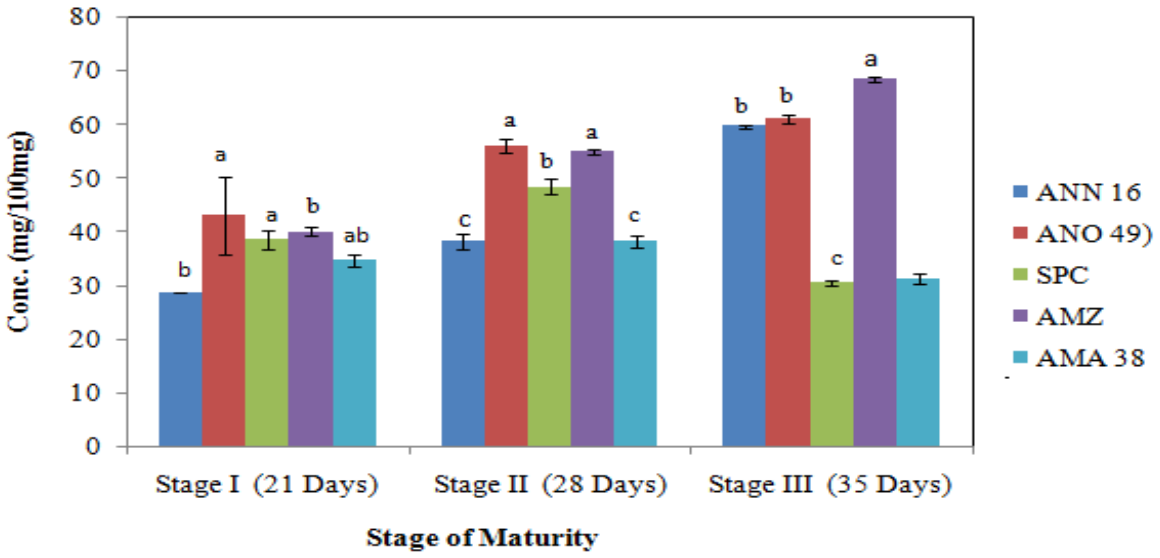
These results resemble the findings of Kitchen and Burns (2006) who found that maximum total oxalate content in dark green bloomsdale was high at 32 days after planting and decreased subsequently as the plants developed towards the vegetative phase. Amanabo *et al*. (2011) also reported an increase in total oxalic acid content in *Amaranths cruentus* from 4.40 ± 0.19 mg/100g at market maturity stage (vegetative stage) to 5.27 ± 0.24 mg/100g at heading (reproductive stage). Abbasi *et al.* (2011) reported that oxalic acid content in amaranth forage decreased from 18.9 g/Kg at 40 days after planting to 10.2 g/Kg dry matter at 60 days after planting.

4.3.1.2 Effect of variety on oxalic acid content

Fig. 9 presents variation of oxalic acid among the five vegetable varieties at each maturity stage. At maturity stage I, variety ANN 16 had lowest oxalic acid content while variety ANO 49 had highest oxalic acid content. At stage II, Varieties ANN16 and AMA 38 had almost same and lowest levels of oxalic acid while variety ANO 49 had the highest concentration of oxalic acid. At stage III of maturity, SPC had the lowest oxalic acid content and variety AMZ had highest concentration of the ant- nutritional factor.

Results from the ANOVA indicate that although all varieties had different oxalic acid concentrations at maturity stage I, varieties ANO 49 and SPC had no significantly different concentrations (Figure 9). Likewise varieties ANN16 and AMZ had statistically same oxalic acid contents at stage I. At stage II, there was no significant difference in oxalic acid contents of varieties ANN16 and AMA 38. Furthermore, at the same stage, varieties ANO 49 and AMZ had statistically same concentration of oxalic acid. At stage III of maturity, varieties SPC, AMZ and AMA 38 had statistically different oxalic acid concentrations while varieties ANN 16 and ANO 49 were statistically not different (Fig. 9). The highest oxalic acid contents were in sample ANO49 within maturity stages I and II

(42.9±0.2mg/100g and 55.9±1.2 mg/100g) and in sample AMZ within stage III (68.3±0.6 mg/100g). Sample ANN16 had lowest oxalate concentrations of 28.7±0.0mg/100g at maturity stage I while sample AMA 38 had lowest oxalic acid contents of 38.1±0.3 mg/100 g and 38 31.1±1.2 mg/100g at maturity stages II and III respectively (Fig. 9 and Appendix 4).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 9: Oxalic acid content (mg/100 DM) in different vegetable varieties within the stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

Several studies support the findings of the current investigation that GLVs contain oxalic acid in varied concentrations depending on the varieties (Nangula *et al*., 2010; and Gupta *et al*., 2005).

4.3.2 Phytic acid content

4.3.2.1 Effect of maturity stage on phytic acid content

Fig. 10 shows variation of phytic acid concentration at the three maturity stages. Phytic acid concentration increased with plant maturity in all vegetable varieties. The increase was markedly higher in AMA 38 from maturity stage II to stage III as compared to other varieties (Fig. 10).

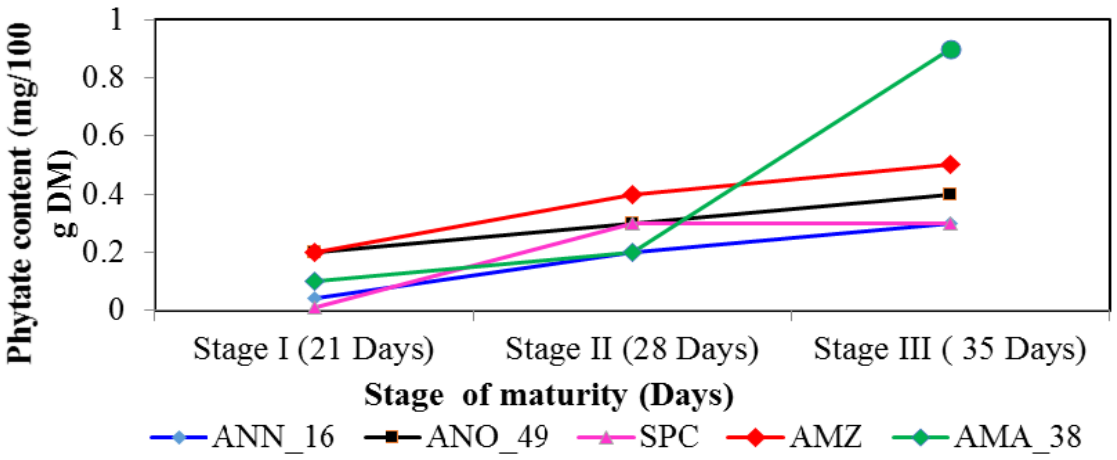


Figure 10: Phytic acid content (mg/100g DM) in different vegetables at different stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM)

The overall results of ANOVA show that phytic acid content increased progressively between stages of maturity in all varieties (Table 5). When the mean values were separated by using Tukey's Honest Test it was revealed that the increases in phytate content in the subsequent stages of maturity were statistically significant ($p < 0.05$) in varieties ANO 49, AMZ and AMA 38 at 95% alpha level. The concentration of phytic acid in SPC at stage I was statistically different from those of stages II and III ($p < 0.05$); but difference in

concentration at stage II and III of maturity was not significant ($p < 0.05$). The lowest phytic acid contents were0.08 ± 0.0 mg/100g in ANN 16, 0.2 ± 0.0 mg/100g in AMA 38 and ANN 16 and 0.3 ± 0.0 mg/100g in SPC at maturity stages I, II and III respectively while the highest values were 0.2 ± 0.0 mg/100g in ANN 49 and AMZ, 0.4 ± 0.0 mg/100g in AMZ and 0.9 ± 0.0 mg/100g in AMA 38 (Table 5).

Table 5: Phytic acid content (mg/100 DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN16	0.08 ± 0.0^{c}	0.2 ± 0.0^{b}	0.3 ± 0.1^{a}
ANO 49	0.2 ± 0.0^{c}	0.3 ± 0.0^{b}	0.4 ± 0.0^{a}
SPC	0.01 ± 0.0^{b}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
AMZ	0.2 ± 0.0^{c}	0.4 ± 0.0^{b}	0.5 ± 0.0^{a}
AMA 38	0.1 ± 0.0^{c}	0.2 ± 0.0^{b}	0.9 ± 0.0^{a}

Data presented as arithmetic means ± SD (n = 3).

Means within the vegetable variety in row with different superscript letters are significantly different at $p < 0.05$.

On the contrary, to these results, Agbaire (2012) found higher phytic acid levels of 4.12mg/100g and 7.39 mg/100g in *Amaranths* and *Solanum* species respectively at market maturity. These differences may be attributed to different harvesting periods which were defined as “market maturity”.

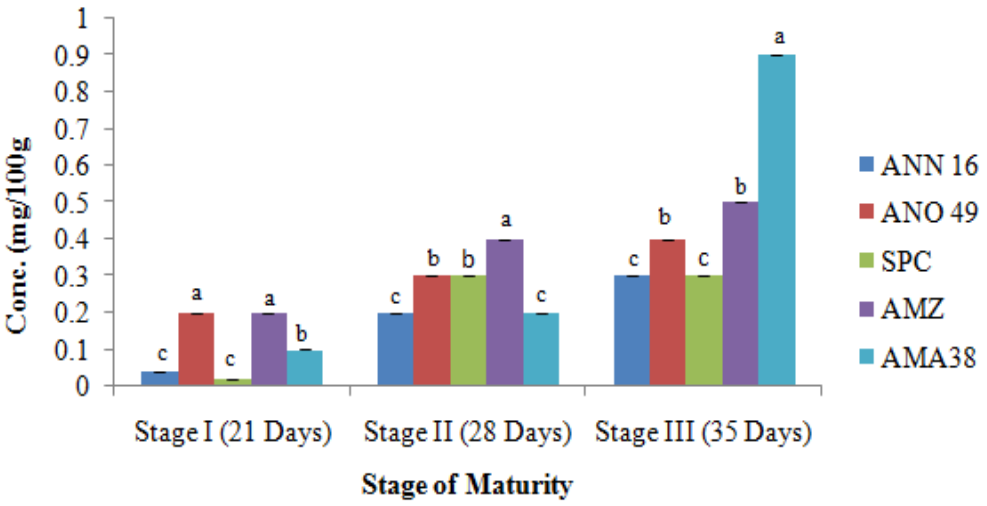
Earlier studies (Muhammad *et al.*, 2010) indicate that phytic acid is broken down by enzyme phytase during seed germination and its concentration increases during seed formation (Raboy *et al.*, 1991).This could explain the increase in phytic acid with plant

maturity in the current study. This implies that maturity stage I was the most appropriate for harvesting the vegetables.

4.3.2.2 Effect of variety on phytic acid content

The effect of variety on phytic acid content within maturity stage is illustrated on Fig.11. At maturity stage I SPC had lowest phytic acid content while variety AMZ had highest levels of phytic acid. At stage II AMA38 had lowest phytic acid levels while AMZ had highest levels of the anti-nutrient. At maturity stage III the lowest and highest phytic acid contents were observed in varieties SPC and AMA 38 respectively (Fig. 11).

At maturity stage I the concentration of phytic acid in varieties ANN 16 and SPC was not significantly different ($p<0.05$). Statistically same concentrations of phytic acid at this stage were also observed in variety ANO 49 and AMZ. At stage II of maturity, varieties ANN 16 and AMA 38 had statistically equal concentrations of phytic acid ($p<0.05$). Varieties ANO 49 and SPC as well had statistically same concentrations at stage II. At maturity stage III varieties ANN 16 and SPC had no significant difference ($p<0.05$) in phytic acid concentration. Likewise, varieties ANO 49 and AMZ had statistically same phytic acid concentrations. At maturity stage I, the highest phytic acid content was 0.2 ± 0.0mg/100g in ANO49 and AMZ whereas the lowest value was 0.08 ± 0.0mg/100g in ANN 16. At maturity stage II AMZ had highest phytic acid value of 0.4 ± 0.0 mg/100g in AMZ while ANN 16 and AMA 38 had lowest phytic acid value of 0.2 ± 0.0 mg/100g at this stage. At maturity stage III, highest phytic acid value was 0.9 ± 0.0 mg/100g in AMZ and the lowest phytic acid content was 0.3 ± 0.0 mg/100g in SPC and ANN16 (Figure 11 and Appendix 5).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 11: Phytic acid content (mg/100 DM) in different vegetable varieties within the stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM 38)

These results are closely related to findings by Gupta *et al*. (2005) who found higher levels of 1.95 mg/100g phytic acid content in *Amaranthus tricolor* at market maturity. Significant variation of phytic acid content among different varieties were also reported by Hossain and Becker (2001) who found that three varieties of Sesbania seeds (*S. aculeate, S. rostrata and S. sesban*) had 0.28 g/100g, 0.16 g/100g and 0.39 g/100g phytic acid content respectively. Phytic acid varies not only among varieties but also among different cultivars of the same variety (Purvika *et al*. 2012).

4.3.3 Nitrate content

4.3.3.1 Effect of maturity stage on nitrate content

Results on nitrate concentration in the vegetables under study at the three maturity stages are presented in Fig. 12. Nitrate content decreased continuously from maturity stages III and I to III in all vegetables except variety ANN 16 which showed almost same nitrate concentration at maturity stages II. Fig. 12 further shows that nitrate content in variety AMA 38 decreased more rapidly when compared with other varieties.

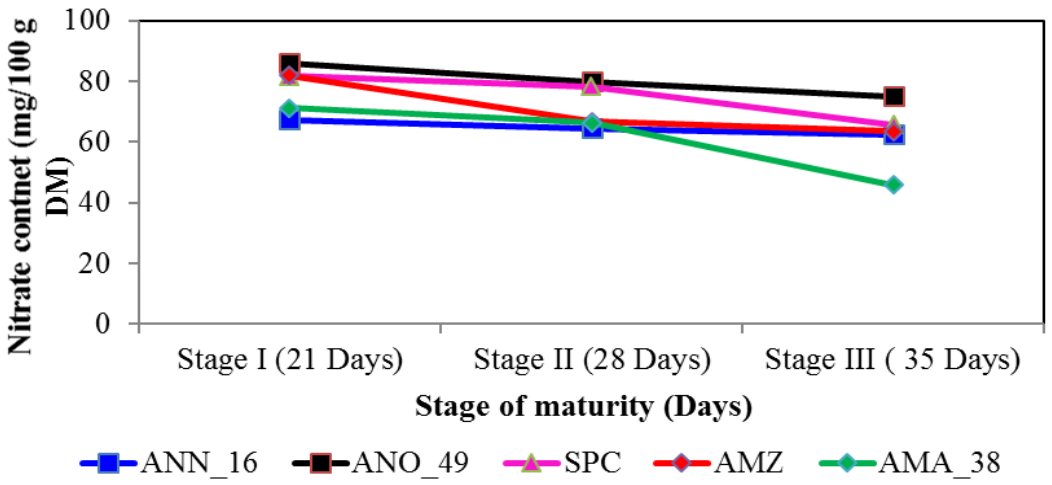


Figure 12: Nitrate content (mg/100 DM) in different vegetables at different stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM)

The overall results of ANOVA for all five vegetable varieties are shown in Table 6. The concentration of nitrate in at least one of the three stages of maturity was statistically different from others ($p < 0.05$). When the mean values were separated by using Tukey's Honest Test it was revealed that the concentrations of nitrate in ANN 16 during stages I and III were different and the difference was statistically significant at 95% level of

confidence ($p < 0.05$). However for the same variety (ANN16), the concentration of nitrate at stage II of maturity was not significantly different from those of stage I and II respectively ($p > 0.05$). As regards to the variety ANO 49, the concentration at stage I was statistically different ($p < 0.05$) from those of stage II and III but for stages II and III it was not statistically different. As for the remaining varieties of SPC,AMZ; and AMA 38, the concentrations of nitrate at stages I and II were not statistically different from each other ($p > 0.05$); but in stage III it was statistically different from those of stage I and II. The highest levels of nitrate concentration at any maturity stage was observed in ANO 49 (86.1 ± 1.1mg/100g, 79.9 ± 0.3mg/100g and 75.0 ± 1.1 mg/100g) at maturity stages I, II and III respectively while lowest nitrate contents were in ANN 16 (67.3 ± 0.7 mg/100 g and 64.5 ± 0. 8 mg/100 g) at maturity stages I and II respectively and in AMA 38 (45.8 ± 0.6 mg/100g) (Table 6).

Table 6: Nitrate content (mg/100 DM) in different vegetables at different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	67.3 ± 0.7^{b}	64.5 ± 0.8^{ab}	62.5 ± 0.7^{c}
ANO 49	86.1 ± 1.1^{a}	79.9 ± 0.3^{b}	75.0 ± 1.1^{b}
SPC	81.7 ± 0.9^{a}	78.1 ± 0.1^{a}	65.8 ± 1.6^{b}
AMZ	81.9 ± 0.4^{a}	66.7 ± 1.0^{b}	63.5 ± 0.7^{c}
AMA 38	71.2 ± 0.1^{a}	66.4 ± 0.6^{b}	45.8 ± 0.6^{c}

Data presented as arithmetic means ± SD (n = 3).

Means within the vegetable variety in row with different superscript letters are significantly different at ($p < 0.05$)

Similar pattern of variation was also observed by Musa *et al.* (2011) who found that nitrate content of *Amaranthus cruentus* decreased from 17.71 mg/100g at market maturity to 7.62

mg/100g at vegetative stage. Abbasi *et al*. (2011) as well found that oxalic acid content in amaranth forage decreased from18.9 g/kg dry mater at 40 days after planting to 10.2 g/kg dry matter at 60 days after planting.

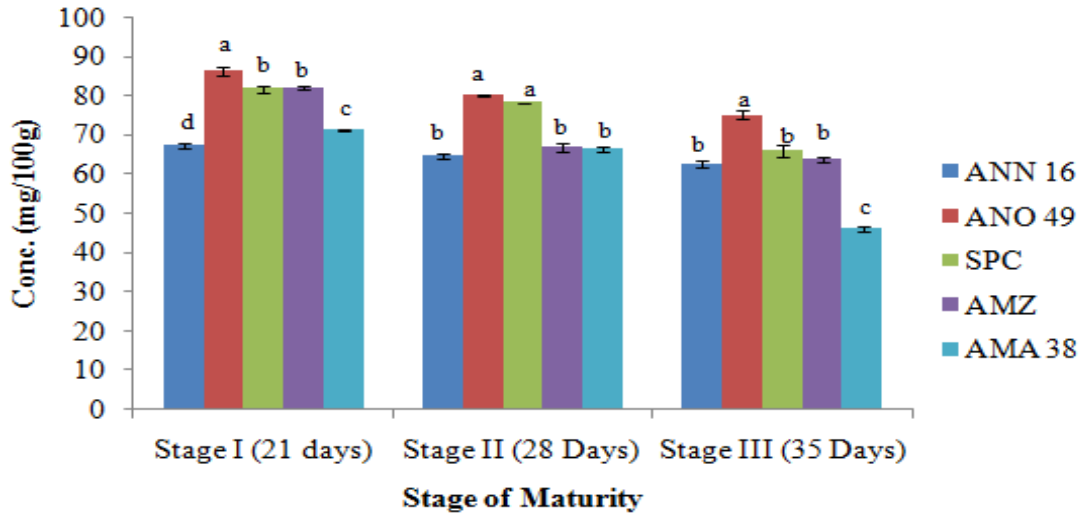
Nitrate is mainly found in cell vacuoles and is transported in the xylem. The xylem carries water and nutrients from the roots to the leaves, whereas the phloem carries the products of photosynthesis from the leaves to the growth points of the plant (Greenwood and Hunt, 1986). This transport system causes the younger leaves to have higher nitrate content than older leaves and storage organs.

4.3.3.2 Effects of variety on nitrate content

Variation of nitrate content among different varieties within each maturity stage is depicted on Fig. 13. Variety ANO 49 had highest nitrate concentrations at all three maturity stages. Lowest nitrate contents were in variety ANN 16 at stage I and II and variety AMA 38 at maturity stage III (Fig.13).

Results from the ANOVA revealed that, at maturity stage I, three varieties ANN 16, ANO 49 and AMA 38 had significant different nitrate contents while varieties SPC and AMZ had no statistically different ($p< 0.05$) nitrate contents. At stage II, varieties ANN16, AMZ and AMA 38 had no significant differences ($p< 0.05$) in their nitrate content. However, these three varieties had significantly lower nitrate content than the other two varieties (ANO 49 and SPC). At maturity stage III varieties ANN 16, SPC and AMZ had no statistically significant ($p > 0.05$) differences in their nitrate contents (Fig.13). Variety ANO 49 had highest nitrate contents of 86.1 ± 1.1 mg/100g, 79.9 ± 0.3 mg/100g and 75.0 ± 1.1 mg/100g at maturity stages I, II and III respectively. Lowest nitrate values were 67.3 ± 0.7 mg/100g and 64.5 ± 0.8 mg/100g in ANN 16 at maturity stages I and II

respectively and in AMA 38 at maturity stage III. The best variety with lowest nitrate content of 45.8 ± 0.6 mg/100g was AMA 38 while the worst variety ANO 49 had highest nitrate contents of 86.1 ± 1.1mg/100g was irrespective of stage of maturity (Fig. 13 and Appendix 6).



Values are expressed as mean ± SD (n=3)

Bars with different letters are significantly different at $p < 0.05$)

Figure 13: Nitrate content (mg/100 DM) in different vegetable varieties within the stages of maturity; ANN 16=African nightshade (Nduruma BG 16), ANO 49 = African nightshade (Olevolosi SS 49), SPC= Spider Plant (Cleome gynandra), AMZ= Amaranths (AMZ, and AMA 38 =Amaranths (Madiira AM)

Great variation in nitrate content among 23 cultivars of endive (*Cichorium endiviae L.*) was also observed by Le Bot and Kirkby (1992). Another study on nitrate contents of green leafy vegetables namely radish, palak and amaranth revealed different concentrations among the varieties (Jana and Moktan, 2012).

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The results of this study confirm that maturity stage at which green leafy vegetables are harvested highly affect their nutritive value. Vitamin C contents in the vegetables investigated increased significantly with maturity whereas variety Spider plant had highest vitamin C content. Therefore for optimal content of vitamin C the vegetables should be harvested during maturity stage III.

Iron content increased with plant age in all samples implying that vegetables should be harvested at older stages and variety Amaranths Madiira EX Zim had the highest iron content. Zinc contents decreased with plant age in all samples and Spider plant was the best variety in zinc content.

The concentration of anti-nutritional factors (oxalic acid and phytic acid) in the vegetables increased with plant age. This signifies that harvesting the vegetables under study at maturity stage I would reduce the level of these anti-nutritional factors. Concentrations of nitrate decreased with plant maturity which implies that harvesting the vegetables in the current study at older maturity stages would have lower levels of nitrate.

5.2 Recommendations

Given the above observations, it is recommended that:

i. When the primary objective of an intervention is to enhance vitamin C intake in target population, then amaranths Madiira EX Zim, African nightshade Nduruma

BG 16 and Olevolossi SS 49 and Spider plants should be harvested at maturity stage III when their vitamin C contents are at maximum levels.

ii. When the vegetables are needed for alleviating iron deficiency, they should be harvested at older stages since iron concentration increased as the vegetables matured. On the contrary the vegetables should be harvested at early stages of maturity when the objective is to alleviate zinc deficiency

5.3 Prospects for Future Studies

Since the current study has confirmed that both nutrient (vitamin C and Iron) and anti-nutritional factors (oxalic acid and phytic acid) increase as vegetables mature, further research should be conducted to investigate a good combination of Amaranths, African nightshade and Spider plant that may bring balanced effects of nutrients and anti-nutritional factors at any maturity stage.

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APPENDICES

Appendix 1: Vitamin C contents (mg/100 g DM) in different vegetable varieties within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN16	49.2 ± 0.0^{b}	49.2 ± 0.0^{d}	115.5 ± 11.6^{c}
ANO 49	29.0 ± 1.5^{c}	41.5 ± 1.3^{e}	105.7 ± 1.6^{cd}
SPC	31.8 ± 1.8^{c}	76.0 ± 2.0^{b}	162.7 ± 1.8^{a}
AMZ	51.6 ± 1.2^{b}	105.5 ± 1.0^{a}	134.6 ± 3.5^{b}
AMA 38	67.1 ± 2.0^{a}	71.2 ± 1.1^{c}	100.4 ± 0.6^{d}

Data presented as arithmetic means ± SD (n = 3)

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p<0.05$.

Appendix 2: Iron contents (mg/100 g DM) in different vegetable variety within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN16	273.5 ± 0.8^{d}	345.5 ± 0.5^{c}	853.0 ± 0.8^{b}
ANO 49	231.1 ± 1.5^{e}	411.4 ± 0.4^{b}	851.0 ± 0.6^{b}
SPC	363.3 ± 3.3^{b}	409.2 ± 3.6^{b}	485.7 ± 3.3^{d}
AMZ	733.2 ± 0.3^{a}	845.9 ± 0.6^{a}	999.0 ± 3.7^{a}
AMA 38	291.8 ± 0.6^{c}	313.2 ± 0.8^{d}	526.0 ± 1.3^{c}

Data presented as arithmetic means ± SD (n = 3)

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p<0.05$.

Appendix 3: Zinc contents (mg/100 g DM) in different vegetable variety within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	72.0 ± 1.8^{b}	54.0 ± 0.0^{b}	43.5 ± 0.9^{b}
ANO 49	58.4 ± 0.9^{c}	53.7 ± 0.4^{b}	44.7 ± 0.9^{b}
SPC	77.9 ± 0.4^{a}	51.6 ± 0.9^{b}	39.2 ± 0.0^{b}
AMZ	46.0 ± 0.9^{d}	46.0 ± 0.8^{c}	37.3 ± 0.0^{b}
AMA 38	62.0 ± 0.9^{c}	57.7 ± 0.9^{a}	39.8 ± 0.9^{b}

Data presented as arithmetic means ± SD (n = 3).

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p < 0.05$.

Appendix 4: Oxalic acid content (mg/100 g DM) in different vegetable variety within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	28.7 ± 0.0^{b}	38.2 ± 1.5^{c}	59.6 ± 0.3^{b}
ANO 49)	42.9 ± 7.2^{a}	55.9 ± 1.2^{a}	60.9 ± 0.9^{b}
SPC	38.4 ± 1.7^{a}	48.3 ± 1.5^{b}	30.5 ± 0.5^{c}
AMZ	$39.9 \pm 0.8a^{b}$	54.9 ± 0.5^{a}	68.3 ± 0.6^{a}
AMA 38	34.6 ± 0.6^{ab}	38.1 ± 0.3^{c}	31.1 ± 1.2^{c}

Data presented as arithmetic means ± SD (n = 3)

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p < 0.05$.

Appendix 5: Phyatic acid contents (mg/100 g DM) in different vegetable variety within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	0.04 ± 0.0^{c}	0.2 ± 0.0^{c}	0.3 ± 0.0^{c}
ANO 49	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.4 ± 0.0^{b}
SPC	0.02 ± 0.0^{c}	0.3 ± 0.0^{b}	0.3 ± 0.0^{c}
AMZ	0.2 ± 0.0^{a}	0.4 ± 0.0^{a}	0.5 ± 0.0^{b}
AMA38	0.1 ± 0.0^{b}	0.2 ± 0.0^{c}	0.9 ± 0.0^{a}

Data presented as arithmetic means ± SD (n = 3)

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p < 0.05$.

Appendix 6: Nitrate contents (mg/100 g DM) in different vegetable variety within the different stages of maturity

Vegetable	Stage of Maturation		
	Stage I	**Stage II**	**Stage III**
ANN 16	67.3 ± 0.7^{d}	64.5 ± 0.8^{b}	62.5 ± 0.7^{b}
ANO 49	86.1 ± 1.1^{a}	79.9 ± 0.3^{a}	75.0 ± 1.1^{a}
SPC	81.7 ± 0.9^{b}	78.1 ± 0.1^{a}	65.8 ± 1.6^{b}
AMZ	81.9 ± 0.4^{b}	66.7 ± 1.0^{b}	63.5 ± 0.7^{b}
AMA 38	71.2 ± 0.1^{c}	66.4 ± 0.6^{b}	45.8 ± 0.6^{c}

Data presented as arithmetic means ± SD (n = 3)

Means between the vegetable variety within the column (stage) with different superscript letters are significantly different at $p < 0.05$.