NUTRIENTS, ANTI-NUTRIENTS AND PHYTOCHEMICAL EVALUATION OF TEN VEGETABLE AMARANTH (*Amaranthus spp*.) VARIETIES AT TWO STAGES OF GROWTH

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Nutrients, Anti-Nutrients and Phytochemical Evaluation of Ten Vegetable Amaranth (*Amaranthus Spp*.) Varieties at Two Stages of Growth

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This thesis is dedicated to my parents, Mr. and Mrs. Nyonje, and my brothers and sisters.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ACRONYM

AOAC	Association of Official Analytical Chemists
DPPH	2, 2-Diphenyl-1-picryl hydrazyl
H1	Vegetative stage
H2	Post flowering stage
HPLC	High Performance Liquid Chromatography
IC_{50}	Equivalent concentration/ concentration at 50% inhibition
JKUAT	Jomo Kenyatta University of Agriculture and Technology
mL	Milliliters
N	Normality
n	Sample size
^{o}C	Degree centigrade
QE	Quercetin Equivalent
rpm	Revolutions per minute
TAE	Tannic Acid Equivalent

ABSTRACT

Vegetable amaranth is widely consumed in Kenya and contributes to the alleviation of food insecurity and malnutrition. Malnutrition is a major challenge facing Kenya in particular and Africa as a whole and the most affected are women and children. Vegetable amaranth has been shown to contain many nutrients and can contribute to the alleviation of malnutrition, and is also reported to have phytochemicals with antioxidant activity that help protect the body from long-term degenerative diseases. However, amaranth vegetable has also been shown to contain some anti-nutrients which may bind nutrients and reduce their bioavailability in the body. There hundreds of amaranth varieties in existent, and new varieties are still being produced, but there is very little information about differences in the nutrients, anti-nutrient and phytochemical levels among the varieties. The objective of this study was to determine the nutrients, anti-nutrient and phytochemical content of ten vegetable amaranth varieties at two growth stages, before and after flowering. The study was carried out in JKUAT. Ten amaranth varieties, eight of which were new varieties, were planted in the JKUAT farm. Harvesting was done at vegetative stage and post flowering stage. The leaves were analyzed for nutrients, anti-nutrients and phytochemicals including total flavonoids, carotenoids and total antioxidant activity. There were significant differences ($P \leq 0.05$)in the nutrient content among the amaranth varieties and at the two stages of maturity. Similarly there was also significant variation ($P \leq 0.05$) in antinutrient and phytochemicals among the ten amaranth varieties and between the maturity stages with the total antioxidant activity increasing significantly at post flowering stage. It was established that various components vary differently as the plant grows older. Variety 5 *(Amaranthus hybridus)*, variety 7 *(Amaranthus dubius)* and variety 8 *(Amaranthus hypocandracus)* were found to be highest in minerals, while variety 5 *(Amaranthus hybridus)*, variety 6 *(Amaranthus hybridus)*, variety 8 *(Amaranthus hypocandracus)* and variety 10 *(Amaranthus dubius)* are the highest in proteins and vitamins.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Amaranth (*Amaranthus spp.*) is one of the Africa leafy vegetables from the family *Amaranthaceae*, and its origin is in Africa and the Latin Americas. It is herbaceous annual crop cultivated for both its leaves which are used as a vegetable and its seeds which are used as a grain. It is also often found growing wild. Amaranth, just as other African Leafy Vegetables (ALV), has been a common food to many African and Kenyan households for a long time. Some of its vernacular names in Kenya include *Ododo* (Luo), *Mchicha* (Swahili) *Terere* (Kikuyu) *Chimboka* (Luhya), *Ekwala* (Teso), *Lookwa* or *Epespes* (Turkana); (Jacob, 2005).

Amaranth vegetables are an important component of the diet as they provide important nutrients, and have been reported to be an excellent source of carotene, iron, calcium, protein and Vitamin C among other nutrients (Schippers, 2000; Janick, 1997; Kauffman and Weber, 1990; Mnkeni, 2005). They have been showed to contain a significant energy value ranging from 27 to 53 kcal/100 g of fresh leaves (Uusikua, 2010). When compared to spinach, leaves of *Amaranthus* contain 3 times more vitamin C, calcium, iron and niacin (Makus, 1984; Mnkeni, 2005). It contains 18 times more beta carotene, 20 times more calcium and 7 times more iron when compared to lettuce (Mnkeni 2005).

However, vegetable amaranth is also known to accumulate various anti-nutrients that might bind the nutrients, reducing their bioavailability in the body. These include phytates, nitrates, tannins and oxalates. Oxalate and phytate are known to chelate mineral elements in the body making them unavailable to the body. Oxalate in combination with calcium leads to the formation of insoluble calcium oxalates which are precipitated and deposited in the kidney to form kidney stones (Prien, 1991). High level of nitrates in vegetables when ingested can be converted to nitrite which can lead

to cancer and metheamoglobinemia or blue-baby disease (Gupta *et al.*, 2000; Macrae, *et al.*, 1997; Oguchi *et al.*, 1996; Takebe and Yoneyame, 1997). Tannins precipitate proteins from aqueous solution by inhibiting its digestive enzymes (Soetan and Oyewole, 2009).

Vegetable amaranths also contain phytochemicals including carotenoids, flavonoids and ascorbic acid that help protect the body from long-term degenerative diseases (Raheena, 2007). The vegetable has been reported to have a high concentration of antioxidant components (Hunter & Fletcher, 2002), and have anti-inflammatory properties (Olumayokun *et al.* 2004). Some anti-nutritional phytochemicals in amaranth such as tannins and phytic acid also exhibit protective effects, thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders (Aletor and Adeogun 1995).

Approximately 60 species of amaranth are recognized, with inflorescences and foliage ranging from purple and red to green or gold. They provide edible leaves while others also provide grains as well (Grubben, 2004). They are also used as ornamentals while others are just weeds (He *et al*, 2002; Kauffman and Weber, 1990). Over 400 varieties and cultivars of amaranth, both vegetable and grain are in existence. Further, new varieties, with unknown nutritional, anti-nutrients and phytochemical characteristics are still being produced.

The objective of this study was therefore to determine the nutrients, anti-nutrient and phytochemical content of ten vegetable amaranth varieties at two stages of growth. In this study, ten different amaranth vegetable varieties under the species *A. blitum, A. hybridus, A. dubius and A. hypochondriacus* were studied. Eight of these varieties are new varieties developed through selection and are yet to be registered by the government, while two are standard varieties from local seed companies, but little is known about their nutritional components.

1.2 Problem statement

Malnutrition has been a major problem in Kenya for a long time. This is mainly protein energy malnutrition, but there is also public health concern of high prevalence of some micronutrient deficiency conditions. According to KENRIK (2004), small children and women in child bearing age are worst hit by protein, calcium, iron, vitamin A, and vitamin C malnutrition. About 60% of children in Kenya below 5 years suffer iron deficiency anemia. According to World Health Organization, in developing countries, every second pregnant woman and about 40% of preschool children are estimated to be anemic. In many developing countries, iron deficiency is aggravated by worm infections, malaria and other infectious diseases like tuberculosis. The major health consequences include poor pregnancy outcome, impaired physical and cognitive development, increased risk of morbidity in children and reduced work productivity in adults. Anemia also contributes to 20% of all maternal deaths. Amaranth vegetable has been documented as a nutritious vegetable and can help in the fight against malnutrition. There are many amaranth varieties in Kenya. Knowledge of the nutritional contents of various varieties of amaranth at different growth stages is an essential factor towards deciding the best stage of harvest for this plant, and also to advise the community on the varieties with the highest nutritional content.

Anti-nutrients are generally present in foods, especially vegetables. Most of them reduce the bioavailability of nutrients. Amaranth is documented to contain various anti-nutrients including tannins, phytates, oxalates and nitrates. Tannins bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids, reducing their bioavailability in the body. Phytate is not digestible to humans; it also chelates and makes unabsorbable certain minerals such as zinc, iron, calcium and magnesium. Oxalates bind calcium making it unavailable for absorption. High oxalate levels may also interfere with carbohydrate metabolism. Nitrates in amaranth leaves are also of concern since it is hypothesized that nitrates may be chemically changed in the

digestive tract into poisonous/carcinogenic nitrosamines. High level of nitrates in vegetables when ingested can be converted to nitrite which can lead to cancer and metheamoglobinemia or blue-baby disease (Gupta *et al*., 2000).

According to Sudhir *et al* (2003), various amaranth varieties contain different amounts of nutrients, anti-nutrient and phytochemicals at different growth stages. There are newly developed varieties of amaranth with unknown nutrient, anti-nutrient and phytochemical contents. Yet there are very few documented studies on the nutrient, anti-nutrient and phytochemical composition of these varieties.

1.3 Justification

Leafy vegetables play a vital role in human wellbeing. It has been established that greens contribute significantly to the daily dietary requirements of calcium and iron among children. Dark green vegetables have been suggested to be significant sources of vitamin A and other nutrients in Africa (Faber *et al.* 2007; Oiye *et al*. 2009; Tchum *et al*., 2009). There is need for diversification of foods and diet to ensure good health and prosperity. In Kenya and even worldwide, many people are conscious about their health status. This propels a need for in-depth studies and improvement of nutritious foods so as to improve and support the people's health status. Amaranth and other African indigenous vegetables become important sources of nutrients to achieve this goal.

Leafy vegetables including amaranth serve as the main source of mineral nutrients, particularly in resource-poor households in low-income countries, since intake of dietary supplements is low (Wambugu and Muthamia, 2009). Amaranth leaves are almost nutritionally similar to beets, Swiss chard and spinach, but are much superior in some nutrients, for example amaranth leaves contain three times more calcium and three times more niacin (Vitamin B3) than spinach leaves (Mnkeni, 2005; Makus, 1984). Amaranth leaves are an excellent source of carotene, iron, calcium, protein, Vitamin C and trace element (Schippers, 2000; Janick, 1997; Kauffman and Weber,

1990; Mnkeni, 2005). Minerals are of great importance in the body, for instance, Ca is important for lactating Mothers in stimulating the milk let-down during breast feeding. Calcium is also essential for skeletal development and helps in the enhancement of strong bones and teeth and therefore recommended especially for infants. Iron is important for red blood cell and a deficiency result into anemia. Vitamin C promotes iron absorption from the small intestine. Iron content of foods is the most important constituent of forming hemoglobin (Olumakaiye, 2011). Iron is also essential for ladies due to the fact that it's a component of hemoglobin hence aiding in restoration of blood lost during birth and menstruation periods. Zinc is required in many enzymatic reactions where it acts as a catalyst and also boosts the human immunity.

Several studies have shown that amaranth is a functional food and contains compounds that help in improving antioxidant status of humans and some immune parameters. In traditional medicine amaranth is especially recommended for people with a low red blood cell count.

Kenya has varying climatic conditions across the country. This, coupled with inefficient food distribution channels has resulted in the existence of different food crops in the different regions. People in a given location become accustomed to the food crops available, and this contributes significantly to their nutritional status. Amaranth grows and matures very fast. Leaves can be harvested within two weeks after sowing, while grains mature in 45-75 days (Jacob, 2005).

Amaranth, being a considerably hardy plant requires minimal inputs in terms of fertilization and other inputs (Shukla and Singh, 2000; Katiyar *et al.*, 2000). This calls for minimum expense and investment in establishing/growing amaranths especially to low income earners. Good nutrition can therefore be attained cheaply by the low income group. Vitamins have major nutritional benefits in the day to day nutrition of humans.

These benefits can be attained only if the harvesting stage is well observed and carried out at the correct time that will ensure their availability optimally.

1.4 Objectives

1.4.1 Broad objective

To determine nutrient, anti-nutrient and phytochemical contents in ten varieties of amaranths at two growth stages.

1.4.2 Specific objectives

- To determine the proteins, beta carotene, Vitamin C, and mineral contents of ten varieties of amaranth before and after flowering.
- To determine oxalates, phytates, tannins and nitrates content of ten different varieties of amaranth before and after flowering.
- To establish the flavonoids, carotenoids and antioxidant activity of ten amaranth varieties before and after flowering.

1.5 Hypothesis

- There is no difference in the proteins, beta carotene, Vitamin C, Ca, Fe, Mg and Zn contents of the ten varieties of amaranth at different maturity stages.
- There is no difference in the oxalates, phytates, tannins and nitrates content of ten varieties of the amaranth at different maturity stages.
- There is no difference in the flavonoids, carotenoids and antioxidant activity of the ten amaranth varieties at different maturity stages.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Amaranth

2.1.1 Origin

Amaranth (*Amaranthus spp.*) is one of the Africa leafy vegetables from the family *Amaranthaceae*. It is believed to have originated in Central Americas but it has spread to other countries such as India, China, Europe, Asia and Africa (Putman *et al*., 1989; Myers, 1998; Mposi, 1999). According to other studies, many species originated in the Andrean region of the South America and are now widely distributed throughout most tropical areas. Nigeria has also been claimed as a centre of diversity of amaranth, corroborated by the prevalent use of local names and the enormous genetic diversity available there. Amaranth is now produced worldwide for both its grains and vegetables. It is also called Amaranth, Tampala, Tassel flower, Flaming fountain plant, Joseph's coat and also include the pigweeds, *Amaranthus hybridus*. It is a large and bushy plant, 0.9-1.3 meters in height. Many Amaranthus species are cultivated as leaf vegetables, cereals or ornamental plants (Dhellot *et al.*, 2006; He, 2002; Schippers, 2000).

2.1.2 Botanical Description

Amaranth is an annual leafy vegetables belonging to the family *Amaranthaceae*. It is a herbaceous plant; short lived, growing up to 1 meter in height. It contains inflorescences that are racemose spikes, which may either be auxiliary or terminal. It is moderately branching, with strongly branched tap root. Its leaves are alternate, long petiolate, simple and entire. Flowers in auxiliary clusters, supper clusters often leafless and in panicled spikes, unisexual, solitary in the axil of a bract, with 2 bracteoles, 3-5 petals

either free stamens as many as petals (male flowers) or ovate or oblong ovary with 2-3 stigmas (female flowers).

The family *Amaranthaceae* has more than 800 species of which about 60 are considered weedy and about 50 are consumed as pot-herbs or leafy vegetables worldwide (Olufaji, 1989; Janick, 1997). Schippers, (2000) noted that all species found in Africa are grown for their leaves. Most grain varieties originated from Americas.

The main species used for grain production are *A. hypochondriacus* (L.); which is also a dual purpose variety; *A. cruentus* (L.), and *A. caudatus* (L.) (Rensburg *et al*., 2004; National Research Council, 1984), and the most popular leafy species are *A. hypochondriacus* (L.), *A. tricolor* (L.), *A. hybridus* (L.), and *A. blitum* (L.) (Rensburg *et al.*, 2004; National Research Council, 1984). There is however no distinct separation between vegetable and grain type species since even the leaves of young grain type plants can be eaten as leafy vegetables.

2.1.3 Environmental Response

Amaranth is grown during both wet and dry seasons, though irrigation is normally required for dry season crops since the rate of transpiration by the leaves is fairly high, frequent applications of water are required, related to the stage of growth of the crop and moisture- retaining capacity of the soil. It is a C_4 plant and this characteristic has contributed to the plants wide geographic adaptability to diverse environmental conditions (Liu and Stutzel, 2004). The process of C_4 carbon fixation is used by few fast growing crops, where they use an especially efficient type of photosynthesis to convert the raw materials of soil, sunlight and water into plant tissues even at high temperatures. Plants that use C_4 carbon fixation pathway tend to require less water than more common C_3 carbon fixation pathway plants (National Research Council, 1984).

Amaranth prefers soils with pH above 6 and soil temperature above 15^0C for establishment. Seeding rates of 1.2 to 3.5 kg seed/ hectare planted to an average depth of 1.3 cm are recommended. Amaranth can however tolerate poor soil fertility and drought, although the tolerance mechanism is not well understood (O'Brien and Price, 1983). Plant quality is, however, poor under stressful conditions. Amaranth also responds well to fertilizers.

2.1.4 Cultivation

The crop is adapted to a wide range of soil conditions. Sandy soil with slight acidity is best suited (Palada and Chang, 2003). A temperature range of 20-30^0C is required for better vegetative growth. It is photoperiod sensitive and most species flower when the day length is shorter than 12 hours (Palada and Chang, 2003).

Amaranth is planted mostly by direct seeding. Seeds are either broadcast or sown in rows on well-prepared seedbeds. Since amaranth seeds are very small, they can be mixed with sand at a ratio of 1 g seed to 100 g sand to make it easier to sow the seed and obtain a uniform stand, cover seed lightly with a layer of compost or rice hulls immediately after broadcast. Amaranth is propagated by seeds. Depending on the cultivar, photoperiod and cultural practices, flowering may start 4 - 8 weeks after sowing then the seeds mature after 3 - 4 months.

Pests and diseases have been reported to be s serious problem in production of amaranth. Pests such as cutworms, aphids, flea beetles and mites, can cause severe losses in *Amaranthus* production if no corrective measures are taken (Mposi, 1999; Palada and Chang, 2003, Stalknecht and Schultz- Schaeffer, 1993).

2.1.5 Amaranth species in Kenya

Several amaranths are found in Kenya, either cultivated or growing in the wild. These species are used for vegetables, grains, ornamental and as medicine.

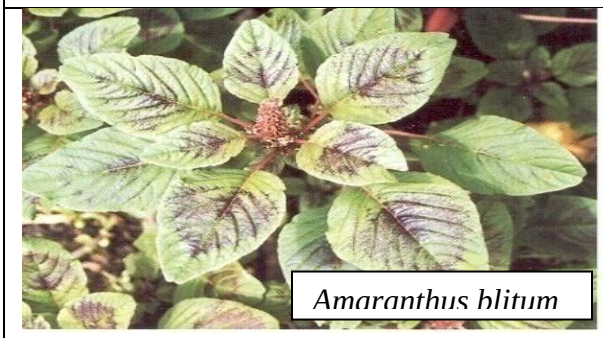
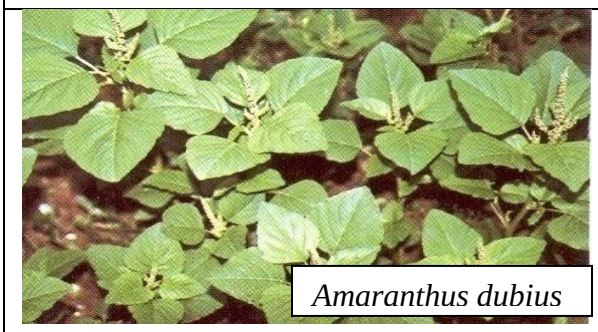
Table 2.1: *Status and use of some amaranth species found in Kenya*

Species	Status	Use
Amaranthus tricolor	Cultivated	Leafy vegetable; ornamental; medicine.
Amaranthus blitum	Wild and cultivated	Leafy vegetable; medicine
Amaranthus hybridus	Cultivated	Leafy vegetable
Amaranthus dubius	Wild and cultivated	Leafy vegetable; medicine
Amaranthus lividus	Wild and cultivated	Leafy vegetable; medicine
Amaranthus cruentus	Cultivated	Grain; leafy vegetable; ornamental; medicine
Amaranthus caudatus	Cultivated	Grain; leafy vegetable; ornamental; medicine
Amaranthus hypochondriacus	Cultivated	Grain; leafy vegetable
Amaranthus graecizans	Wild and cultivated	Grain; ornamental.
Amaranthus spinosus	Wild	Fodder; leafy vegetable; medicine.
Amaranthus thunbergii	Wild	Leafy vegetable

Table adapted from Grubben and Denton, (2004)

The figure below show the pictures of some amaranth varieties produced in Kenya and their geographical distribution.

Variety	Distribution

 *Amaranthus blitum* *Photo by Mel Oluoch*	• The most widely grown African amaranths • Commonly found in Kenya and Uganda • Origin-Africa
 *Amaranthus blitum* *Photo by Mel Oluoch*	• This is the type grown in Uganda
 *Amaranthus dubius* *Photo by M.O. Abukutsa-Onyango*	• Commonly found in Kenya and in Uganda and Sychelles • Origin-C. America

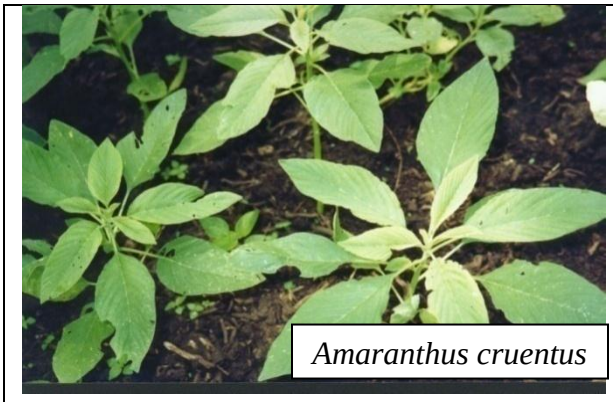
 *Photo by M.O. Abukutsa-Onyango*	• Commonly found in the Nairobi area in Kenya and other E. African countries, Cameroon • Sometimes treated as a sub-species of *A. hybridus* • Origin- C. America

Plate 1: pictures and distribution of some common varieties.

2.2 Nutrients of Amaranths

2.2.1 Overview

Green leafy vegetables including amaranth occupy an important place among the food crops as they provide adequate amounts of many vitamins and minerals for humans.

Table 2.2: *The approximate composition of grain and uncooked leaves of amaranth (100 g portions).*

Component	Vegetable	Grain
Moisture	86.9 g	9.0 g
Ash	2.6 g	2.6 g
Calcium	267 mg	490 mg
Iron	3.9 mg	--
Phosphorus	67 mg	477 mg
Potassium	411 mg	--
Protein	3.5 g	15.0 g
Total carbohydrates	6.5 g	63.0 g
Fat	0.5 g	7.0 g

Fiber	1.3 g	2.9 g
Calories	36	391
Vitamin A(beta carotene)	6100 IU	0
Vitamin C (ascorbic acid)	80 mg	3.0 mg
Riboflavin	0.16 mg	0.32 mg
Niacin	1.4 mg	1.0 mg
Thiamine (B1)	0.08 mg	0.14 mg

Source: J.N. Cole, Amaranth: from the Past, for the Future (1979).

They are rich sources of carotene, ascorbic acid, riboflavin, folic acid and minerals like calcium, iron and phosphorus (Fasuyi, 2006). Amaranth has also been shown to be a good source of gluten free protein. Amaranth vegetables therefore become very important in the efforts to alleviate Protein Energy Malnutrition and micronutrient malnutrition.

2.2.2 Protein

Amaranth may be a promising source of protein to those who are gluten sensitive, because unlike the protein found in grains such as wheat and rye, its protein does not contain gluten. According to Schippers, (2000), amaranth vegetable is rich in lysine, an essential amino acid that is lacking in diets based on cereals and tubers. Protein is one of the major macronutrients, containing carbon, hydrogen, oxygen and nitrogen, while some also contain sulphur and phosphorus. The building units of protein are amino acids, which are joined by peptide linkages to form protein. Amino acids are water soluble, crystalline and insoluble in organic solvents. Proteins on hydrolysis break down to polypeptides and finally into amino acids. The amount as well as the type of protein taken in diet is equally important as the digestibility and absorbability vary between different proteins.

The functions of proteins in the body include general growth, maintenance & repair of body tissues, energy source, essential for synthesis of protoplasm, enzymes e.g. globular proteins and hormones, production and maintenance of body proteins and is also required for the supply of the essential amino acids which cannot be synthesized in the body. Low protein diets in experimental animals cause anemia, hypoalbuminemia and edema. Infants with improper weaning and fed on low protein diets suffer from physical and mental retardation along with anemia, hypoprotenemia, conjunctivitis edema and fatty liver. This is known as 'kwashiorkor'. These infants are frequently prone to infection and the mortality is high in such untreated children. Protein deficiency is generally observed with calorie deficiency and this condition is called 'protein calorie malnutrition'.

Vegetables such as amaranth has been shown to contain some amounts of protein (Muriuki *et al*., 2014), which becomes very important when dealing with Protein Energy Malnutrition especially among vegetarians and the poor who cannot afford the animal protein sources. Amaranth leaves have been shown to contain 17.5 to 38.3% dry matter as protein of which 5% is lysine (Oliveira and De Carvallo, 1975). Kadoshnikov *et al.*, (2005) reported that the protein content of amaranth vegetables accumulates maximally at the blossoming phase of the plant, and ranges between 17.2- 32.6% on dry weight basis of various samples.

2.2.3 Beta-carotene

Beta-carotene is a type of pigment found in plants, especially carrots and colorful vegetables. The name beta-carotene is derived from the Latin name for carrot. It gives yellow and orange fruits and vegetables their rich hues. Beta-carotene is also used as a coloring agent for foods such as margarine.

The Recommended Dietary Allowance (RDA) for vitamin A is 2-3,000 IU depending on the individual. Beta-carotene is converted to vitamin A (retinol) by the body.

Vitamin A is needed for good vision and eye health, for a strong immune system, and for healthy skin and mucus membranes. While large amounts of vitamin A in supplement form can be toxic, the body will convert only as much vitamin A from beta-carotene as it needs. That means beta-carotene is considered a safe source of vitamin A.

Like all other carotenoids, beta-carotene is an antioxidant. It protects the body from damaging molecules called free radicals. Free radicals cause damage to cells through a process known as oxidation. Over time, this damage can lead to a number of chronic illnesses. Some therapeutic uses of beta carotene include prevention of chronic illnesses, decreases sun sensitivity, treatment of age related macular degeneration, treatment of metabolic syndrome, treatment of oral leukoplakia; a condition in which white lesions form in your mouth or on your tongue; and Scleroderma, a connective tissue disorder characterized by hardened skin, due to low levels of beta-carotene in blood.

The richest sources of beta-carotene are yellow, orange, and green leafy fruits and vegetables (such as carrots, amaranths, spinach, lettuce, tomatoes, sweet potatoes, broccoli, cantaloupe, and winter squash). In general, the greater the intensity of the color of the fruit or vegetable, the more beta-carotene it contains.

2.2.4 Vitamin C (Ascorbic acid)

Vitamin C is a water-soluble vitamin, meaning it cannot be stored in the body. It has to be obtained from food, including citrus fruits, broccoli, and tomatoes. Vitamin C is an antioxidant, along with beta-carotene, and many other plant-based nutrients. Antioxidants block some of the damage caused by free radicals, substances that damage DNA.

Signs of vitamin deficiency include dry and splitting hair; gingivitis (inflammation of the gums) and bleeding gums; rough, dry, scaly skin; decreased wound-healing rate,

easy bruising; nosebleeds; and a decreased ability to ward off infection. A severe form of vitamin C deficiency is known as scurvy. The Recommended Dietary Allowance (RDA) for vitamin C is 60-200 mg; 60 mg is the bare minimum to prevent scurvy. Low levels of vitamin C have been associated with a number of conditions, including high blood pressure, gallbladder disease, stroke, some cancers, and atherosclerosis, the build-up plaque in blood vessels that can lead to heart attack and stroke. Getting enough vitamin C from your diet, by eating lots of fruit and vegetables, may help reduce the risk of developing some of these conditions. There is no conclusive evidence that taking vitamin C supplements will help or prevent any of these conditions.

Vitamin C plays a role in protecting against heart disease**,** high blood pressure**,** common cold**,** cancer**,** osteoarthritis**,** age-related macular degeneration**,** pre-eclampsia**,** asthma. It is also important in boosting immune system function**,** maintaining healthy gums**,** improving vision for those with uveitis (an inflammation of the middle part of the eye)**,** treating allergy-related conditions, such as asthma, eczema, and hay fever (called allergic rhinitis)**,** reducing effects of sun exposure, such as sunburn or redness (called erythema)**,** alleviating dry mouth, particularly from antidepressant medications (a common side effect from these drugs)**,** healing burns and wounds**,** decreasing blood sugar in people with diabetes**,** some viral conditions, including mononucleosis -- although scientific evidence is lacking, some doctors may suggest high-dose vitamin C to treat some viruses

Dietary sources of vitamin C are fruits and vegetables. Vitamin C is sensitive to light, air, and heat, so you'll get the most vitamin C if you eat fruits and vegetables raw or lightly cooked.

2.2.5 Calcium

Green vegetables including amaranth are one of the major sources of calcium in the diet. Most of the calcium in the body is present in the bones and teeth; only a small

amount (1%) is present in blood/body fluids. It is present either in combination with protein or as calcium ion. Thyroid and parathyroid hormones maintain the concentration of calcium in blood. During dietary deficiency, the calcium present in the bones maintains the blood calcium levels.

The dietary calcium absorption is only 20-30%, and this depends on factors such as vitamin D levels, any renal insufficiency, hypothyroidism and interaction with other diet components like phytates and oxalates. The availability of calcium is reduced as it binds to form insoluble calcium phytate/oxalate. The calcium absorption across the intestinal mucosa is by both active and passive transport.

Calcium is required for blood clotting, for contraction of the muscles and for many enzymatic activities. Calcium loss leads to a condition is known as osteoporosis. Recommended Dietary Allowances for calcium ranges from 400-1000 mg per day depending on age, sex and physiological conditions, and if dietary calcium sources are insufficient, the calcium required is taken from bones.

2.2.6 Iron

It is the major component of pigment hemoglobin in red blood cells, (which is essential for the transportation of oxygen; accounting for approximately 0.1% mineral content in the body. Iron, to a smaller extent, is also present in muscle as myoglobin (muscle protein) and as cell enzymes (cytochromes). Rest is stored in the liver, spleen and bone marrow (as iron binding protein- ferritin and haemosiderin). Green leafy vegetables amaranth, colocasia leaves, mustard leaves, pulses like soybean lentil, cow pea, cereals and millets are rich sources of iron.

The absorption of dietary iron by the body is small. Many factors affect rate of absorption-such as source of iron, presence of anti nutrients and presence of vitamin C

in the diet which promotes absorption as it reduces ferric ion to the absorbable ferrous state.

Iron is a major component of pigment hemoglobin in red blood cells. With a prolonged iron deficiency, the hemoglobin falls below normal and the condition is known as anemia. Iron deficiency anemia is an important nutritional problem in our country. It is observed that women especially pregnant women (60-70%) and children are suffering from iron deficiency anemia. Different factors contribute to it -poor iron absorption from our diets, various worm infestations and blood losses.

The minerals including iron content in vegetables have been documented to depend on species, variety, plant age, production techniques, besides other environmental factors (Chweya and Nameus, 1997; Lisiewska *et al.*, 2006; Rickman *et al.*, 2007). The recommended dietary allowance for iron is 28-38 mg for adults.

2.3 Anti-Nutrients in Amaranths

2.3.1 Overview

Vegetables contain anti-nutritional factors that can affect the availability of nutrients to the human body. These anti-nutritional factors interfere with metabolic processes and reduce the bioavailability of nutrients from plants or plant products used as human foods (Abara, 2003; Agbaire and Emoyan, 2012). Plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytates, trypsin inhibitors and nitrates) which are known as secondary metabolites and are biologically active (Soetan and Oyewole, 2009).

Some of the reported anti-nutritional factors in amaranth are phenolics, saponins, tannins, phytic acid, oxalates, protease inhibitors, nitrates and polyphenols. Of these, oxalates, phytates and nitrates are of more concern.

Cooking/ heat processing has decreases to a substantial extent, the levels of hydrogen cyanide, tannins and phytate (Habiba, 2002; Embaby, 2010, 2011). The levels of phytate and tannins are also decreased significantly after cooking (Fagbemi *et al*., 2005; Embaby, 2011). According to Yadav and Sehgal (2002), blanching of green leafy vegetables induces moderate losses of 5- 15% of phytate in vegetables.

2.3.2 Phytates

Phytic acid, also known as inositol hexasphosphate or phytate when in the salt form, is the storage form of phosphate in many plant tissues. It is not digested by humans and is therefore not a dietary source of inisitol or phosphate. They bind iron, zinc, calcium and magnessium. In presence Ca^{2+} and Mg^{2+}, it forms insoluble complexes which are not readily absorbed by the gastrointestinal tract (Akande *et al*., 2010; Agbaire and Emoyan, 2012). On germination of grains, the phytate reduces due to enzymatic breakdown of phytate that improves iron availability.

According to Oke (1969) a phytate diet of 1- 6% over a long period of time decreases the bioavailability of mineral elements in mono gastric animals. Phytic acid acts as a strong chelator forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability (Erdman, 1979). Phytate is associated with nutritional diseases such as rickets and osteomalacia in children and adult, respectively.

Phytic acid as a phytochemical, however, has several beneficial properties including; anti-cancerogenous, antioxidant anti-inflammatory, lowering cholesterol level, blood glucose lowering. (Watzl and Leitzmann, 1999). Phytic acid is used as an acidulant for pH adjustment in foods and beverages as it does not affect the original taste; it also has potential to prevent color degradation in food or beverage including anthocyanin. Phytic acid is one of the most potent natural iron chelator and has strong bacteriostatic and antioxidant action (Graf and Eaton, 1990; Graf *et al*, 1987). Phytic acid is found to have similar iron-chelating properties as desferrioxamine, a drug commonly used to kill

germs, tumor cells or to remove undesirable minerals from the body (Hawkins *et al*., 1993).

2.3.3 Oxalates

Amaranth is one of the vegetables that have been documented to accumulate high amounts of oxalic acid (USDA, 1984). The amount of oxalic acid is roughly the same as that found in spinach (*Spinacia oleracea*) and chard (*Beta vulgaris var. cicla*). Excessive amounts of oxalic acid may reduce the availability of certain minerals in the body, most notably calcium. This could be a concern especially if calcium intake levels are low to begin with, or if foods high in oxalic acid are consumed on a regular basis over long periods of time. Oxalates occur in many plants where it is synthesized via incomplete oxidation of carbohydrates.

In the body, oxalic acid combines with divalent metallic cations such as calcium (Ca^{2+}) and iron (II) (Fe^{2+}) to form crystals of the corresponding oxalates which are then excreted in urine as minute crystals. These oxalates are known to form insoluble calcium oxalate with calcium thereby preventing the absorption and utilization of calcium by the body hence causing diseases such as rickets and osteomalacia (Ladeji *et al*., 2004; Agbaire, 2012).

Accumulation of this insoluble compound in the renal glomeruli leads to the formation of renal calculi and kidney damages (Nwachukwu and Obi, 2007; Maikai and Obagaiye, 2007). Accumulation of oxalates appear to be related nitrate assimilation and cation-anion imbalance (Fasett, 1973)

Oxalates have a possible role in pest resistance, similar to the functions of protease inhibitors and tannins in legume seeds. Calcium oxalate, an important constituent in leaf extract of elephant foot yam (*Amorphophalus campanulata)* has been reported to block growth and aflatoxin biosynthesis in *Aspergillus flavus.* (Prasad *et al*., 1994).

Metabolism and absorption of oxalates

Oxalate combine with calcium to form calcium oxalate in the lumen; making calcium unavailable for absorption. The calcium oxalate is later excreted in feaces.

Free or soluble oxalate is absorbed by passive diffusion in the colon in humans (Hughes *et al*., 1992; Modigliani *et al*., 1978). Other studies also suggest that the small intestine is the major absorption site rather than the colon (Prenen *et al*., 1984).

It has been estimated that about 2-5% of the total oxalates administered is absorbed in the body; while its absorption is higher at lower doses (Finch, *et al*., 1981).

Toxic effects of oxalates

Minimum doses that can lead to death are 4-5 g of oxalate (Fasset, 1973); whereas other studies show that 10-15 g is the usual dose that causes fatalities. Ingestion of oxalic acid results in corrosion of the mouth and the gastrointestinal tract; gastric hemorrhage; renal failure and haematuria (Concon, 1988). High oxalate levels may interfere with carbohydrate metabolism.

Oxalate content has been reported to increase as plant ages (Yoshikawa et al., 1988), and occasionally, it can accumulate up to 15% of the total dry weight. Other studies suggest that the accumulation of oxalates in plant tissues could be attributed to a shift in equilibrium towards biosynthesis rather than to degradation (Hitomi and Tamaki, 1992). Accumulation of oxalate also appears to be related to nitrate assimilation and cation-anion imbalance (Fasset, 1973), studies have shown that photorespiration contributes to the biosynthesis of oxalate in spinach, and the CO_2 enrichment may lower the rate of oxalate biosynthesis and increase ascorbate content

Effects of oxalates on bioavailability of minerals

Oxalates inhibit calcium absorption by binding it to form calcium oxalate (Haeney *et al*., 1988). Adverse effects are considered in terms of oxalate: calcium ratio, (Fasset, 1973), which varies and can be classified into:

i. Those with ratio greater than two e.g. spinach, rhubarb, beet, sorrel, cocoa.
ii. Those with ratio of approximately one e.g. potatoes, amaranth.
iii. Those with ratio less than one e.g. cabbage, cauliflower, green beans, peas.

Oxalates also cause mineral imbalances in the body. Intake of oxalates plus fibre causes negative balance of calcium, magnesium, zinc, and copper (Kelsay *et al.*, 1979). Decreased mineral balance may be due to the combined effects of high fibre intake and oxalic acid, but this may be only a transient response (Kelsay, 1987).

2.3.4 Nitrates

Nitrates in amaranth leaves are a concern since it is hypothesized that nitrates may be chemically changed in the digestive tract into poisonous/carcinogenic nitrosamines. High level of nitrates in vegetables when ingested can be converted to nitrite which can lead to cancer and metheamoglobinemia or blue-baby disease (Gupta *et al*., 2000; Macrae, *et al*., 1997; Oguchi *et al.*, 1996; Takebe and Yoneyame, 1997). Boiling the leaves like spinach or chard 5-10 minutes, then discarding the water alleviates both oxalate and nitrate problems (Ogbadoyi *et al*., 2006). Regardless, research has shown that consumption of 200 grams of cooked amaranth poses no health problems. (Grubben, 2004).

Nitrate Accumulation & Poisoning: amaranth is one of the crops known to accumulate nitrates. This mostly happens when soil fertility is very high e.g. as a result of adding nitrogen fertilizer), and when the process of photosynthesis is slowed down by factors such as herbicide, drought, or frost. Nitrates are mostly accumulated in the plant tissues and not in the seeds.

Infants have bacteria in their digestive system which convert nitrates to nitrite. Nitrites bind to the hemoglobin in blood, robbing it of the ability to carry oxygen. Since hemoglobin's function is to carry oxygen, the net effect is oxygen starvation. However, in older humans, the acid level in the digestive system rises and kills the bacteria. There is also a possibility that Vitamin A may (or may not) help counteract the poisoning.

Nitrates also combine with some proteins to make nitrosamines, which may cause cancer. Whether or not they cause cancer, Vitamin C will prevent the nitrosamines from forming.

Vegetables with nitrate levels of 1000 – 4000 mg/kg are classified as high nitrate content (Anjana *et al*., 2007). The acceptable daily intake (ADI) of nitrate is 3.65 mg/kg for 60 kg body weight (219.00 mg/day). Various processing methods can be adopted to reduce the nitrate content of vegetables to the acceptable levels (Ogbadoyi *et al.*, 2011).

2.3.5 Tannins

Tannins are condensed bitter plant polyphenolic compounds which are present in high amounts in seed coats of most legumes, and certain fruits and vegetables including amaranth. Tannins may precipitate proteins from aqueous solution by inhibiting digestive enzymes (Soetan and Oyewole, 2009) and have been found to interfere with digestion by displaying anti-trypsin and anti-amylase activity. Tannins chelate iron and zinc irreversibly and interfere with their absorption.

Major classes/polymer of tannins includes the hydrolysable tannins and non-hydrolysable or condensed tannins; which are both found in plants. Tannins are distributed throughout the plant kingdom and are common in both gymnosperms and angiosperms. The most abundant polyphenols are the condensed tannins, found in virtually all families of plants, and compromising up to 50% of the dry weight of leaves.

Tannins have traditionally been considered anti-nutritional but it is now known that their beneficial or anti-nutritional characteristics depend on their chemical structure and dosage. Condensed tannins can inhibit digestion by binding to plant proteins, making them more difficult to digest. They also interfere with protein absorption and digestive enzymes. Other studies show that tannin, also known as proanthocyanidins, posses various properties such as antioxidants, anti-aging, anti-apoptotic, anti-inflammatory, anti-carcinogenic as well as anti-atherosclerosis and cardiovascular protection.

Uses of tannins

They are an important ingredient in the process of tanning leather. Mimosa, oak and other have traditionally been the primary source of tannery tannin, though inorganic tannin agents are also in use today and accounts for 90% of the worlds leather production (Marion and Thomson 2006).

Tannins produce different colours with ferric chloride according to the type of tannin. Iron gall ink is produced by treating a solution of tannins with iron (II) sulphate.

It is a component in a certain type of industrial adhesive developed jointly by the Tanzania Industrial Research and Development Organization and Forintek Labs Canada (Bisanda *et al*. 2003). Pinus radiata tannins have also been investigated for the production of wood adhesives (Li, and Maplesden, 1998). Condensed tannins and hydrolysable tannins appear to be able to substitute synthetic phenols in phenol-formaldehyde resins for wood particleboard.

Medical uses and potential

Tannins may be effective in protecting the kidneys. When incubated with red grape juice and red wines with a high content of condensed tannins, the poliovirus, herpes

simplex virus, and various enteric viruses are inactivated. Tannins have shown potential anti-viral, antibacterial and antiparasitic effects.

2.4 Phytochemicals in Amaranth

2.4.1 Overview

Phytochemicals are compounds found in plants that are not required for normal functioning of the body, but have a beneficial effect on health or play an active role in amelioration of diseases. There have been claims by some scientists that many of the diseases afflicting human beings are the result of lack of phytonutrients in their diet. Phytonutrients have various health benefits, for example, they may have antimicrobial, anti-inflammatory, cancer preventive, antidiabetic and antihypertensive effects to mention but a few. The phytochemical constituent of a plant will often determine the physiological action on the human body (Pamplona-Roger, 1998).

2.4.2 Carotenoids

Amaranth vegetable have been documented to contain higher carotenoids content than most vegetables. Carotenoids are yellow, red and orange pigments present in many fruits and vegetables. In dark green leafy vegetables, carotenoids are masked with the presence of chlorophyll. Leafy vegetables are a rich source of many carotenoids (Kimura and Rodriguez-Amaya, 2003; De Oliveira and Rodriguez-Amaya, 2007). More than 700 carotenoids have been identified in nature, with β - carotene being the most familiar carotenoid. The most commonly studied include lutein, zeaxanthin, lycopene, *β*-carotene, *α*-carotene, and *β*-cryptoxanthin (McLauren and Frigg, 2002). Besides the well-known provitamin A activity of some carotenoids (Tanumihardjo, 2008), they act as powerful antioxidants and are believed to protect the body against free radical attack and hence reduce the incidence of cataracts, heart disease and other degenerative diseases (Krinsky, 1993). Carotenoids including α -carotene, β carotene and β-

cryptoxanthin can be converted into Vitamin A, while lycopene, lutein, and zeaxanthin have no vitamin A activity.

It is not clear which specific carotenoids are most important in reducing the cancer risk. Previously, scientists believed that β-carotene had important cancer prevention properties. In several recent studies, however, β -carotene supplements did not lower the risk of cancer. The antioxidant activity of carotenoids differs among the different compounds. Studies have shown that the singlet oxygen quenching capacity of lycopene is twice that of β- carotene and ten times that of tocopherol. Several studies on the bioavailability of β -carotene from vegetables in the human diet have shown that in broccoli it ranges from 22-24%, in carrots 19-34%, and in leafy vegetables it ranges from 3-6%. Studies have also shown that combination of fatty foods with carotenoid rich vegetables enhanced carotenoids uptake. Most recent studies have shown that the bio availability of lycopene from tomato has increased dramatically by heat treatment in the presence of oil. For example, lycopene was found to be more bio-available from tomato paste than from fresh tomato due to heat treatment and presence of oil content in the paste. It has also been shown that lutein, which has no vitamin A activity is five times more readily available than β-carotene. Carotenoids are powerful antioxidant that may reduce the incidence of age-related diseases such as cancer and coronary heart disease.

Food carotenoids are usually C_{40} tetraterpenoids built from eight C_5 isoprenoid units, joined so that the sequence is reversed at the center. Although commonly thought of as plant pigments, carotenoids are also encountered in some animal foods. Animals are incapable of carotenoid biosynthesis, thus their carotenoids are diet derived, selectively or unselectively absorbed, and accumulated unchanged or modified slightly into typical animal carotenoids. Leaves have a strikingly constant carotenoid pattern, the main carotenoids being lutein (about 45%), beta-carotene (usually 25–30%), violaxanthin (15%), and neoxanthin (15%) (Britton, 1991).

The carotenoid composition of foods are affected by factors such as cultivar or variety; part of the plant consumed; stage of maturity; climate or geographic site of production; harvesting and postharvest handling; processing and storage (Rodriguez-Amaya, 1993; Gross, 1991; 1987).

Carotenoids are insoluble in water and soluble in organic solvents, such as acetone, alcohol, ethyl ether, chloroform, and ethyl acetate. They are readily soluble in petroleum ether, hexane, and toluene; xanthophylls dissolve better in methanol and ethanol. Crystalline carotenoids may be difficult to dissolve in the above solvents but do dissolve in benzene and dichloromethane (Schiedt and Liaaen-Jensen, 1995).

Solubility of both ß-carotene and the xanthophyll lutein in tetrahydrofuran has been shown to be excellent (Craft and Soares, 1992). Carotenoids in solution obey the Beer-Lambert law—their absorbance is directly proportional to the concentration. Thus, carotenoids are quantified spectrophotometrically.

The highly unsaturated carotenoid is prone to isomerization and oxidation. Heat, light, acids, and adsorption on an active surface promote isomerization of *trans* carotenoids, their usual configuration, to the *cis* forms. This results in some loss of color and provitamin A activity. Oxidative degradation, the principal cause of extensive losses of carotenoids, depends on the availability of oxygen and is stimulated by light, enzymes, metals, and co-oxidation with lipid hydroperoxides. Formation of epoxides and apocarotenoids (carotenoids with shortened carbon skeleton) appears to be the initial step. Subsequent fragmentations yield a series of low-molecular-weight compounds similar to those produced in fatty acid oxidation. Thus, total loss of color and biologic activities are the final consequences.

2.4.3 Flavanoids

Flavonoids are a ubiquitous group of polyphenolic substances which are present in most plants. They are a class of secondary metabolites. They were previously referred to as vitamin P from the mid 1930s to early 1950s but the term fell out when it was realized that these substances did not have the properties of vitamins (Mobh, 1938). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-neoplastic, antiviral, anti-thrombotic and vasodilatory activities (Miller, 1996). Good correlation between the total flavonoids content and antioxidant activity has been show, (Ayoola *et al*, 2008), indicating that the flavonoids contribute in free radical scavenging. The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the above actions as oxidative damage is implicated in most disease processes. Indeed laboratory research on flavonoids and other antioxidants suggest their use in the prevention and treatment of a number of these diseases. Traditional uses for fever and toothache suggest possible anti-inflammatory properties, use as cardio tonic, hypertension and improved mental alertness suggests possible anti-thrombotic and vasodilatory properties. These are in accordance with some of the properties of flavonoids. Flavonoids are normally classified into various classes:

Flavonols: Flavonols include quercetin, kaempferol, fisetin, and myricetin. Quercetin is the most important flavonoid in vegetables. It has been detected in onion, and to a lesser extent in tomato and bean. Kaempferol, myricetin, and fisetin have been detected in onion, lettuce, and endive. Many investigators have studied the effect of flavonols on carcinogenesis and cardiovascular diseases in human and laboratory animals. Several mechanisms have been proposed for the mode of action of these compounds including inhibition of estrogen binding in mammalian cells, induction of the phase II enzyme quinone reductase in murine hepatoma cells, antioxidant protection from oxygen radicals and induction of apoptosis.

Flavones: Flavones include apigenin and luteolin. Flavones have been detected in conjugated form in celery, tomato, brinjal, garlic and onion. The mechanism of action of flavones on chronic diseases is similar to that of other flavonoids. They were proposed to function primarily as antioxidants by conserving α-tocopherol content of low-density lipo-proteins and membrane lipids in the reduced state.

Isoflavonoids: Isoflavonoids include diadzein and genistein and have limited distribution in vegetables. They exist mainly in legumes including soybean, lentil and chickpea. Small quantities have been detected in vegetables such as broccoli, asparagus, alfalfa sprouts, okra and mushrooms. A health benefit of iso-flavonoids has been due to the phytestrogenic response of genistein. As a phytoestrogen, genistein is believed to block estrogen perception by actively competing for binding sites or by blocking estrogen synthesis. Studies have confirmed the role of early exposure to estrogen in reducing hormone dependent and chemically induced cancers. Studies have shown that Asian women who consumed a diet rich in soy products had relatively low risk of breast cancer.

2.4.4 Antioxidant nutrients

Antioxidant is a term widely used but rarely defined. It can be defined as any substance that when present at low concentrations, compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell & Gutteridge, 1995). This definition was further simplified as “any substance that delays, prevents or removes oxidative damage to a targeted molecule (Halliwell & Gutteridge, 2007). A generic definition of an antioxidant is not experimentally constructive unless it is associated with the notion of the oxidant that has to be neutralized (Azzi *et al*., 2004). Antioxidants protect the body from adverse biological reactions involving oxygen. The body's natural metabolism involving oxygen also generates a host of toxic compounds/reactive by products called “free radicals", which are highly unstable and

destructive. Left unchecked, these free radicals can attack lipids in cell membranes, destroy cellular enzymes and even damage the genetic material deoxyribonucleic acid (DNA). If the injury to DNA by free radicals is not immediately repaired, the damaged DNA is replicated in new cells and deterioration occurs. It is this gradual deterioration that may be responsible for ageing and the development of heart disease, cancer and cataracts. Fortunately the body has a natural defense system against cellular damage by free radicals, which comprise primarily of antioxidant nutrients (such as vitamin E, vitamin C, and certain carotenoids), which are assisted by a variety of phyto-chemicals from the diet.

Antioxidants counteract, or neutralize, the harmful effects of free radicals. Free radicals and reactive oxygen species are no longer seen only as destructive but also as messengers involved in intracellular and intercellular signaling (Bartosz, 2005; 2009; Halliwell, 2006). These antioxidants act as scavengers for these tree radicals and reactive oxygen species, thereby preventing them from disrupting the chemical stability of the cells. A variety of external factors such as inflammation, cigarette smoke, air pollutants, radiation (X-rays and ultra-violet rays), carcinogens and excessive tension and exercise can promote free radical formation in our body. Consequently, individuals exposed to these sources of oxidants would require a greater supply of dietary antioxidants. Orange and yellow fruits and vegetables, as well as green leafy vegetables, are rich sources of carotenoids. Citrus fruits, strawberries, green peppers, tomatoes, and kiwi are examples of foods high in vitamin C. Good sources of vitamin E include nuts and seeds, wheat germ, and vegetable oils. With the exception of vitamin E, these antioxidants are widely' distributed in fruits and vegetables.

The antioxidant effects in plants are mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids, tannins and phenolic diterpenes (Polterait, 1997). Oxidative damage is implicated in most disease processes such as cardiovascular

disease, cancer, inflammatory conditions, asthma, liver disease and macular degeneration (Willcox *et al*., 2004).

Vegetables amaranth has been shown to have good antioxidant activity, which in turn prevents the body from harmful radicals hence prevent some chronic diseases. The antioxidant potential of the extracts would be responsible for the prevention of the cardiovascular and neurodegenerative diseases (Heim *et al*., 2002) and of the bones diseases (Govindarajan *et al.*, 2005). It would prevent also cancers (Kawanishi *et al.*, 2001). Ethanolic extract of *A.dubius* was found to show good inhibition of lipoxygenase, with an IC_{50} of 69.4 µg/mL at the concentration of 100 µg/mL (Uma and Odhav, 2008). Aqueous extracts of *Amaranthus hybridus* have been shown to have anti-anemic activity on rabbits treated with the phenyl hydrazine HCl with the amount of 30 mg/kg (Ogbe *et al.,* 2012). Studies carried out by Ozsoy *et al*. (2009) could show that other species of the same family as *Amaranthus lividus* stems with leaves and flowers seem to be good sources of natural antioxidants. Oloyede et al., (2013) reported greatest concentration of antioxidants at maturity stage in *A. cruentus* and *Celosia argentea.*

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site

The study was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) between May - August 2013. JKUAT is in Juja, Central Kenya under the geographical coordinates 1^0 11' 0" S, 37^0 7' 0" E. The area is majorly covered by cambisols and vertisols, the average annual rainfall is 856 mm, and the mean annual temperature is 18.9^0C (Muchena *et al.,* 1978). The crops were grown in JKUAT farm and the analysis done in the Food Science laboratory in JKUAT.

Table 3.1: *Experimental material*

Var. No	Variety name	Status	Botanical name	Comments
1	Abuku amaranth 1	Advanced line	*Amaranthus blitum*	Being evaluated by KEPHIS
2	Abuku amaranth 2	Advanced line	*Amaranthus blitum*	Being evaluated by KEPHIS
3	Abuku amaranth 3	Advanced line	*Amaranthus hybridus*	Being evaluated by KEPHIS
4	Abuku amaranth 4	Advanced line	*Amaranthus hybridus*	Being evaluated by KEPHIS
5	Abuku amaranth 5	Advanced line	*Amaranthus hybridus*	Being evaluated by KEPHIS
6	Abuku amaranth 6	Advanced line	*Amaranthus hybridus*	Being evaluated by KEPHIS
7	Abuku amaranth 7	Advanced line	*Amaranthus dubius*	Being evaluated by KEPHIS
8	Abuku amaranth 8	Advanced line	*Amaranthus hypocandracus*	Being evaluated by KEPHIS
9	Natures best amaranths	Standard variety	*Amaranthus hybridus*	Safari seed Co. Ltd
10	Amaranths (terere)	Standard variety	*Amaranthus dubius*	Simlaw seed Co.

3.2 Experimental design and treatments

The experiment was laid in randomized complete block design (RCBD) and was replicated three times. The treatments comprised of ten different amaranth varieties. Varieties 1- 8 were new varieties produced by a researcher, while variety 9 and 10 were standard varieties obtained from Safari Seed Company and Simlaw Seed Company, respectively. The other treatment was two plant maturity stages at which harvesting of the leaves was to be done, the vegetative stage and post flowering stage.

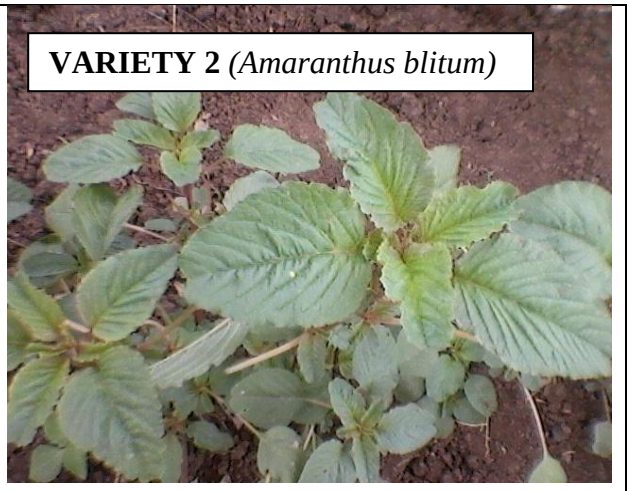
3.3 Crop establishment

The ten varieties were planted on a 10 m by 5 m plot at JKUAT farm, in rows of 50 cm apart. Blocks were 2 m long and the distance between blocks was 1 m. The seeds were directly sown by drilling in furrows of about 1 cm deep. Organic manure was also applied uniformly at a rate of 10 ton/ ha during planting. Rainfall and irrigation were the sources of water for the crops. In the weeks where there was no rain, the plants were irrigated twice a week. Two weeks after planting, thinning was done to an intra-row spacing of 10 cm. Shallow cultivation using jembes and hand pulling of weeds was carried out to keep the plot weed free (Grubben, 2004).

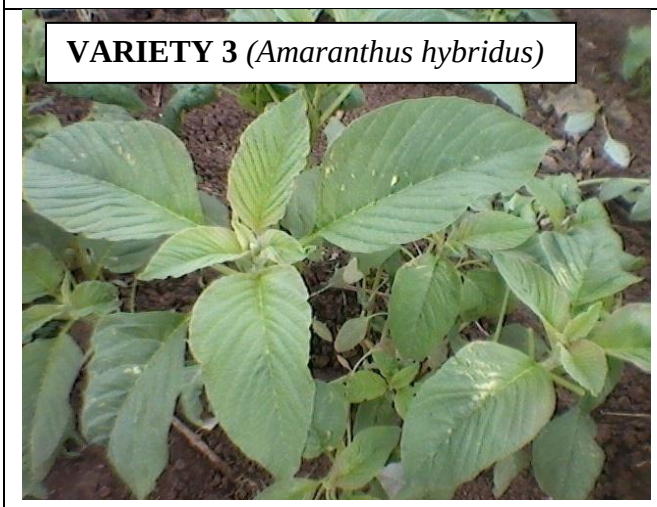
3.4 Harvesting/ sample collection

Leaves were harvested at the vegetative stage, four weeks after planting and after flowering, seven weeks after planting. The leaves were harvested by hand plucking. They were then put in polythene bags and stored in a cold room at 10^0 C awaiting analysis.

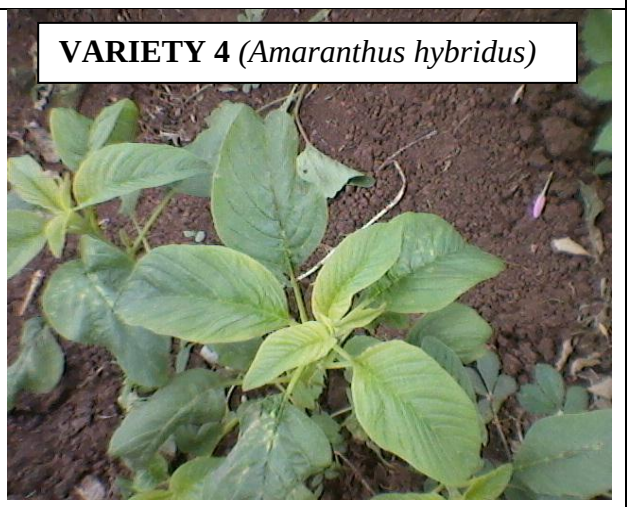
VARIETY 1 *(Amaranthus blitum)*



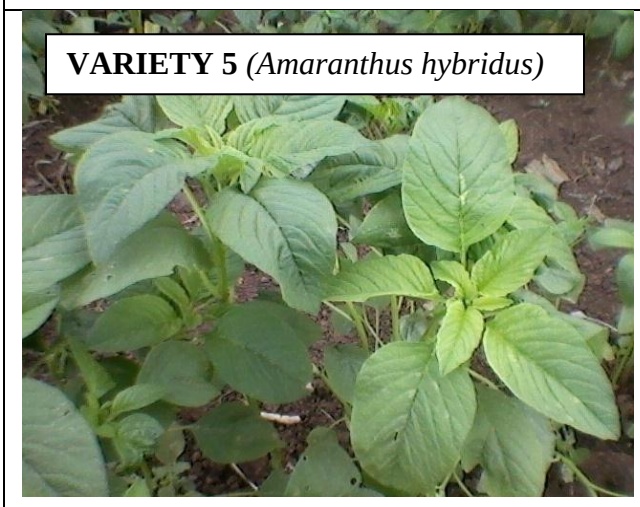
VARIETY 2 *(Amaranthus blitum)*



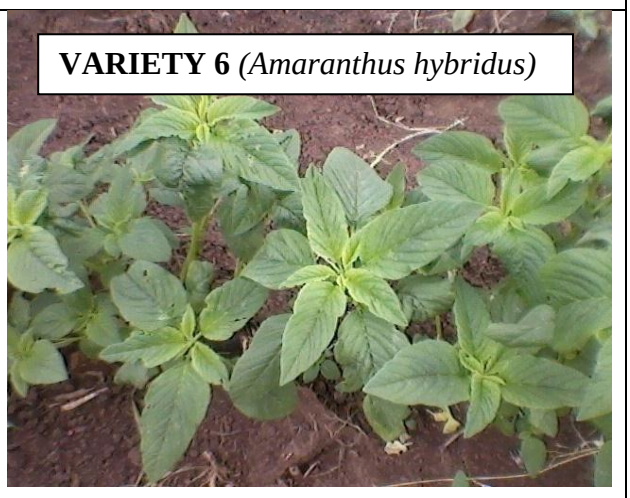
VARIETY 3 *(Amaranthus hybridus)*



VARIETY 4 *(Amaranthus hybridus)*



VARIETY 5 *(Amaranthus hybridus)*



VARIETY 6 *(Amaranthus hybridus)*

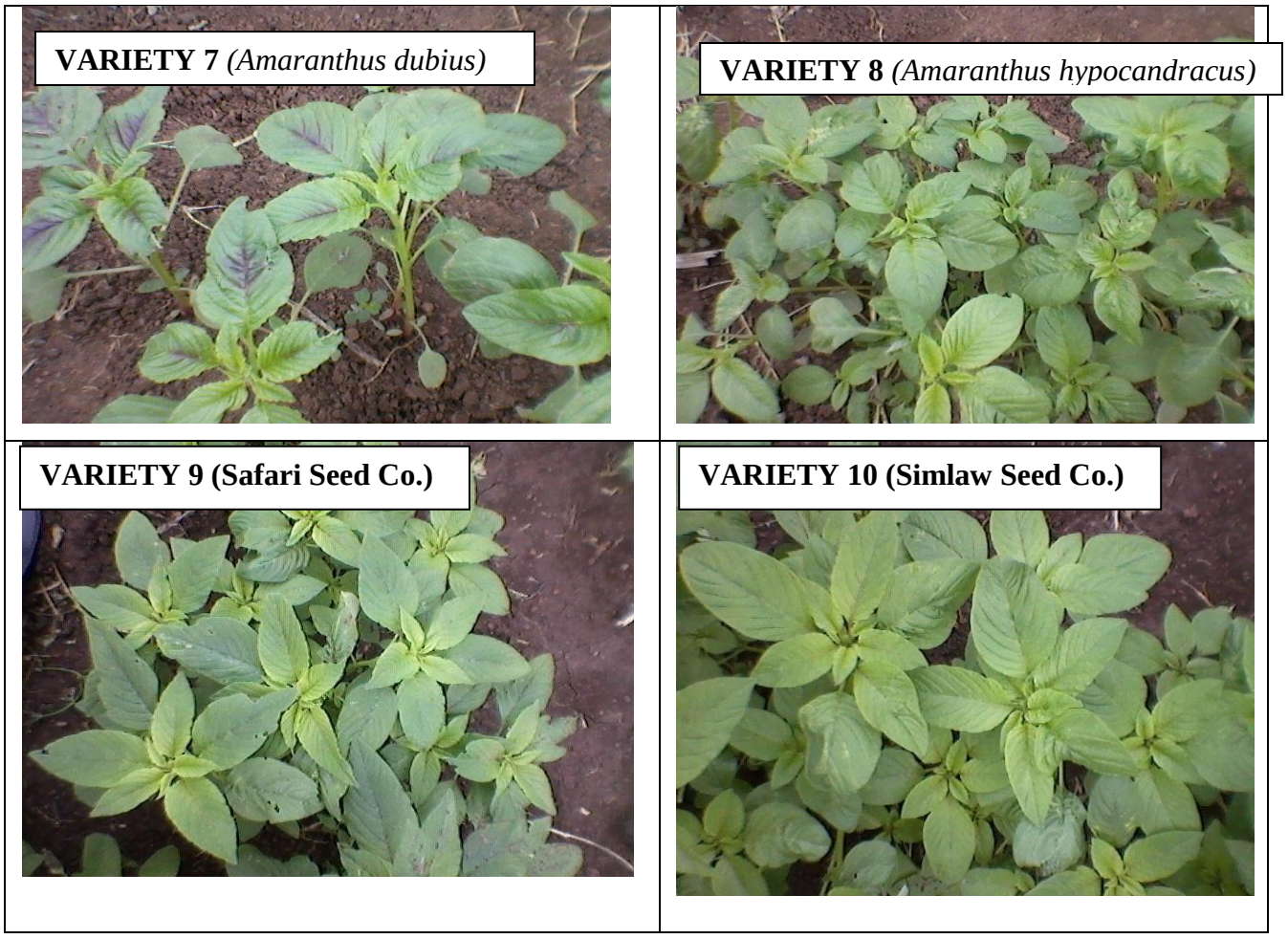


Plate 2: Pictures of the ten amaranth varieties at vegetative stage

Plate 3: Amaranth vegetables at post flowering stage

3.5 Nutrient Analysis

Nutrient analysis was done including proximate composition (moisture, ash and protein content), beta carotene, vitamin C, calcium, iron, zinc and magnesium.

3.5.1 Determination of moisture

Moisture was determined according to AOAC method 925.10-32.10.03 (AOAC, 1995). About 5 g of fresh sample was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a moisture oven and the temperatures adjusted to 105^0C. The samples were dried for 3 hours and cooled in a

dessicator and weighed. The amount of moisture in the samples was calculated using the formula:

$$\%\ Moisture = \frac{(wt\ before\ drying - wt\ after\ drying)}{Sample\ wt} \times 100$$

3.5.2 Determination of ash

Ash content was determined by incinerating in a muffle furnace (AOAC, 1995) method 923.03-32.1.05. About 5 g of fresh sample was weighed into a clean and weighed crucible, and charred by heating in a fume hood till smoking ceased. The charred samples were then transferred to a muffle furnace and temperature increased gradually to 550^{o}C. The samples were the allowed to ash to completion. Temperature was reduced, samples removed and cooled in a descicator before weighing. The amount of ash was calculated using the formula:

$$\%Ash = \frac{(wt\ of\ crucible\ with\ ash - wt\ of\ empty\ crucible)}{sample\ wt} \times 100$$

3.5.3 Determination of Protein

Protein was determined using the semi-micro kjeldal method (AOAC, 1995), specification 950.46 method 20.87-37.1.22. Approximately 2 g of sample was weighed into a digestion flask together with a combined catalyst of 5 g potassium sulphate and 0.5 g of copper sulphate and 15 mL of sulphuric acid. The mixture was heated in a fume hood till the digest color turned blue. This signified the end of the digestion process.

The digest was cooled, transferred to 100 mL volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalyst was also made. Exactly 10 mL of diluted digest was transferred into the distilling flask and washed with distilled water. 15 mL of 40% NaOH was added and this also washed with distilled water. Distillation was done to a volume of about 60 mL distillate. The distillate was titrated using 0.02 N HCL to an orange color of the mixed indicator, which signified the end point. The nitrogen in the sample was calculated as:

$$\%\ Nitrogen = \frac{(V1 - V2) \times N \times F \div (V \times 100)}{Sample\ wt}$$

Where: V_1 is the titre for sample in ml, V_2 is titre for blank in mL; N= normality of standard HCL (0.02); f= factor of std HCL solution; V= volume of diluted digest taken for distillation (10 mL); S= weight of sample taken for distillation (1 g). The protein content was then calculated as:

$$\%Protein = Nitrogen \times Protein\ factor(6.25)$$

3.5.4 Determination of Beta carotene

Beta carotene content was analyzed using column chromatography and UV Spectrophotometer. Extraction was done by acetone and petroleum ether as described by Rodriguez-Amaya and Kimura, (2004).

Approximately 2 grams of fresh sample was weighed, chopped finely and placed in a mortar with about 10 mL of acetone. This was thoroughly ground and the acetone extract transferred into 100 mL volumetric flask. The residue was again extracted with 10 mL acetone and the extract was added to the contents of the volumetric flask. The extraction with acetone was continued until the residue no longer gave color. The combined extract was made to a volume of 100 mL with acetone.

Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator. The residue was dissolved with 10 mL petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance was read at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Beta carotene standard was also prepared to make a calibration curve.

3.5.5 Determination of Ascorbic acid

The ascorbic acid content in the samples was determined by HPLC method (Vikram *et al*, 2005).

About 2 g of sample was weighed and extracted with 0.8% metaphosphoric acid. This was made to 20 mL of juice. The juice was centrifuged at 10000 rpm. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. This was passed through 0.45 µ filter and 20 µL injected into the HPLC machine. Various concentrations of ascorbic acid standards were also made to make a calibration curve. HPLC analysis was done using Shimadzu UV-VIS detector. The mobile phase was 0.8% metaphosphoric acid, at 1.2 mL/min flow rate and wavelength of 266.0 nm.

3.5.6 Determination of Minerals

Determination of minerals was done by dry ashing and atomic absorption spectrophotometer (AAS), according to AOAC, (1995); Osborne and Voogt, (1978). The minerals that were determined were calcium, iron, zinc and magnesium.

Clean dry crucible was weighed and about 5 grams of sample weighed into it. The crucibles was placed on a hot plate under a fume hood and the temperature increased slowly until smoking cease and the samples were thoroughly charred. They were then

put in muffle furnace and temperature increased gradually to 250^0 and heated for 1 hour. The temperature were increased to 550^0 and incinerated to complete ashing. The temperature was then decreased to $300^{0,}$ the crucibles removed and cooled to room temperature. The ash was transferred quantitatively to 100 mL beaker using 20 mL of 1N HCL, then heated at 80-90^0C on a hot plate for 5 minutes. This was then transferred to 100 mL volumetric flask and filled to the mark using 1N HCL. Insoluble matter was filtered and the filtrate kept in a labeled polyethylene bottle. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS). The various mineral standards were also prepared to make the calibration curve.

3.6 Anti-nutrient analysis

Anti-nutrients analyzed were phytates, oxalates, nitrates and tannin.

3.6.1 Determination of Phytates

Phytates was determined using HPLC (Camire and Clydesdale, 1982). Approximately 0.5 g of sample was extracted with 10 mL of 3% H_2SO_4. Contents were filtered and the filtrate transferred to a boiling water bath for 5 minutes followed by 3 mL of $FeCl_3$ solution (6 mg ferric iron per mL in 3% H_2SO_4) added. Contents were heated for 45 minutes to complete precipitation of the ferric phytate complex. They were then centrifuged at 2500 rpm for 10 minutes and the supernatant discarded. The precipitate was washed with 30 mL distilled water, centrifuged and the supernatant discarded. A 3 mL of 1.5 N NaOH was added to the residues and the volume brought to 30 mL with distilled water. Contents were heated for 30 minutes in a boiling water bath to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50 mL volumetric flask. The precipitate was rinsed with 10 mL distilled water, centrifuged and the supernatant added to the contents of the volumetric flask. This was micro filtered and kept awaiting HPLC analysis. HPLC analysis was

done using Shimadzu Refractive Index Detector (RID- 6A). The mobile phase was 0.005 N sodium acetate in distilled water, at a flow rate of 0.5 µL/min.

3.6.2 Determination of Oxalates

Determination of oxalates was done by HPLC (Libert, 1981) with modifications suggested by Yu *et al*, (2002). A 0.5 g fresh weight of sample was homogenized in 4 mL of 0.5N HCL. The homogenate was heated at 80^0 C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25 mL About 3 mL of the solution was withdrawn and centrifuged at 12000 rpm for 10 minutes. About 1 ml of supernatant was passed through a micro filter (0.45µ) before HPLC analysis. Standards were prepared at varying concentrations for quantification. HPLC analysis was done using Shimadzu UV-VIS detector, Hypsil C18 column (5µ M, 4.6 mm *250 mm) equipped waters 550 was used as the static phase and the mobile phase was a solution 0.01 N H_2SO_4. Flow rate was 0.6 mL min^{-1}, pressure of 62 kgf and detection wavelength of 221 nm.

3.6.3 Determination of Nitrates

The nitrate content in the test samples was determined by the calorimetric method using salicylic acid (Cataldo *et al.,* 1975). About 500 mg of fresh sample was weighed and put in a test tube, and 10 mL of hot (90-95^0C) distilled water was added. The closed tubes were placed in a water bath at 80^0C for 30 minutes and shaken. The samples were cooled and centrifuged at 4500 rpm. Supernatant was decanted and weighed to determine the exact volume of extract. Chlorophyll in leaf extract was removed by adding 0.5 g $MgCO_3$ to the supernatant, and centrifuged again. The supernatant containing the nitrate extract was treated with 2 N NaOH and a combination of Salicylic acid and H_2SO_4 in a ratio 1:20 w: v. Standards were also prepared using sodium nitrate. Absorption was measured at 410 nm with UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan).

3.6.4 Determination of Tannins

Tannin content was determined by the Folin-Denis calorimetric method described by Kirk and Sawyer (1998).

Qualitative analysis: preliminary analysis was carried out to establish the presence of tannins in the samples. About 0.5 g of dried powdered samples was boiled in 20 mL of water in a test tube and then filtered. A few drops of 1.0% ferric chloride were then added. A brownish green or blue-black coloration indicated presence of tannins.

Quantitative analysis: A 5 g of sample was ground in a mortar, dispersed in 50 mL distilled water and shaken. The mixture was allowed to stand for 30 minutes at 28^0 C before it was filtered through Whatman filter paper No. 42. Various concentrations of standard tannic acid were made. Four mL of distilled water were placed in different test tube for samples and standards. To this, 1 mL of sample extract/standard tannic acid was added. To each, 0.5 mL of Folin Ciocateau solution (10 mL of 2 N Folin Ciocateau solution in 100 mL distilled water) was added. Then 1.5 mL of 7.5% Na_2CO_3 solution was added to each. This was then topped up with distilled water. Their respective absorbance was measured in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan) at 720 nm using the reagent blank to calibrate the instrument at zero.

3.7 Determination of Phytochemicals

The phytochemicals determined were flavonoids, total carotenoids and vitamin C. Additionally, the free radical scavenging activity was also determined. This antioxidant activity results from the activity of the various phytochemicals.

3.7.1 Determination of total carotenoids

Total carotenoids were extracted using acetone and analyzed using column chromatography and UV Spectrophotometer, (Rodriguez-Amaya and Kimura, 2004).

Approximately 2 grams of fresh sample was weighed, chopped finely and placed in a mortar with about 10 mL of acetone. This was thoroughly ground and the acetone extract transferred into 100 mL volumetric flask. The residue was again extracted with 10 mL acetone and the extract was added to the contents of the volumetric flask. The extraction with acetone was continued until the residue no longer gave color. The combined extract was made to a volume of 100 mL with acetone.

Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator. The residue was dissolved with 10 mL petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance was read at 450 nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Total carotenoid was calculated using the formula:

$$Total\ carotenoids\ content\ (\mu/g = \frac{A \times Volume(mL) \times 10^4}{A^{1\%}_{1cm} \times Sample\ wt\ (g)}$$

Where A= absorbance; volume = total volume of extract (25 mL); $A^{1\%}_{1cm}$ = absorption coefficient of β−carotene in Petroleum Ether (2592).

3.7.2 Sample extraction of flavonoids and antioxidants

Sample extraction for analysis of flavonoids and antioxidant activity was done as described by Harborne (1973). About 5 g of dried and crushed samples were weighed into a 250 mL conical flask and about 100 mL methanol added. The flask was closed securely using parafilm and covered with aluminum foil. The samples were put in a shaker and shaken for about 3 hours. They were then kept in the dark and left to extract for 72 hours.

After 72 hours, the samples were filtered through Whatman No. 4 filter paper, and then the filtrate concentrated in a vacuum evaporator to a volume of 20 mL the extract was transferred into vial bottles and securely stoppered.

3.7.3 Determination of Flavonoids

Qualitative analysis: A preliminary qualitative analysis was first carried out to establish the presence of flavonoids. This was done according to the method of Harborne (1973). Five mL of dilute ammonia solution was added to a portion of aqueous filtrate of extract followed by addition of concentrated H_2SO_4. A yellow coloration observed indicated the presence of flavonoids. The yellow coloration disappeared on standing.

Quantitative analysis: Aluminum chloride colorimetric method was used for determination of flavonoids (Jagadish *et al.,* 2009). In a 10 mL volumetric flask, 4 mL of distilled water and 1 mL of plant extract were added. After 3 minutes, 0.3 mL of 5 % sodium nitrite solution was added. After 3 minutes, 0.3 mL of 10 % aluminum chloride was added. After 5 minutes, 2 mL of 1 M sodium hydroxide was added and the volume made up to 10 mL with water. Absorbance was measured at 415 nm using UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The amount of total flavonoids was calculated from Calibration curve of standard prepared from quercetin.

3.7.4 Determination of free radical scavenging activity

The radical scavenging activities of the plant extracts against 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) radical (Sigma-Aldrich) were determined by UV spectrophotometer at 517 nm (Molyneux, 2004). The following concentrations of the extracts were prepared, 0.01, 0.1, 1.0, 2.0 and 5 mg/mL in methanol (Analar grade). Vitamin C was used as the antioxidant standard at concentrations of same as the extract concentrations. One mL of

the extract was placed in a test tube, and 3 mL of methanol was added followed by 0.5 mL of 1 mM DPPH in methanol. A blank solution was prepared containing the same amount of methanol and DPPH. Methanol was used to zero the spectrophotometer and the absorbance was read at 517 nm after 5 minutes in UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). The radical scavenging activity was calculated using the following formula:

$$\%\ Inhibition\ of\ DPPH = \frac{(A_B - A_A)}{A_B} \times 100$$

Where A_B is the absorption of blank sample and A_A is the absorption of tested extract solution.

The results were expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) determined from a plot of % inhibition of DPPH versus concentration of extract.

3.8 Data management and analysis

The data was analyzed statistically using analysis of variance at 5% level of significance using the statistical software Genstat Release 14.1, to determine whether there was significant difference in nutrients, anti-nutrients and phytochemicals among the ten varieties. The means that were significantly different were separated using Duncan's Multiple Range Test (DMRT). T-test was used to determine the difference between the two maturity stages.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Proximate content of the amaranth varieties

4.1.1 Moisture content

There was significant difference in the moisture content among the varieties. There was, however, no significant ($P \leq 0.05$) difference in the moisture content of nine amaranth varieties, while variety 5 had significantly lower moisture content ($P \leq 0.05$). At vegetative stage, the moisture content of the ten amaranth varieties ranged from a minimum of 86.7 g/100 g to a maximum of 87.7 g/100 g with a mean of 87.5 g/100 g (Table 4.1). At post flowering stage, moisture content ranged from 77.4 g/100 g to 84.1 g/100 g with a mean of 80.6 g/100 g.

There was a general trend of decreasing moisture content from vegetative to post flowering stage, which can be attributed to the increase in the dry matter content. The A. *blitum* varieties (Variety 1 and 2) generally had the highest moisture contents, while varieties 9 and 10 had the lowest moisture contents at both the vegetative stage and post flowering stage. This shows that these varieties are more succulent compared to the other eight varieties.

4.1.2 Ash content

There were significant differences ($P \leq 0.05$) among the amaranth varieties in the ash content. There was a significant increase in ash content from vegetative stage to post flowering stage from mean of 2.7% -3.3% (Table 4.1). The ash content at the vegetative stage ranged between 2.4% to 3.1% with a mean of 2.7% (Table 4.1). At post flowering stage the ash content ranged from 2.7% to 4.5% with a mean of 3.3%. There was significant difference ($P \leq 0.05$) in ash content due to varieties.

Table 4.1: *Proximate composition of the amaranth varieties at vegetative and post flowering stage*

Variety	Moisture (%)		Ash (%)		Proteins (%)	
	H1	H2	H1	H2	H1	H2
1	87.7± 0.14 ^{b}	82.7± 0.28 ^{f}	2.57± 0.15 ^{b}	2.73± 0.09 ^{a}	3.5± 0.3 ^{bc}	4.1± 0.13 ^{a}
2	87.4± 0.03 ^{b}	84.1± 0.22 ^{g}	2.71± 0.05 ^{c}	2.87± 0.08 ^{bc}	3.4± 0.3 ^{abc}	3.9± 0.13 ^{a}
3	87.4± 0.13 ^{b}	79.4± 0.23 ^{b}	2.85± 0.02 ^{d}	3.33± 0.04 ^{d}	3.2± 0.1 ^{ab}	5.3± 0.04 ^{c}
4	87.6± 0.15 ^{b}	81.8± 0.31 ^{e}	2.67± 0.06 ^{bc}	2.81± 0.05 ^{b}	3.5± 0.3 ^{abc}	5.9± 0.12 ^{e}
5	86.7± 0.33 ^{a}	80.4± 0.48 ^{c}	2.41± 0.02 ^{a}	2.92± 0.02 ^{bc}	3.7± 0.3 ^{c}	5.3± 0.09 ^{c}
6	87.6± 0.47 ^{b}	79.7± 0.15 ^{b}	2.39± 0.01 ^{a}	2.83± 0.37 ^{b}	3.5± 0.2 ^{abc}	4.4± 0.3 ^{b}
7	87.5± 0.23 ^{b}	82.0± 0.07 ^{e}	3.05± 0.04 ^{e}	3.12± 0.17 ^{cd}	3.6± 0.2 ^{c}	5.2± 0.1 ^{c}
8	87.4± 0.48 ^{b}	81.2± 0.38 ^{d}	2.84± 0.03 ^{d}	3.45± 0.37 ^{d}	3.1± 0.1 ^{a}	5.6± 0.05 ^{d}
9	87.6± 0.27 ^{b}	77.7± 0.21 ^{a}	2.95± 0.12 ^{de}	4.11± 0.13 ^{e}	3.4± 0.04 ^{abc}	5.2± 0.08 ^{c}
10	87.6± 0.26 ^{b}	77.4± 0.32 ^{a}	2.93± 0.00 ^{de}	4.51± 0.17 ^{f}	3.8± 0.1 ^{c}	5.2± 0.1 ^{c}
LSD (5%)	**0.51**	**0.49**	**0.12**	**0.32**	**0.36**	**0.23**
Mean	**87.5**	**80.6**	**2.79**	**3.3**	**3.4**	**4.9**

H1*- vegetative stage,* ***H2****-post flowering stage; Means within the same column with different superscripts were significantly (*P≤0.05*) different. Values are presented as Mean± SD,* ***n=3.***

The ash results in this study were similar to those reported by Muriuki *et al.,* (2014), which ranged between 2.23% - 3.30% in *A. albus*, *A. hybridus* and *A. dubius*. The increase in the ash content at the post flowering stage can be attributed to the increase in the dry matter content as the plants grow older, which is also illustrated by the results of

moisture content in this study. The amount of ash represents the amount of mineral matter in the vegetable.

4.1.3 Protein content

There was a significant difference ($P \leq 0.05$) in the protein levels among the ten varieties. There was a significant increase the protein level in all the varieties as the plant grew older, from a mean of 3.4% at vegetative stage and 4.9% at post flowering stage (Table 4.1). Variety 10 and variety 4 had significantly higher proteins at vegetative stage and post flowering stage respectively.

The protein levels found in this study are similar to 3.4% reported by Gupta *et al.*, (2005). Oguche (2011) also reported values of 3.5% protein in *Amaranthus aquatic*. This could account for 6-10% of the daily protein requirement. The amaranth varieties in this study can be classified as superior in terms of protein contents, compared to other vegetables, e.g. cauliflower, carrot, cabbage, lettuce, spinach which are reported to have between 0.9% - 2.1% proteins (Hanif *et al.*, 2006). This therefore shows that these vegetable amaranth varieties can be an important source of proteins especially to vegetarians and to the poor. Amaranth leaves have been reported to be an excellent source of protein, with its maximal accumulation in the blossoming phase (Kadoshnikov *et al.*, 2005). The increase in protein content as the plants mature can be due to the increase in dry matter content.

4.2 Vitamin content in the amaranth samples

4.2.1 Beta carotene content

There was significant difference ($P \leq 0.05$) in beta carotene content in the amaranth leaves both due to variety and maturity stage. At vegetative stage, the amounts of beta carotene ranged between 5.3 – 6.8 mg/100 g (Table 4.2), which decreased to between

2.9 – 4.2 mg/100 g at post flowering stage in the various varieties. The mean decreased from 6.1 mg/100 g at vegetative stage to 3.6 mg/100 g at post flowering stage.

Table 4.2: *Vitamin composition in 100 g fresh weight of the amaranth varieties at vegetative and post flowering stage*

Variety	**Beta carotene(mg)**		**Ascorbic acid (mg)**	
	H1	**H2**	**H1**	**H2**
1	6.6 ± 0.08 ^{de}	3.2 ± 0.07 ^{b}	117.1± 3.9 ^{e}	152.7± 4.1 ^{a}
2	6.7 ± 0.30 ^{de}	3.2 ± 0.14 ^{b}	74.8± 4.9 ^{b}	155.0± 3.6 ^{ab}
3	5.4 ± 0.05 ^{ab}	2.9 ± 0.04 ^{a}	68.6± 0.7 ^{a}	151.6± 3.4 ^{a}
4	5.4 ± 0.03 ^{ab}	4.1 ± 0.06 ^{f}	76.6± 0.6 ^{b}	150.1± 1.7 ^{a}
5	6.7 ± 0.07 ^{de}	4.2 ± 0.05 ^{f}	85.1± 3.5 ^{cd}	149.8± 4.8 ^{a}
6	6.8 ± 0.14 ^{e}	4.1 ± 0.02 ^{f}	82.1± 3.4 ^{c}	176.2± 0.4 ^{c}
7	6.2 ± 0.03 ^{c}	3.7 ± 0.06 ^{e}	66.4± 2.3 ^{a}	175.9± 1.7 ^{c}
8	6.5 ± 0.01 ^{d}	3.1 ± 0.07 ^{b}	88.8± 3.7 ^{d}	159.6± 1.1 ^{b}
9	5.6 ± 0.13 ^{b}	3.4 ± 0.03 ^{c}	85.5± 0.5 ^{cd}	150.9± 0.6 ^{b}
10	5.3 ± 0.01 ^{a}	3.5 ± 0.01 ^{d}	84.9± 1.1 ^{cd}	158.1± 0.5 ^{b}
LSD (5%)	**0.19**	**0.11**	**4.87**	**4.76**
Mean	**6.1**	**3.6**	**83**	**158**

H1*- vegetative stage,* ***H2****-post flowering stage; Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD,* ***n=3***

The beta carotene contents of the varieties in this study translate to about 10,000 IU at vegetative stage and about 6,000 IU at post flowering stage (conversion;$1IU \cong$

0.0006 mg). If taken in the raw state, these varieties can provide the full recommended daily allowance of beta carotene to all individuals. The results in this research were similar to those reported by Gupta *et al*. (2005), of 5.41 mg/100 g in fresh leaves of *Amaranthus tricolor.* The beta carotene contents of the samples also compare favorably with the values reported in *Amarathus hybridus* L. leaves of 3.29 mg/100 g (Akubugwo *et al.,* 2008). As the amaranths mature, the pigmentation of the leaves tend to reduce. This explains the reduced amounts of beta carotene at post flowering stage. Beta carotene from plants is normally converted to vitamin A (retinol) with the help of enzymes from the liver. It is needed for healthy skin, protects against infections, antioxidant and immune booster, essential for night vision

4.2.2 Vitamin C/ Ascorbic acid content

There was significant difference ($P \leq 0.05$) in ascorbic acid content due to variety at both stages of maturity. Amounts of ascorbic acid increased significantly as the plants matured. At vegetative stage, the ascorbic acid content ranged from a minimum of 68.6 mg/100 g in Variety 3 to a maximum of 117.1 mg/100 g in Variety 1, with a mean of 83.0 mg/100 g. There was a significant increase ($P \leq 0.05$) due to maturity, with the mean vitamin C content rising to 158 mg/100 g at the post flowering stage.

Vitamin C content in Variety 7 increased by 163%; from 66.4 mg/100 g at vegetative stage to 175.9 mg/100 g at post flowering stage (Table 4.2). Variety 1, which had the highest amount at vegetative stage, had the least increase of 30% up from 117.1 mg/100 g at vegetative stage to 152.7 mg/100 g.

The overall amounts are similar to reported values of 135 mg/100 g (Abukutsa-Onyango, 2003). Consumption of 100 g raw amaranth leaves can provide up to 270% of vitamin C daily requirement. Vitamin C (ascorbic acid) is however highly sensitive to air, light, temperatures and is also soluble in water. Hence most of it, 57% - 78% has been reported to be lost during cooking of vegetables. Shorter cooking time is therefore

recommended to avoid greater losses of this vitamin. Human beings cannot synthesize vitamin C, which is a very important antioxidant. Vitamin C is an important co-factor in protein chemistry and has many other uses. It strengthens immune system, fights infections, makes collagen, keeping bones, skin and joints firm and strong, antioxidant, protecting against cancer and heart disease.

4.3 Mineral content of the amaranth varieties

4.3.1 Calcium content

There was significant difference ($P \leq 0.05$) in calcium content due to variety at both stages of maturity. At the vegetative stage and the post flowering stage, the calcium content in the amaranth leaves varied from 225.2 – 399.7 mg/100 g, and 361.3 - 557.9 mg/100 g, respectively (Table 4.3). The calcium content also significantly increased due to maturity ($P \leq 0.05$) from a mean of 305.1 mg/100 g at the vegetative stage to that of 456.2 mg/100 g at the post flowering stage. This has potential to provide up to 50% of the recommended daily value of 1000 mg of calcium.

The results suggest that most calcium accumulation in amaranth happens as the plant enters reproductive stage. Yang and Keding (2009) reported similar results in five amaranth species; *A. blitum*, *A. tricolor*, *A. cruentus*, *A. dubius* and *A. viridis*. These results were considerably lower than those reported by Makobo *et al*., (2010) which showed 2693.1 mg/100 g at week 3 and 89% reduction at week 8 in *Amaranthus cruentus*; and was higher than 44.15 mg/100 g reported by Marschner (1986). The big difference can be attributed to differences in climatic conditions and difference varieties.

4.3.2 Iron content

There was significant difference (P≤0.05) in iron content due to variety, and also due to maturity stage (Table 4.3). The iron content ranged from a minimum of 22.8 mg/100 g in Variety 1 to a maximum of 33.7 mg/100 g in Variety 7 at the vegetative stage.

Table 4.3*: Calcium and iron content of the amaranth varieties at both vegetative and post flowering stage in 100 g fresh weight*.

Variety	**Calcium (mg)**		**Iron (mg)**	
	H1	**H2**	**H1**	**H2**
1	281.1±8.3 ^{bc}	501.9± 9.5 ^{f}	22.8± 2.5 ^{a}	13.8± 0.9 ^{ab}
2	288.3±4.5 ^{c}	429.8± 5.6 ^{c}	32.1± 1.1 ^{de}	22.4± 0.5 ^{de}
3	399.7±4.4 ^{f}	459.2± 1.4 ^{e}	26.6± 1.5 ^{c}	12.5± 0.6 ^{a}
4	270.9±3.1 ^{b}	361.3± 1.3 ^{a}	23.9± 2.1 ^{ab}	19.9± 1.0 ^{c}
5	225.2±4.7 ^{a}	495.2± 9.3 ^{f}	35.4± 1.5 ^{f}	24.0± 1.8 ^{e}
6	315.6±7.3 ^{d}	441.7± 2.3 ^{d}	30.9± 0.7 ^{d}	22.0± 0.7 ^{d}
7	353.0±12.9 ^{e}	513.6± 2.3 ^{g}	33.7± 0.5 ^{ef}	23.3± 1.9 ^{de}
8	314.1±7.1 ^{d}	557.9± 0.9 ^{h}	31.6± 0.5 ^{de}	20.1± 0.4 ^{c}
9	289.9±6.8 ^{c}	378± 5.0 ^{b}	25.7± 0.5 ^{bc}	14.3± 0.5 ^{ab}
10	312.8±2.4 ^{d}	423.7± 2.4 ^{c}	26.5± 1.6 ^{c}	15.6± 0.5 ^{b}
LSD (5%)	**10.65**	**8.95**	**2.47**	**1.84**
Mean	**305.1**	**456.2**	**28.9**	**18.8**

H1*- vegetative stage,* ***H2****-post flowering stage; Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD, n=3*

The corresponding range at the post flowering stage was a minimum of 12.5 mg/100 g in Variety 3 and a maximum of 24.0 mg/100 g in Variety 5. The iron content decreased from 28.9 mg/100 g to 18.8 mg/100 g in the vegetative and post flowering maturity stages, respectively.

These show that with good bioavailability, these vegetables can provide enough iron to meet the daily value of 18 mg. These values are almost similar to those reported by Abukutsa (2010), which were 16.0 mg/100 g and 20.1 mg/100 g fresh weight of amaranths grown at two different sites. Akubugwo *et al.,* (2008) also reported 13.2 mg/100 g of iron in shredded *Amaranthus hybrid L.* leaves while Gupta *et al.,* (2005) reported 15.1 mg/100 g fresh leaves of *Amaranthus tricolor*. Adequate intake of the vegetable could provide the body with the recommended daily intake of 18 mg/day of iron for normal adult (Tietz *et al*., 1994). Iron is an essential trace element for hemoglobin formation, normal functioning of the central nervous system and energy metabolism. (Shills and Young, 1988; Ishida *et al*., 2000).

4.3.3 Zinc content

There was significant difference ($P \leq 0.05$) in zinc content due to variety. The zinc content also significantly decreased as the amaranth matured from the vegetative to the amaranth stage. Zinc amounts ranged between 1.4 mg/100 g and 2.3 mg/100 g among the varieties at vegetative stage, with a mean of 1.8 mg/100 g (Table 4.4). After flowering the zinc content ranged from 0.7 to 1.1 mg/100 g, with a mean of 0.9 mg/100 g.

Variety 8 had the highest zinc at vegetative stage while variety 3 and variety 7 had the highest amounts of zinc at post flowering stage. These results are similar to those reported by Muriuki *et al.,* (2014) which ranged between 0.6- 1.67 mg/100 g in fresh leaves of five amaranth species. These results show that the varieties can provide about 7% of the recommended daily allowance for zinc if 100 g of the amaranth leaves is

consumed. Zinc is involved in the normal functioning of the immune system. Zinc is normally required in the body in trace amount, and therefore regular consumption of vegetable amaranth can assist in preventing adverse effects of zinc deficiency such as growth retardation.

Table 4.4*: Zinc and Magnesium content of the amaranth varieties at both vegetative and post flowering stage in 100 g fresh weight*.

Variety	**Zinc (mg)**		**Magnesium (mg)**	
	H1	**H2**	**H1**	**H2**
1	1.4± 0.2 ^{a}	0.7± 0.03 ^{a}	166.9± 1.4 ^{c}	307.0± 2.1 ^{g}
2	1.4± 0.2 ^{a}	0.7± 0.03 ^{a}	206.1± 4.1 ^{i}	264.4± 1.1 ^{b}
3	1.6± 0.4 ^{ab}	1.1± 0.03 ^{f}	179.5± 2.0 ^{ef}	259.9± 4.8 ^{a}
4	1.8± 0.1 ^{bc}	0.8± 0.03 ^{b}	176.5± 2.5 ^{e}	275.9± 0.8 ^{d}
5	1.9± 0.2 ^{c}	0.8± 0.02 ^{c}	172.0± 0.6 ^{d}	299.9± 1.7 ^{f}
6	1.9± 0.02 ^{cd}	1.0± 0.01 ^{e}	150.1± 2.3 ^{a}	278.3± 2.9 ^{d}
7	1.5± 0.02 ^{a}	1.1± 0.05 ^{f}	183.1± 1.1 ^{g}	335.1± 1.2 ^{h}
8	2.3± 0.03 ^{e}	0.9± 0.00 ^{d}	182.4± 2.4 ^{fg}	303.8± 0.8 ^{fg}
9	2.1± 0.2 ^{d}	0.7± 0.01 ^{a}	194.2± 1.2 ^{h}	269.7± 1.5 ^{c}
10	1.8± 0.04 ^{bc}	0.8± 0.02 ^{b}	163.4± 0.5 ^{b}	294.2± 1.8 ^{e}
LSD (5%)	**0.21**	**0.04**	**3.18**	**3.93**
Mean	**1.8**	**0.9**	**177.4**	**288.8**

H1*- vegetative stage,* ***H2****-post flowering stage; Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD, n=3*

4.3.4 Magnesium content

There was a significant variation ($P \leq 0.05$) in the magnesium content due to variety at both vegetative and post flowering stages. The mean magnesium content in the amaranth leaves increased significantly from a mean of 177.4 mg/100 g at the vegetative stage to a mean of 288.8 mg/100 g at the post flowering stage (Table 4.4).

Varieties 2 and 7 had the highest amounts of magnesium at vegetative and post flowering stage respectively, while variety 6 and 3 had the lowest amounts of magnesium at vegetative stage and at post flowering stage. The values of magnesium in this research are similar to 249.92 mg/100 g reported by Asaolu *et al.,* (2012) in *A. hybridus*. This can account for about 40- 70% of the recommended daily allowance of magnesium if 100 g of these amaranths is consumed. Magnesium is an essential mineral in human nutrition and is involved in many biological functions and can also be used to ease many conditions such as muscle spasm and tension, pain and physical symptoms of stress.

4.4 Anti-nutrient content of the amaranth varieties

4.4.1 Phytate content

There were also significant differences ($P \leq 0.05$) in the phytate contents among the ten varieties at both vegetative and post flowering stages. The amounts of phytates in the samples ranged between 119.0- 194.7 mg/100 g at vegetative stage and 226.2-295.3 mg/100 g at post flowering stage. There was a significant increase ($P \leq 0.05$) in the mean phytate content from 157.5 mg/100 g at vegetative stage to 247.4 mg/100 g at post flowering stage (Table 4.5).

It was observed that Variety 4 had the highest phytate content and Variety 3 had the lowest phytate content in both maturity stages. Phytate is the storage form of phosphates

in plants, and the results of this research show that there is high accumulation of these phosphates as the plants mature. Phytate acts as a strong chelator forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability (Erdman, 1979).

Table 4.5: *Phytate and oxalate contents in 100 g of the Fresh Amaranth Leaves at vegetative and post flowering stage.*

Variety	Phytates (mg)		Oxalates (mg)	
	H1	**H2**	**H1**	**H2**
1	135.1± 0.3 ^{c}	256.6± 0.5 ^{h}	590.7± 27.2 ^{h}	936.2± 12.3 ^{d}
2	178.8± 0.4 ^{f}	238.7± 0.2 ^{e}	467.1± 11.7 ^{bc}	659.6± 8.5 ^{a}
3	119.8± 0.4 ^{a}	226.2± 0.1 ^{a}	482.4± 21.1 ^{cd}	768.2± 24.2 ^{b}
4	194.7± 0.8 ^{i}	295.3± 0.1 ^{j}	507.0± 19.8 ^{def}	948.9± 15.1 ^{d}
5	128.0± 0.8 ^{b}	242.5± 0.2 ^{f}	551.7± 7.7 ^{g}	678.6± 14.9 ^{a}
6	187.3± 0.1 ^{g}	268.9± 0.5 ^{i}	642.1± 13.9 ^{i}	757.9± 23.9 ^{b}
7	193.4± 0.9 ^{h}	229.9± 0.3 ^{b}	533.7± 5.9 ^{eg}	843.2± 19.5 ^{c}
8	119.0± 0.4 ^{a}	231.1± 0.2 ^{c}	506.2± 10.3 ^{de}	738.8± 19.7 ^{b}
9	167.2± 0.5 ^{e}	250.9± 0.2 ^{g}	453.1± 23.3 ^{b}	832.8± 22.3^{c}
10	151.3± 0.6 ^{d}	233.6± 0.8 ^{d}	352.6± 7.6 ^{a}	737.1± 10.4 ^{b}
LSD (5%)	**1.07**	**0.72**	**26.33**	**31.76**
Mean	**157.5**	**247.4**	**508.6**	**790.1**

H1*- vegetative stage,* ***H2****-post flowering stage. Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD, n=3*

Phytate is associated with nutritional diseases such as rickets and osteomalacia in children and adults, respectively. According to Watzl and Leitzmann, (1999), phytate is also a beneficial phytochemical and has antioxidant activity in the body.

4.4.2 Oxalate content

There were significant differences ($P \leq 0.05$) in the oxalate content among the ten varieties. At vegetative stage, the amount of oxalates ranged between 352.6-642.1 mg/100 g and between 659.6-948.9 mg/100 g at post flowering stage. There was also a significant increase in the oxalate levels as the plant matured, from 508.6 mg/100 g to 790.1 mg/100 g, at vegetative and post flowering stages, respectively (Table 4.5).

The oxalate values in this research were similar to 690 mg/100 g oxalates reported by Gupta *et al.,* (2005) in fresh leaves of *A. tricolor*. These values are above the permissible levels of 250 mg/100 g fresh weight (Oguchi *et al*., 1996); but much lower than the minimum doses that can lead to death, which is 4-5 g of oxalate (Fasset, 1973). However, other studies show that boiling the vegetable for five minutes and discarding the water eliminates most of the oxalates. In the body, oxalate combine with calcium to form insoluble calcium oxalate, which is later excreted from the body with faeces and urine. This prevents the absorption and utilization of calcium by the body hence increasing the risk for diseases such as rickets and osteomalacia (Ladeji *et al*., 2004; Agbaire, 2012).

4.4.3 Nitrate content

Nitrate contents varied significantly among the varieties at both the vegetative and post flowering stages. At vegetative stage, the nitrate content varied between 164.4- 205.2 mg/100 g and between 101.3- 137.7 mg/100 g at post flowering stage with significant differences ($P \leq 0.05$) among the varieties. There was also significant decrease ($P \leq 0.05$)

in the mean nitrate content from 182 mg/100 g at vegetative stage to 121 mg/100 g at post flowering stage (Table 4.6).

Table 4.6: *Nitrate and Tannin contents in 100 g of the Fresh Amaranth Leaves at vegetative and post flowering stage.*

Variety	**Nitrates (mg)**		**Tannins (mg TAE)**	
	H1	**H2**	**H1**	**H2**
1	175.1± 0.5 ^{cd}	101.3 ± 1.3 ^{a}	81.9 ± 0.8 ^{ab}	106.5 ± 1.1 ^{b}
2	203.9± 1.2 ^{f}	110.6 ± 5.1 ^{b}	91.2 ± 0.9 ^{d}	117.3 ± 0.8 ^{d}
3	193.7± 2.9 ^{e}	124.9 ± 0.2 ^{d}	80.9 ± 1.9 ^{ab}	102.1 ± 0.2 ^{a}
4	196.2± 0.3 ^{e}	121.6 ± 2.5 ^{cd}	90.9 ± 0.7 ^{d}	114.3 ± 0.2 ^{c}
5	183.7± 2.9 ^{d}	133.0 ± 0.6 ^{e}	83.3 ± 2.6 ^{b}	103.3 ± 1.1 ^{a}
6	205.2± 0.7 ^{f}	139.1 ± 1.3 ^{e}	86.4 ± 0.6 ^{c}	106.5 ± 0.7 ^{b}
7	170.5± 1.0 ^{bc}	120.9 ± 0.7 ^{c}	80.1 ± 1.5 ^{a}	107.9 ± 2.5 ^{b}
8	166.4± 3.7 ^{ab}	110.1 ± 1.7 ^{b}	82.1 ± 1.9 ^{ab}	107.4 ± 1.1 ^{b}
9	164.4± 2.6 ^{a}	120.4±1.3 ^{c}	96.2 ± 1.0 ^{e}	116.3 ± 0.8 ^{cd}
10	170± 0.5 ^{abc}	137.7±1.1 ^{e}	94.2 ± 0.6 ^{e}	124.7 ± 3.2 ^{e}
LSD (5%)	**5.52**	**3.66**	**2.53**	**2.53**
Mean	**182.1**	**121.9**	**86.7**	**110.6**

H1*- vegetative stage,* ***H2****-post flowering stage,* ***TAE****- Tannic acid equivalent. Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD, n=3*

4.4.4 Tannin content

Qualitative analysis showed the presence of tannins in the amaranth leaves.

The tannin content of the amaranth varieties varied significantly among the varieties ($P \leq 0.05$). The tannin values ranged between 80.1-96.2 mg/100 g at vegetative stage and 102.1-124.7 mg/100 g at post flowering stage, respectively (Table 4.6). There was significant increase ($P \leq 0.05$) in mean tannin content from 86.7 mg/100 g before flowering to a mean of 110.6 mg/100 g after flowering.

The tannin results in this project were found to be lower than those reported by Omobolanle (2013) of 877 mg/100 g TAE in *Amaranthus cruentus*. The results were however in agreement with those reported by Gupta *et al.*, (2005), of 106 mg/100 g in fresh leaves of *Amaranthus tricolor.* Tannins are polyphenols with molecular weight greater than 500 and they have the ability to precipitate proteins from aqueous solutions. Tannins bind proteins, hence interfering with their bioavailability. Other studies have also shown tannins as a phytochemical and having some antioxidant activity in the body.

4.5 Phytochemical content of the amaranth varieties

4.5.1 Total carotenoid content

There were significant differences ($P \leq 0.05$) in carotenoid content among the varieties at both maturity stages. The total carotenoids content in the samples ranged from 39.1-47.8 mg/100 g at vegetative stage and 41.2-47.3 mg/100 g after flowering (Table 7). However, there was an increase in the total carotenoid content from a mean of 42.5 mg/100 g at vegetative stage to a mean of 43.7 mg/100 g at post flowering stage, the increase was not significant (Table 7).

These results are almost similar to 35.17 mg/100 g reported in *Amaranthus tricolor* by Gupta *et al*., (2005). Carotenoids are yellow, red and orange pigments present in many fruits and vegetables. In the diet they act as powerful antioxidants and are believed to protect the body against free radical attack and reduce the incidence of cataracts, heart disease and certain cancers (Kumar and Rao, 2012).

Table 4.7: *Flavonoid, carotenoid content and Total Antioxidant Capacity of the Amaranth Leaves at vegetative and post flowering stage.*

Variety	Total carotenoids(mg)		Flavonoid (mg/100g QE)		Antiox activity: IC_{50} (mg/ml)	
	H1	H2	H1	H2	H1	H2
1	44.7± 1.1 ^{ef}	41.2± 1.3 ^{a}	4284.0± 19.2 ^{i}	2330.1± 2.8 ^{i}	2.3± 0.17 ^{b}	1.2± 0.1 ^{ab}
2	47.8± 2.1 ^{g}	41.5± 0.5 ^{a}	3368.1± 4.9 ^{g}	1929.5± 12.5 ^{c}	2.3± 0.29 ^{b}	1.5± 0.4 ^{b}
3	39.5± 0.8 ^{ab}	42.0± 0.8 ^{a}	3629.9± 15.3 ^{h}	1697.5± 13.2 ^{b}	2.5± 0.36 ^{b}	1.0± 0.05 ^{a}
4	39.1± 1.1 ^{a}	44.2± 1.5 ^{b}	3026.5± 5.6 ^{c}	1975.9± 16.1 ^{d}	2.2± 0.17 ^{ab}	1.3± 0.2 ^{ab}
5	45.4± 0.4 ^{f}	46.5± 0.1 ^{c}	3170.1± 7.0 ^{e}	2279.5± 20.7 ^{h}	2.4± 0.11 ^{b}	1.2± 0.0 ^{ab}
6	43.2± 0.5 ^{de}	44.2± 0.2 ^{b}	3002.9± 2.7 ^{b}	2065.7± 10.4 ^{e}	2.3± 0.15 ^{b}	1.3± 0.05 ^{ab}
7	41.6± 0.7 ^{cd}	42.6± 0.2 ^{a}	2819.9± 12.5 ^{a}	2198.7± 17.3 ^{g}	2.3± 0.2 ^{b}	1.2± 0.0 ^{ab}
8	41.3± 0.1 ^{bcd}	41.9± 0.8 ^{a}	3201.0± 8.4 ^{f}	2289.7± 10.4 ^{h}	1.9± 0.2 ^{a}	1.4± 0.2 ^{ab}
9	41.3± 1.2 ^{bcd}	45.9± 0.5 ^{c}	3048.9± 4.9 ^{d}	1446.5± 10.3 ^{a}	2.2± 0.15 ^{ab}	1.1± 0.3 ^{ab}
10	40.4± 0.5 ^{abc}	47.3± 0.5 ^{c}	2994.8± 7.6 ^{b}	2117.5± 10.3 ^{f}	2.5± 0.2 ^{b}	1.0± 0.1 ^{a}
LSD (5%)	**1.78**	**1.43**	**18.22**	**21.61**	**0.34**	**0.36**
Mean	**42.5**	**43.7**	**3254.6**	**2033.9**	**2.3**	**1.2**

H1*- vegetative stage,* ***H2****- post flowering stage,* ***IC_{50}****- concentration at 50% DPPH inhibition,* ***QE****- quercetin equivalent. Means within the same column with different superscripts were significantly (P≤0.05) different. Values are presented as Mean± SD,* ***n=3****.*

4.5.2 Flavonoid content

Preliminary phytochemical screening of the methanolic extract showed the presence of flavonoids.

There were significant differences ($P \leq 0.05$) in the flavonoid content of the ten amaranth varieties at both vegetative and post flowering stage. At vegetative stage, flavonoid content ranged between 2819.9-4284.0 mg/100 g quercetine equivalent (QE), and 1446.5-2330.1 mg/100 g QE at post flowering stage. There was significant decrease in the flavonoid content, from 3255 mg/100 g at vegetative stage to 2034 mg/100 g at post flowering stage (Table 7).

These flavonoid values are relatively lower compared to those reported by Olajire and Azeez (2011) which were 69.67 mg/ g (6967 mg/100 g) in *Amaranthus caudatus* and much higher than those reported by Akubugwo *et al*., (2008). Flavonoids have been shown to have antibacterial, anti-inflammatory, anti-allergic, anti-neoplastic, antiviral, antithrombotic and vasodillatory activities (Miller, 1996). The potent antioxidant activities of flavonoids have been suggested to be responsible for many of the above actions, as oxidative damage is implicated in most disease processes.

4.5.3 Antioxidant activity/ Free radical scavenging activity

There was significant difference ($P \leq 0.05$) in antioxidant activity among the ten varieties at the two maturity stages. At vegetative stage, the antioxidant activity ranged from IC_{50} of 1.9 to 2.5 mg/mL while at post harvest stage, the values ranged from IC_{50} of 1.0 to 1.5 mg/mL. There was no significant difference ($P \leq 0.05$) in the antioxidant activity of nine varieties (Table 7). Variety 8 had significantly higher antioxidant activity (lowest IC_{50} value, 1.9 mg/mL) than other varieties but was not significantly different ($P \leq 0.05$) with variety 4 and 9 at 2.2 mg/mL At post flowering stage, there was a significant rise in the

antioxidant activity of all the vegetables to a mean IC_{50} of 1.2 mg/mL from a mean of 2.3 mg/mL.

The lower the IC_{50} value the higher the FRSA/antioxidant power. The antioxidant activity results in this study were found to be similar to those reported by Ouedraogo *et al.,* (2011), of 1.26 mg/mL in *Amaranthus dubius*, 1.43 mg/mL in *Amaranthus graecizans*, 1.50 mg/mL in *Amaranthus hybridus* and 2.73 mg/mL in *Amaranthus virdis*. The leaf extract of amaranth exhibited a good potential to act as a free radical scavenger. However the IC_{50} for DPPH inhibition for amaranths (1.0-2.5 mg/mL) was comparably lower to that of Vitamin C (0.004 mg/mL) which is a known free radical scavenger.

High % inhibition of DPPH is an indication of high free radical scavenging activity (FRSA) of the extract. The methanol extracts of the amaranth varieties showed potential of free radical scavenging activity against DPPH, which was, however, considerably lower compared to vitamin C as a free radical scavenger.

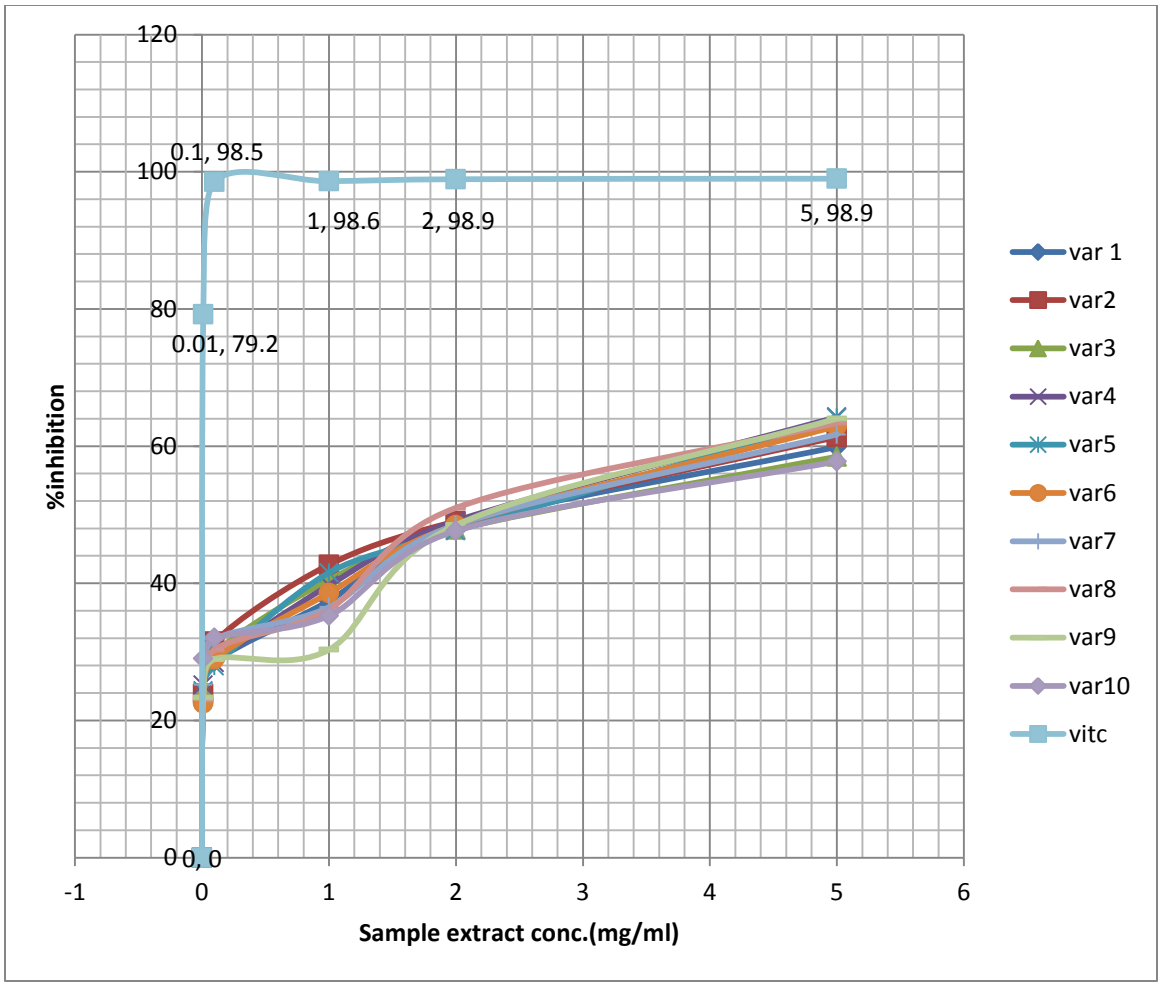


Figure 2*: Inhibition (%) of DPPH against concentration of extracts of amaranth leaves harvested at vegetative stage and Vitamin C*

At vegetative stage, maximum inhibition was achieved at a high concentration of 5 mg/mL (57.7-64.2%) for the amaranth leaves compared to 0.1 mg/mL (98%) for Vitamin C (Figure 1). At post flowering stage, maximum inhibition was achieved at extract concentration of 5 mg/mL (63.1-71.8%) in the different varieties, and at 0.1 mg/mL (98%) for Vitamin C (Figure 2). Hence a higher concentration of amaranth leaves will be required to achieve maximal inhibition of DPPH compared to Vitamin C.

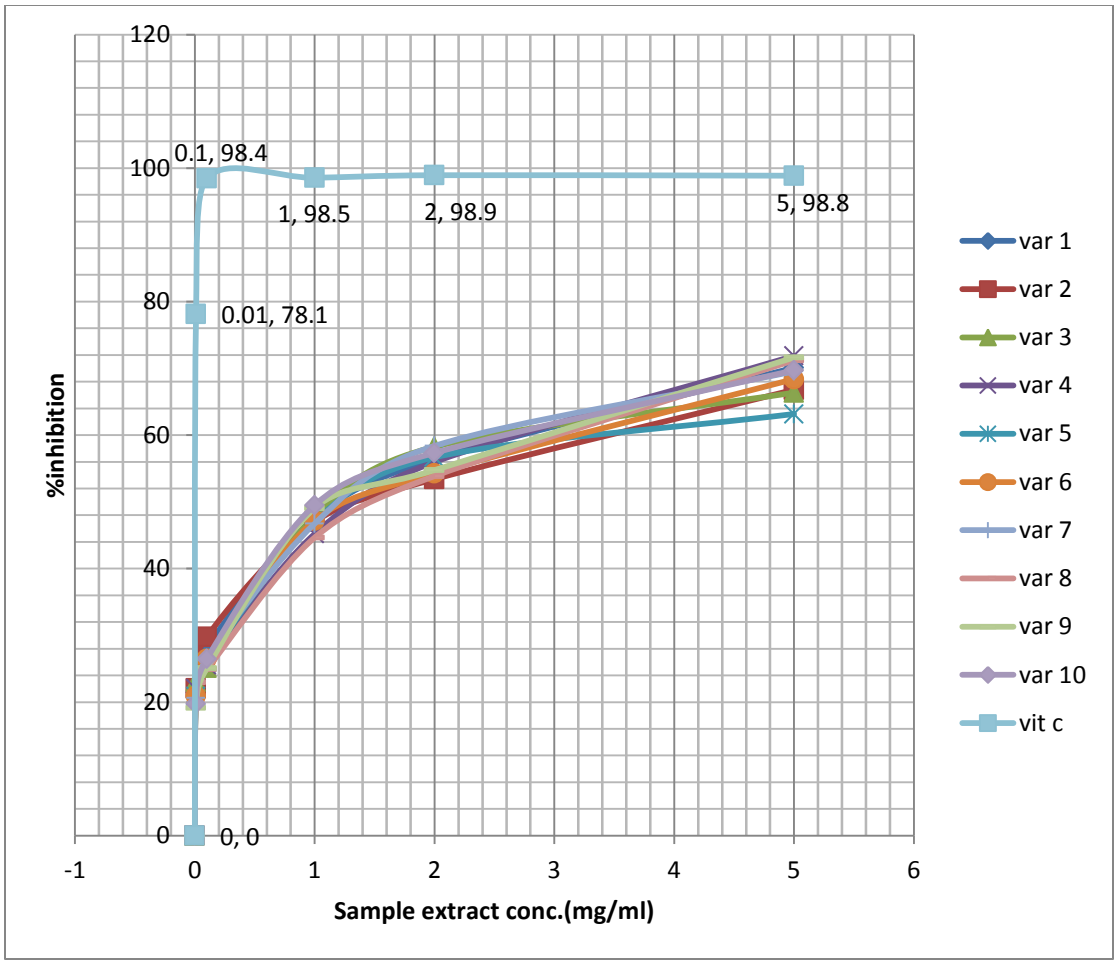


Figure 2: *Inhibition (%) of DPPH against concentration of extracts of amaranth leaves harvested at post flowering stage and Vitamin C*

The antioxidant activity is contributed to by the various phytochemicals with antioxidant activity. These include vitamin C, carotenoids and flavonoids. However, vitamin C is heat labile, it is also easily dissolved and most of it may have been lost during sample extraction for the antioxidant activity analysis. The increase in the antioxidant activity at post flowering stage can be attributed to other components such as tannins and phytic acid which have been shown to have antioxidant activity.

5.0 CONCLUSIONS AND RECOMMENDATION

5.1 Conclusion

There were significant differences (P≤0.05) in the nutrient content among the amaranth varieties and at the two stages of maturity. Variety 5 *(A. hybridus)*, variety 7 *(A. dubius)* and variety 8 *(A. hypocandracus)* had the highest minerals, while variety 5 *(A. hybridus)*, variety 6 *(A. hybridus)*, variety 8 *(A. hypocandracus)* and variety 10 *(A. dubius)* had the highest proteins and vitamins.

Similarly there was also significant variation (P≤0.05) in anti-nutrient content in the ten amaranth varieties both due to variety and due to maturity stage. Variety 3 *(A. hybridus)* was found to be lowest in phytates and tannins, while Variety 1 (*A. blitum)* was high in nitrates. The anti-nutrients were found to accumulate at post flowering stage except nitrates.

Phytochemicals also varied significantly (P≤0.05) among the varieties and between the maturity stages with the total antioxidant activity increasing significantly at post flowering stage. Variety 1 (*A. blitum)* had the highest flavonoids which was significantly high at vegetative stage.

5.2 Recommendation

Further studies can be done on effects of cooking and processing methods such as drying on the various nutritional components of these amaranth vegetables, and the best method that preserves nutrients and destroys anti-nutrients be established.

There is potential for selection of amaranth varieties to promote the utilization of the varieties with high nutrient content and/or low anti-nutrient levels. Selection can also be

done for specific varieties with relatively high antioxidant activities. There is also potential to plan the harvesting to be done at a maturity stage of the amaranth that provides leaves with relatively high specific nutrients, or low anti-nutrients.

Studies on composition of other indigenous vegetables can also be done and compared with that of amaranth to establish the most nutritious vegetables.

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APPENDICES

APPENDIX 1: ANOVA TABLES

Variate: antiox_Activity

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.19467	0.09733	2.44	
replication.*Units* stratum					
variety	9	0.90133	0.10015	2.51	0.046
Residual	18	0.71867	0.03993		
Total	29	1.81467			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.06467	0.03233	0.72	
replication.*Units* stratum					
variety	9	0.59333	0.06593	1.47	0.233
Residual	18	0.80867	0.04493		
Total	29	1.46667			

Variate: ascorbic_acid

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	26.511	13.256	1.64	
replication.*Units* stratum					
variety	9	5413.55	601.505	74.42	<.001
Residual	18	145.479	8.082		
Total	29	5585.54			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	8.47	4.235	0.55	
replication.*Units* stratum					
variety	9	2744.8	304.977	39.47	<.001
Residual	18	139.085	7.727		
Total	29	2892.35			

Variate: ash

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.00559	0.0028	0.54	
replication.*Units* stratum					
variety	9	1.0933	0.12148	23.44	<.001
Residual	18	0.0933	0.00518		
Total	29	1.1922			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.11382	0.05691	1.59	
replication.*Units* stratum					
variety	9	11.6123	1.29026	35.99	<.001
Residual	18	0.64531	0.03585		
Total	29	12.3715			

Variate: beta_carotene

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.06053	0.03026	2.33	
replication.*Units* stratum					
variety	9	10.5286	1.16984	90.09	<.001
Residual	18	0.23374	0.01299		
Total	29	10.8229			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.02081	0.0104	2.74	
replication.*Units* stratum					
variety	9	5.739	0.63767	167.99	<.001
Residual	18	0.06833	0.0038		
Total	29	5.82814			

Variate: calcium

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	245.05	122.53	3.18	
replication.*Units* stratum					
variety	9	60461	6717.89	174.41	<.001
Residual	18	693.3	38.52		
Total	29	61399.3			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.

replication stratum	2	17.34	8.67	0.32	
replication.*Units* stratum					
variety	9	103094.5	11454.94	420.41	<.001
Residual	18	490.45	27.25		
Total	29	103602.3			

Variate: carotenoids

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	1.945	0.972	0.90	
replication.*Units* stratum					
variety	9	217.922	24.214	22.40	<.001
Residual	18	19.455	1.081		
Total	29	239.322			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.258	0.129	0.18	
replication.*Units* stratum					
variety	9	135.692	15.0769	21.53	<.001
Residual	18	12.602	0.7001		
Total	29	148.552			

Variate: flavonoids

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	30.6	15.3	0.14	

replication.*Units* stratum					
variety	9	4912568.2	545840.9	4837.37	4912568.2
Residual	18	2031.1	112.8		
Total	29	4914629.9			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	661.3	330.6	2.08	
replication.*Units* stratum					
variety	9	2162716.1	240301.8	1514.30	<.001
Residual	18	2856.4	158.7		
Total	29	2166233.7			

Variate: iron

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	3.193	1.596	0.77	
replication.*Units* stratum					
variety	9	507.44	56.382	27.09	<.001
Residual	18	37.457	2.081		
Total	29	548.09			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.720	0.360	0.31	
replication.*Units* stratum					
variety	7	504.926	56.103	48.53	<.001
Residual	14	20.807	1.156		

Total	23	526.454			

Variate: magnessium

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	24.94	12.47	3.63	
replication.*Units* stratum					
variety	9	6748.883	749.876	218.05	<.001
Residual	18	61.902	3.439		
Total	29	6835.725			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	2.449	1.224	0.23	
replication.*Units* stratum					
variety	9	14786.97	1642.997	312.02	<.001
Residual	18	94.783	5.266		
Total	29	14884.21			

Variate: moisture

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.01981	0.0099	0.11	
replication.*Units* stratum					
variety	9	2.19588	0.24399	2.68	0.036

Residual	18	1.63946	0.09108		
Total	29	3.85515			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.20033	0.10016	1.22	
replication.*Units* stratum					
variety	9	126.748	14.0831	171	<.001
Residual	18	1.48241	0.08236		
Total	29	128.43			

Variate: nitrates

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	16.05	8.03	0.77	
replication.*Units* stratum					
variety	9	6709.62	745.51	71.92	<.001
Residual	18	186.59	10.37		
Total	29	6912.26			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	14.111	7.055	1.54	
replication.*Units* stratum					
variety	9	13413	1490.34	326.31	<.001
Residual	18	82.21	4.567		

Total	29	13509.4			

Variate: oxalates

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	1209.2	604.6	2.57	
replication.*Units* stratum					
variety	9	170611.5	18956.8	80.49	<.001
Residual	18	4239.3	235.5		
Total	29	176060.0			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	266.4	133.2	0.39	
replication.*Units* stratum					
variety	9	262928.0	29214.2	85.20	<.001
Residual	18	6171.9	342.9		
Total	29	269366.3			

Variate: phytates

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.3187	0.1593	0.40	
replication.*Units* stratum					
variety	9	25270.4258	2807.8251	7091.22	<.001

Residual	18	7.1272	0.3960		
Total	29	25277.8717			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.4072	0.2036	1.15	
replication.*Units* stratum					
variety	9	12495.0472	1388.3386	7857.45	<.001
Residual	18	3.1804	0.1767		
Total	29	12498.6348			

Variate: protein

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.03731	0.01866	0.42	
replication.*Units* stratum					
variety	9	1.26623	0.14069	3.16	0.018
Residual	18	0.80049	0.04447		
Total	29	2.10403			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.05209	0.02604	1.45	
replication.*Units* stratum					
variety	9	11.7877	1.30974	73.1	<.001
Residual	18	0.32251	0.01792		

Total	29	12.1623			

Variate: tannins

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	1.196	0.598	0.28	
replication.*Units* stratum					
variety	9	955.862	106.207	48.91	<.001
Residual	18	39.088	2.172		
Total	29	996.146			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	5.100	2.550	1.18	
replication.*Units* stratum					
variety	9	1397.081	155.231	71.63	<.001
Residual	18	39.008	2.167		
Total	29	1441.190			

Variate: zinc

h1

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.25368	0.12684	7.72	
replication.*Units* stratum					
variety	9	2.28823	0.25425	15.48	<.001
Residual	18	0.29572	0.01643		

Total	29	2.83763			

h2

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
replication stratum	2	0.0004200	0.0002100	0.32	
replication.*Units* stratum					
variety	9	0.6694700	0.0743856	113.66	<.001
Residual	18	0.0117800	0.0006544		
Total	29	0.6816700			

APPENDIX 2: ANOVA SUMMARY

	F pr		e.s.e		s.e.d		l.s.d		s.e		Cv%	
	H1	**H2**	**H1**	**H2**	**H1**	**H2**	**H1**	**H2**	**H1**	**H2**	**H1**	**H2**
Antiox	0.046	0.233	0.11	0.12	0.16	0.17	0.34	0.36	0.19	0.21	8.7	17.2
Ascorbic acid	0.001	0.001	1.64	1.60	2.32	2.27	4.87	4.76	2.84	2.78	3.4	1.8
Ash	0.001	0.001	0.04	0.10	0.05	0.15	0.12	0.32	0.07	0.18	2.6	5.9
Beta carotene	0.001	0.001	0.06	0.03	0.09	0.05	0.19	0.10	0.11	0.06	1.9	1.7
Calcium	0.001	0.001	3.58	3.01	5.07	4.26	10.65	8.95	6.21	5.22	2.0	1.1
Flavonoids	0.001	0.001	6.13	7.27	8.67	10.29	18.22	21.61	10.62	12.6	0.3	0.6
Iron	0.001	0.001	0.83	0.62	1.17	0.87	2.47	1.84	1.44	1.07	5.0	5.7
Moisture	0.036	0.001	0.17	0.16	0.24	0.23	0.51	0.49	0.30	0.28	0.3	0.4
Nitrates	0.001	0.001	1.85	1.23	2.62	1.74	5.52	3.66	3.22	2.13	1.8	1.7
Oxalates	0.001	0.001	8.86	10.69	12.53	15.12	26.33	31.76	15.35	18.52	3.0	2.3
Phytates	0.001	0.001	0.36	0.24	0.51	0.34	1.07	0.72	0.62	0.42	0.4	0.2
Proteins	0.018	0.001	0.12	0.07	0.17	0.10	0.36	0.22	0.21	0.03	6.1	2.7
Tannins	0.001	0.001	0.85	0.85	1.20	1.20	2.53	2.53	1.47	1.47	1.7	1.3
Zinc	0.001	0.001	0.07	0.01	0.10	0.02	0.21	0.04	0.12	0.02	7.3	3.0
Carotenoids	0.001	0.001	0.60	0.48	0.84	0.68	1.78	1.43	1.04	0.83	2.5	1.9
Magnesium	0.001	0.001	1.07	1.32	1.51	1.87	3.18	3.93	1.85	2.29	1.0	0.8