

**NUTRITIONAL DIVERSITY OF LEAFY AMARANTH
(*Amaranthus*) SPECIES GROWN IN KENYA**

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**Nutritional diversity of leafy amaranth (*Amaranthus*) species grown
in Kenya**

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Science Degree in Food Science and Nutrition in the Jomo Kenyatta
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DECLARATION

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

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DEDICATION

To my parents, Mr. Johnson Muriuki, Mrs. Mary Muriuki and Mrs. Susan Mathenge, my brother and sisters, my husband Mr. Samuel Kihuni and my lovely son Jabali Mathenge. They have not only encouraged me but also supported me every step of my study and research process. Their continual support and motivation gave me additional strength to work towards achieving this dream. In addition, they have been an inspiration to me in carrying out this study. I love you all.

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ABBREVIATIONS AND ACRONYMS

AA	--	Ascorbic acid
AAS	--	Atomic Absorption Spectrophotometry
AOAC	--	Association of Official Analytical Chemists
AIDS	--	Acquired Immune Deficiency Syndrome
Ca	--	Calcium
CE	--	Catechin Equivalent
Conc	--	Concentration
DCIP	--	Dichlorophenol indophenol
DPPH	--	Diphenyl picryl hydrazyl
DWB	--	Dry weight basis
FAO	--	Food and Agriculture Organization
FST	--	Food Science and Technology
Fe	--	Iron
GAE	--	Gallic Acid Equivalent
H₂SO₄	--	Sulphuric acid
HCL	--	Hydrochloric acid
HPLC	--	High performance liquid chromatograph
ILV's	--	Indigenous Leafy Vegetables
IPGRI	--	International Plant Genetic Resources Institute
JKUAT	--	Jomo Kenyatta University of Agriculture and Technology
KARI	--	Kenya Agricultural Research Institute
NAOH	--	Sodium hydroxide
QE	--	Quercetin Equivalent
UV	--	Ultra violet
Zn	--	Zinc

ABSTRACT

Malnutrition is a major problem in Kenya affecting about 50% of the population. Utilization of nutritious food crops of African origin in mitigating malnutrition has been hindered by production and farming practices. Amaranth is one of the underutilized but nutritious food crops. Despite the large genetic diversity of the amaranth species grown in Kenya, there is inadequate information on their nutritional differences and how they can be tapped in mitigating hidden hunger. The current study aimed at determining the nutritional diversity of the most common amaranth species grown in Kenya. A baseline survey was conducted in Bondo, Kieni East, Lugari, Luanda and Sabatia regions of Kenya to identify the most popular amaranth varieties. Five of the most popular varieties namely; *A. dubius*, *A. hybridus*, *A. cruentus*, *A. albus* and *A. hypochondriacus* were selected and planted in the Jomo Kenyatta University of Agriculture and Technology experimental farm under the same agronomic conditions. Leaves from the 5 varieties were harvested at 4-6 weeks after planting. The samples were analyzed for their proximate composition, vitamin C, β carotene, antinutrient and phytochemical content. The samples were subjected to blanching (80°C for 3 minutes and 65°C for 25 minutes) prior to solar-drying. The effect of processing on vitamin C and β carotene was determined. The protein (3.37%), zinc (1.67mg/100g), β carotene (23.66mg/100g) and total phenolics (3.59GAE/100mg) content, were significantly ($p < 0.05$) high in *A. cruentus* than in the other species. The, calcium (336.47mg/100g) and iron (18.64mg/100g) content were significantly ($p < 0.05$) higher in *A. dubius* than in the other species. The antinutrient content in all the species was not significantly

different ($p < 0.05$). Blanching at 80°C for 3 minutes prior to solar drying led to better retention of vitamin C and β carotene than solar drying without prior blanching. According to this study, the most nutritious amaranth species is *A. cruentus*.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Amaranth is the common name for the domesticated species of the genus *Amaranthus* (family *Amaranthaceae*). It is one of the oldest food crops in the world (Gigliola camaggio, 2012). *Amaranthus* is one of the most promising plant genus and it consists of approximately 70 species 40 of which are native to the Americas 17 are mainly vegetable species three are grain while others are weedy (Andreas et al., 2011). Amaranth is a multipurpose crop whose leaves and grains are tasty and of high nutritional value, additionally it can be cultivated as an ornamental plant (Venskutonis & Kraujalis, 2013). The genus *Amaranthus* has received considerable attention in many countries because of the high nutritional value of some species that are important sources of food, either as vegetable or grain (Srivastava, 2001).

Vegetable amaranth has been used in China for over 400 years, it is commonly found in the Caribbean and Africa, grain amaranth was cultivated and revered by the Aztecs of Mexico, the Mayas of Central America and the Inca of South America (O'Brien & Price, 2008). Amaranth plants grow as weed and are available abundantly during the rainy season in Haryana State. Its use in the United States is limited to canned imports for ethnic uses, primarily in the New York City area (Singh et al., 2001).

In Kenyan rural areas, amaranth is known as a traditional vegetable which can grow in open fields. It exhibit the highest diversity of species exploited as traditional vegetables(Wambugu & Muthamia, 2009). At least every ethnic group has a name for amaranth; it is called *terere* in Kikuyu, *Mchicha* in Kiswahili, *Omboga* in Luhya *Ododo* in Luo, *Sikukuu* or *Chepkuratian* in Pokot, *Lookwa* or *Epespes* in Turkana , and *Ekwala* in Teso(Jacob alemu, 2005). Amaranth is mostly grown for its edible leaves which are a regular food component of most local community diets in the country; however, some of the species have edible seeds. It is one of the few

plants whose leaves are eaten as a vegetable while the seeds are used in the same way as cereals. There is no distinct separation between the vegetable and grain types since the leaves of young plants grown for grain can be eaten (Kariuki et al., 2013).

Previously there has been aggressive promotion of exotic types of vegetable resulting to abandonment of indigenous vegetable. However recently, due to the realization of their high nutritive and medicinal value and low input requirement, there has been intensified awareness creation resulting in increased production, consumption and marketing of these vegetables(Wambugu & Muthamia, 2009). In Kenya, the volume of production of vegetable amaranth have increased over the last few years in response to the growing urban vegetable demand(Onyango & Imungi, 2007). Vegetable amaranth is found in many supermarkets and green grocers' stores in the urban centers of Kenya. More than 90% of the supply of the vegetables to these outlets is normally from farms that are within the environs of the urban center (Moraa, 2008).

1.2 Problem statement and justification

About 50% of Kenyans are malnourished. They are faced with both under nutrition and over nutrition the former being more common, particularly in children, pregnant women and lactating women. Kenya is also faced with micronutrient deficiency especially vitamin A and Iron deficiency. Malnutrition is a major direct and indirect cause of infant and childhood mortality and morbidity; nutritional problems also reduce the productivity of a country. There is therefore a need to identify nutrient-rich foods that can be produced inexpensively to meet the nutrient requirements of everyone and especially the vulnerable groups. Amaranth is a fast growing plant, is easily cultivated in gardens and fields and is drought tolerant and richer in nutrients compared to many exotic vegetables consumed in Kenya. There are many cultivars and land races of amaranth in cultivation, but it is not known which of these has the highest nutritional impact. As such it is of interest to map out the nutritional diversity of the different species of leafy amaranth found in Kenya as a criterion for deciding its production, consumption and marketing.

1.3 Objectives of the study

1.3.1 Overall objective

To determine the nutritional diversity and health benefits of vegetable amaranth species grown in Kenya.

1.3.2 Specific objectives

1. To identify the most common leafy amaranth species found in different regions of Kenya.
2. To determine the macronutrient and micronutrient content of the selected species of the leafy amaranth.
3. To determine the content of selected phytochemicals (phenols, catechin and quercetin) components in different amaranth species.
4. To determine the content of selected antinutrients (oxalates and phytates) of the selected species of leafy amaranth.
5. To assess the effect of blanching and solar drying on the vitamin C and β carotene content of the selected species of the leafy amaranth.

1.4 Hypothesis

This study tested the following hypotheses;

1. H_{01} : There exists similar amaranth species in all regions in Kenya.
2. H_{02} : There exist no significant differences in the macronutrient and micronutrient content of different vegetable amaranth species.
3. H_{03} : There exist no significant differences in the phytochemical content of different vegetable amaranth species.
4. H_{04} : There exist no significant differences in the antinutrient content of different vegetable amaranth species.
5. H_{05} : There is no effect of blanching and solar drying on the vitamin C and β carotene content of amaranth during.

1.5 Significance of the study

The study has generated knowledge on the nutritional diversity of leafy amaranth species. This is significant because it shows that each species is different in its nutrient profile. These differences are important especially during nutritional interventions as one can select the superior source of the nutrient of concern. There is inadequate information on the phytochemical content of amaranth in Kenya which this study has highlighted. The findings are useful to community health workers in sensitizing the community on the existence of different amaranth varieties that contribute different nutrients. The community can use this information to produce amaranth for different nutritional purposes which will contribute to dietary diversity and nutritional security.

The study also provides findings on the effects of processing on selected micronutrients. This information is important to food processors especially in new product development and modification of products targeting various groups of people.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Description of the Amaranths

Amaranthus, collectively known as amaranth, is an annual or short lived perennial plant distributed worldwide in warm, humid regions (“Amaranth,” 2015). Amaranths are botanically distinguished by their small chaffy flowers, arranged in dense, green or red, monoecious or dioecious inflorescences, with zero to five perianth segments and two or three styles and stigmata, and by their dry membranous, indehiscent, one-seeded fruit (Schippers, 2000). *Amaranthus* uses the C4-cycle photosynthetic pathway which enables it to be uniquely efficient in utilizing sunlight and nutrients at high temperatures (O’Brien & Price, 2008). Plants that use the C4 carbon fixation pathway tend to require less water than the more common C3 carbon-fixation pathway plants (*Amaranth*, 1984). *Amaranth* has often been referred to as drought tolerant (Andreas et al., 2011), this is probably due to the ability of the genus to grow under a wide range of climatic conditions coupled with its competitive ability which permits cultivation with minimum management (Shukla et al., 2006).

In addition, amaranth grows quickly, requires little inputs and can be harvested within a short time (4-6 weeks after planting) (Shukla et al., 2010), it does not have a high nitrogen requirement like maize (O’Brien & Price, 2008). According to Palada & Chang, (2003), *Amaranthus* grows best in loam or silty-loam soil with good water holding capacity although it can also grow on a wide range of soil type, soil moisture levels and soil pH. It is also known to be a low management crop that can grow in poor soils but studies have shown that its yields could be improved by application of fertilizers (Palada & Chang, 2003).

2.2 Amaranth species

Amaranthus comprises about 70 species, of which about 40 are native to the Americas. It includes at least 17 species with edible leaves and 3 grain amaranths (Grubben, 2004a). Among the species commonly found in Kenya are, *A. dubius* and *A. hybridus*, which is grown as vegetables while *A. cruentus* is grown for its grain and *A. hypochondriacus* is dual purpose (O'Brien & Price, 2008).

Table 2.1 shows some of the main uses of the common species grown in Kenya, though most are not used exclusively for one purpose, the grain types can also be used as vegetables in their early stages (O'Brien & Price, 2008).

Table 2.1: Common Amaranth species grown in Kenya (O'Brien & Price, 2008)

Variety	Leafy	Grain
<i>A. dubius</i>	√	
<i>A. albus</i>		√
<i>A. hybridus</i>	√	
<i>A. cruentus</i>		√
<i>A. caudatus</i>	√	
<i>A. hypochondriacus</i>	√	√

2.2.1 Description of some species

According to the figures below, different species have different physical appearance and characteristics; this is useful in determining which variety to use for what purpose especially for the local people who do not have knowledge of the scientific names of the different species.

Amaranthus cruentus (Figure 2.1) is an annual herb that grows up to 2m tall; it is often reddish tinted throughout with stout and branched stems. The leaves are long with petioles arranged spirally without stipules, they are deeply lobed and oval shaped with a sub-acute apex that ends abruptly in a sharp point. Seeds are egg-shaped with a narrow end at the base, whitish to yellowish or blackish in colour (Grubben, 2004c)



Figure 2.1: *A. cruentus*

Amaranthus cruentus was domesticated as a grain amaranth in Central America from the weed *Amaranthus hybridus* L. from around 6000 years ago. It is a widespread traditional vegetable in all countries of tropical Africa and very important in many lowland areas e.g. in southern Nigeria, DR Congo, Kenya and Tanzania. It is also an important vegetable in

many tropical areas outside Africa (Grubben, 2004a).

The main use of *Amaranthus cruentus* in the past was for its grain, however presently, its leaves are consumed as a vegetable dish or used in sauces; the leaves and tender stems are cut and fried and eaten with cereals and tubers (Grubben, 2004c).

Amaranthus hypochondriacus L. (Figure 2.2) is an annual herb, that grows up to 2 m tall, often reddish tinted throughout; stem stout, branched, with multicellular hairs. Leaves are long but wider in the middle, very lobed, arranged spirally, simple, without stipules with a blunt tip. It has thick branches, large and complex, with many lateral, perpendicular, thin branches. Seed are very deep red, dark-seeded



Figure 2.2 *A.hypochondriacus*

form of the species (Jansen, 2004).

It has its origin in Mexico and was considered almost as important as corn and potatoes (Nee Repo, 2011). It is the most robust and highest-yielding grain amaranth and is rich in carbohydrates, plant protein, iron and vitamins, especially A and C (Nee Repo, 2011) Though this species has been described earlier as a grain type, it can be grown as a

leaf vegetable (O'Brien & Price, 2008).

Amaranthus dubius (Figure 2.3) is an annual herb, which grows up to 150 cm tall; stems are slender to stout and branched (Fern, 2014). It has distinctive dark-green, broad, oval shaped, ridged leaves (Jacob alemu, 2005). Leaves are arranged spirally, simple, without stipules; lamina is oval shapes, sometimes the center of the lamina is red. Seed are lens shaped and black and are extremely small (Grubben, 2004c).



Figure 2.3: *A. dubius*

It is widespread throughout the humid lowland tropics. It originates from tropical America and is found in several African and Central American countries especially African lowland areas. The main use of *Amaranthus dubius* is as a cooked leaf vegetable. (Grubben, 2004b) The product is dark green and tender but its taste somewhat neutral. In Kenya its leaves are cooked together with bitter leaf vegetables such as nightshades (*Solanum* spp.), *Cleome gynandra* L. or *Launaea cornuta* the leaves easily become soft after 5–10 minutes cooking in lightly salted water (Grubben, 2004b). It is commonly sold in Kenyan seeds companies for commercial and domestic production (Jacob 2004).

Amaranthus hybridus (Figure 2.4) is a herb that grows up to 1.5 m tall, it has an unbranched light green stem. The upper stem terminates in an elongated panicle of

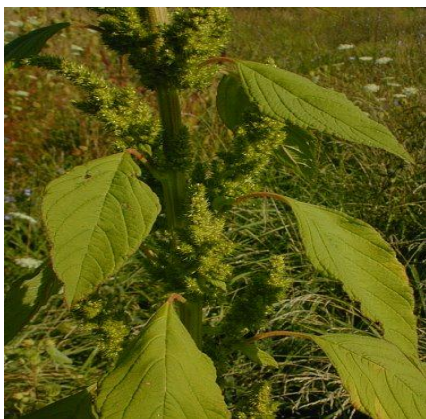


Figure 2.4: *A. hybridus*

spikes with sma--ll green flowers. It has small flattened seeds, dark brown or black, circular, and shiny. They are produced in great abundance. The alternate leaves are up to 5½" long and 3" across becoming slightly smaller as they ascend the central stem and are oval shaped (Grubben, 2004c). The uppermost leaves are smaller, long but wide in the middle. It has predominately green foliage, although they may be red tints

along the margins of the leaves and elsewhere. The petioles are quite long, causing the leaves to droop downward. (*Amaranth*, 1984) It originated from tropical America; it is now spread throughout tropical areas. It grows wild on moist ground, in waste places, or along roadsides, thrives in altitudes up to 1,300m. This weedy species is one of the most common leafy vegetables (*Amaranth*, 1984).

A. albus (Figure 2.5) is also known as tumbleweed or white Amaranth can be distinguished from *Amaranth spp.* (Amaranths) by its white stems and small light green leaves; the foliage has a pale appearance overall (“White Amaranth (*Amaranthus albus*),” 2014). Originating in South America, tumbleweed amaranth is introduced throughout North America and nearly worldwide. It is an annual plant that, after senescing, breaks off at the base of the stem and, blown by the wind, may roll great distances, scattering its seeds far and wide (“*Amaranthus albus* (tumbleweed amaranth): Go Botany,” 2011) it grows up to 0.6-0.9 m high but sometimes as high as 1.8 m (Dibble, 2012). The whitish and rigid stems are branching and almost hairless they are round or slightly furrowed. Occasionally, plants are tinged red or purple.



Figure 2.5: *A. albus*

The leaves egg-shaped, 10-50 mm long and 2-20 mm wide with conspicuous pale veins. Each leaf is light green, with the top wider than bottom and smooth or slightly undulate along the margins. Sometimes the leaves have yellowish or reddish tints (Atlas of Living Australia, 2014). The side branches often develop at right angles (90°) from the central stem. It has small round seed which are dark, reddish brown to black, shiny, and somewhat

flattened and about 1 mm in diameter (“White Amaranth (*Amaranthus albus*),” 2014)

There are substantial differences that species of a given vegetable will possess. In addition to variety and genetic strain differences with respect to weather, insect and

disease resistance, species of a given vegetable will differ in size, shape, time of maturity, and resistance to physical damage.

2.3 Food uses

Amaranth grain is milled for flour or popped; the leaves of both the grain and vegetable types may be eaten raw or cooked. Amaranths grown principally for vegetable use have better tasting leaves than the grain types. Mostly, vegetables are consumed in cooked form, or are eaten with the main dish of cereals or tubers (Reddy & Love, 1999). Traditionally in arid regions, the leaves are dried and the leaf powder is used in sauces during the dry season (Yadav & Sehgal, 2002). The leaves easily become soft after 5–10 minutes cooking in lightly salted water. Moreover, most people cook amaranth in ample water and discard the cooking water containing soluble nitrate and oxalate (Grubben, 2004c). Consumers also indicate that other traditional vegetables are less attractive than the *Amaranthus* spp. since some have a bitter taste (Onyango, 2010) .

2.4 Role of amaranth in nutrition

2.4.1 Protein content

Amaranthus leaves have been reported to contain 17.5 – 38.3 % protein (dry-weight basis) of which 5 % is lysine; an essential amino acid that is lacking in most diets based on cereals and tubers (Mnkeni et al., 2007). Leaf-protein levels (dry-weight basis) have been reported as 27% for *Amaranthus blitum*, 28% for *Amaranthus hybridus*, 30% for *Amaranthus caudatus*, and 33 % for *Amaranthus tricolor*.) (Mnkeni et al., 2007). The cooked leaves (not mixed with other foods) have been reported to contain about 8% protein, 4% carbohydrates (Maundu et al., 1999)

The protein quality of the amaranth leaf-nutrient concentrate (determined by amino acid composition, digestibility, and nutritional effectiveness) is excellent. It serves as an inexpensive rich source of protein and dietary fiber (Shukla et al., 2010). The protein has a high content of sulphur-containing amino acids (methionine, cysteine), which makes it a good combination with cereals (Grubben, 2004c).

2.4.2 Micronutrients

Vegetable amaranth is also an important source of vitamins, especially pro vitamin A, the lack of which results in a most serious nutritional deficiency in the tropics and leads to blindness in thousands of children each year (Singh et al., 2001). The leaves are also a good source of vitamin C, K and folate (Moraa, 2008). When compared to spinach, *Amaranthus spp* contains 3 times more vitamin C, calcium, iron and niacin (Mnkeni et al., 2007). It contains 18 times more vitamin A, 20 times more calcium and 7 times more iron when compared to lettuce. *Amaranthus* leaves have high levels of carotene and micronutrients such as sodium, copper, manganese, chloride (Mnkeni et al., 2007).

2.4.3 Phytochemical content

In addition to being a significant source of vitamins and minerals, amaranth also contributes to the intakes of other phytochemicals such as phenolic compounds (Imungi, 1999) and isothiocyanates (group of glucosinolates), which possess strong antioxidant properties, and have been reported to help in the prevention and suppression of diseases such as cancer, arteriosclerosis and aging (Robbins, 2003). Earlier studies have established the abundance of antioxidants in *Amaranthus* leaves (Ali & Oba, 2010) and that there was a general trend towards increased antioxidant activity with increased total phenolic content in *Amaranthus tricolor* L (Ali, & Oba, 2008).

As antioxidants, phenolic compounds act as free radical scavengers and act to prevent diseases which follow free radical mechanism in humans (Stevenson & Hurst, 2007). Among the group of flavonoids, quercetin has been reported to be a strong antioxidant (Hertog et al., 1992). It has been found to chelate metals, scavenge oxygen free radical and prevent oxidation of low density lipoprotein in *in vitro* studies (Khandaker et al., 2008).

2.4.4 Fibre and antinutrient content

Vegetable amaranths are recommended as a good source of fibre for patients with constipation (Grubben, 2004c). Amaranths in addition to their high nutritional value also accumulate high levels of anti-nutritional factors such as oxalates. Research indicates that oxalate plays various functional roles in plants, including calcium regulation, plant protection and detoxification of certain metals (Gupta et al., 2005). It is well established from the studies carried out by many workers on animals that oxalates present in foods interfere with the assimilation of calcium (“Food Toxicants, Naturally Occurring,” 2005).

Amaranth also contains phytic acid which, though considered as an antinutritional factor, is a common storage form of phosphorus in seeds and in few tubers and fruits (Mziray et al., 2001). The complexing of phytic acid with nutritionally essential minerals are responsible for the antinutritional activity. Phytic acid interferes with Ca, Fe, Mg and Zn absorption because of its ability to chelate divalent cationic minerals (Oboh, 2005). These anti nutrients like phytic acid, oxalates, pro-anthocyanidins, tannins and dietary fibres reduce the bio availability of the nutrients (Akindahunsi & Salawu, 2005).

2.5 Role of amaranthus in food security

Communities in developing countries have in the past depended greatly on the large diversity of traditional crops for food and as a source of nutrition. These foods were, however, edged out of many diets, especially of the urban populations by the introduction of exotic substitute (Mwangi & Kimathi, 2006). However, due to very recent spirited campaigns by governmental, non-governmental organizations and private individuals on the superiority of traditional foods in terms of nutrition, health and taste, they are slowly creeping back into diets (Mwangi & Kimathi, 2006). *Amaranthus spp*, is one of the traditional vegetables that have been

successfully re-introduced into the diets of most of the people in developing countries of Sub-Saharan Africa and Asia (IPGRI, 2003) .

Traditional vegetables possess more familiar tastes to the communities than their exotic counterparts (Akindahunsi & Salawu, 2005). Consequently, improvement of traditional crops such as amaranths through research and development could produce an easy and cost-effective way of eliminating malnutrition and promoting the people's health as well as attaining food security (Njume et al., 2014). Opportunities exist in Kenya as well as other developing countries to use traditional leafy vegetables (TLVs) to expand the local food base, improve the health of the population, enhance food security and generate income (IPGRI, 2003).

2.6 Processing of amaranth vegetable

During harvesting season, there is a huge loss in leafy vegetables, their highly perishable nature demands processing for longer availability (Negi & Roy, 2003). To extend the shelf-life, different ways of preserving traditional vegetables such as amaranth have been developed (Makobo et al., 2010). The technique of dehydration is probably the oldest method of food preservation practiced by humankind. The removal of moisture prevents the growth and reproduction of microorganisms causing decay and minimizes many of the moisture-mediated deteriorative reactions. It brings about substantial reduction in weight and volume, minimizing packing, storage, and transportation costs and enables storability of the product under ambient temperatures (Gupta et al., 2013) .

Amaranth is available only for a short period and thus can be dried, and the dried amaranth leaves can be used for supplementing cereal based diets as they are rich in protein, calcium and iron (Singh et al., 2001). Prior to drying, vegetables can be blanched, blanching is a unit operation prior to freezing, canning, or drying in which fruits or vegetables are heated for the purpose of inactivating enzymes; modifying texture; preserving color, flavor, and nutritional value; and removing

trapped air (Reyes et al., 2004). This heat treatment depends upon the specificity of vegetables, the objectives that are followed and the subsequent processing / preservation methods (Sagar & Suresh, 2010). However, the various processing techniques have been reported to alter both the nutrient, antinutrient and antioxidant property of some commonly consumed plant foods(Oboh,2005).

2.7 Summary of literature review

Malnutrition affects a great percentage of the Kenyan population. Lack of dietary diversity and lack of knowledge on nutritional contents of various foods is one of the causes of malnutrition. Malnutrition increases the morbidity and mortality rates especially in children women and the elderly. If not addressed, these deficiencies will set a vicious cycle of disease and malnutrition, which will have an impact on the economic development.

Currently food based approaches such as consumption of indigenous vegetables can be used to combat malnutrition. Amaranth is one of the indigenous underutilized vegetables in Kenya despite having high content of key micronutrients. Besides, amaranth does not only provide nutritional benefits but also phytochemicals which are important in improvement of health. There exist differences in the nutrient content of different varieties of Amaranth. Amaranth can also be used to produce value added products by use of various processing methods. However, studies have shown that processes such as, blanching at different temperatures and solar drying have effects on nutrients especially micronutrients, as such, it is important to map out the effects of processing on the nutrients in amaranth, which would help in determining the best method for retention of nutrients.

CHAPTER THREE

MATERIALS AND METHODS

The figure (3.1) below is a flow diagram of the activities carried out during the study.

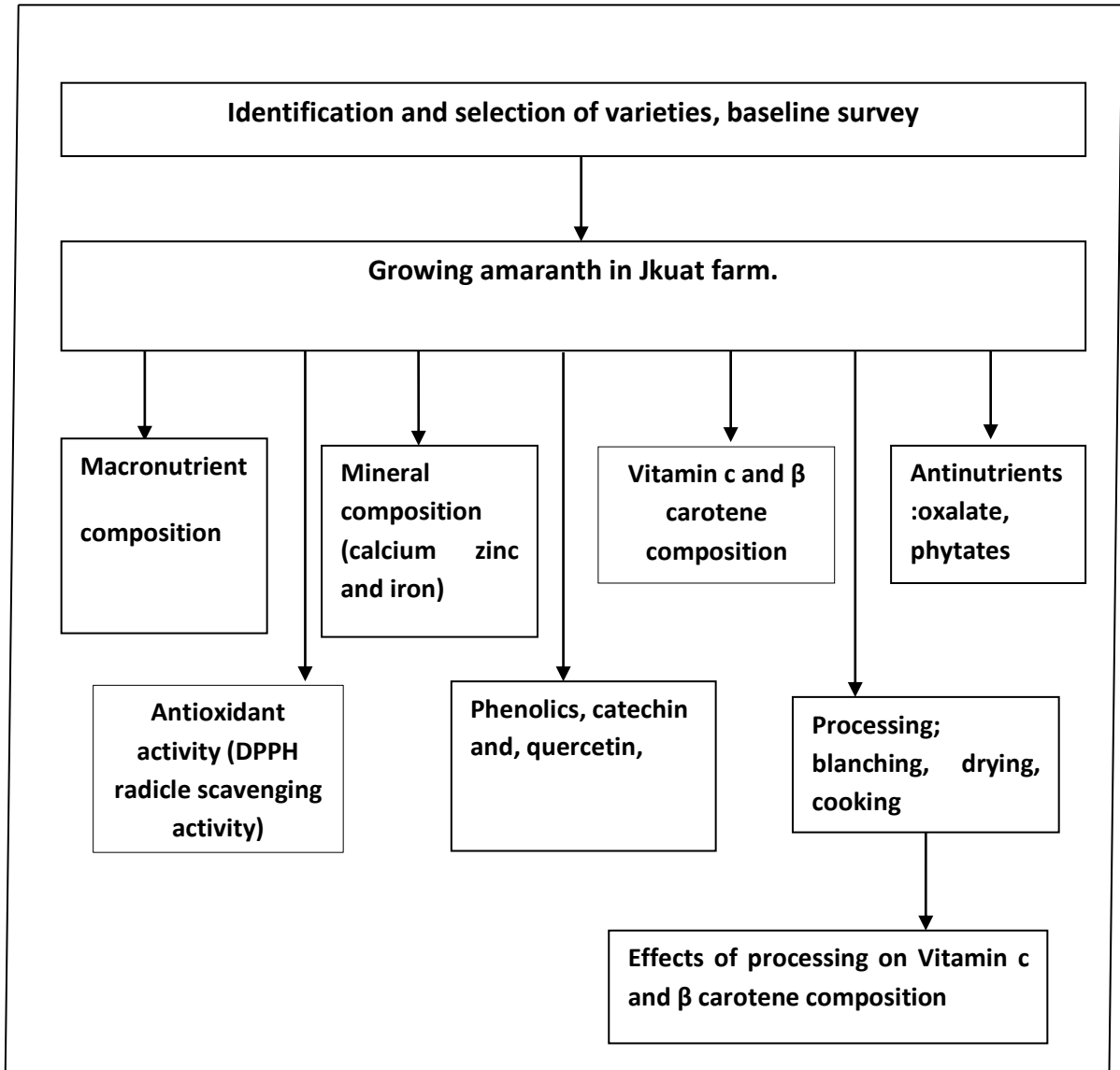


Figure 3.1: A schematic overview of the research design

3.1 Identification and selection of varieties

3.1.2 Baseline Survey

A baseline survey which adopted a cross-sectional design was carried out. This was done in order to identify the common amaranth cultivars grown, to determine planting and management and other production characteristics of amaranth, and to determine the existing knowledge of the crop. The survey was conducted in five regions namely: Bondo, Kieni East, Luanda, Lugari and Sabatia. These areas were purposively selected since they are the common amaranth growing regions in Kenya. Information on the amaranth farmers in the five regions was obtained from the Ministry of Devolution and Planning; poverty and eradication commission. Data was collected using a structured questionnaire (Appendix 1) and focus groups discussions (Appendix II) in each of the regions. A total of 128 amaranth farmers were interviewed. The information gathered was used to contextualize the research agenda.

3.2 Planting of amaranth leaves, sample preparation and analysis

3.2.1 Preparation of samples

The most common species identified from the survey were: *A. cruentus*, *A. dubius*, *A. hybridus*, *A. albus*, *A. hypochondriacus*. Seeds of these species were obtained from AVRDC gene bank Tanzania and planted at JKUAT research farm.

An experimental plot of ¼ acre was acquired at the JKUAT research farm for planting the vegetables. Farm workers were engaged to manually prepare the land for planting. A completely randomized block design was used in planting the species. The seeds of the five amaranth species were planted in blocks of 3 m by 3 m and spacing of 60cm (between rows) by 15cm (between vegetables) No artificial fertilizers were used but farm yard manure was applied at planting. Irrigation was undertaken every 3 days using overhead sprinklers while weeding was carried out weekly.

Mature leaves of each of the species were harvested between 4-6 weeks after planting. They were then cleaned by removing all adhering dirt, fibrous or aged leaves and damaged parts. The young and succulent leaves were then separated from their branches and analyzed.

3.6 Determination of nutrient content

3.6.1 Moisture content determination

Moisture was determined according to AOAC methods specification 950.46, method 925.10-32.10.03 (AOAC, 1995). About 5g of ground sample was accurately weighed into a moisture dish and transferred to a hot-air oven previously heated to around 105°C. Drying was done for one hour. Final weight of sample was taken after drying and cooling in a desiccator. Percentage moisture was calculated as follows:

$$\% \text{ moisture} = \left(\frac{W_1 - W_2}{W_1} \right) \times 100$$

W_1 = Weight of sample before drying

W_2 = Weight of sample after drying

3.6.2 Protein content analysis

Protein was determined using the semi-micro Kjeldal method, specification 950.46. Method 20.87-37.1.22 (AOAC, 1995). Approximately 2g of sample was weighed into a digestion flask together with a combined catalyst of 5g K_2SO_4 and 0.5g of $CuSO_4$ and 15ml of concentrated H_2SO_4 . The mixture was heated in a fume hood till the digest colour turned blue. The digest was cooled and transferred to 100ml volumetric flask and topped up to the mark with deionized water. A blank digestion with the catalysts was also made and 10ml of diluted digest was transferred into the distilling flask and washed with about 2ml of distilled water. Approximately 15 ml

of 40% NaOH was then added and this was also washed with 2ml of distilled water. Distillation was done to a volume of about 60ml distillate. The distillate was titrated using 0.02N HCl to an orange colour of the mixed indicator, which signified the end point.

$$\%N = \frac{(V_1 - V_2) \times N \times f \times 100}{(V_1 \times \frac{100}{S})}$$

Where: V_1 is titer for sample in ml, V_2 is titer for blank in ml

N is normality of standard HCl solution (0.02), f is the factor of the standard HCl solution, V is the volume of diluted digest taken for distillation (10ml),

S is weight of sample taken (1g),

% protein = Nitrogen x 6.25

3.6.3 Fat content analysis

Determination was done using the soxhlet method 920.85-32.1.13,(AOAC, 1995) . The extraction flask was heated to constant weight at 105°C for 1 h then cooled to room temperature in a desiccator. This gave intermittent extraction of oil with excess of fresh condensed organic solvent to be used. Approximately 5g of sample was weighed accurately into extraction thimbles and initial weight of extraction flasks taken. The thimble was place in an extraction apparatus Fat extraction was done using ethyl ether in soxhlet apparatus for 8 hours. The extraction solvents were rota evaporated and the fat extracted dried in a hot air oven for 15 minutes before the final weight of flasks with extracted oil taken.

$$\%Fat = \frac{\text{weight of fat extracted} \times 100}{\text{weight of sample}}$$

3.6.4 Ash and mineral content analysis

Ash content was determined by incinerating in a muffle furnace (AOAC, 1995), method 923.03-32.1.05). Sample weights of about 5g were weighed in pre-conditioned crucibles. The sample was charred by a flame before being incinerated at 550⁰C in a muffle furnace, to the point of white ash. The residues were cooled in a desiccator and the weights taken.

$$\% \text{ crude ash} = \frac{\text{weight of ash}}{\text{weight of sample}} \times 100$$

The atomic absorption spectrophotometer (AAS) method was used to determine iron calcium and zinc content (AOAC, 1995). 15 ml of 1:1 HCL: water was added to the cooled ash sample and topped up to 100ml with distilled water. The ash was transferred quantitatively into a beaker using the HCL water mixture. It was then heated to 80-90⁰c on a hot plate for 5 minutes and transferred to a 100ml volumetric flask and filled up to the mark with HCL and mixed well and filtered. A standard solution of the minerals, at different concentrations was prepared and was subjected to atomic absorption spectrophotometer (Model A A.6200, Shimadzu, Corp., Kyoto, Japan).

3.6.5 Dietary fibre

Crude fibre was determined according to method 920.86.32.1.15 (AOAC, 1995). Two grams of sample was weighed into a 500ml conical flask. About 200ml of boiling 1.25% H₂SO₄ was added and boiling done for 30 minutes under reflux condenser. Filtration was done in slight vacuum with Pyrex glass filter (crucible type) and the residue washed to completely remove the acid with boiling water. About 200ml of boiling 1.25% NaOH was added to the washed residue and boiling done under reflux for another 30 minutes. Filtration was done again using the same glass filter previously used with the acid. The residue was rinsed with boiling water followed by 1% HCl and again washed with boiling water to rinse off the acid from the residue. The residue was then washed twice with alcohol and three times with

ether. It was dried in a hot- air oven at 105⁰C in a porcelain dish to a constant weight (W₁). Incineration was done in a muffle furnace for 3 hours at 550⁰C. The dish was then cooled in a desiccator and the final weight (W₂) taken. This is the formula that was used to calculate percentage crude fibre:

$$\% \text{ crude fibre} = \frac{(W_1 - W_2)}{W} \times 100$$

Where, W₁ is weight of acid and alkali and digested sample

W₂ –the weight of incinerated sample after acid and alkali digestion

W- Weight of sample taken

3.6.6 Determination of β-carotene

β-carotene was determined according to method (AOAC, 1998). Approximately 2g of vegetable sample was weighed accurately. It was then placed in a mortar with about 10ml of acetone and ground thoroughly. The acetone extract was then transferred to a 100ml volumetric flask and the residue extracted again with 10ml of acetone and transferred to the volumetric flask. The extraction with acetone was repeated until the residue no longer gave color to acetone. The combined extract was then made to the 100ml mark. 25ml of the extract was evaporated to dryness on a rotary vacuum evaporator (Bibby Sterilin Ltd, RE 100B, UK) and the residue dissolved in about 1ml petroleum ether. The solution was introduced into chromatographic column and eluted with petroleum ether.

Beta-carotene went through the column as a yellow pigment and was collected to 25ml volume in the volumetric flask with petroleum ether. Five solutions of standards with concentrations between 0.5µg/ml and 2.5µg/ml were prepared from a stock solution containing 2.5µg/ml pure β- carotene. The absorbance values of the solution were determined at 440 nm using UV is spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan) and plotted against their corresponding concentration to give a standard curve. The β-carotene content of the amaranth was then calculated per 100g of the material.

3.6.7 Determination of Vitamin C

The method described by Vikram et al, (2005) was followed; in determination of Vitamin C, 2g of the fresh sample was extracted with 10ml 0.8% metaphosphoric acid. The extract was centrifuged at 10,000 rpm for 10 minutes at 4⁰c, the supernatant was filtered through a filter paper. About 1 ml filtrate was diluted with 10ml of 0.8% metaphosphoric acid and filtered with 0.450 Millipore filter. 30µl of the juice were injected into the HPLC. Based on the peak areas and the corresponding retention times of 8 minutes the concentration was calculated from the standard graphs as mg/100g. Column C₁₈(ODS 15cmx 46mm) was used, the mobile phase was 0.8% metaphosphoric acid and flow rate 0.5ml/min, a UV detector set at 254nm and a working pressure < 110kgf/cm² was used.

3.7 Determination of phytochemicals

3.7.1 Total phenolics assay

The total phenolics were determined by an assay described by Shetty et al, (1995). Briefly, one milliliter of ethanolic extract was transferred into a test tube and mixed with 5ml of distilled water. To each sample 0.5ml of 0.2N (v/v) Folin-Ciocalteu reagent was added and mixed. After 5min, 1.5ml of 5% Na₂CO₃ was added to the reaction mixture and allowed to stand for 60 min. The absorbance was read at 765nm. The absorbance values were converted to total phenolics and were expressed in milligrams equivalents of gallic acid per grams dry weight (DW) of the sample. Standard curves were established using various concentrations of gallic acid in 95% ethanol.

3.7.2 Catechin and Quercetin Assay

The colorimetric aluminum chloride method was used. About 1 ml of extract was added to 4ml of water, 0.3 ml of 5% sodium nitrite was then added. After 2 minutes 0.3ml of 10% aluminum chloride was added and allowed to stand for 6 minutes. Approximately 2ml of 1M sodium hydroxide was then added and the solution

topped up with 10ml distilled water. The absorbance was read at 415nm against catechin and quercetin standards.

3.8 Determination of antioxidant activity

The radical-scavenging activity was determined using diphenyl picryl hydrazyl (DPPH). This provides information on the reactivity of the test compounds with a stable free radical and gives a strong absorption band at 517nm in the visible region. Fifty grams of the fresh leaves were put in a flask was covered with 500ml ethanol and allowed to stand for 48 - 72 h. It was then filtered through Whatman filter paper No. 1 and distilled using rotary evaporator (Bibby Sterilin Ltd, RE 100B, UK) at 60°C until ethanol- free liquid was obtained. The resulting extracts were then subsequently labelled as ethanol extracts and preserved at 5°C in airtight bottles until further use (Alanís, Calzada, Cervantes, Torres, & Ceballos, 2005). The following concentrations of the extracts were prepared, 0.05, 0.1, 0.5, 1.0, 2.0 and 5 mg/ml in ethanol in cuvette placed in the spectrophotometer (Analar grade). Vitamin C was used as the antioxidant standard at the same concentrations as the extract. One ml of the extract was placed in a test tube, and 3ml of methanol added followed by 0.5ml of 1 mM DPPH in methanol.

The mixture was shaken vigorously and left to stand for 5 min. A blank solution was prepared containing the same amount of methanol and DPPH. The absorbance of the resulting solution was measured at 517 nm with a UV-vis spectrophotometer (UV mini 1240 model, Shimadzu Corp., Kyoto, Japan). All tests were run in triplicate and the radical scavenging activity was then calculated using the following formula:

$$\% \text{ inhibition} = \{ [Ab - Aa] / Ab \} \times 100$$

Where: Ab = absorption of the blank sample

Aa = absorption of the extract.

3.9 Determination of Anti nutrients

3.9.1 Oxalates Determination

This was done by HPLC analysis method (Xu, 2006). Aliquots of 0.2–0.5 g, sample was homogenized in 1–4 ml of 0.5 N HCl. The homogenate was heated at 80 °C for 10 min with intermittent shaking. To the homogenate distilled water was added up to a volume of 5–25 ml. About 2–3 ml of the solution was withdrawn and centrifuged at 12 000 rpm for 10 min. About 1 ml of supernatant was passed through a filter (0.45 µm) before HPLC analysis. Standards were prepared at varying concentrations for quantification. Hypsil C₁₈ column (5 µM, 4.6 mmx250 mm) equipped Waters 550 was used as the static phase and the mobile phase was a solution containing 0.5% KH₂PO₄ and 0.5 mM TBA (tetrabutylammonium hydrogen sulphate) buffered at pH 2.0 with orthophosphoric acid. Flow rate was 1 ml min⁻¹ and detection wavelength was at 220 nm.

3.9.2 Determination of phytates

This was done by HPLC analysis method of phytic acid (Camire & Clydesdale, 1982). Approximately 50mg sample was extracted with 10ml of 3% H₂SO₄. The extracts were filtered and the filtrate transferred to a boiling water bath (BWB) for 5min followed by addition of 3ml of FeCl₃ solution (6mg ferric iron per ml in 3% H₂SO₄). The mixture was then heated for 45min to complete precipitation of the ferric phytate complex. The mixture was then centrifuged at 2500 rpm for 10 min and the supernatant discarded. Precipitate was washed with 30ml distilled water, centrifuged and the supernatant discarded. Three (3) ml of 1.5N NaOH was added to the residues and the volume brought to 30ml with distilled water. The mixture was then heated for 30min in order to precipitate the ferric hydroxide. Cooled samples were centrifuged and the supernatant transferred into a 50ml volumetric flask. The precipitate was rinsed with 10ml distilled water, centrifuged and the supernatant added to the contents of the volumetric flask.

About 20µl of the supernatant was injected into a HPLC fitted with a C-18 (5µm) Chromosorb column at 30°C and an RID detector. The mobile phase was 0.005N

sodium acetate in distilled water, flowing at $0.5\mu\text{lmin}^{-1}$. A stock solution of the standard containing 10 mg/ml of sodium phytate (Inositol hexaphosphoric acid $\text{C}_6\text{H}_6(\text{OPO}_3\text{Na}_2)_6+\text{H}_2\text{O}$) was used.

3.10 Effect of blanching and solar drying on vitamin C and β carotene

The vegetables were subjected to different processing methods to determine the effects of processing on vitamin C and β carotene. Fresh amaranth leaves were collected from the farming site. The leaves were rubbed gently with a clean napkin to remove all soil and dust before blanching. Effects of blanching were determined by subjecting the vegetables to different blanching time temperature combinations. Marked inactivation of peroxidase has been reported to occur at temperatures of above 70°C , with the enzyme being completely inactivated after 3 minutes at 80°C (Valdir & Lourenço, 1998). Thus, the vegetables were blanched at 80°C for 3 minutes. The low temperature long time equivalent of 80°C for 3 minutes was blanching at 60°C for 25 minutes the blanched samples were further solar dried for 4 days and boiled.

Solar drying in a solar drier with a temperature of 45°C for 3 days without prior blanching or cooking was done to establish the effects of solar drying on the vitamin C and β carotene, 200g of the solar dried sample were then cooked by boiling in 200 ml water for 10 minutes to determine subsequent losses of β carotene and vitamin C during cooking of solar dried samples. Fresh non- treated amaranth was used as the control.

3.11 Data Analysis

The data obtained from the survey was analysed using MS excel. Data on knowledge of amaranth, planting and management, production characteristics and value addition of amaranth was described using descriptive statistics (mean and percentages) and summarized using bar graphs, pie charts and frequency tables. Effect of species on nutrient content data were subjected to analysis of variance

using the GLM procedure of SAS software version 9.2 for ANOVA (*SAS Institute Inc.*, 2008). Data on the nutrient content of the five species was subjected to one way analysis of variance (ANOVA). Tukey's studentized range test was done to measure statistical difference in the nutrient content of the five species at 5% level of significance.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Baseline survey.

The information gathered during the baseline survey was used to understand the gaps in knowledge and technology in amaranth production and utilization. The socio economic characteristics were evaluated as shown below

4.1.1 Social economic characteristics of respondents

Most (51%) of the amaranth producers in the surveyed regions were female aged 26years to over 40 years old as shown in (Figure 4.1). In Bondo and Lugari production was done predominantly by men (50 and 100%) respectively (Figure 4.2). Majority of the amaranth farmers had primary level of education. In Bondo, the number of farmers with primary education and those with secondary education was equal (37%). In Kieni East region, only 25% of the farmers had studied up to university with the majority having studied up to primary level of education 71 %.

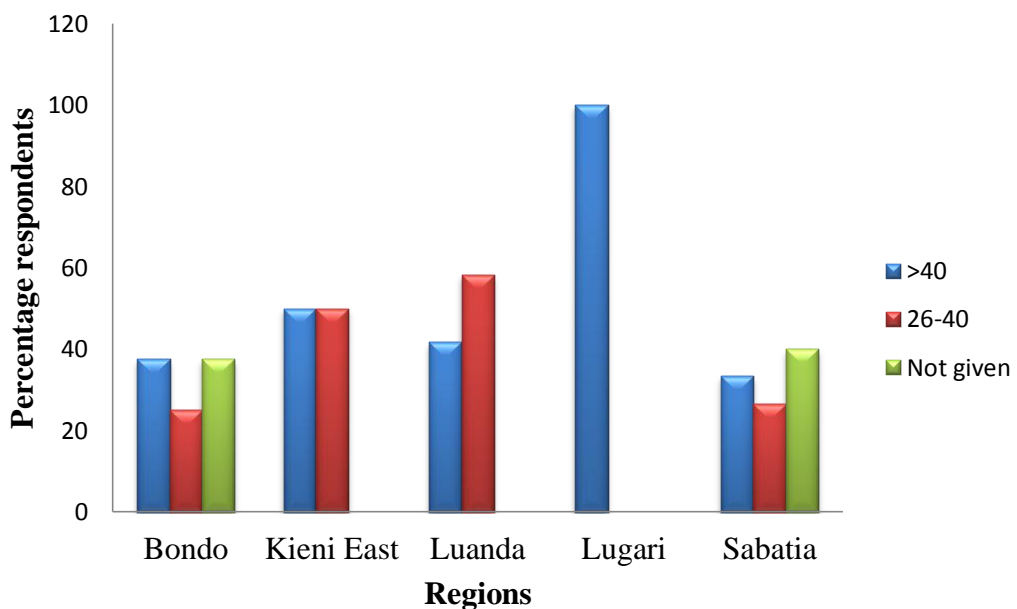


Figure 4.1. Proportion of farmers' in age engaged in amaranth production

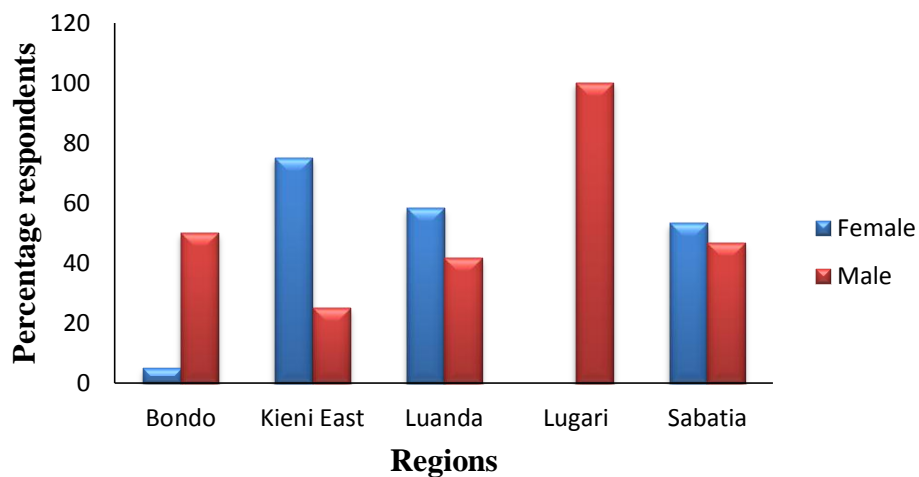


Figure 4.2 Proportion of farmers' in gender engaged in amaranth production

4.1.2 Knowledge on amaranth

Majority of farmers in all the regions and especially Kieni East (100%) started growing amaranth since their childhood, however, in Lugari 66% of the farmers had not grown amaranth since childhood (Figure 4.3). The key sources of knowledge on amaranth were family, school and research projects. (Figure 4.4)

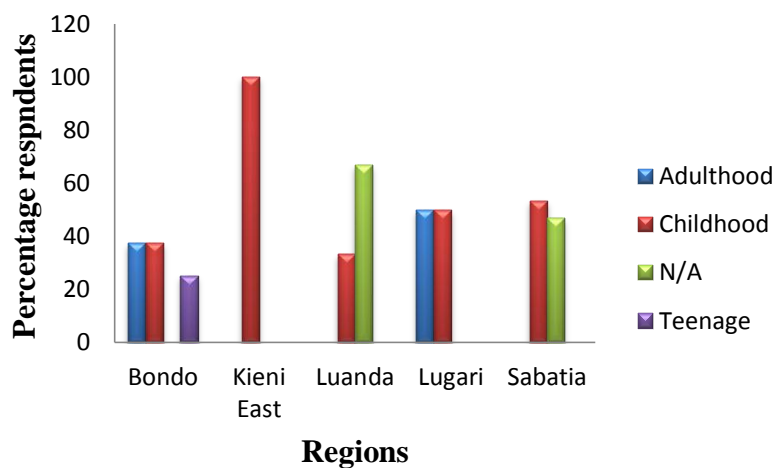


Figure 4.3 Proportion of farmers and their experience in amaranth production

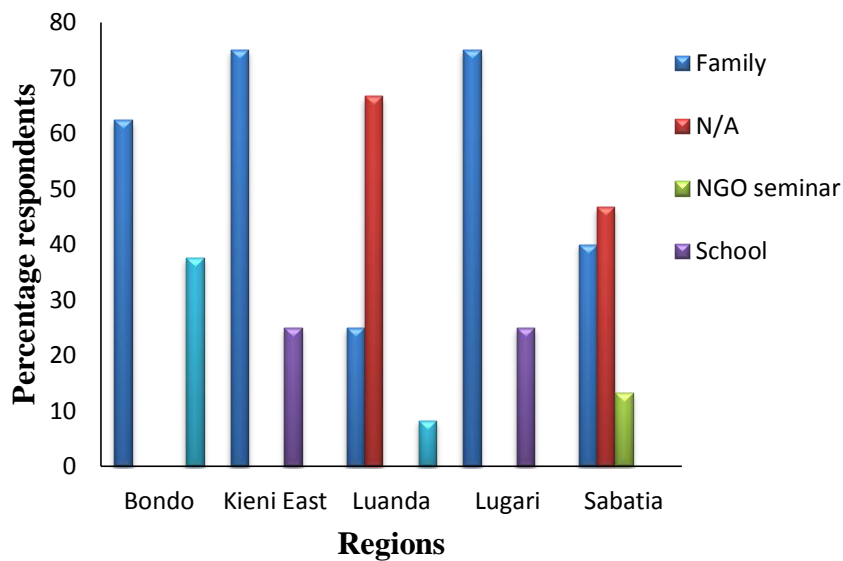


Figure 4.4 Proportion of farmers and their source of information

4.1.3 Types of Amaranth cultivars grown

There were different names of amaranth vegetables varieties existing in the five regions. Table 4.1 shows the different names used in referring to various amaranth species. ‘Terere’ was the predominant name for the variety grown in Kieni East region. Two main names Chimboga and Tsimboga (25 and 8.3%) were used for species grown in Luanda, while a significant number of farmers (66%) did not have any particular name. On the other hand, Bondo and Sabatia farmers did not have any particular name for the cultivars they grew. In Lugari the names of the varieties grown were, Litoto, Livokoyi, Toto and Tsimboga.

In Bondo the most preferred variety was the tall and white variety (25%), with (50%) reporting not to any particular preference. The most (50%) preferred variety in Kieni East was the golden variety for its exuberant production of grains and leaves. Majority of the farmers (66%) in Luanda were not specific to any particular cultivar while farmers in Sabatia (53%) didn’t seem to know the name of their preferred cultivar. There were varying reasons for preference, they included; broad

leaves, production of large quantities of grain, tasty leaves and beautiful colours especially during the flowering stage.

Table 4.1: Common Amaranth cultivars grown in the five regions

District	Scientific name	Local name of variety grown	Percentage respondents
Bondo	<i>A. albus</i> <i>A. hybridus</i>	Not known	100
Kieni East	<i>A. dubius</i>	Terere	100
Luanda	<i>A. dubius</i> <i>A. hypochondriacus</i> , <i>A. cruentus</i>	Chimboga, Tsimboga, Not known	25, 8.3, 66.7
Lugari	<i>A. hybridus</i> , <i>A. blitum</i> , <i>A. albus</i> , <i>A. hypochondriacus</i>	Litoto, Livokoyi, Toto, Tsimboga	25, 25, 25, 25
Sabatia	<i>A. albus</i>	Not known	100

4.1.4. Land preparation

Hand tillage was a common mode of land preparation in the five regions sampled; moreover, it was the exclusive method in Kieni East region as shown in figure 4.5. A majority of farmers (75%) prepared their land using animal ploughs in Bondo while in Lugari both hand tilling and animal ploughing were common with majority (75%) opting for animal ploughs. The use of machinery in land preparation was not common, this could be due to the fact that amaranth plots are small in size which does not justify the use of machinery, additionally low income levels and rough terrain does not allow for the use of machines. Incorporation of manure was also part of land preparation before seed sowing.

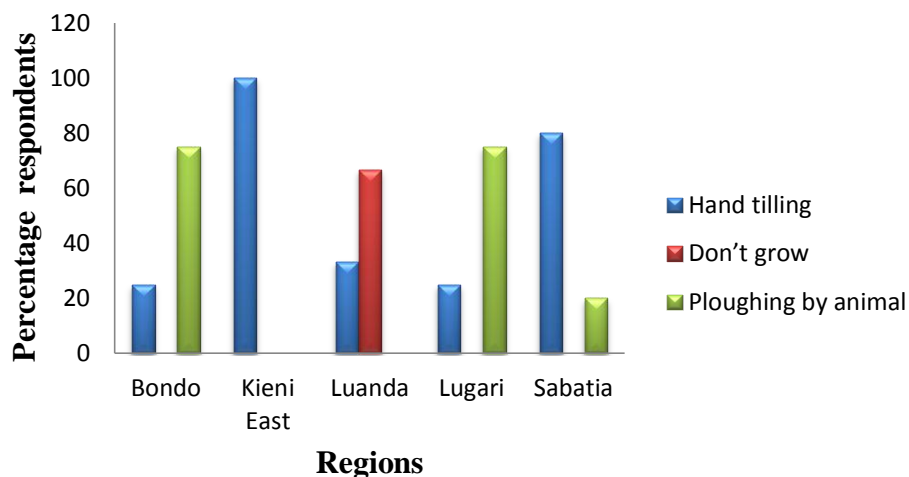


Figure 4.5: Main land preparation methods used by farmers

4.1.5. Planting and management of amaranth

Majority of the farmers in the sampled regions plant amaranth twice per year, a situation that is mainly dictated by rainfall patterns. (Figure 4.6). However, in Lugari an equal number of farmers (45%) planted twice and thrice, these farmers relied on irrigation in addition to the rainfall. The production cycle of most of the amaranth varieties is three to four weeks this makes it impossible to plant amaranth every month. Unreliable rainfall was the main reason why the farmers did not plant the crop on time.

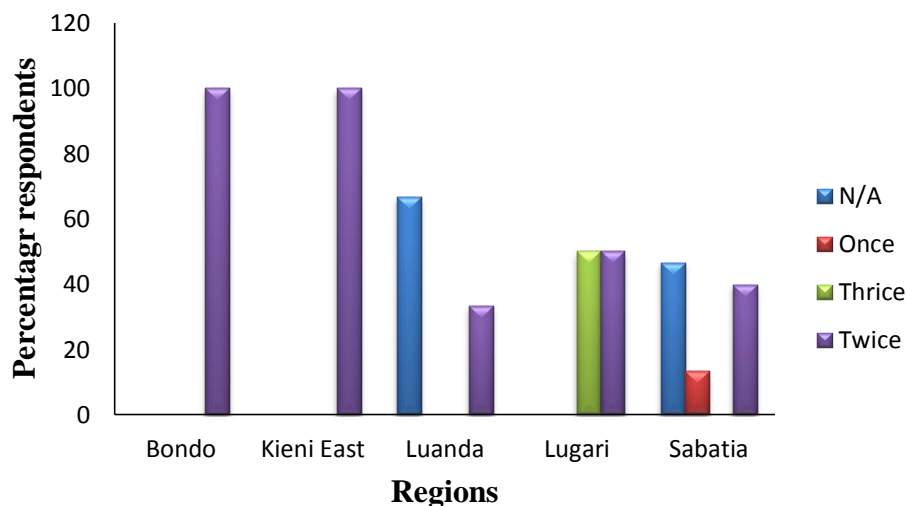


Figure 4.6: Proportion of farmers and frequency of amaranth production per year

Broadcasting was the main method of planting in Kieni East (100% of farmers) and Bondo (75% of farmers). Line sowing was mainly used in Lugari and Sabatia (10 and 43% respectively). The use of organic fertilizer was more common in the five regions than the use of inorganic fertilizer. Lugari had the highest percentage (83%) of farmers using organic fertilizer while Bondo had the highest (37%) of farmers using inorganic fertilizer. (Figure 4.7) Reasons for preferring the organic fertilizer to the inorganic ones included the fact that organic fertilizer especially in the form of wood ash is important in repelling insects and also promoting fast growth, it's readily available and easy to make especially manure. A few farmers reported that amaranth grown using inorganic fertilizer does not taste very well. Other management practices include weeding, tilling, mulching, thinning and the use of herbicides.

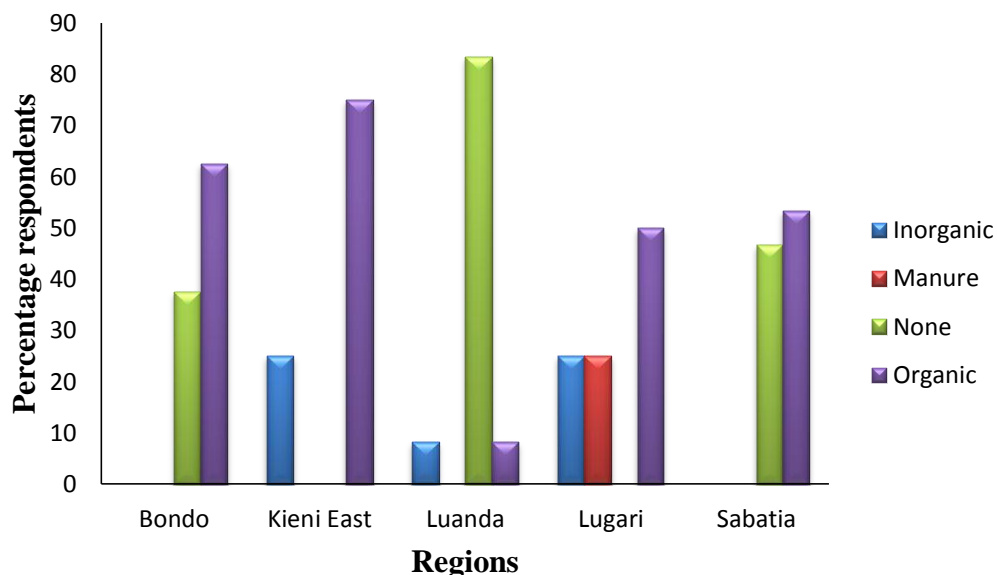


Figure 4.7: Type of fertilizer used in the five regions

4.1.6 Harvesting and maturity indices

Majority of the farmers reported that amaranth takes 2 months to mature (Bondo 37%, Kieni East 83%, Luanda 25%, Lugari 67% and Sabatia 40%) as shown in figure 4.8. Maturity index is dependent on the final use, the vegetables types take approximately one month while the grain types take three months. The three most important amaranth vegetable harvesting maturity indices observed by farmers in these regions were; Plant height, leaf size and color (Figure 4.9). The harvesting methods applied for amaranth vegetable were not different in the five surveyed regions. In all regions, direct plucking of the leaves was the major method employed in harvesting. Uprooting of the entire plant was only practiced in Luanda, Lugari and Sabatia at a small scale.

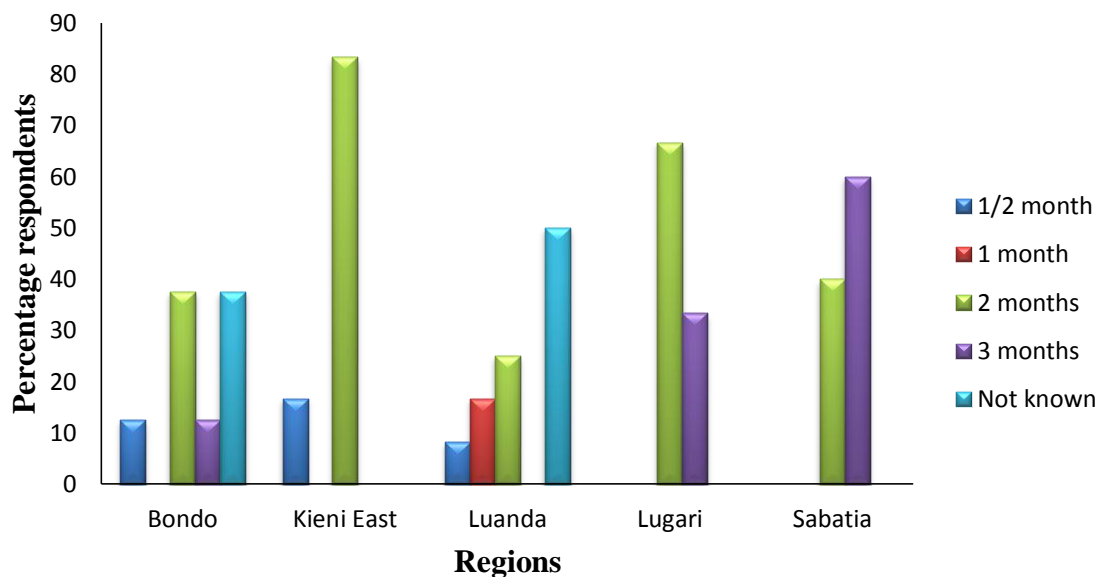


Figure 4.8: Maturity period for amaranth in the selected regions

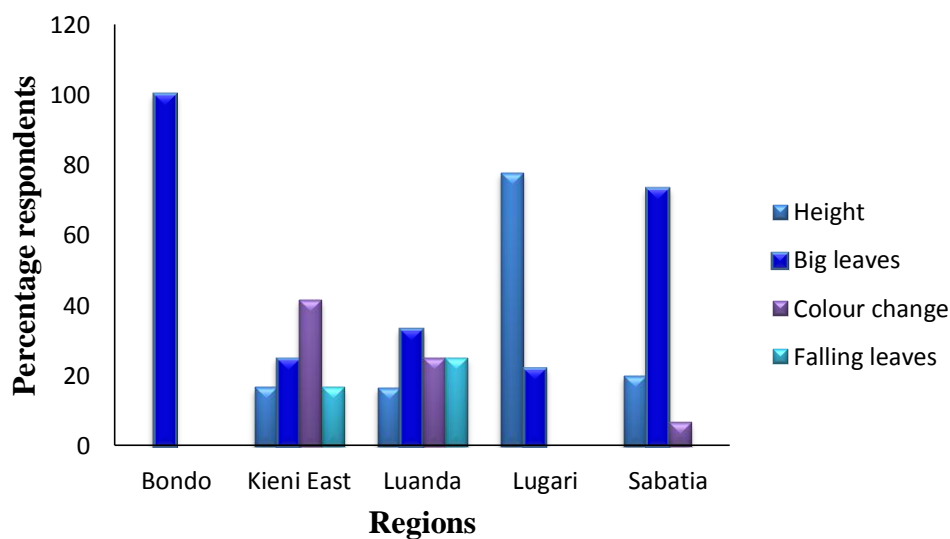


Figure 4.9: Harvesting method of amaranth vegetable

4.1.7 Yield estimates

Most respondents in the five regions did not know the yield estimates (Kieni East 100%, Luanda 66%, Lugari 77%, Sabatia 53%). This could be attributed to the fact that most of the amaranth produce is meant for local consumption, which does not

necessitate accurate measurement of yield. However, some farmers reported to get yields of 1-3kg of produce per quarter of acre of land.

4.1.8 Storage methods applied on vegetable amaranth

Gunny bags were used by many farmers as the most preferred method of storage; other methods that were occasionally observed include blanching, sun drying and the use of traditional granaries as shown in figure 4.10.

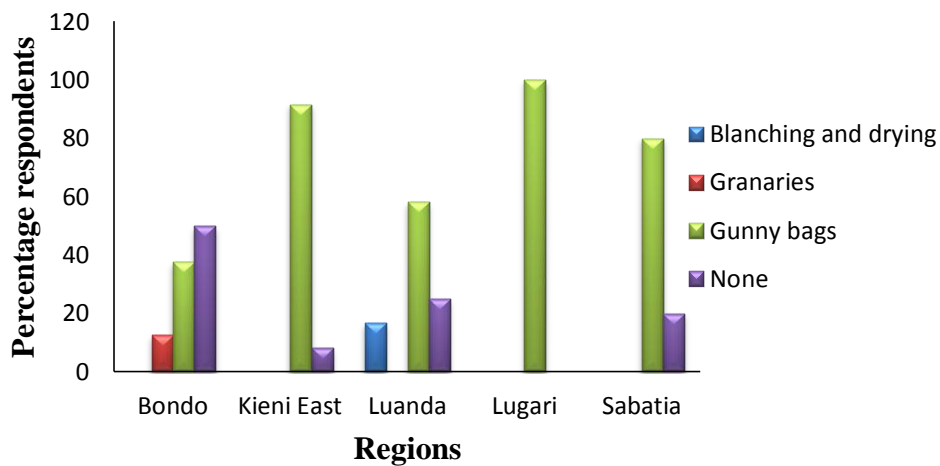


Figure 4.10: Storage methods applied in vegetable amaranth

4.1.9 Benefits of amaranth production

Amaranth is grown predominantly as a cash crop in Lugari, Sabatia and Bondo regions this is due to its fast growth and short production life cycles which ensure monthly income to farmers. In Kieni and Luanda it's grown as a food crop. A few farmers in Kieni East and Sabatia grow amaranth as part of food and as a nutritional security crop. Amaranth is a crop that majority of the farmers agreed had varied nutritional benefits. These benefits included provision of minerals, vitamins and dietary fibre. All the amaranth growers interviewed agreed that amaranth farming has a great future.

4.1.10 Conclusion of the baseline survey.

The data obtained from the baseline survey was important in identifying the most common species which were grown in the five regions. The survey also generated information on production characteristics such as; land preparation, planting, weeding and management of amaranth in the farm as practiced in the five regions. This information was used to contextualize these practices during planting and management of amaranth species in the farm. The amaranth species were then analyzed for their nutrient content.

4.2 Nutrient content of the selected species of the leafy amaranth

4.2.1 Proximate composition

The proximate composition of the five species was determined. The results are displayed in Table 4.2. The moisture content was ranging between (79.29-82.52%) on wet weight basis. *A. cruentus* exhibited significantly lower moisture content and significantly ($P<0.05$) higher protein content compared to the other species.

Protein content of the vegetables ranged from (2.3-3.37%). This is in agreement with studies by Sheela et al. (2004) who got values of 3.6% of protein in *A. spinosus* species grown in India. Kwenin, et al. (2011) found higher values (4.46%) of protein in *A. cruentus* while Asibbey-Berko & Tavie, (1999) reported lower protein values of 2.1% in *A. incarvutus* in Ghana. The protein content of *A. cruentus* (3.37%) is higher than some of vegetables commonly consumed in Kenya. For example, Hanif et al. (2006) found crude protein content ranging from 0.9% to 2.1% in cauliflower, carrot, cabbage, lettuce, spinach. Uusiku, (2010) reported 3% protein in pumpkin leaves. *A. cruentus* species can be used in place of these vegetables since it has higher protein content.

Table 4.2: Proximate composition of the Amaranth vegetables

	<i>A.albus</i>	<i>A.hybridus</i>	<i>A.cruentus</i>	<i>A.dubius</i>	<i>A.hypochondriacus</i>
Moisture	81.29 ± 0.45 ^a	81.52±0.26 ^a	79.29± 1.26 ^b	82.53±0.16 ^a	82.44± 0.60 ^a
Protein	2.35± 0.06 ^d	3.34±0.06 ^b	3.37 ±0.02 ^a	2.74±0.06 ^c	3.34 ±0.09 ^b
Fat	1.59 ±0.03 ^b	2.53 ±0.06 ^a	1.62 ± 0.03 ^b	1.88 ± 0.07 ^b	2.63± 0.11 ^a
Ash	2.35± 0.13 ^b	2.2 ±0.34 ^b	1.52± 0.02 ^c	3.30±0.04 ^a	1.21±0.13 ^c
Crude fibre	2.31± 0.23 ^a	2.85± 0.55 ^a	3.01± 0.29 ^a	2.53± 0.17 ^a	2.49± 0.09 ^a

Values are given as means of three replicates ± Standard error of the mean. Means with different small letters within a row are significantly different at 5% level of significance.

Protein deficiency is one of Kenya's main nutritional problems which affect a large proportion of the poor urban populations, an adult requires 0.8g/kg body weight of protein in a day, this is on average about 46-56g (WHO, 2002). Therefore consumption of 100g *A. cruentus* would contribute 10% of the daily requirement according to the results of this study, There were significant differences ($p < 0.05$) in protein content in the species studied. *A. albus* had the lowest protein and was significantly differently from the rest. The protein content of *A. hybridus* and *A. hypochondriacus* were not significantly ($p < 0.05$) different. *A. cruentus* had the highest protein content and was also significantly different from the rest.

The fat content of *A. hybridus* ($2.53 \pm 0.1\%$) and *A. hypochodriacus* ($2.63 \pm 0.11\%$) was significantly higher than the other species (Table 4.2). The values got in this study were similar to those of Sheela et al., (2004) who reported 1.4% fat in amaranth. This was also in consonance with Funke, (2011) who documented 2.31% fat in amaranth vegetables in Nigeria. These values are lower compared to the fat content reported for black nightshade (8 mg/100g) by (Gqaza et al., 2013). The levels of fat were not significantly different in *A. albus*, *A. cruentus* and *A. dubius* but were significantly ($P < 0.05$) lower than values of *A. hybridus* and *A. hypochondriacus*.

The ash content ranged from (1.21-3.3.0%), which is comparable to that of *Amaranth incarvutus* (1.4%) as documented by Asibbey-Berko & Tavie, (1999). A study by Gigliola, (2012) also indicated similar ash values of (2.8-3.8%) in amaranth vegetables. The ash content reported in this study are in the same range as those of some commonly consumed vegetables, for example, Emebu & Anyika, (2011) reported (1.33%) ash in kales (*Brassica oleracea*) while Gqaza et al, (2013) reported 1.8% ash in *Solanaum nigrum* vegetables. The ash content is an index of the mineral content therefore from the levels of ash obtained from this study it shows that the vegetables could be a good source of minerals.

The fibre content ranged from (2.31-3.0mg/100g) with *A. cruentus* having highest content (3.01 ± 0.29 mg/100g). These values were higher than the figure that was reported by Funke, (2011) (0.77 %) crude fibre in amaranth. These values are in the same range as those of *Corchorus olitorius* (2.10 %) and *C. ochroleuca* (1.2 %) (Mibei, 2011). However, the differences in the content of crude fiber among the species were not significantly difference ($P < 0.05$).

On average an adult require 14g, of dietary fibre respectively (Institute of Medicine (U.S.), 2005). Consumption of 100 grams of fresh amaranth could contribute 15% of the recommended daily allowance. Dietary fibre is important in reducing the risk of colon cancer, constipation, diabetes and reducing absorption of cholesterol (Ishida et al., 2000). Consumption of dietary fiber improves blood glucose control in diabetes, it also plays a role in prevention and treatment of obesity (Anderson et al., 2004).

4.2.2. Minerals composition

This study revealed significant ($P < 0.05$) inter-species differences in the mineral levels (Table 4.3). *A. dubius* was found to have the highest levels of iron (18.64 ± 0.23 mg/100g) while *A. hypochondriacus* recorded the lowest iron contents (9.55 ± 0.15 mg/100g). Values of iron obtained in the current study agree with findings of Onyango, (2010) who reported 16 mg/100g iron content in *A. hypochondricus*. Raja et al. (1997) reported iron contents of 13.43 mg/100 g in amaranth sold in Dar es Salaam, while Srivastava, (2001) reported a range of 12.23-14.55mg/100g iron in *Amaranth* species in India. A study by Akubugwo et al. (2007) also indicated 13.58 mg/100g in *A. hybridus* in Ghana. However, a study by Chege, (2012) found the iron content in fresh *Amaranthus cruentus* leaves to be 8.47 mg/100 g in Kenya this is slightly lower than values obtained in this study.

Table 4.3: Mineral composition of the amaranth vegetables in mg/100g

	<i>A.albus</i>	<i>A.hybridus</i>	<i>A.cruentus</i>	<i>A.dubius</i>	<i>A.hypochondriacus</i>
Calcium	235.19±0.89 ^b	198.48±5.08 ^c	222.69±1.75 ^b	336.47± 0.99 ^a	131.06± 3.14 ^d
Zinc	1.40± 0.03 ^a	0.89±0.35 ^c	1.67± 0.03 ^a	0.60± 0.05 ^c	1.36±0.04 ^a
Iron	11.42± 0.10 ^b	10.57± 0.06 ^c	11.61± 0.30 ^b	18.64±0.23 ^a	9.55±0.15 ^d

Values are given as means of three replicates ± SEM. Means with different small letters within a row are significantly different ($P < 0.05$). SEM= Standard error of the mean

The iron contents are high compared to some vegetables consumed in Kenya. For example, the iron content of spinach (*Spinacia oleracea*) is about 1.7 mg/100 g edible portion (FAO, 2004). Hanif et al. (2006) documented 1.4mg/100g and 1.1mg/100g of iron in carrot and lettuce respectively.

A.dubius had the highest iron content. The content of iron in *A. albus* and *A. cruentus* were not significantly different from each other but were significantly from the rest. *A.hybridus*, *A. dubius* and *A. hypochondriacus* had significance differences in their iron content. The best source of iron in order of amount is *A.dubius*, *A. cruentus*, *A.albus*, *A. hybridus*, and *A. hypochondriacus*.

Human body requires iron for the synthesis of haemoglobin, myoglobin, for transporting the oxygen, and for the formation of heme enzymes and other iron-containing enzymes which are particularly important for energy production, immune defense and thyroid function (FAO/WHO, 2001). It is especially important to women of reproductive age, pregnant women and young children who are vulnerable to iron deficiency anemia. The daily iron requirements for children range between 7-15mg/day and women of reproductive age require 15-18mg/day while pregnant women require 27mg/day (FAO/WHO, 2001). Thus, consumption of 100g

of amount of amaranth leaves from the species *A. dubius* would meet 100% of the daily requirements if the iron is highly bio-available.

The levels of zinc ranged from 0.6-1.67mg/100g (Table 4.3). *A. cruentus* had the highest zinc content (1.67 ± 0.03 mg/100g) while *A. dubius* had the lowest content but was not significantly different from *A. hybridus*. These values are lower than the values obtained by Raja et al. (1997) who reported values of 4.08 mg/100 g for amaranth collected in various markets in Dar es Salaam; Onyango, (2010) obtained values of up to 6.3mg/100g in *A. hypochondriacus* sold in various supermarkets in Kenya. However, these values are higher than those recorded in other vegetables. For example Mibei, (2011) documented zinc content of 0.1mg/100g in spider plant, Van Jaarsveld et al. (2014) also documented lower values (0.42 mg/100g) in cowpeas leaves but similar values of zinc (0.75mg/100g in pumpkin leaves.

A. cruentus, *A. hypochondriacus* and *A. albus* were not significantly different in their zinc content but had significantly higher zinc content than *A. hybridus* and *A. dubius*. In order of amount the best sources of zinc are *A. cruentus*, *A. albus*, *A. hypochondriacus*, *A. hybridus* and *A. dubius*.

Zinc is present in all body tissues and fluids. It is necessary for a wide range of biochemical, immunological and clinical functions (FAO/WHO, 2001). Zinc is a stabilizer of the structures of membranes and cellular components. It plays a biochemical function by being an essential component of a large number of zinc-dependent enzymes, particularly in the synthesis and degradation of carbohydrates, lipids, proteins and nucleic acids (Guansheng Ma, 2008). Zinc plays a central role in the immune system. These biochemical functions of zinc are responsible for its unique role for growth and development (FAO/WHO, 2001).

A. dubius had the highest calcium content (336.47 ± 0.99 mg/100g) and *A. hypochondriacus* had the lowest (131.06 ± 3.14 mg/100g) (Table 4.3). Calcium was higher in all the five species, compared to other commonly consumed

vegetables. For example, Hanif et al., (2006) found 76 mg/100g in spinach and 52mg/100g in cabbage, (Mibei, 2011) reported a calcium content of 94.1mg/100g in spider plant.

Calcium is essential for the full activity of many enzymes such as nitric oxide synthase, protein phosphatases, and adenylate kinase and is required as a component of the human diet. It is also necessary for nerve transmission, muscle contraction, glandular secretion, contraction and dilation of blood vessels, and rebuilding of bone tissue (Whitfield & Chakravarthy, 2001). Chronic calcium deficiency contributes to a reduction in bone mass and the development of osteoporosis. RDA is 1000mg/day for adults aged 19-50years (FAO/WHO, 2001).

The calcium levels were significantly different in all the species except in *A. albus* and *A. cruentus* which were not significantly different from each other. The best source of calcium in order of amount is *A. dubius*, *A. albus*, *A. cruentus*, *A. hybridus*, and *A. hypochondiacus*.

4.3 Effect of processing methods on the nutrient content of the leafy amaranth

4.3.1 The effects of processing on β carotene content on five amaranth species

Fresh *A.hybridus* and *A.albus* recorded the highest amount of β carotene (26.89 ± 2.45 and 24.64 ± 1.24 mg/100g) respectively while *A. dubius* and *A. hypochondriacus* had the lowest levels with (17.32 ± 0.123 and 19.94 ± 1.18 mg/100g) (Table 4.4). This was in agreement with studies by Nambiar and Sharma, (2014) who found β carotene values of 21.35 mg/100g in amaranth species. Gupta et al. (2013) found slightly higher β carotene values (31.7 mg/100g) in *Amaranthus gangeticus* while Raja et al. (1997) reported slightly lower values (18.67 mg/100g) for amaranth in India. However, Negi and Roy, (2003) reported higher values of β carotene (59.4 mg/100g) in amaranth in India.

Under normal storage, vegetables undergo changes in their nutritional and organoleptic properties; these changes are due to the presence of peroxidases enzymes. The process of blanching destroys peroxidases but is deleterious to nutrients such as vitamin C and β carotene.

Table 4.4: Effect of processing on β carotene (mg/100g) on five amaranth species

	Fresh	Blanching at 80⁰c for 3 minutes	Blanching at 60⁰c for 25 minutes	Solar drying	Solar dried and cooking	Blanching at 80⁰c for 3 minutes then solar drying	Blanching at 80⁰c for 3 minutes solar drying and cooking	Blanching at 60 degrees for 25 minutes then solar drying	Blanching at 60 degrees for 25 minutes then solar cooking
<i>A.albus</i>	24.64±1.24 ^{ba}	17.96±1.12 ^a	8.77±1.02 ^{ab}	2.77 ±0.69 ^a	0.33 ±0.0 ^c	5.04±0.47 ^b	N D	3.0±0.68 ^b	N D
<i>A.hybridus</i>	26.89±2.45 ^a	18.23±1.23 ^a	10.50±0.23 ^b	3.03±0.59 ^a	2.99± 0.11 ^a	5.77±0.57 ^a	0.81±0.04 ^a	3.21±0.41 ^b	N D
<i>A.cruentus</i>	23.66±1.56 ^a	9.95 ±1.45 ^c	5.89±0.12 ^a	2.08± 0.08 ^c	1.84±0.04 ^b	5.85±0.21 ^a	0.47±0.04 ^b	2.64 ±0.12 ^b	0.02± 0.03 ^b
<i>A.dubius</i>	17.32±0.12 ^{bc}	12.27±0.23 ^c	7.05±0.45 ^b	2.25±0.1 ^c	1.85±0.06 ^b	6.26 ±0.33 ^a	0.88±0.06 ^a	2.09±0.04 ^a	0.71± 0.04 ^a
<i>A.hypochondriacus</i>	19.94 ±1.85 ^{bc}	15.53 ±1.23 ^b	7.52±1.25 ^b	1.65±0.59 ^b	N D	4.07±1.9 ^c	N D	0.52±0.01 ^c	N D

Values are given as means of three replicates ± Standard error of the mean. Means with different small letters within a column are significantly different at 5% level of significance ND = Not detected

Blanching at 80°C for 3 minutes led to a loss of 22-57% of the β carotene. A study by Negi and Roy, (2003) indicated similar losses of up to 51% in fenugreek during blanching. *A. cruentus* had the least amounts of β carotene 9.95 ± 1.45 mg/100g after blanching at 80°C for 3 minutes. However, *A. hybridus* still had the highest amount (18.23 ± 1.23 mg/100g) of β carotene after blanching.

A loss of 59-75% was experienced after blanching for 65°C for 25 minutes. *A. cruentus* had the lowest amount of β carotene (5.89 ± 0.12 mg/100g) after blanching at 65°C for 25 minutes while *A. hybridus* had the highest amount (10.50 ± 0.23 mg/100g). Thermal treatment to carotenoids in the presence of oxygen results in the formation of volatile compounds and larger non-volatile components (Bonnie & Choo, 1999). This explains the losses of β carotene experienced during blanching. Additionally pro-longed exposure to high temperatures (as in blanching at 65°C for 25 minutes) can lead to production of isomers or oxidation products resulting to lower carotene levels (Robert et al., 2012). The results indicated that there was a greater loss of β carotene during blanching at 65°C for 25 minutes compared to blanching at 80°C for 3 minutes.

Solar drying alone caused losses of β carotene 88-91%. This was in consonance with (Kiremire et al. (2010) who reported β carotene losses of 86.5% in *A. dubius* during solar drying. The least amount of β carotene recorded after solar drying were those of *A. cruentus* and *A. hypochondriacus* (2.08 ± 0.08 and 1.65 ± 0.59 mg/100g) respectively as shown in Table 4.4.

During the solar drying process, photo oxidation of both trans- and cis carotenoids takes place resulting into epoxidation (Djuikwo, 2011). The high loss of β -carotene during drying could have been caused by oxidation and was catalyzed by light. The solar dried samples were then cooked, this led to a further loss of up to 98%. *A. hybridus* had the highest amounts of β carotene (2.99 ± 0.11 mg/100g) after solar drying and cooking.

Solar drying the samples that were blanched at 80°C for 25 minutes caused a 63-79% loss, *A.dubius* had the highest amounts (6.26 ±0.03 mg/100g) of β carotene after blanching for 80 °C for 25 minutes then solar drying while *A.hypochondriacus* had the least amounts (4.07±1.9mg/100g). When the samples were boiled, the β carotene levels in *A. albus* and *A.hypochondriacus* were not detectable *A. hybridus* *A.cruentus* and *A.dubius* had little amounts of between 0.4-0.8mg/100g (Table 4.4). Solar drying the samples that were blanched at 60°C for 25 minutes also caused a great β carotene loss of >87%. *A. hybridus* had the highest amounts of 3.21 ±0.41 mg/100g after blanching at 60 degrees for 25 minutes and solar drying, very minimal levels were detected in *A.cruentus* while *A. dubius*. *A. hypochondricus* exhibited complete β carotene destruction when the vegetables were blanched at 60°C for 25 minutes and then solar dried. *A. hybridus* had the highest retention of β carotene content after solar drying and blanching. *A. albus* and *A.hypochondriacus* had the highest losses of β carotene after blanching at both temperatures and solar drying.

Carotenoids are associated with different proteins depending on the variety of *amaranth* (Clarissa *et al.*, 2004). The carotenoid-protein complexes are denatured by the heating of vegetables. It could be assumed that heating led to a destruction of carotenoid-protein complexes and membranes of chromoplasts. The carotenoids were then degraded by oxygen and high temperature (Seybold *et al.*, 2004). When the blanched samples were solar dried, β carotene was further destroyed by light causing even greater losses.

Fruits and vegetables are a good source of β carotene. This is a carotenoid that the body converts to vitamin A (retinol). Vitamin A is important in vision, in cell differentiation, maintaining the integrity of the epithelial tissue and immune system, gene expression, reproduction, embryonic development growth and also in reproduction (FAO/WHO, 2001). Deficiency of vitamin A causes xerophthalmia. Intake of carotenoid from foods is associated with lower risk of several chronic diseases (WHO, 2000).

In this study, fresh *A. hybridus*, *A. cruentus* and *A. albus* recorded the highest β carotene content, all processes that involved blanching prior to solar drying recorded high losses of β carotene of above 89%. *A. hypochondriacus* had significantly ($P < 0.05$) lowest levels of β carotene after processing.

4.3.2 The effect of processing on vitamin C content on five amaranth species

The levels of vitamin C ranged from (413-802mg/100 g). Fresh *A. dubius* had vitamin C in significantly ($P < 0.05$) high amounts (802.63 ± 4.12 mg/100g) while fresh *A. cruentus* had significantly ($P < 0.05$) low amount (413.66 ± 2.13 mg/100g) as shown in (Table 4.5). A study by Yadav and Sehgal, (1997) indicated similar amounts of AA ranging from 624.1-629.0 mg/100 g in *A. tricolor*. Additionally, Nicholas, (2011) recorded mean AA content of *A. hypochondriacus* leaves as (443.90-619.21 mg/100 g) while that of *A. cruentus* leaves was (440.07-598.13 mg/100 g). Findings from this study are also in agreement with Onyango, (2010) and Mnkeni et al., (2007) who reported vitamin C values of 496.5-630.9 mg/100 and 627 mg/100 respectively in amaranth leaves.

Blanching at 80°C for 3 minutes led to a loss of between (13-46%). Gupta et al, (2008) found higher losses (80%) of vitamin C after blanching amaranth at 80°C for 4 minutes. (Negi & Roy, 2003) recorded higher losses (60%) when blanching amaranth for 95°C for 3 minutes. The species with the highest vitamin C after blanching at 80°C for 3 minutes was *A. dubius* (647.17 ± 1.45 mg/100g) while *A. hybridus* and *A. hypochondriacus* had the lowest (355.41 ± 1.2 mg/100g) and (384.49 ± 2.01 mg/100g) respectively (Table 4.5.). Blanching for 60°C for 25 minutes led to 85-90% loss of vitamin C. Agbemafla et al, (2012) recorded similar vitamin C losses of 42% to 85%. *A. dubius* had the highest amounts (98.37 ± 1.78 mg/100g) after blanching at 60°C for 25 minutes while *A. hypochondriacus* had the least amounts (52.25 ± 1.67 mg/100g).

Table 4.5: Effect of processing on vitamin C mg/100g on five amaranth species

	Fresh	Blanching at 80 ⁰ c for 3 minutes	Blanching at 60 ⁰ c for 25 minutes	Solar drying	Solar dried and cooking	Blanching at 80 ⁰ c for 3 min then solar drying	Blanching at 80 ⁰ c for 3 min then solar drying then cooking	Blanching at 60 degrees for 25 min then solar drying for 4 days	Blanching at 60 degrees for 25 min then solar drying then cooking
<i>A.albus</i>	670.55±2.56 ^b	505.72±2.12 ^b	90.88±2.01 ^c	44.05 ±0.43 ^a	5.45±0.94 ^a	7.86± 0.09 ^a	0.68± 0.05 ^b	ND	ND
<i>A.hybridus</i>	670.13±1.95 ^b	355.41±1.2 ^d	65.86±1.23 ^d	36.48±0.5 ^c	1.81±0.09 ^b	5.25±0.05 ^b	0.53±0.05 ^b	ND	ND
<i>A.cruentus</i>	413.66±2.13 ^d	356.88±2.87 ^c	58.562±1.45 ^c	39.37 ±0.32 ^b	1.487± 0.13 ^b	2.84 ±0.13 ^d	0.11 ±0.01 ^c	ND	ND
<i>A.dubius</i>	802.63±4.12 ^a	647.17±1.45 ^a	98.37±1.78 ^a	44.04 ±0.50 ^a	4.11 ±0.29 ^a	7.63 ± 0.07 ^a	1.46± 0.03 ^a	ND	ND
<i>A.hypochondriacus</i>	542.9±1.23 ^c	384.49±2.01 ^d	52.25±1.67 ^b	32.19 ±0.32 ^d	1.1 ±0.01 ^b	3.75 ±0.09 ^c	ND	ND	ND

Values are given as means of three replicates ± Standard error of the mean. Means with different small letters within a column are significantly different at 5% level of significance. ND = Not detected

Solar drying alone caused a loss of between 90-94% of ascorbic acid. *A. albus* and *A. dubius* had significantly ($P < 0.05$) high amounts (44.05 ± 0.43 and 44.04 ± 0.50 mg/100g respectively) of vitamin C after solar drying. When the samples were blanched under the two blanching conditions (80°C at 3 minutes and at 65°C at 25 minutes) and solar dried, a loss of $> 98\%$ was recorded. *A. albus* and *A. dubius* had significantly ($P < 0.05$) high amounts (7.86 ± 0.09 mg/100g and 7.63 ± 0.07 mg/100g) respectively when the vegetables were blanched at 80°C for 3 minutes and then solar dried, while *A. hypochondriacus* had the lowest amounts (3.75 ± 0.09 mg/100g). No vitamin C was detected when the vegetables were blanched at 65°C for 25 minutes and then solar dried, additionally, when the blanched and solar dried samples were cooked there was no vitamin C that was detected.

Vitamin C content was high in fresh leaves of *A. dubius*, and *A. hybridus*. Its thermo-labile nature was demonstrated by the huge loss and a significant destruction upon blanching and drying. The process of blanching combined with solar drying had a near complete loss of vitamin C with destruction being more than 98% regardless of whether the blanching was done at high temperature short time or low temperature long time. However, slightly lower values of 93% of vitamin C were destroyed during the process of solar drying without prior blanching. Blanching alone caused losses of between (13-90%) while blanching at high temperature short time (80°C for 3 minutes) was a more effective in retaining vitamin C content as compare to low temperature short time (65°C for 25 min). These results agree with findings of Mathooko and Imungi 1994 who reported a loss of 80% ascorbic acid during cooking. Mziray *et al.*, (2000) reported 87.4% reduction of ascorbic acid content in *A. hybridus*. during solar drying.

Vitamin C also known as ascorbic acid is one of the most important vitamins in human nutrition that is supplied by fruits and vegetables. Ascorbic acid is available in reduced form (L-ascorbic acid) and oxidized form (L dehydroascorbic acid). It is

a powerful dietary antioxidant; it is water-soluble, thermo-labile and is easily oxidized (Ball, 1998). The enzyme ascorbic acid oxidase which catalysis the oxidation of ascorbic acid to dehydroascorbic acid is denatured by high temperatures and is sensitive to air (Acisevk, 2009).

Vitamin C plays an important role in the manufacturing of collagen, which is a connective tissue that holds bones together. It is used to maintain the integrity of the membranes by protecting molecules such as proteins, lipids carbohydrates and nucleic acids from damage by free radicals and reactive oxygen species and reactive nitrogen species that are generated during normal metabolism and through exposure to toxins and pollutants (Hanif et al., 2006). It also modulates the absorption, transport and storage of iron (Ball, 1998).

4.4 Phytochemical composition in amaranth

Phytochemicals are bioactive non-nutrient plant compounds in plant foods. More than 5000 phytochemicals have been identified. This study focused on only three which are common in amaranth that is: total phenols, and flavonoids (quercetin and catechin). The results indicated the presence of flavonoids i.e. catechins and quercetin, and phenolics compounds (Table 4.6), *A. cruentus* was found to have significantly higher ($P < 0.05$) levels of all the three phytochemicals compared to all the other species. Total phenolics (TP) ranged from (1.68 - 3.59mg GAE/100g). *A. hypochondriacus* and *A. dubius* had significantly ($p < 0.05$) lower phenolics with (1.68 ± 0.001 , and 2.22 ± 0.19 mg GAE/100g respectively). Quercetin and catechin contents were highest in *A. cruentus* (14.28 ± 0.86 mg QE/100g and 7.15 CE/100mg respectively) (Table 4.6.) *A. dubius* exhibited the lowest quercetin level of (4.69 ± 0.45 mg QE/100g) which was not significantly different from *A. hypochondricus*. On the other hand, the catechin level in *A. hypochondriacus* was the lowest (3.63 ± 0.2 mg QE/100g). Nana et al, (2012) reported 7.55 ± 1.18 mg (GAE)/100 g total phenolics and 2.90 ± 0.25 (QE)/100 mg in *A. cruentus*, this study

reveals similar values of TP but lower quercetin level in *A. cruentus*. The values of TP in *A. hybridus* obtained in this study were much lower than those of Nana et al, (2012) who reported a value of 8.30 ± 0.52 mg (GAE)/100g. Additionally, Nana et al, (2012) reported lower quercetin content (4.33 ± 0.27 (QE)/100g) in *A. hybridus*..

Table 4.6: Phytochemical composition of the amaranth vegetables in GAE, CE, and QE mg/100g

	<i>A.albus</i>	<i>A.cruentus</i>	<i>A.dubius</i>	<i>A.hybridus</i>	<i>A.hypochondriacus</i>
Phenols(GAE)	2.24± 0.07 ^b	3.59± 0.01 ^{ab}	2.22± 1.19 ^b	2.77± 0.02 ^a	1.68±0.01 ^c
Catechins(CAE)	5.55±0.18 ^a	7.15± 0.63 ^a	3.75±0.39 ^b	6.05±0.10 ^a	3.63±0.2 ^b
Quercetin(QE)	12.55±1.04 ^a	14.28± 0.86 ^a	4.69± 0.45 ^b	13.76±1.16 ^a	6.5± 0.71 ^b

Values are given as means of three replicates ± Standard error of the mean. Means with different small letters within a row are significantly different at 5% level of significance.

According to Bhagwat et al, (2011) onions are one of the best source of quercetin and contain about 28 mg QE/ 100g. *A.cruentus* would provide half the amount produced by onions which makes it a better source compared to other commonly consumed vegetables such as cabbage which have (0.28 mg QE/ 100g) and kales (11 mg QE /100g) (Bhagwat et al., 2011).

A. hypochondricus had significantly (P<0.05) lowest amounts of total phenols while the catechins and quercetin content in *A. dubius* and *A. hypochondricus* were not significantly different and were the lowest compared to other species.

Phytochemicals such as phenolic compounds and flavonoids possess strong antioxidant properties and have been implicated in the prevention of cancer and cardiovascular disease, diabetes and aging (Hertog et al., 1992).Phytochemicals

help prevent oxidative damage to large biomolecules such as DNA, lipids and proteins by neutralizing free radicals. Oxidative damage of biomolecules increases the risk of cancer, cardiovascular diseases and diabetes.

Intake of phytochemicals such as flavonoid is significantly inversely associated with mortality from coronary artery disease and inversely related with incidence of myocardial infarction (Hertog et al., 1992). Flavonoids have also been reported to have medicinal properties such as antimicrobial and antioxidant properties (Bhagwat et al., 2011). A study in Finland showed that intake of apples and onions, both high in quercetin, was inversely correlated with total mortality and coronary mortality (Knekt et al., 1996). Other studies have also indicated that the total intake of flavonoids is inversely correlated with the plasma total cholesterol and low-density lipoprotein (LDL) cholesterol concentrations (Liu, 2003). These phenols reduce the risk of heart disease and certain types of cancer. However some of the phenols lower the digestibility of protein (Bhagwat et al., 2011). The presence of phytochemicals in amaranth shows that besides providing nutrients amaranth has additional nutritional properties hence its consumption should be encouraged.

4.5. Antioxidant activity

The ethanol extracts of *A.cruentus* had significantly ($P < 0.05$) highest DPPH radical inhibition activity (87.71%) while the least was *A.dubius* 64.5% (Figure 4.11). Figure 4.12 illustrates the absorbance versus the concentration curves of the ethanolic extracts of the five varieties.

The decrease in absorbance as the concentration increase was taken as a measure of the extent of radical scavenging activity. *A.cruentus* had the highest decrease in absorbance when the concentration was increased and also had the highest radical scavenging effects. The scavenging effects of the extracts increased as the concentration increased. However, when the concentration of the extracts were

greater than 1mg/ml the increase in the activity was marginal. All the five extracts had significantly ($p < 0.05$) higher DPPH inhibition than ascorbic acid.

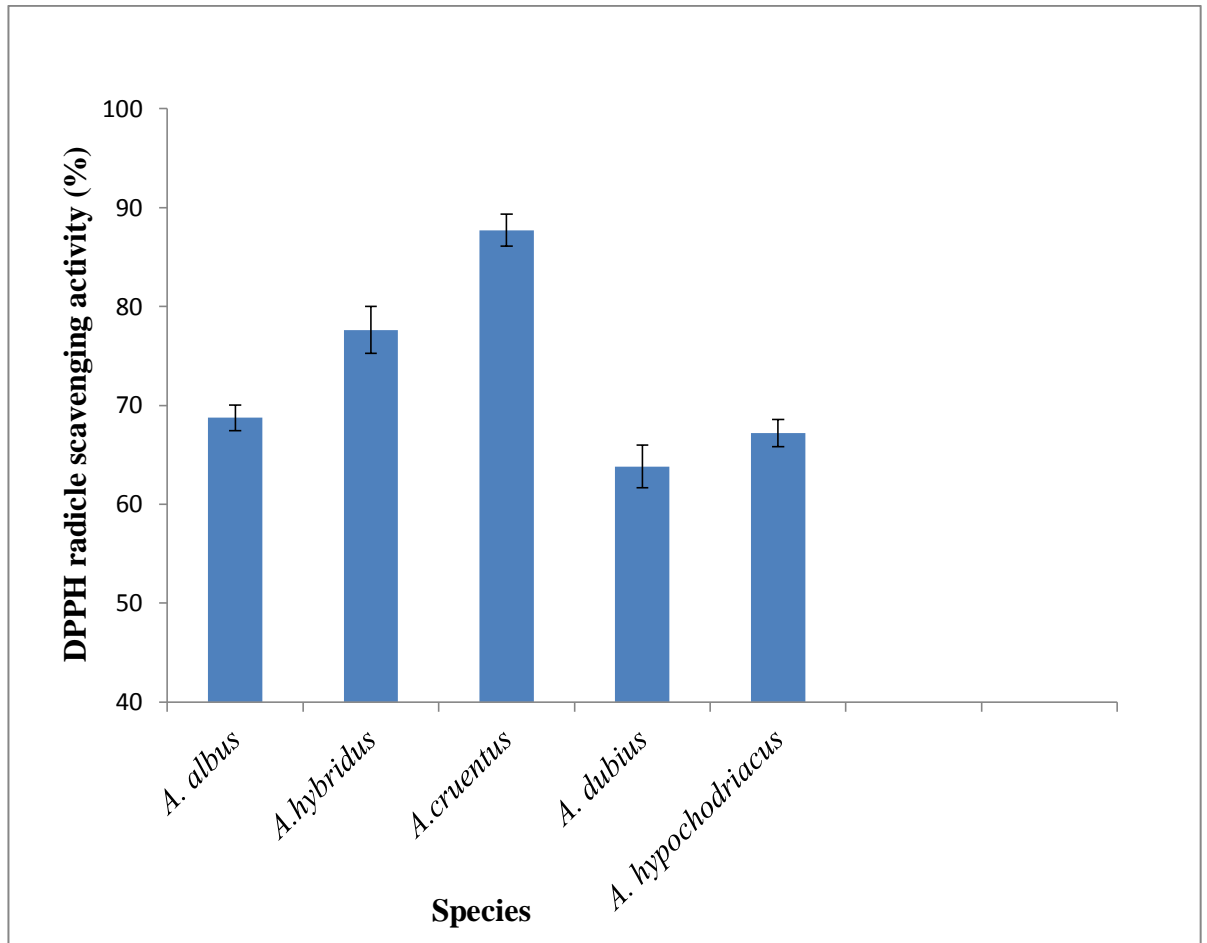


Figure 4.11.: DPPH radical scavenging activity of ethanolic extracts of five amaranth species. Stem height =SEM (Standard error of the mean).

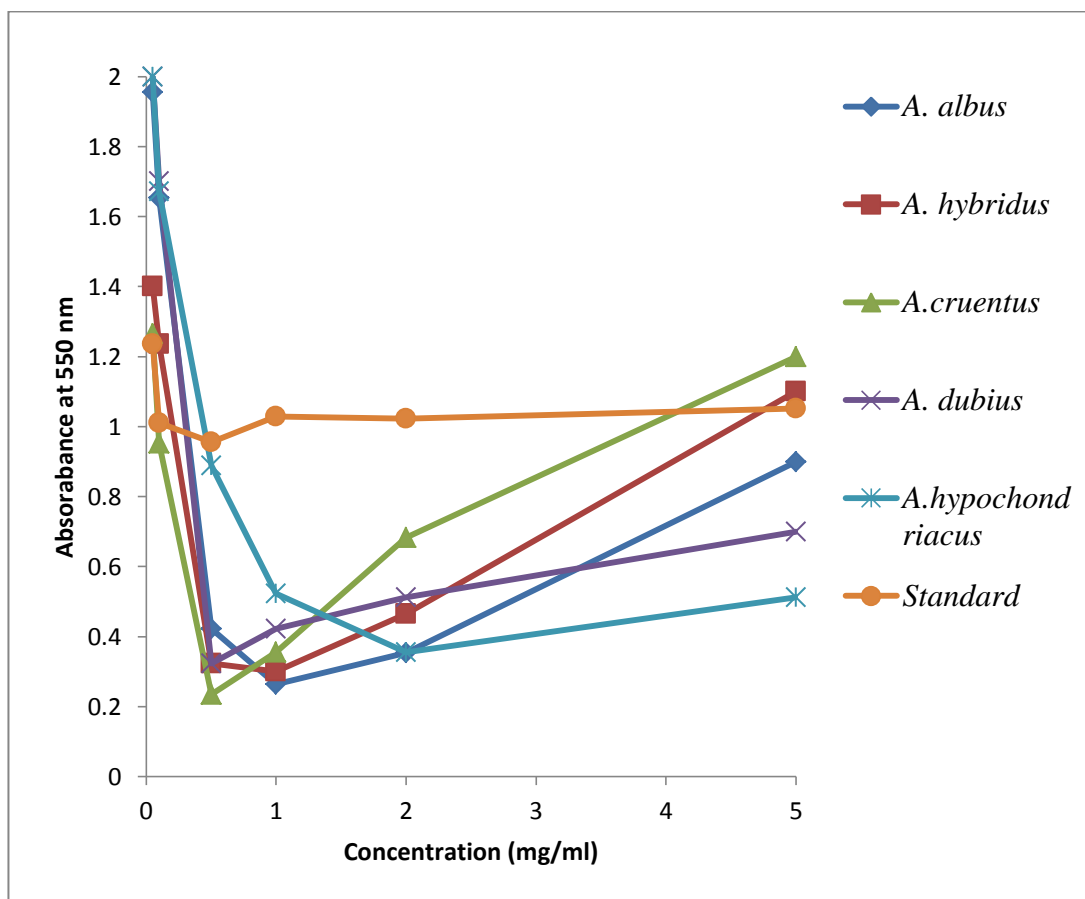


Figure 4.12: Changes in absorbance of ethanolic extracts of fresh amaranth leaves with Concentration (mg/ml).

Human cells are constantly exposed to a variety of oxidizing agents which are produced during metabolic activities within cells; some are present in food, in water and also in air. An overproduction of oxidants can cause oxidative stress which causes damage to biological molecules which leads to cell impairment or death eventually leading to chronic disease (Thomas *et al.*, 2011).

To prevent the harmful effects of excess oxidants sufficient amounts of antioxidants have to be consumed. Therefore, the need to know the composition of antioxidants in food is imperative. The antioxidant activity was examined using DPPH by comparing the free radical scavenging activity of amaranth ethanolic extract, with ascorbic acid which is a known antioxidant. Antioxidants are extracted in higher amounts in ethanolic/ methanolic solvents (Sultan *et al.*, 2009).

Ethanollic extracts of the different amaranth species had significant scavenging effects and all reported a significantly ($P < 0.05$) higher percentage of DPPH inhibition than ascorbic acid which was 63.9% the results indicated that the DPPH scavenging activity of *A. cruentus*, *A. albus* and *A. hybridus* was directly proportional to the total phenolic content in them. However, *A. hypochondriacus* showed high DPPH scavenging activity with lower total phenolic content than *A. dubius* and *A. albus* extracts. Other findings by Khandaker et al, (2008) have established the abundance of antioxidants in *Amaranthus* leaves and that there exists a general trend towards increased antioxidant activity with increased total phenolics content.

4.6 Antinutrients content in amaranth

Antinutrients are substances that interfere with the digestion, absorption and assimilation of nutrients. This study focused on oxalates and phytates which are the main antinutrients found in vegetables. In all the species analyzed, the phytates and oxalates levels were less than 2%, and the levels in the five different species were not significantly different ($p < 0.05$) from each other (Table 4.7).

Table 4.7: Antinutrient composition of the amaranth vegetables mg/100g

	<i>A.albus</i>	<i>A.cruentus</i>	<i>A.dubius</i>	<i>A.hybridus</i>	<i>A. hypochondriacus</i>
Oxalates	1.56±0.03 ^a	1.65± 0.08 ^a	1.79±0.09 ^a	1.95± 0.35 ^a	0.06 ±0.02 ^a
Phytates	0.37± 0.06 ^a	0.07±0.004 ^a	0.063±0.02 ^a	0.075±0.01 ^a	0.06±0.01 ^a

Values are given as means of three replicates ± SEM. Means with the similar small letters within a row are not significantly different ($P < 0.05$). SEM= Standard error of the mean

The levels of oxalate were in the range (0.06-1.95mg/100g). The amount of oxalates obtained in this study were in the range of those recorded by Imungi, (1999) who reported values of 1.8 mg/100g in amaranth while (Onyango, 2010) reported lower values 0.5 mg/100g and 0.6mg/100g in *A. hypochondriacus*.

Oxalic acid renders minerals unavailable to animals by forming water insoluble precipitates salts with potassium, it also precipitates calcium forming insoluble calcium oxalate this reduces absorption of dietary calcium (Habtamu & Negussie, 2014). Oxalic acid also binds on to iron and magnesium reducing their absorption. Levels toxic to humans have been indicated to be 2 to 5 g of oxalic acid per day for those populations consuming low levels of calcium (Habtamu & Negussie, 2014). If oxalic acid is consumed in large quantities it can cause calcium deficiencies even when the diet is sufficient in calcium. For infants and children who are experiencing active formation of bones and teeth, this situation is more critical, and also in older persons who can suffer calcium resorption from bones leading to osteoporosis (Grubben, 2004c). The maximum safe intake of oxalic acid is 5g which would be contained in about 2kg of fresh amaranth vegetables, an amount which is much more than the average amaranth consumed in a day.

The levels of phytates were in the range (0.063-0.37mg/100g) there was no significant ($p < 0.05$) differences in the phytate content of the species. Values obtained in this study are in agreement with values reported by Agbaire, (2012) of 0.32mg/100g in *Amaranthus hybridus* in Nigeria. However this values are lower than those reported by Gupta et al, (2005) of 1.95mg/100g in *Amaranthus tricolor* in India while reported. Phytate occurs as storage form of phosphorus, and is found in some plant seeds and in many roots and tubers. Phytates forms protein -phytic complexes thereby decreasing the bioavailability of protein in the body (Habtamu & Negussie, 2014). Phytate also forms insoluble complexes with essential elements such as zinc, iron and calcium and protein, thereby decreasing the bioavailability of these nutrients in the body (Habtamu & Negussie, 2014). Consumption of food high in phytates is associated nutritional disorders such as rickets and osteomalacia in

children and adults respectively. Consumption of 1-6g of phytates over a long period of time decreases the bioavailability of mineral in monogastric animals (Agbaire, 2012). The levels of phytates in amaranth species reported in this study (< 2mg) are below the toxic levels and would not cause any significant decrease in bioavailability of minerals and proteins.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

- Within the limits of this study, the most common amaranth cultivars grown in Kenya are: *A. dubius*, *A. hybridus*, *A. cruentus*, *A. albus* and *A. hypochondriacus*.
- This study indicates that the amaranth species found in Kenya are a good source of key nutrients. The species exhibited significant differences in their protein, fibre, fat and ash content. *A. cruentus* is a superior source of protein compared to the other four species.
- The calcium, iron, zinc, β carotene and vitamin C content differed significantly in all the varieties. *A. dubius* is a relatively superior source of iron than the other amaranth species. *A. cruentus* is a good source of zinc while *A. albus* is a good source of calcium.
- *A. cruentus* which had the highest total phenols, quercetin and catechin, also had the highest antioxidant properties compared to the rest of the species.
- The phytates and oxalates found in amaranth were not significantly different in all the species.
- Blanching and solar drying of the vegetables caused losses in β carotene and vitamin C. According to this study, the higher retention of β carotene and vitamin C was experienced when the vegetables are blanched at 80⁰C for 3 minutes as opposed to 65⁰C for 25 minutes.
- Blanching prior to solar drying increased the retention of β carotene and vitamin C during solar drying.

5.2 Recommendations

- In terms of nutritive value species can be adopted in the following order, *A. dubius*, *A. cruentus*, *A. albus*, *A. hybridus* and *A. hypochondriacus*.
- The agriculture extension workers should train the communities on production and management of amaranth to improve nutrition security.
- The nutritionists should create awareness of the nutrition benefits of amaranth species in the communities and health centers.
- *A. dubius*, and *A. cruentus* vegetables can be used in nutrition interventions since they are superior in micronutrients and phytochemicals than the other species
- Better processing, preparation and storage methods should be investigated for better retention of vitamin C and β carotene.
- The nutrient content of other species should be investigated.

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APPENDICES

Appendix I: Structured Questionnaire

QUESTIONNAIRE

A BASELINE SURVEY FOR AMARANTH PRODUCTION

CHARACTERISTICS AND VARIETIES

QUESTIONNAIRE FOR FARMERS

(FILL IN ONE FORM FOR EACH FARMER)

DISTRICT _____

Qnr No. _____

APPENDIX I: QUESTIONNAIRE

SECTION A: DEMOGRAPHIC STATISTICS

1. Your Name _____
2. Are you: (please tick as necessary) Male Female
3. Indicate the bracket from which your age falls?
 1. Between 18-25() 2. Between 25-40 years () 3. Over 40 years ()
4. What is your level of education?
 1. Primary () 2. Secondary () 3. College () 4. No formal education ()
5. What is your occupation?
 1. Employed () 2. Business woman () 3. Farmer () 4. House wife ()

SECTION B: KNOWLEDGE ON AMARANTH

6. Do you grow amaranth?

Yes [] No []

7. Where did you get the knowledge from?

- 1. Family ()
- 2. School ()
- 3. Research projects ()
- 4. NGO ()
- 5. N/A ()

8. At what age did you start growing amaranth?

- 1. As a child ()
- 2. At teenage ()
- 3. As an adult ()
- 4. N/A

9. How many varieties of amaranth do you grow?

.....
.....

What are their local names?

.....
.....

State some of the methods you use for preparing land for amaranth cultivation

.....
.....
.....
.....

SECTION C: CULTURAL PRACTICES AND MANAGERMENTS OF AMARANTH

10. How do you prepare land used for growing amaranth

.....
.....
.....
.....

11. How many times do you plant amaranth in a year?

- 1. once ()
- 2. Twice ()
- 3. Thrice ()
- 4. N/A ()

12. Which method do you use for planting?

.....
.....
.....
.....

13. Do you use any fertilizer

- Yes [] No []

14. If yes which type of fertilizer do you use?

- Inorganic () organic ()
Manure () none ()

15. How long does amaranth leaves take to mature?

.....
.....

16. What are the maturity indices of amaranth vegetables?

.....
.....
.....
.....

17. How and where do you store amaranth vegetables?

.....
.....
.....
.....

SECTION C: BENEFITS AND CHALLENGES OF GROWING AMARANTH

18. Are there any economic benefits of growing amaranth?

Yes ()

No ()

If yes which ones? -----

19. What are the nutritional and medicinal benefits of eating amaranth

.....
.....

Appendix II: Focus group discussion guide

**A BASELINE SURVEY FOR AMARANTH PRODUCTION
CHARACTERISTICS AND VARIETIES
FOCUS GROUP: DISCUSSION GUIDE
FOR FARMERS**

DISTRICT _____

1. What do you know about amaranth?

a. What are the names given to amaranth in this community?

.....
...
.....
...

b. Where and how is it grown?

.....
.....

c. What are some of its uses?

.....
.....
.....

2. What are the benefits of growing amaranth?

.....
.....
.....

3. Do you think that amaranth farming has a future? Do you think it's likely to improve the nutrition status and food security of this community? If not, why

not.....
.....
.....
.....

4. Of all the things we've discussed today, what would you say are the most important issues you would like to express about amaranth?

.....
.....
.....
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