PHENOTYPIC BASED CHARACTERIZATION OF NUTRITIONAL AND BIOACTIVE TRAITS OF AMARANTH VEGETABLE FOR IMPROVED IRON BIOAVAILABILITY AND ACCEPTABILITY

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Phenotypic Based Characterization of Nutritional and Bioactive traits of Amaranth Vegetable for Improved Iron Bioavailability and Acceptability

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DECLARATION

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

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DEDICATION

This work is dedicated to my parents, Mr. Manason Nyonje and Mrs. Fleria Nyaugo-Nyonje and my brothers and sisters, Collins, Irene, Evans and Ephie for their support and encouragement during my studies.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic absorption spectrophotometer
ABA	Abscisic acid
AIV	African Indigenous Vegetables
ALV	African leafy vegetables
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
bp	Base pairs
cDNA	Complementary deoxyribonucleic acid
cm	Centimeter
cm^2	centimeters square
cm^3	Cubic centimeters
CRD	Completely randomized design
Ct	Cycle threshold
3D	Three- dimensional
o	Degrees
^oC	Degree Celsius
DMRT	Duncan multiple range test
DNA	Deoxyribonucleic acid
DW	Dry weight
EA	East Africa
FAO	Food and Agriculture Organization
FGD	Focus group discussion
g	Gram
µg	Microgram
GGP	Guanosine diphosphate-galactose phosphorylase
GGPP	Geranylgeranyl Pyrophosphate
GLO	Glycolate oxidase
GME	Guanosine diphosphate-mannose 3,5-epimerase

h	Hour
HPLC	High performance liquid chromatography
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KII	Key Informant Interview
kgf	kilogram force
LC-MS	Liquid Chromatography – Mass Spectrum
LCYB	Lycopene beta cyclase
LCYE	Lycopene epsilon cyclase
L	Litre
µL	Microlitre
LSD	Least significant difference
mg	Milligram
mL	Millilitre
mm	Millimeter
min	Minutes
µm	Micrometer
nm	Nanometre
n	Number of items/sample size
NIR	Near infrared
ns	Not significant
ppm	Parts per million
PSY	Phytoene synthase
RCBD	Randomized complete block design
RNA	Ribonucleic acid
rpm	Rotations per minute
Rt	Retention time
RT-qPCR	Real time quantitative polymerase chain reaction
s	Seconds

SSA	Sub-Saharan Africa
TDHS	Tanzania Demographic and Health Survey
TNNS	Tanzania National Nutrition Survey
r	correlation coefficient
VTC2	Vitamin C Defective 2
WHO	World Health Organization

ABSTRACT

The amaranth vegetable has been shown to be rich in micronutrients as well as many bioactive compounds. The crop, however, has different species and varieties which possess different nutritional attributes. Though the vegetable has anti-nutrients that reduce mineral bioavailability, it is tolerant to harsh environmental conditions which may affect nutrient accumulation positively or negatively. Varied cooking methods may also affect nutrient retention and bioavailability. The objective of this study was to determine how varietal differences, environment and cooking affects nutritional composition of amaranth vegetables and also to develop nutritionally improved recipes that enhance nutrient retention and iron bioavailability of these vegetables. The study sought to understand how morphological phenotypes, environmental stress in form of drought and cooking habits relate to the nutrient composition of amaranth. The study involved ten accessions of amaranth from four different species: *Amaranthus hypochondriacus, Amaranthus cruentus, Amaranthus blitum and Amaranthus dubius*. The seeds were obtained from the World Vegetable Centre in Tanzania and Taiwan. Morphological characterization of the lines was done by precision phenotyping technology using Phenospex field scan. Nutritional laboratory analysis was also carried out and the association between the traits determined. The amaranth accessions were also grown in a greenhouse and subjected to drought stress. The effect of drought stress on nutrient accumulation as well as the effect on the transcription of biosynthesis genes of selected components were then determined. Surveys on consumer preference and utilization practices for amaranth vegetables were carried out in Kenya and Tanzania through focus group discussions and key informant interviews to explore the knowledge, attitudes and practices around amaranth. Nutrient retention of amaranth vegetable dishes prepared using traditional food preparation methods identified from the survey were evaluated. Iron bioavailability of traditional and improved amaranth dishes was determined. Recipes were then developed, based on themes that help improve diversity, including nutrient retention, mineral bioavailability and other nutritional benefits from the vegetables. The accessions of the *A. dubius* species showed significantly higher nutritional content compared to the accessions of other species. Greenness of the leaves was found to be positively correlated with oxalate and vitamin C content, while the hue of the leaves correlated with the content of some carotenoids. Oxalates were reduced under drought stress, while components with anti-oxidative properties including vitamin C, carotenoids, flavonoids and phenolic acids were enhanced under the stress. Changes in accumulation of calcium, iron and zinc were not significant. Significant changes in transcription of biosynthetic genes for oxalate, vitamin C and carotenoids were also observed, with a positive correlation between vitamin C change and the level of expression of its biosynthetic gene, GDP-L-galactose phosphorylase (GGP/VTC2). Preference for amaranth vegetables differed with region as well as individuals. *A. dubius* and *A. blitum* were the most commonly consumed varieties in Kenya; while *A. dubius* and *A. hypochondriacus* were the most common in Tanzania. The consumer preference for species was mostly based on taste and availability. Cooking methods were varied among consumers and included boiling, frying and steaming. Most respondents considered taste rather than nutrition when cooking. Some of the commonly used cooking practices included prolonged cooking, which reduces nutritional value. Incorporating vitamin C, adding an iron rich vegetable and boiling of the vegetable significantly improved iron bioavailability. Incorporating lemon juice enhanced dialysable iron of the selected recipe by up to 66%. Sixteen (16) recipes were developed based on 5 nutritional themes. The themes addressed nutritional and utilization related factors of amaranth, and also applied principles from the iron bioavailability enhancement study to improve nutritional quality of amaranth vegetables. Sensory evaluation in Kenya and Tanzania showed high acceptability of the recipes. This study concludes that varietal differences significantly affect the nutritional content in amaranth vegetables, and some morphological phenotypes such as hue and greenness of leaves are associated with certain nutritional components. Drought stress, affects metabolite biosynthesis and accumulation in amaranth. Finally, there exists varied preferences, attitudes and utilization practices of amaranth in Kenya and Tanzania. The study recommends the adoption of improved amaranth cooking methods in order to increase consumption and maximize the nutrition benefits from amaranth vegetables.

CHAPTER ONE

INTRODUCTION

1.1 Study background

The amaranth vegetable (*Amaranthus* spp.) is one of the African indigenous vegetables (AIVs), believed to have their secondary center of origin in Africa. The vegetable is in the *Amaranthaceae* family under the genus *Amaranthus* and consists of about 50-70 species (Stetter & Schmid, 2016). For many years, AIVs, including amaranth have suffered neglect (Abukutsa-Onyango, 2011; Mbwambo *et al*., 2015), with the young consumers and urban dwellers equating the vegetables to traditional lifestyles (Matenge *et al*., 2012). However, their consumption is currently on the rise, owing to recent research on the indigenous vegetables which has shown that the vegetables are rich in vitamins, minerals and bioactive compounds, and are also easy to cultivate and cook (Karanja *et al*., 2012; Krause *et al*., 2019).

Amaranth vegetables provide a rich and cheap source of micronutrients including vitamins and minerals which are important in diet (Jimoh *et al*., 2018). Apart from being rich in nutrients, these vegetables also have several antioxidant phytochemicals which protect the body from long-term degenerative diseases (Sarker & Oba, 2019b). Extracts from almost all plant parts of amaranth seem to have medicinal benefits. This could be due to the high antioxidant activity and anti-inflammatory properties of the various species (Peter & Gandhi, 2017). Phytochemical analysis of edible parts of various *Amaranthus spp*. have established the presence of active compounds including alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, amino acids, vitamins, minerals, terpenoids, lipids, betaine, catechuic tannins and carotenoids (Nana *et al*., 2012; Vithya & Jayshree, 2017).

However, the amaranth vegetable (*Amaranthus spp*.) is also known to accumulate various anti-nutrients that might bind the nutrients, reducing their bioavailability in the body. These include oxalates, nitrates and tannins (Nyonje *et al*., 2014). It has also been reported that some plant foods with high dietary fiber content, including amaranth are low in bioavailable calcium, which may be due to the presence of other inhibitors in the food (Amalraj & Pius, 2014). Oxalic acid and its salts have been shown to have deleterious effect on human nutrition and health mostly by decreasing absorption of some mineral nutrients and aiding the formation of kidney stones (Olawoye & Gbadamosi, 2017).

Among the agronomic characteristics of amaranth is the great genetic and phenotypic variability, which is observed in several plant characteristics such as inflorescence type, seed color, precocity, protein content of the seed and resistance to pests and diseases (Akaneme & Ani, 2013; Erum *et al*., 2012). Wide nutritional variations among various amaranth varieties also exist (Nyonje *et al*., 2014). The associations between phenotypes with the nutritional traits of the vegetables is still not clear. Amaranth is also a hardy crop and can tolerate wide environmental conditions considerably well.

Nutritional benefits from amaranth vegetables could depend on several factors, including species (Nyonje *et al*., 2014), production practices and cooking method used (Castanheira, 2016). Certain beliefs and negative attitudes have over the years led to neglect of vegetable amaranth. For instance, the vegetable was previously seen as a weed and a poor man's food (Achigan-Dako, Sogbohossou and Maundu, 2014; Maseko, Id and Tesfay, 2017; Olusanya *et al.*, 2021). Certain attitudes may also exist that lead to increased consumption of these vegetables. Change in attitude, proper preparation and utilization of amaranth vegetables could help in dealing with the problem of micronutrient malnutrition also known as the hidden hunger.

Even with the vast level of knowledge on the nutritional value of amaranths, more research is still needed on several aspects including; the nutrient differences of local species and varieties and the variation in the nutrients with respect to the environmental conditions; as well as the impact of processing and cooking methods commonly used in Africa on the nutritional value of species so as to suggest food preparation methods that ensure good nutrient retention and mineral bioavailability.

The aim of this research was to determine the morphological and nutritional traits of selected amaranth accessions and test associations between these traits, to determine the effect of drought stress on nutrients, metabolites and on transcription of their selected biosynthesis genes in leaf amaranth accessions. The study also intended to assess consumer awareness, attitudes, and utilization practices of amaranth in Kenya and Tanzania. Another focus of this study was to evaluate the nutrient retention of amaranth vegetable dishes prepared using some traditional recipes, and also to enhance the iron bioavailability of amaranth dishes using food preparation methods, and develop nutritionally enhanced amaranth-based recipes.

1.2 Statement of the Problem

Micronutrient malnutrition (hidden hunger) is a major challenge in Sub-Saharan Africa (SSA) (Biesalski, 2013). According to FAO 2020, the prevalence of undernourishment in SSA and East Africa (EA) was 22% and 27.2% respectively; and this situation is projected to worsen and be at 29.4% and 33.6% prevalence in SSA and EA, respectively, by 2030 (FAO *et al*., 2020)[i]. Children under the age of five years and women of child bearing age appear to be worst hit by malnutrition. In Kenya chronic malnutrition affects 26% of children under five (USAID, 2017). In some areas of Kenya 76% of children have been reported to have been anemic at least at one point since birth (Kao *et al*., 2019). About 27.2% of Kenyan women in reproductive age have anemia (Global Nutrition Report, 2019). This hidden hunger could be linked to the high rates of morbidity and mortality among children and women especially in rural areas. Malnutrition, especially micronutrient deficiencies (e.g., vitamin A and iron), also remains a problem in Tanzania in general. It has been reported that in Tanzania, chronic malnutrition affects about 31.8% of children under five and 10% of women between 15-49 years; while three in every five children, and 28.8% of women are anemic (TDHS, 2016; TNNS, 2018).

Among the major causes of malnutrition in Sub-Saharan Africa is limited dietary diversity (Aboagye *et al*., 2021; Khamis *et al*., 2019; Thompson & Meerman, 2014). High rates of micronutrient malnutrition are also caused by low intake of micronutrient-dense fruits and vegetables. This could be as a result of low availability of these healthy foods, and low access to seeds of AIVs such as amaranth (Croft *et al*., 2018). According to a study carried out in Western Kenya by Musotsi *et al*., (2017), inaccessibility to AIVs and the high market prices are the major constraints to consumption of the vegetables. There is also limited use of genetic diversity coupled with limited research due to underinvestment in the vegetable sector, particularly the traditional vegetables (Ebert, 2020; van Zonneveld *et al*., 2021).

Even with the noticeable increase in the consumption of the indigenous vegetables, micronutrient malnutrition is still a problem especially iron, vitamin A, zinc and iodine, (Kamenwa, 2017). This could be due to low micronutrient bioavailability in the body caused by high anti-nutrient content in the green leafy vegetables such as oxalates in amaranth, which binds to certain mineral nutrients reducing their bioavailability (Onyango *et al*., 2012). Oxalates bind calcium and iron making them unavailable for absorption. High oxalate levels may also interfere with carbohydrate metabolism. Tannins bind to and precipitate proteins and various other organic compounds including amino acids, reducing their bioavailability in the body (Olawoye & Gbadamosi, 2017).

There are many varieties of amaranth utilized which differ significantly in nutrients, anti-nutrients and bioactive compounds (Nyonje *et al*., 2014). Most breeders focus mostly in improving agronomic traits rather than nutritional attributes. Despite phenotypic variations being used to estimate the genetic diversity in many crops such as amaranth, there are no criteria of relating the vegetable morphological characteristics to their contents of (anti)nutrients and bioactive compounds. It is possible that the nutritional components may be associated with some morphological features of the vegetables.

Sub-optimal food preparation methods could also have a negative effect on the bioavailability of the important vegetable components. There are varied preparation methods of these vegetables, some of which lead to loss of the important nutrients (Lee *et al.*, 2018), and the effects of these preparation methods on anti-nutrients, nutrient bioavailability and bio-accessibility is still not clear.

1.3 Justification

A sustainable way to reduce malnutrition is to improve availability, accessibility and utilization of micronutrient-rich foods as part of balanced diets that include fruit and vegetables. African indigenous vegetables including amaranth have a great potential in reducing micronutrient malnutrition. Several studies have shown that African indigenous vegetables such as amaranth are very rich in nutrients as well as phytochemicals which help to reduce prevalence of long-term degenerative diseases (Mampholo *et al*., 2016; Nyonje *et al*., 2014). Such findings could be part of the contributing factors to an increasing trend in consumption of indigenous vegetables such as amaranth among communities in Kenya and the whole of East Africa. The persistent cases of malnutrition even vegetable consumers could be partly due to anti-nutrients present in the vegetables, which reduce the bio-accessibility and bioavailability of important nutrients (Samtiya *et al*., 2020).

Food based approaches including dietary diversification, nutrient retention and nutrition education can be a very effective method of eliminating micronutrient deficiencies. Ochieng' *et al.,* (2017) reported that promotion and demand creation activities of traditional vegetables are important elements in increasing dietary diversity of children under five and women in reproductive age. Programs such as food fortifications are being implemented to deal with micronutrient malnutrition, but combining this with locally available nutrient rich foods such as traditional leafy vegetables can be more effective (Kruger *et al*., 2015). Since these vegetables are rich in nutrients and bioactive compounds, strategies such as provision of recipes that enhance consumption of African indigenous vegetables in adequate amounts could contribute to improved nutrition security and reduced risk of degenerative diseases (Birol *et al*., 2015).

Amaranth displays wide phenotypic diversity, drought stress tolerance as well as very good nutritional quality. There is a wide genotypic variations in amaranth, which could be due to frequent inter-specific and inter-varietal hybridizations (Suresh *et al*., 2014). Proper understanding of the genetic diversity and trait variations in plant populations provides a very useful tool in plant breeding. Existence of high genetic variability in amaranth has also been reported (Gerrano *et al*., 2014), with high significant differences in phenotypic characteristics. Nutritional and morphological analysis of amaranths informs breeders and other concerned scientists on which amaranth accessions/cultivars could be promoted or used in breeding low anti-nutrients and high nutrient (Achigan-Dako *et al*., 2014).

Selection and promotion of high nutrient amaranth varieties as well as complementary cooking methods that destroys anti-nutrients as much as possible and at the same time keeps nutrients and improve micronutrient bioavailability as much as possible are essential to open up the full potential of amaranth to improve dietary quality. Appropriate food preparation and recipes can enhance nutrient retention and reduce anti-nutrient effects.

1.4 Objectives

1.4.1 Overall Objective

To establish the effect of varietal difference, water stress and cooking methods on the nutritional composition, retention and bioavailability in amaranth vegetable.

1.4.2 Specific Objectives

1. To determine the association between phenotypic characteristics and (anti)nutritional attributes of amaranth accessions.

2. To determine the effect of water stress on accumulation of biochemical components of amaranth lines and on expression of some biosynthetic genes.
3. To assess the preparation and utilization methods of amaranth vegetables in Kenya and Tanzania.
4. To determine the effect of food preparation methods on the nutritional quality and iron bioavailability of amaranth vegetable.
5. To develop nutritionally improved recipes that enhance nutrient retention, bioavailability and consumer acceptability.

1.5 Hypotheses

1. Phenotypic characteristics of amaranth are not significantly associated with their nutritional attributes
2. Water deficit stress does not significantly affect nutrient accumulation and expression of biosynthetic genes in amaranth.
3. The preparation and utilization methods of amaranth vegetable does not significantly differ between consumers in Kenya and Tanzania.
4. There is no significant effect of food preparation method on the nutritional quality and iron bioavailability of amaranth dishes.

1.6 Research questions

1. Are morphological characteristics of amaranth associated with nutritional attributes?
2. Does water deficit stress affect nutrient accumulation and expression of biosynthetic genes in amaranth vegetables?
3. What are the preparation and utilization methods of amaranth vegetables in Kenya and Tanzania?
4. How do food preparation methods affect the nutritional quality and iron bioavailability in amaranth vegetables?

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Green leafy vegetables provide vital nutrients required for human health and wellbeing. These include amino acids, vitamins, essential fatty acids, minerals and dietary fiber (Arasaretnam *et al*., 2018; Beaulah *et al*., 2020; Natesh *et al*., 2017; Punchay *et al*., 2020). It also has significant socioeconomic benefits, for instance, farmers in the tropics and subtropics, mostly women, grow and harvest green leafy vegetables to supplement household income. In rural areas, traditional leafy vegetables play an important role as a nutritional source, and are available all-year round. Green leafy vegetables are usually considered the cheapest source of food for vitamins and micronutrients supplementation to combat nutrients deficiencies (Natesh *et al*., 2017).

In Africa, there are several green leafy vegetables with primary or secondary origins being in the continent (Abukutsa Onyango, 2010; Maseko *et al*., 2017; Mnzava *et al*., 1999; Schippers, 2000). These vegetables are collectively referred to as African Indigenous vegetables (AIVs). Some of the most common AIVs in East Africa are amaranth, nightshade, spider-plant, cowpeas among others. Some of the vegetables are also used as herbal and medicinal plants in various cultural and traditional settings for many different ailments (Ishiekwene *et al*., 2019; Kamble & Jadhav, 2013; Mokganya & Tshisikhawe, 2019).

Despite the food diversity in Africa, the continent still experiences the triple burden of malnutrition encompassing undernutrition, overnutrition and micronutrient deficiencies (Ahinkorah *et al*., 2021; Global Nutrition Report, 2019). Further reports show that not a single country in the Africa region is on course to meet targets for anemia in women of reproductive age, low birth weight, diabetes and obesity in men and women (Global Nutrition Report, 2020).

Dietary approaches have been proved to be one of the most effective ways to deal with malnutrition. It entails various strategies as shown in Figure 2.1 below:

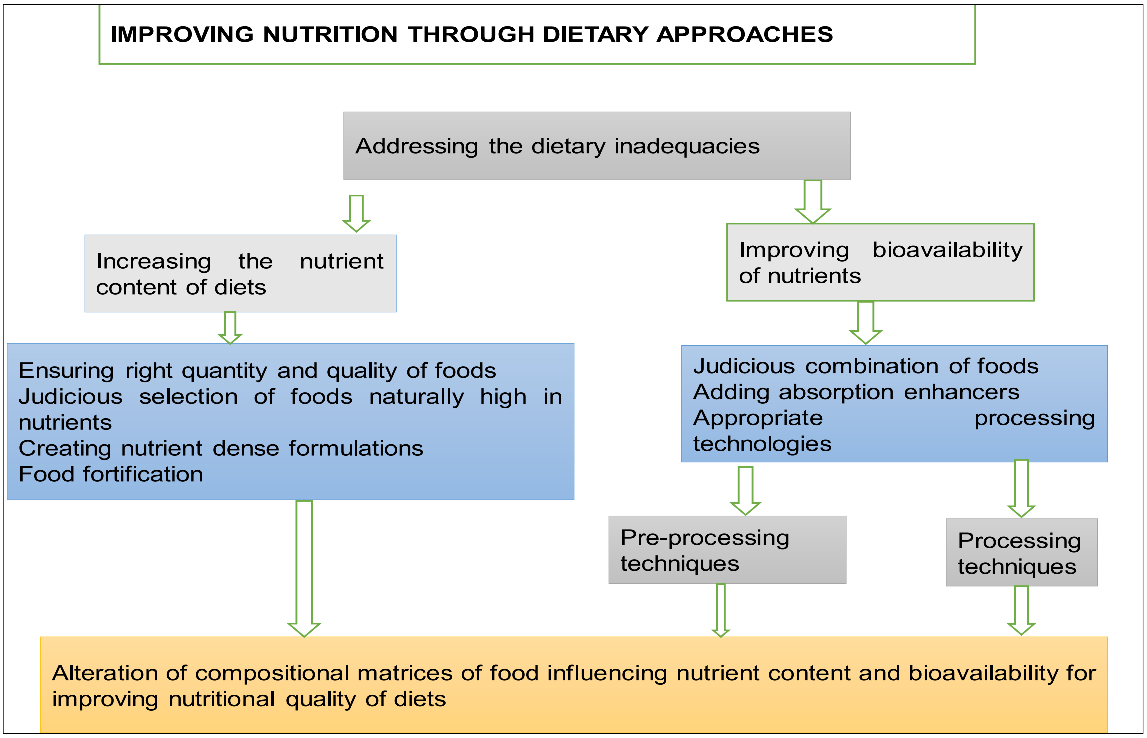

Figure 2.1: Dietary approaches to deal with malnutrition

Source: Prakash (2014)

Leafy vegetables have been reported to be nutrient rich, and therefore present an important component in the fight against malnutrition (Wong, 2017). The presence of anti-nutritional factors such as nitrates, oxalates, phytates and tannins in green leafy vegetables can affect micronutrients absorption and thus, make the latter unavailable (Samtiya *et al.,* 2020). Thermal processing of leafy vegetables through boiling, cooking and blanching before consumption help in reducing the level of anti-nutrients. There is scope for research to include these vegetables, especially amaranths in mainstream agri-food systems, to explore different varieties and possibility of adopting agronomic practices that will reduce the concentration and effect of anti-nutritional factors in green leafy vegetables as well as enhance their nutritive value (Natesh *et al*., 2017).

2.2 Amaranth overview

2.2.1 Introduction

Amaranth (*Amaranthus spp.*) is one of the Africa leafy vegetables from the family *Amaranthaceae*; a "sister" group to the family Chenopodeaceae, which include beets, Swiss chard and spinach, with which they share some nutritional attributes. It is one of the most popular in Africa, as it is the most commonly harvested and consumed African leafy vegetable across the continent (Achigan-Dako *et al*., 2014). It is herbaceous annual crop cultivated for both its leaves which are used as a vegetable and its seeds which are used as a grain. It is also often found growing wild. Amaranth, just as other African Leafy Vegetables (ALV), has been a common food to many African and Kenyan households for a long time. It is also called Tampala, Tassel flower, Flaming fountain plant, Joseph's coat and the pigweed. Some of its vernacular names in Kenya include *Ododo* (Luo), *Mchicha* (Swahili) *Terere* (Kikuyu) *Chimboka* (Luhya), *Ekwala* (Teso), *Lookwa* or *Epespes* (Turkana). Other vernacular names used across Africa include *Tete (*Nigeria*), Hanekam* (Afrikaans), *Thepe* (Sesotho), *Imbuya* (IsiZulu) and *Vowa* (Tshivenda).

There are many varieties of amaranths differing in many different aspects including morphology, nutrient content, seed color etc. It has high genetic diversity as well as phenotypic plasticity (Rastogi & Shukla, 2013). This makes it very well adaptable to a wide range of environmental conditions. Because it undergoes C4-cycle of photosynthesis, amaranth can sustain high photosynthetic activity and water use efficiency under high temperatures and high radiation intensity, making it an ideal crop for abiotic stress conditions under changing climates (Tsutsumi *et al*., 2017). Vegetables, including amaranths, contain nutritional factors which include nutrients and health promoting phytochemicals, as well as anti-nutritional factors which are health inhibiting or toxic phytochemicals (Natesh *et al*., 2017).

2.2.2 Origin and Classification

Amaranth (*Amaranthus spp.*) is believed to have originated in Mesoamerica and South Americas (Ebert *et al*., 2011), but it has spread to other countries such as India, China, Europe, Asia and Africa. It is one of the oldest food crops with evidence of its cultivation showing as early as 6700 BC. According to other studies, many species originated in the Andean region of the South America and are now widely distributed throughout most tropical areas (Adhikary *et al.*, 2020; Das, 2016). In Africa, Nigeria has been claimed as a center of diversity of amaranth, corroborated by the prevalent use of local names and the enormous genetic diversity available there. Amaranth currently consists of very many species and is being produced worldwide for both its grains and vegetables (Achigan-Dako *et al.*, 2014). Current research shows that amaranth is one of the leading priority vegetables in Africa.

2.2.3 Varieties of amaranths

Breeding efforts have developed three types of amaranth: a leaf type, dual type for leaf and grain and a grain type (Hanson, 2016). The most popular amaranth species are *A. hypochondriacus* (L.) which is also a dual-purpose variety – for both grain and leaves, *A. dubius, A. cruentus*, *A. tricolor* (L.), *A. hybridus* (L.), and *A. blitum* (L.). There is however no distinct separation between vegetable and grain type species since even the leaves of young grain type plants can be eaten as leafy vegetables.

The family *Amaranthaceae* has approximately 60 recognized species (Suresh *et al*., 2014), which are consumed as pot-herbs or leafy vegetables worldwide. *Amaranthus* species have been separated into four principal groups: cultivated which are the improved species or cultivars, wild and weedy which are growing in the wild, racial which are based on geographic morphological patterns and landrace or accession which are populations from specific locations (Thapa & Blair, 2018).

2.2.4 Uses of Amaranth vegetables

Amaranth vegetables are mostly used as leafy vegetable. Its tender leaves and stem are used as food in many countries in Africa as infusions, salads, sauces, soups; singly or mixed with other vegetables or legumes (Achigan-Dako *et al*., 2014).

Other studies have shown that amaranth leaves flour can be used as a component of green pasta to improve proteins supply (Namrata *et al*., 2021), in particular, the essential amino acid, lysine content, which is the most deficient in wheat flour. Experiences in the usage of amaranth leaves flour in pasta, in the ratio of 250 g/1000 g of wheat flour, have shown very good performance in pasta manufacturing, cooking quality and taste; so amaranth leaves seem to be successful used in green pasta (Namrata *et al*., 2021).

Apart from its uses as a vegetable, it has also been used as an effective alternative to drug therapy in people with hypertension and cardiovascular disease (CVD) (Martinez-Lopez *et al*., 2020; Nardo *et al*., 2020).

2.3 Nutritional and bioactive components of amaranth

Amaranth vegetables can be considered to be an inexpensive source of minerals and certain vitamins in the diet (Achigan-Dako *et al*., 2014). The vegetable provides an alternative source of many nutrients in developing countries as well as several health promoting bioactive compound (Venskutonis & Kraujalis, 2013). They have been reported to be an excellent source of carotene, iron, calcium, protein and Vitamin C among other nutrients. They have been shown to contain a significant energy value ranging from 27 to 53 kcal/100 g of fresh leaves (Uusiku *et al*., 2010). When compared to spinach, leaves of *Amaranthus* contain 3 times more vitamin C, calcium, iron and niacin. It contains 18 times more beta carotene, 20 times more calcium and 7 times more iron when compared to lettuce.

The nutritional composition of amaranth vegetables have been documented to depend on species, variety, plant age, production techniques, besides other environmental factors (Nyonje *et al*., 2014). According to Makobo *et al.,* (2010), amaranth nutritional values can vary depending on environment, genotype and other adaptations (Makobo *et al*., 2010). The vegetables has also been reported to be rich in lysine and methionine, essential amino acid that are lacking in diets based on cereals and tubers (Beswa *et al*., 2016). Kadoshnikov *et al.,* (2008) reported that the protein content of amaranth vegetables accumulates maximally at the blossoming phase of the plant, and ranges between 17.2- 32.6% on dry weight basis of various samples (Kadoshnikov *et al*., 2008).

Amaranths vegetables also contain phytochemicals including carotenoids, flavonoids, phenolics and ascorbic acid that help protect the body from long-term degenerative diseases (Li *et al*., 2015). The vegetable has been reported to have a high concentration of antioxidant components, and have anti-inflammatory properties (Schröter *et al.*, 2019). Several types of flavonoids including rutin, nicotiflorin and isoquercetin; several phenolic acids have been reported in amaranth as well as betalains which are reported to only occur in certain species (Niveyro *et al.*, 2013). Betalains have been shown to be abundant in the colorful species, *Amaranthus tricolor*, while it is low in the dominantly green species such as A. *hypochondriacus* (Khanam & Oba, 2013).

Aside from the leaves, amaranth seeds also exhibit a valuable mix of nutritional characteristics with higher amounts of proteins and oils compared to conventional cereals such as corn and sorghum (Palavecino *et al*., 2016). The seeds also contain good fatty acid profile with high amounts of oleic acid, which is important for prevention and management of cardiovascular diseases, hypertension, diabetes and other non-communicable diseases (Sánchez-López *et al*., 2020; Tang & Tsao, 2017). Amaranth grain has also been shown to be a good source of protein to those who are gluten sensitive, because unlike the protein found in grains such as wheat and rye, its protein does not contain gluten.

Some anti-nutritional phytochemicals in amaranth such as tannins and phytic acid also exhibit protective effects, thus making them to serve a dual purpose of reducing some essential nutrients and protecting the body against a number of biochemical, physiological and metabolic disorders (Akin-Idowu *et al.*, 2017).

2.4 Anti-nutritional factors in Amaranths

Anti-nutrients are plant phytochemicals which have the ability to bind other nutrients, reducing their bioaccessibility and bioavailability in the body. These undesirable chemical substances referred to as anti-nutrients are abundant in both cultivated and wild plant species (Natesh *et al*., 2017). The quantity and the distribution of these chemical compounds vary with plant genera and species. Being an anti-nutritional factor is not an intrinsic characteristic of a compound but depends on the digestive process of the ingesting animal (Thakur *et al*., 2019). The level of adversity is largely dependent on the diet pattern and method of processing involved before the consumption of the specific plant food. Some of the anti-nutrients commonly found in amaranth are oxalates, nitrates and tannins (Natesh *et al*., 2017).

Like other green vegetables such as spinach, vegetable amaranth is also known to accumulate various anti-nutrients that might bind the nutrients, reducing their bioavailability in the body (Agbaire, 2012). These include phytates, nitrates, tannins and oxalates. Oxalate and phytate are known to chelate mineral elements in the body making them unavailable to the body (Akter *et al*., 2020). In amaranth leaves, the major anti-nutrients of health concern include oxalates and nitrates.

Oxalates: Oxalic acid [(COOH) 2] is the simplest and most acidic of the dicarboxylic organic acids (Penniston, 2014). When in combination with its salts or minerals, it forms oxalates. Oxalic acid and other antinutrients are present in the cell sap of many of the green leafy vegetables (Olusanya *et al*., 2021). Depending on plant species, oxalates can occur as insoluble salts of calcium, magnesium and iron, and soluble salts of potassium and sodium or as a combination of these two forms.

In the body, oxalic acid combines with divalent cations to form crystals of corresponding oxalates. Iron oxalates cause significant oxidative damage and diminish iron stores needed for red blood cell formation (Chukwuebuka and Chinenye, 2015). Insoluble oxalates are excreted in feaces. Whereas, soluble oxalates affect the human body by forming a strong chelate with dietary calcium and other minerals rendering the complex unavailable for absorption and assimilation. This insoluble calcium oxalate in the crystal form is stored in the kidney causing serious health-related problem called kidney stone (Radek & Savage, 2009). The adverse effect of calcium absorption is higher when the ratio of oxalate: calcium is more than 9:4 (Radek & Savage, 2009; Shkembi & Huppertz, 2022). The distribution of oxalic acid is uneven in plants and varies among species. Generally, the amount of oxalate is high in leaves followed by seeds and less in stems (Tuazon-Nartea & Savage, 2013). Hence, consumption of leafy vegetables is more of a concern when there is a risk of high oxalic acid concentration.

Oxalate in various forms is taken up ubiquitously by most vascular plants, which exploit the calcium-binding potential of oxalate primarily to regulate intracellular pH and calcium concentrations but also, as in the case of plants with crystalliferous cells, as a feeding deterrent against certain insects (Penniston, 2014). Plants also synthesize oxalate via oxidation of glycolate and glyoxylate and, in some cases, ascorbic acid (Liu *et al*., 2015).

Processing methods to reduce anti nutrients

Heat treatment is the most effective method to reduce the anti-nutritional factors present in green leafy vegetables so far. Boiling the leaves of amaranths for about 5-10 minutes, then discarding the water has been shown to alleviate both oxalate and nitrate problems. According to Yadav & Sehgal (2003), blanching of green leafy vegetables like amaranth induces moderate losses of 5- 15% of anti-nutrients in vegetables (Yadav & Sehgal, 2003). A study done by Amalraj and Pius (2014) also showed that cooking of vegetables reduces oxalate levels by up to 50%.

Cooking and blanching help in the removal of anti-nutrients through rupturing the plant cell wall followed by leaching out of soluble compounds into the blanching medium (Natesh *et al*., 2017). The levels of phytic acid and oxalic acid can be effectively reduced by cooking and blanching methods. However, this practice could also lead to the leaching out of nutritional elements (Mepba *et al*., 2007). A study conducted on leaves of amaranth *(Amaranthus tricolor)*, bathua *(Chenopodium album)*, fenugreek *(Trigonella foenum grecum)* and spinach *(Spinacia oleracia)* for reducing anti nutrients reported blanching alone between 10 and 15 minutes resulted in significant reduction in phytic acid. Oxalic acid content was reduced by both blanching and cooking whereas drying and storage did not significantly affect anti-nutrition factors in green leafy vegetables (Wakhanu *et al*., 2016).

2.5 Phenotyping of amaranth

Phenotype refers to a set of traits that can be observed by direct inspection, or by use of analytical tools; it can also be described as the interaction between the genotype and the environment (Johannsen, 2014; Walter *et al*., 2015). These interactions affect growth, development, structural and physiological traits of plants and can in turn be associated with accumulation of various components in plants. Changes in plant internal phenotypes also determine external phenotypes of the plant including morphology, biomass and yield (Zhao *et al.*, 2019). There is wide variability in amaranth morphological characteristics with respect to leaf colour, stem color and inflorescence color (Shah, *et al.*, 2018). Several morphological traits such as leaf color have been utilized in diversity analyses of various African indigenous vegetables including amaranth (Costa *et al*., 2013); however, these morphological traits have a disadvantage in that they are limited in number and are also subject to modifications due to environmental conditions. Although some reports on the nutritional attributes of amaranth are available, there is a lot of research that is continuing on breeding, with major focus on agronomic characteristics. This may also affect the nutritional qualities of the new accessions. Existence of high phenotypic variability in amaranth has also been reported (Gerrano *et al*., 2014), with high significant differences in morphological characteristics. The wide phenotypic variations in amaranth could be due to frequent inter-specific and inter-varietal hybridizations (Suresh *et al*., 2014). The current need for improved food quality should amplify the interest in breeding for high nutritious crop cultivars with easily identifiable morphological feature.

Phenotyping is the quantitative description of plant's anatomy, ontogeny, physiology and biochemical properties (Walter *et al*., 2015). Traditional plant phenotyping systems relied on visual scoring of phenotypic characteristics by experts, a process that is very laborious and time consuming and can also generate expert bias as well as experimental bias (Liu *et al*., 2020). Over the past few years, high-throughput phenotyping approaches have been used to measure plant phenotypic traits, but most of these approaches have only been applied under controlled environments (Zhou *et al*., 2019). Some of the phenotyping technologies involve the use of imaging sensors including RGB imaging, spectroscopy imaging, fluorescence imaging, thermal imaging and three dimensional imaging (Li *et al*., 2020; Liu *et al*., 2020). High throughput phenotyping platforms (HTPP) are currently emerging, integrating non-invasive imaging technologies, spectroscopy, image analysis and high-performance computing. Some of these methods can be used under normal field conditions, permits time series measurements and can take several measurements of hundreds of plants per day (Li *et al*., 2020). The high throughput phenotyping refers to sensing and quantifying plant traits rapidly, non-destructively, regularly and with a high precision (Choudhury *et al*., 2019). It involves the characterization of the cascade of changes that occur after gene transcription that leads to formation of proteins. These changes define plant phenotypic traits such as metabolites and morphological or architectural parameters (Walter *et al*., 2015).

Morphological aspects of amaranth such as leaf width and leaf length have been shown to correlate with yield (Dinssa *et al*., 2019). A positive correlation between vegetable yield with leaf width, leaf length and plant height was also reported, with a negative correlation between vegetable yield with the number of branches (Tejaswini *et al*., 2017). Such correlation with nutritional attributes could also help in selection and breeding. Large leaved amaranths may be expected to be more photosynthetically active than small leaved species, hence may be expected to contain higher nutrients. In other studies, increase in biomass production, which is closely related to increased leaf area, has been reported to result in lower photosynthetic rates because of increased shading (Bugbee, 2016). The broad leaved species may as well be expected to have high evapotranspiration, but interestingly has high preference by farmers, especially *A. dubius*, indicating presence of traits for low moisture adaptation (Dinssa *et al*., 2019). Variability in morphological and nutritional characteristics can provide valuable information for amaranth breeders to select and breed cultivars with low anti-nutrients such as oxalate, and high nutrients and anti-oxidant compounds. Correlation between two of more morphological characteristics with nutrients can also provide a suitable means for indirect selection for high nutrients.

2.6 Amaranth and drought stress

Amaranth (*Amaranthus spp.*) has been shown to be one of the most tolerant to abiotic stress of all the African indigenous vegetables. It has high genetic diversity as well as phenotypic plasticity (Rastogi & Shukla, 2013); making it very well adaptable to a wide range of environmental conditions. Because it undergoes C4-cycle of photosynthesis, amaranth can sustain high photosynthetic activity and water use efficiency under high temperatures and high radiation intensity, making it an ideal crop for abiotic stress conditions under changing climates (Wang & Ebert, 2012).

Stress resistance could be defined as the ability of a plant to survive unfavorable factor, and even grow in the presence of that factor (Kulakow & Hauptli, 2018). Drought stress can be defined as moderate loss of water that results in closure of stomata, hence limiting transpiration and gas exchange. This type of stress can largely affect physiological processes in the plant as the plant responds through acclimatization or resistance (Olowolaju *et al.* 2018). Amaranth cultivars exhibit morphological, physiological as well as biochemical changes when subjected to water stress (Sarker & Oba, 2018c). They grow well under heat and drought stress, they are also fairly tolerant to a variety of unfavorable abiotic conditions, including salinity, acidity, or alkalinity, making them well suited to subsistence agriculture (Sogbohossou *et al*., 2015). Some mechanisms that permit amaranth to conserve its supplies under low water conditions include stomatal closure, cuticular barriers to water loss and decrease in transpiring surface. These mechanisms in turn decrease growth as transpiration and photosynthesis rates are reduced (Kulakow & Hauptli, 2018). Amaranth is also able to maintain turgor pressure due to osmotic adjustments during low water potentials, the C4 metabolism however helps to maintain high photosynthetic rates under high temperatures, while inhibiting photorespiration and increasing water use efficiency. The plant also has a tap root system which enables it to reach for water in deeper parts of the soil. Coupled with high nutritional value, the adaptability characteristics makes amaranth a potential crop for significant impacts on the problem of malnutrition (Achigan-Dako *et al*., 2014).

Genetic changes under drought stress

For a better understanding of plant response to drought, an analysis of gene expression changes and secondary metabolites is essential. Genes related to drought stress are expressed differently in order to regulate plant physiological processes during such stress conditions. The biosynthetic pathways of important metabolites in amaranth such as oxalate, vitamin C and beta carotene could be affected, either causing reduction or increase in certain components; hence affecting the nutritional properties of the vegetable. A study on the effect of heat stress in *Amaranthus hybridus* showed that the stress not only affects growth parameters such as biomass, but also negatively affects important phytochemical attributes of the plant (Olowolaju *et al*., 2018).

Biosynthetic pathways of some plant metabolites such as oxalates have not been well explained. But some genes that are responsible for the metabolic processes have been documented (Cai *et al*., 2018). The study by Cai *et. al*., (2018) on spinach also showed that oxalate accumulation in the vegetables is regulated by a complex regulatory mechanism which are varied in different varieties. Biosynthesis of oxalate have been postulated to relate to various pathways, (Figure 2.2) including photorespiration, tri-carbocyclic-acid (TCA) cycle via isocitrate which is converted to glyoxylate by isocitrate lyase; and photosynthetic pathways via ascorbate peroxidase enzyme (APX) and oxaloacetate (Cai *et al*., 2018; Igamberdiev & Eprintsev, 2016).

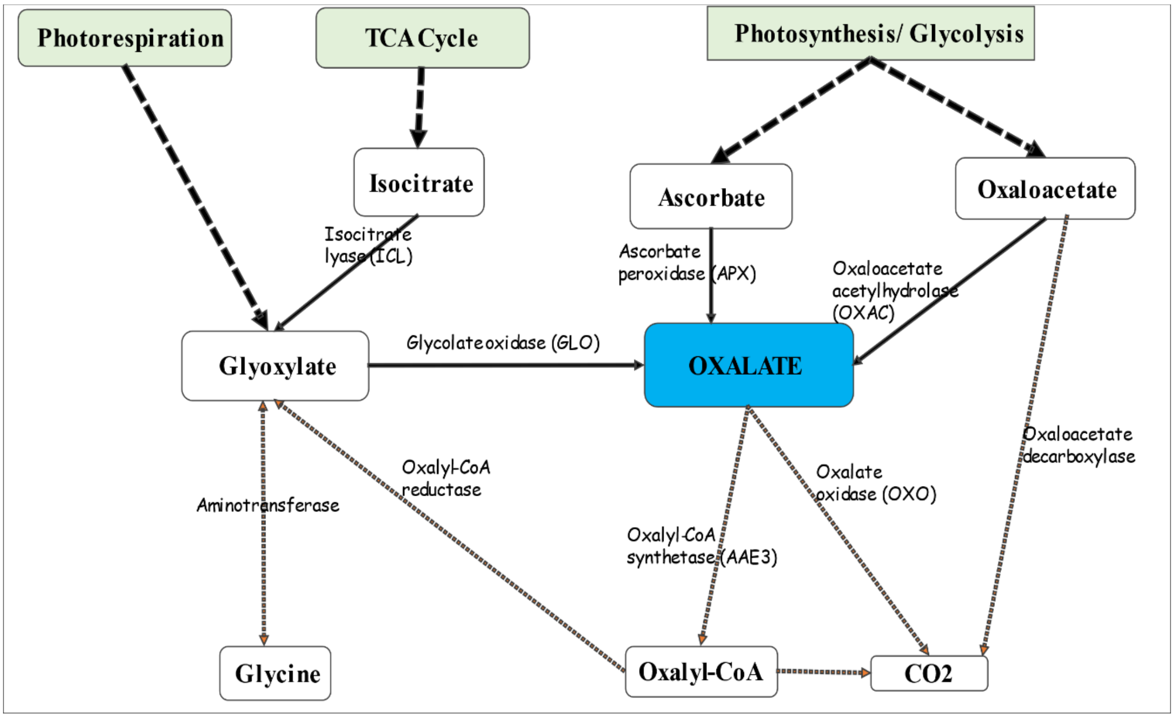

Figure 2.2: Oxalate Biosynthetic and Degradation Pathway

Source: (Cai *et al*., 2018; Igamberdiev & Eprintsev, 2016)

Oxidation of glyoxylate by the enzyme glycolate oxidase (GLO) is the most evident way of oxalate accumulation (Igamberdiev & Eprintsev, 2016; L. Yu *et al*., 2010). The glyoxylate is produced in plants from photorespiration and isocitrate pathway; the latter being the dominant in oxalate biosynthesis (Miyagi *et al*., 2013) as photorespiration rarely occurs in some plants.

Vitamin C biosynthesis takes place through various pathways, however, the L-galactose or the Smirnov-Wheeler pathway is one of the most important pathways for its biosynthesis in plants (Shiri & Zebarjadi, 2018).

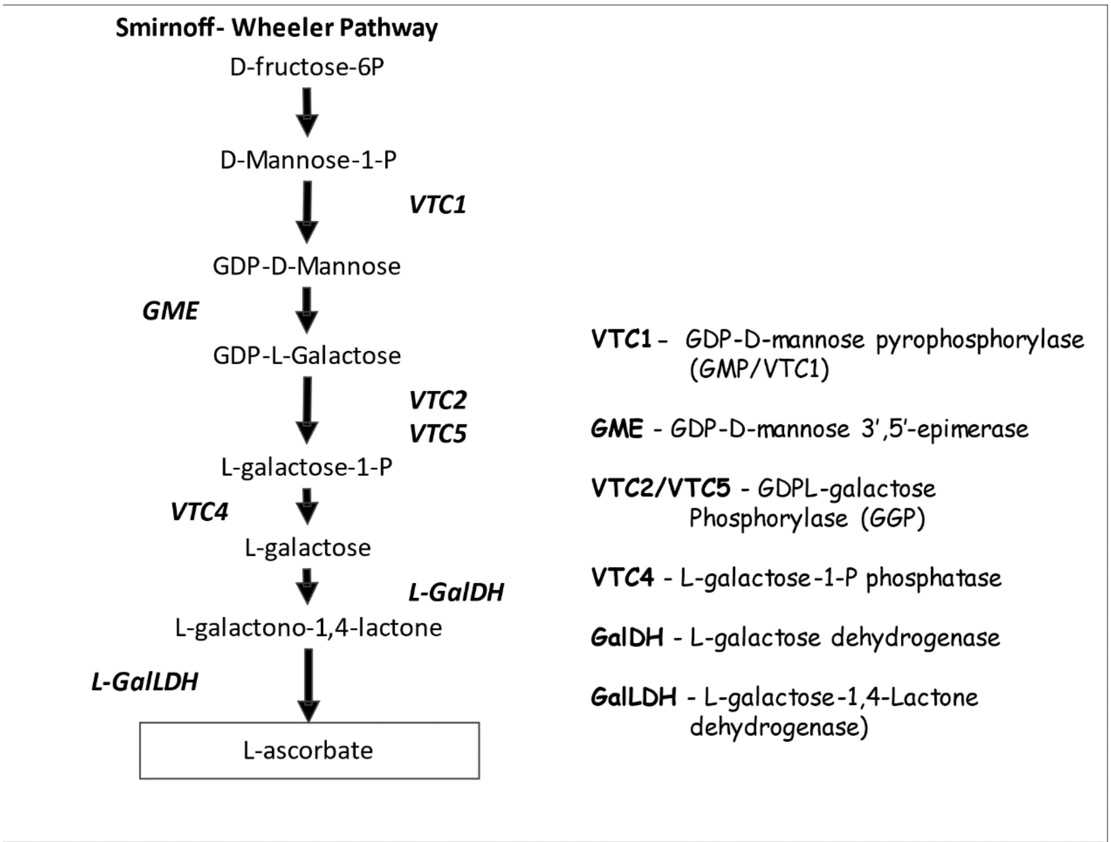

Figure 2.3: Vitamin C pathway (Smirnoff Wheeler pathway/ L-Galactose pathway)

Adapted from: (Seminario *et al*., 2017; Smirnoff, 2018)

The pathway consists of GDP-D-mannose and contains L-galactose arrangement (Figure 2.3). Several transformation, gene expression and mapping studies have suggested that the concentration of ascorbic acid in plants is majorly regulated by enzyme GDP-L-galactose phosphorylase (VTC2/GGP) and GDP-D-mannose 3',5'-

epimerase (GME) (Laing *et al*., 2017; Laing *et al*., 2015; Yoshimura *et al*., 2014). The GGP gene, also known as VTC2, has been found to be a key enzyme in the biosynthetic pathway of L-ascorbic acid as it is responsible for conversion of GDP-L-galactose to L-galactose-1-P in the first step of the Smirnov-Wheeler pathway. The gene has also been used in different plants for increasing vitamin C content (Shiri & Zebarjadi, 2018). Increase in expression of GGP in plants can therefore indicate a significant increase in the concentration of ascorbate in the tissues.

Carotenoids is a group of natural pigments largely produced by plants, algae, phototropic bacteria and some mycetes (Enfissi *et al*., 2017). The biosynthesis of these pigments in green plants take place within the chloroplast, especially in the inner envelope and the thylakoid bilayer. Biosynthesis of carotenoids begins with condensation of two molecules of geranylgeranyl pyrophosphate (GGPP) by the enzyme phytoene synthase (PSY) to form phytoene. This is considered as the rate-limiting step of carotenoid biosynthesis (Sun *et al*., 2018). The phytoene then undergoes sequential dehydrogenation processes yielding lycopene (Enfissi *et al*., 2017). The biosynthetic pathway then begins to branch after lycopene has been formed. Lycopene E cyclase (LCYE) acts on one end leading to the formation of a carotene, while Lycopene b cyclase (LCYB) works on the other end to form b carotene (R. Zhang *et al*., 2021). This makes LCYB emblematic in the formation of beta carotene. They act as accessory pigments to maximize light harvesting efficiency of photosystems (Schweiggert & Carle, 2017); the photo-protectors, shielding against photo-oxidation of the cell constituents and detoxifying the triplet state chlorophyll molecules and the highly reactive oxygen species (ROS) produced by photosynthesis (Hashimoto *et al*., 2016); intermediates in the synthesis of abscisic acid (ABA) and strigolactones (SLs), key phytohormones regulating plant development and environmental stress responses (Al-Babili & Bouwmeester, 2015; Hou *et al*., 2016). Besides their functions in plants, carotenoids are also essential in the human diet owing to their nutrition and health benefits. Apart from being good source of provitamin A, they also possess antioxidant activity, which helps lower the risks of long-term degenerative diseases. Research interests on these pigments has led to the identification and characterization of genes and enzymes involved in their biosynthetic and catabolic core reactions (Nisar *et al*., 2015). Study results point to the fact that the enzymes involved in carotenoid metabolism are encoded by nuclear genes, synthesized in the cytosol, translocated within plastids, and sorted to specific organelle sub-domains depending on plastid type and morphology (Sun *et al*., 2018).

2.7 Bioavailability and Bioaccessibility of Vegetable Nutrients

Bioavailability refers to the ability of the body to digest and absorb the mineral in the food consumed (Fekadu, 2013). Bioavailable portion of food can be defined as a fraction of the compound that is released from the food matrix into the gut, and is available for absorption through the intestinal cells and assimilation into the body cells (Cardoso *et al*., 2015). Bioaccessibility on the other hand is the fraction of a compound that is released from the food matrix in the gastrointestinal tract and thus becomes available for intestinal absorption. It can be affected by the amount of food constituent present in the gut as a consequence of its release from the solid food matrix. One of the main determinants of nutrient bioavailability from a food is the processing it undergoes, as this can have negative or positive effects (Cilla *et al*., 2018).

Factors that affect bioavailability and bioaccessibility include the form in which the nutrient exists in a food, the amounts of the nutrient in the food and presence of other nutrients or non-nutrients which may increase while some may reduce bioavailability (Platel & Srinivasan, 2015). Other factors that determine bioavailability of micronutrients in amaranth include cooking, blanching as well as other additives used during cooking (Olaide *et al*., 2018). The presence of anti-nutritive compounds such as oxalates, nitrates and tannins in amaranth and other dark green leafy vegetables have been reported to reduce micronutrient absorption, whereas other compound like some vitamins and antioxidants enhance absorption of certain minerals (Nomkong *et al*., 2019).

Bioavailability of vegetable iron

Aside from the influence by the food composition and matrix, bioavailability of iron from vegetables is very low and variable (Rodriguez-Ramiro *et al*., 2019). This is also because of the non-heme nature of iron from plant sources. Reports show that iron bioavailability from plant diets range between 5-12% (Hurrell & Egli, 2010).

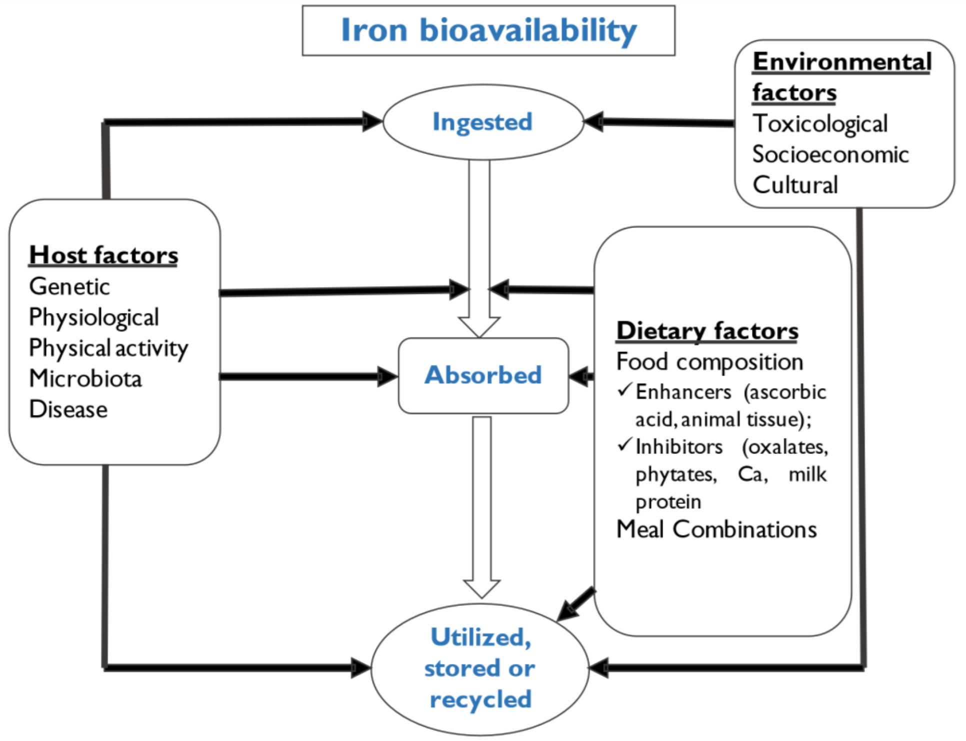

Figure 2.4: Factors affecting iron bioavailability from food.

Adapted from: (Blanco-Rojo & Vaquero, 2019)

The nutritional quality of vegetables in terms of mineral nutrient contents depends on their quantity as well as their bioavailability. Blanching and cooking increases availability of minerals such as iron, calcium and zinc and to some extent also reduces anti-nutrient contents (Achigan-Dako, Sogbohossou and Maundu, 2014). Several studies have shown that amaranth vegetable is a good source of iron, calcium, zinc, potassium and magnesium among others, with appreciable amounts carotenes and vitamin C. The total amount of nutrients in a food does not however reflect the available amounts to the body via absorption as only a certain quantity is bioavailable (Jafari & McClements, 2017). Previous studies have reported that preparing amaranth vegetables with other ingredients rich in lutein, vitamin C, polyphenols and lycopene enhances the stability of beta carotenes in amaranth. Sulphur-compounds-rich Allium species, food acidulants and lime, organic acids, animal products, beta carotene rich vegetables, and pungent spices enhances bioavailability and absorption of micronutrients from foods (Platel & Srinivasan, 2016).

Studies have shown that ascorbic acid is one of the most potent iron bioavailability enhancer, the mechanism of which involves formation of soluble iron-ascorbate complexes which remain soluble in the intestine, as well as reduction of ferric iron (Fe^{3+}) to ferrous (Fe^{2+}) which favors absorption (Blanco-Rojo & Vaquero, 2019).

2.8 Vegetable consumption patterns and food habits in Kenya and Tanzania

African Indigenous vegetables (AIVs) have been consumed in Kenya for a long time, the most common types of these vegetables grown and consumed in Kenya include Amaranth, African nightshade, cowpeas, spider plant, pumpkins, jute mallow and slender leaf (Abukutsa Onyango, 2010). Despite their nutritional, economic, and environmental benefits, the production and consumption of traditional vegetables in Tanzania and other countries in sub-Saharan Africa remains considerably low in most areas due to factors including cultural values, human perceptions, and lack of consumer awareness about their benefits (Afari-Sefa *et al*., 2016; Faber *et al*., 2010).

The consumption of the different types of indigenous vegetables is greatly influenced by cultural backgrounds, hence some types and varieties are only associated with specific communities (Croft *et al*., 2014). The acceptability of the vegetables has been shown to be higher in elderly people majorly due to their experience in cooking, consumption and other utilization of the vegetables. There is also growing consumption in younger people which indicates that there might be other factors which determine preference (Ayanwale *et al*., 2016). A study conducted in Kenya indicated a higher consumption intensity of leafy AIVs in rural dwellers compared to urban dwellers with a mean of four and two times a week, respectively (Gido *et al*., 2017a). A study carried out in Tanzania showed that strong cultural food beliefs and taboos still exist among communities, which strongly influence attitudes towards traditional vegetables (Kansiime *et al*., 2018). They concluded that changing perceptions and food habits may be a slow process because food has important psychological associations with the family and community.

Consumer preferences of different vegetables is also dependent on sensory characteristics including appearance, taste, smell, texture; which is also the most important quality that is considered when making other food choices (Dias *et al*., 2012). A study done by Hiscock *et al.,* (2018) showed significant variations in sensory characteristics between different genotypes of amaranth, though the overall liking of the amaranth vegetables was similar. They however noted that there was no clear connection between specific species and their sensory properties, as genotypes from same species showed both similar and contradictory sensory properties.

Amaranth is mostly utilized as a vegetable, which involve eating of leaves and tender stems in fresh, steamed, stir fried, as soup, stewed or pureed form (Ebert *et al*., 2011). The most commonly consumed Amaranth species in Kenya is *Amaranthus cruentus* (Croft *et al*., 2014), which is generally consumed as a vegetable with *ugali*. The vegetables are either cooked singly or mixed with other vegetables such as spiderplant. Amaranth is believed to "*add blood*" (improves levels of hemoglobin) and therefore very preferred vegetables especially by pregnant women (Kariuki *et al*., 2017).

2.9 Cooking/ preparation of amaranth vegetables

Cooking of vegetables improves edibility, and also induce significant changes in physical characteristics, chemical composition, biological characteristics and bioavailability of vegetable nutritional components. Most vegetables undergo the cooking process prior to consumption on the basis of convenience and taste preferences rather than retention of nutrients and health promoting compounds (Hossain *et al.,* 2017). Cooking of vegetables destroys microorganisms and reduces anti-nutrients thereby increasing the safety; it also enhances digestibility of food and bioavailability of nutrients. The nutritional value and bioavailability of the nutrients may be decreased or increased depending on the cooking method used. Boiling of vegetables has also been reported to allow soluble oxalates to leach into the cooking water, whereas pan-frying needs further consideration as the process may concentrate oxalates in the cooked food (Savage & Klunklin, 2018). Habwe (2012) recommended that the best practices for vegetable preparation methods that preserve micronutrients should involve short cooking times, adding vegetables to boiling water rather than cold water before heating, covering of vegetables while cooking, boiling the vegetables in just enough amount of water, and boiling before frying rather than intensive frying. Additionally, modified preparation methods with enhancing ingredients provide better results in improving iron bioavailability in vegetables than traditional preparation methods (Habwe, 2012).

In Western Kenya, vegetable amaranth is mostly cooked in combination with other vegetables including spider plant and nightshade, and the cooking process involves boiling for about 40 minutes then frying in onions and oil with optional addition of milk or cream (Musotsi *et al*., 2017).

CHAPTER THREE

PHENOTYPIC CHARACTERIZATION AND NUTRIENT ASSOCIATION IN AMARANTH VEGETABLE ACCESSIONS

Summary

Amaranth (*Amaranthus spp.*) is an important leafy vegetable and grain crop which is a good source of nutrients and bioactive compounds. It is widely consumed in several parts of the world. However, varieties of different *Amaranthus* species show great phenotypic variation and may also have different nutritional attributes. This study aimed to phenotype vegetable amaranth accessions of four different species (*Amaranthus hypochondriacus*, *Amaranthus cruentus, Amaranthus dubius* and *Amaranthus blitum*) and relate the morphological phenotypes to the nutritional attributes of the leaves. Ten selected amaranth accessions from the four species were obtained from the World Vegetable Center collections in Arusha and Taiwan were subjected to a high throughput phenotyping system to determine morphological traits including digital biomass, greenness, plant height and hue of the leaves using 3D scanning. These morphological phenotypes were correlated with the nutritional traits including carotenoids, flavonoids, vitamin C, minerals and oxalate contents of the various accessions. The analysis of variance for both morphological and nutritional traits showed significant ($P \leq 0.05$) difference for most traits recorded between the accessions. *A. blitum* (AM1909) had significantly lower values of the daily increases in morphological traits compared to other accessions. Significant correlations were also recorded among the various morphological traits. Amaranth accession AM1908 (*A. dubius*) and AM1910 (*A. dubius*) had the highest content of most nutrients, while AM1902 (*A. hypochondriacus*) and AM1903 (*A. hypochondriacus*) had the lowest nutrient content. Significant correlations were observed between greenness with oxalate and vitamin C contents, as well as between hue values and carotenoids. We conclude that only some of the morphological traits of amaranth are associated with the nutritional content of the leaf. Based on the findings, we recommend that leaf color be used as breeding trait indicator that is associated with some nutritional attributes.

3.1 Introduction

The family *Amaranthaceae* consists of about 70 species (Stetter & Schmid, 2016). Amaranth species are classified into three categories: vegetable amaranth e.g., *A. tricolor*; grain amaranth e.g. *A. hypochondriacus* and *A. cruentus*; and weed amaranth including *A. dubius*, *A. hybridus, A. spinosus, A. viridis*. The classification which is mostly based on the inflorescence features (Das, 2012). These different species have been shown to have great genetic variability and differences in phenotypic characteristics (Gerrano *et al*., 2014), particularly in terms of inflorescence type, leaf color, growth habit, as well as resistance to pests and diseases (Akaneme & Ani, 2013; Erum *et al*., 2012; Kachiguma *et al*., 2015). Features such as leaf area of amaranth varieties also differ due to the different leaf shapes and plant sizes among the species. The wide genotypic variation in amaranth species could be due to frequent inter-specific and inter-varietal hybridizations (Suresh *et al*., 2014). Other morphological traits including plant height and leaf color also differ among species and varieties.

The amaranth vegetables provide a rich and cheap source of nutritional components including protein, dietary fibers, vitamins and mineral that are important in the human diet (Sarker *et al*., 2015b; Sarker *et al*., 2020). Apart from being rich in nutrients, the vegetable also contains several phytochemicals including pigments, phenolic acids and flavonoids having antioxidant activity to protect the human body from long-term degenerative diseases (Sarker *et al*., 2019; Sarker & Oba, 2018e, 2018c). Extracts from almost all plant parts of amaranth seem to have medicinal benefits, probably due to the high antioxidant activity and anti-inflammatory properties (Peter & Gandhi, 2017). Phytochemical analysis of edible parts of various *Amaranthus* species have established the presence of bio-active compounds including alkaloids, flavonoids, glycosides, phenolic acids, steroids, saponins, amino acids, vitamins, minerals, terpenoids, lipids, betalain, catechin, tannins, carotenoids, quercetin, kaempferol, beta carotene, betacyanins and betaxanthins (Sarker & Oba, 2020e). Wide variation in different bioactive compounds has been reported across amaranth varieties (Sarker *et al*., 2019).

The amaranth vegetable exhibited wide adaptability to abiotic stresses like drought (Sarker & Oba, 2018b, 2018c) and salinity (Sarker & Oba, 2020e).

Phenotyping is defined as the process of determining the observable characteristics of an organism that are produced by the interaction of the genotype and the environment, including morphological, developmental or biochemical characteristics (Demidchik *et al*., 2020; Johannsen, 2014; Walter *et al*., 2015). Most traditional phenotyping technologies are labor intensive, subjective, time consuming and destructive to plants (Chen *et al*., 2014).

This study employed non-destructive three-dimensional (3D) laser scanning of plants using a Phenospex field scan device (PlantEye F500, Phenospex, Heerlen, The Netherlands). This is a high-precision with ultra-high throughput phenotyping equipment, fitted with sensors that move on a gantry on top of the plant canopies taking data sets for each plant three times per day. The 3D laser scanning with multispectral imaging capability of the phenospex, captures morphological plant features as well as reflectance in 4 wave lengths (near infrared (NIR), red, blue and green) to generate precise and objective based plant parameters data in real time in a non-destructive manner. Leaf area, angle and inclination, plant size and digital biomass were determined based on the analysis of the 3D data point clouds, obtained for each individual plant, based on the reflectance of NIR laser light. Physiological indices were calculated based on the reflectance of red, green, blue and infrared light (Hatfield & Prueger, 2010). The Phenospex Field Scan device was set up outdoors under field conditions and each data point recorded had a time stamp that linked it to continuously monitored weather conditions.

Phenotypic parameters can in turn be associated with accumulation of various components in plants. We hypothesized that owing to the morphological differences observed between various Amaranth species, variation in nutritional traits may be associated with certain phenotypic characteristics. The associations between morphological characteristics with nutritional traits could provide a simple selection criterion for more nutritious plants for both vegetable producers and consumers. The aim of this study was to determine the morphological and nutritional traits of selected amaranth accessions and test associations between these traits.

3.2 Materials and Methods

3.2.1 Study site and Experimental Design

The experiment was conducted under open field conditions at World Vegetable Center research farm, located in Shanhua, Taiwan (latitude 23°06'49.2" N, longitude 120°17'49.7" E). The experimental site had loamy clay soil. The study involved ten accessions of amaranth from four different species: *A. hypochondriacus, A. cruentus, A. blitum and A. dubius*.

Table 3.1: Amaranth Accessions

Accession No.	Entry Name	Species	Genotype name/No.
AM1901	Abuku-Amar-5	*A hypochondriacus*	AbukuAm5
AM1902	RW-AM-16-ES13-6	*A. hypochondriacus*	RW-AM-16-ES13-6
AM1903	AH-TL-Sel	*A. hypochondriacus*	AVAM1605
AM1904	Abuku-Amar-6	*A. hypochondriacus*	AbukuAm6
AM1905	Madiira 1	*A. cruentus*	EX-Zim (VI060290)
AM1906	SSCFS-002	*A. cruentus*	SSCFS-002
AM1907	Madiira 2	*A. cruentus*	AM-38 (VI060470)
AM1908	UG-AM-23	*A. dubius*	VI062426
AM1909	Blitum-Ke	*A. blitum*	VI050997
AM1910	A-GARE	*A. dubius*	VI050448

The seeds were obtained from World Vegetable Centre offices in Tanzania and Taiwan (Table 3.1).

The seeds were first planted in a tray under greenhouse conditions. Two weeks old seedlings were then transplanted in September – October 2019 into a field fitted with the Phenospex field Scan Equipment Three blocks of 10 plants for each entry were planted in a RCBD design and subjected to Phenospex 3D scanning to measure morphological characteristics over a period of 3 weeks.

3.2.2 Crop management and harvesting

The plants were grown on raised beds with farrows between the beds/rows. No fertilizers were applied to the soil. Furrow irrigation was carried out by applying water along the furrows twice every week. During the growth period, morphological analysis was done. The leaves were harvested 3 weeks after transplanting (5 weeks after sowing) and the nutritional attributes were determined in the laboratory.

3.2.3 Morphological characterization

The morphological phenotypes were evaluated and recorded using a Phenospex Field Scan equipment. The investigated traits included digital biomass of the plants, average greenness of the leaves, height of the plant, hue of the leaves and 3D-leaf area.

The Phenospex Field Scan device was fitted with two set of scanners (PlantEye F500, Phenospex, Heerlen, The Netherlands) which were moved over the field on a gantry. The system generated 3D point clouds of the crop canopy from which several morphological characteristics of the plants were extracted using the Phena software (Phenospex). In this experiment, the equipment was set to take three measurements per day. The equipment functionality is as described by Vadez *et al*., (2015).

The Phenospex Field Scan system included a weather station that continuously monitored environmental conditions such as temperature, relative humidity, light and wind speed.

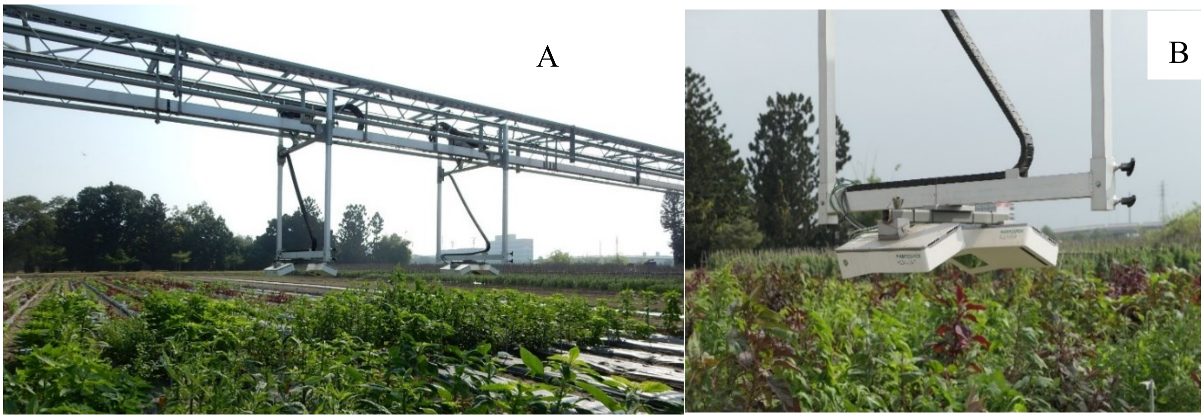

Figure 3.1: Picture of Phenospex. A- field view, B- Pair of phenospex cameras

All the data were accessed, visualized and analyzed through the web-based software interface HortControl (Phenospex).

3.2.4 Nutritional characterization

Leaves for nutritional characterization were harvested 5 weeks after sowing. Nutritional traits of the leaves were determined by chemical analysis for all the samples. The analysis was done for antinutrient including oxalates and nutrients including carotenoids, vitamin C, calcium, iron, zinc and flavonoids in the analytical laboratory using standard protocols. The leaves were first separated (destalked), washed, and then freeze dried. The results of the nutritional components were reported on fresh weight basis. Freeze drying was done to obtain information on the nutrient content as present in the field grown plants for direct comparison with the morphological traits that were recorded in the field.

Reagents

All reagents used were of analytical reagent (AR) grades. The chemicals were purchased from Merck and Sigma–Aldrich Co. (St. Louis, MO, USA).

Determination of oxalate content

Oxalate content of the samples was determined by Libert's HPLC method of oxalate analysis (Libert, 1981) with modifications according to Yu *et al.*, (2010). A 0.5 g fresh weight of sample was homogenized in 4 mL of 0.5N HCL. The homogenate was heated at 80 ^{0}C for 10 minutes with intermittent shaking. To the homogenate, distilled water was added up to a volume of 25 mL. About 3 mL of the solution was withdrawn and centrifuged at 12000 rpm for 10 minutes. About 1 mL of supernatant was passed through a micro filter (0.45 µ) before HPLC analysis. Standards were prepared at varying concentrations for quantification. HPLC analysis was done using Shimadzu UV-VIS detector. Hypsil C18 column (5µ M, 4.6 mm *250 mm) equipped waters 550 was used as static phase and mobile phase was a solution 0.01 N H_2SO_4. Flow rate was 0.6 mL min^{-1}, pressure of 62 kgf and detection wavelength of 221 nm.

Determination of carotenoids

Extraction was done according to Rodriguez (2001). Exactly 0.1 g of freeze-dried sample was weighed into a vial. Then 0.6 mL distilled water and 4.4 mL acetone was added, mixed and shaken for 30 min. The mixture was centrifuged at 12000 rpm for 10 min and 2.0 mL of supernatant transferred to 10 mL test tube. Nitrogen gas was then used to dry the samples at 36 °C. To the dried sample, 100 µL of Tetrahydrofuran (THF) and 1900 µL Methanol (Merck LC grade) was added and mixed. The solution was then filtered through a 0.22 µm membrane and 20 µL injected into HPLC for analysis. Separation and identification of carotenoids was performed using a HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelength between 210 - 700 nm. The static phase was a C 30 Column (YMC^{TM} Carotenoid 3.0 µm, 4.6 mm × 150 mm). The running conditions were set at 30°C using a gradient at 1.3 mL/min from 0% to 1% THF in methanol at 0 - 15 min, 1% to 25% THF in methanol at 15 – 25 min, 25% to 70% THF in methanol at 25 – 50 min, and the final 100% THF at 50 – 60 min. Identification of sample carotenoids was performed by comparing retention time and light absorption spectra (350 nm – 700 nm) of known standards. The peak areas were calibrated against known amounts of standards.

Determination of vitamin C

The vitamin C content was determined by UV spectroscopy based on coupling 2,4-

dinitrophenylhydrazine (DNPH) with ketonic groups of dehydroascorbic acid through the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP), as described by Hanson *et al*., (2004). About 20 grams of blended samples was homogenized with 80 mL of 5% metaphosphoric acid and centrifuged at 7000 rpm for 10 mins. Two mL of the supernatant was transferred into 20 mL test tube followed by addition of 0.1 mL of 0.2% 2,6-DCPIP sodium salt in water, 2 mL of 2% thiourea in 5% metaphosphoric acid and 1 mL of 4% 2,4-DNPH in 9 N H_2SO_4. The mixtures were kept in water bath at 37 ^oC for 3 h followed by an ice bath for 10 min. To the mixtures, 5 mL of 85% sulphuric acid was added and kept at room temperature for 30 min before reading at OD 520 nm. Commercial L- (+)-ascorbic acid was used for calibration.

Determination of minerals

Mineral contents (Ca, Fe, Zn) were analyzed by strong acid digestion followed by atomic absorption spectrophotometry (AAS) (AOAC, 2016). The minerals that were determined are calcium, iron and zinc. A 0.3 g of freeze-dried sample was mixed with 5 mL of 36 N H_2SO_4 in a digestion flask. The flasks were placed in a digester at 300 ^oC for 2-3 hrs. The contents were then cooled to about 150 ^oC and 2 mL of 30% hydrogen peroxide (H_2O_2) added. The tubes were placed in the digester at 300 ^oC for further 30 min till the mixture was transparent. The mixture was then cooled to about 40 ^oC and diluted with 50 mL distilled water. The absorbance of the solutions was read by Atomic Absorption Spectrophotometer (AAS) at their respective wavelengths. The various mineral standards were also prepared to make the calibration curve.

Determination of flavonoids and phenolic Acids

Flavonoids and phenolic acids were determined simultaneously by methanolic extraction, separated by LC-MS and quantified according to Zhang *et al*., (2016). To 0.1 g of freeze-dried sample in a glass vial, 5 mL of extraction solution containing 1% formic acid in 80% methanol was added. The mixture was shaken for 30 minutes then centrifuged at 10000 rpm for 3 minutes. Two mL of the supernatant was mixed with 2 mL of 2.4 M HCL in 20% methanol. This was incubated for 15 minutes then filtered through 0.45 μm membrane. Exactly 30 μm was then auto-injected into HPLC. Separation and identification of flavonoids was performed using a HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelength between 210 - 600 nm. The static phase was Agilent Zorbax ODS (SB-C18) Column, 4.6 x 150 mm, 3.5 μm. The running conditions were set at a temperature of 40 °C using a gradient at 0.7 mL/min flow rate. The mobile phase was 1% formic acid in acetonitrile. Identification of sample flavonoids was done by comparing retention time and light absorption spectra of known standards. Calibration curve was made using peak areas of standards for calculation.

3.2.5 Data Analysis

The morphological and nutritional data were subjected to analysis of variance (ANOVA). The means of the trait values of the amaranth accessions were compared and separated by Duncan's Multiple Range Test (DMRT) using the least significance difference (LSD) at 0.05 probability level. The correlation coefficients were computed using Pearson Correlation coefficients to determine the degree of trait association.

3.3 Results

3.3.1 Morphological traits

Results were analyzed for five morphological traits including digital biomass of the plants per block, average greenness of leaves, plant height, average hue of the leaves and 3D-leaf area of the plants in the block. These traits are considered by vegetable producers as indicators of yield and nutrient richness. Average daily changes in these traits as well as one-point values at the time of harvest are reported in Table 3.2.

Table 3.2: Average daily changes in the morphological characteristics of the amaranth accessions

Accession	Biomass (cm^3)	Greenness Index	Height (mm)	Hue (^{0})	Leaf area (cm^2)
AM1901	109233.89^{c}	0.005^{abc}	30.98^{ab}	0.67^{d}	1052.15^{e}
AM1902	56966.18^{a}	0.003^{ab}	34.99^{abc}	0.24^{b}	620.85^{cd}
AM1903	67160.84^{a}	0.004^{ab}	29.66^{a}	0.30^{b}	526.81^{cd}
AM1904	50619.66^{a}	0.006^{bc}	33.62^{abc}	0.33^{b}	429.75^{bc}
AM1905	56883.66^{a}	0.006^{bc}	53.11^{d}	0.32^{b}	262.91^{b}
AM1906	90252.25^{b}	0.006^{bc}	40.12^{bc}	0.54^{c}	640.31^{d}
AM1907	173344.38^{e}	0.004^{ab}	42.55^{c}	0.36^{b}	1085.43^{e}
AM1908	208206.67^{f}	0.008^{c}	66.81^{e}	0.73^{d}	1701.75^{f}
AM1909	51405.08^{a}	0.002^{a}	30.67^{a}	0.03^{a}	37.95^{a}
AM1910	128461.68^{d}	0.004^{ab}	42.38^{c}	0.70^{d}	936.43^{e}
Mean	99253.43	0.005	40.48	0.42	729.43
LSD	17428.22	0.003	8.48	0.12	192.27

Values are presented as Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD = Least Significant difference at 5% level of significance.*

The daily changes for the digital biomass and leaf area were recorded for the whole block; while those for greenness, plant height and hue represent the average values for each plant. The average daily changes in the morphological characteristics as captured by the Phenospex system for a period of 3 weeks as presented in Table 3.2. The values for all the characteristics were positive, indicating that all the parameters had an increasing trend throughout the growth period. Some accessions did not differ significantly in the daily changes of the morphological traits, especially greenness (Table 3.2).

Differences in the magnitudes of correlation coefficients varied for all the characteristics of the Amaranth accessions (Table 3.3).

Table 3.3: Correlation coefficients of the daily changes in morphological characteristics

	Dig biomass	Greenness Index	Height	Hue	Leaf area
Dig. biomass	1.00				
Greenness Index	0.31	1.00			
Height	0.65*	0.66*	1.00		
Hue	0.68*	0.54*	0.48	1.00	
Leaf area	0.92*	0.42	0.58*	0.80*	1.00

*Values are presented as correlation coefficients, r, at 95% confidence level. * represents significance.*

There was significant ($P \leq 0.05$) correlations between daily increase in traits except for greenness with leaf area and digital biomass; as well as that of height with hue. Highly significant and positive correlation was observed between leaf area and biomass daily increases.

The results in Table 3.4 are the means of the various morphological characteristics as recorded on the day of harvest. There were significant ($P \leq 0.05$) differences among the amaranth accessions in all the morphological characteristics that were measured. The digital biomass of the plants was recorded in cm^3 values, representing the volume of the canopy, not of the plant. Accession AM1907 (*A. cruentus*) had the highest digital biomass per block while AM1909 (*A. blitum*) had the lowest biomass among the accessions that were used in this study (Table 3.4). Among the accessions, AM1908 (*A. dubius*) had the darkest shade of green while AM1909 (*A. blitum*) and AM1905 (*A. cruentus*) had the lightest green leaves. Height of the plant was recorded in mm, measuring from the pot-height (ground level) to the highest tip of the plant. The height of the amaranth accessions ranged between 615 mm in AM1909 (*A. blitum*) to 840 mm in AM1901 (*A. hypochondriacus*). Height differences among some accessions were not significant ($P \leq 0.05$). Accessions AM1903 (*A. hypochondriacus*), AM1904 (*A. hypochondriacus*) and AM1906 (*A. cruentus*) were not significantly different, and this was also the case with AM1905 (*A. cruentus*), AM1907 (*A. cruentus*) and AM1908 (*A. dubius*) (Table 3.4).

Table 3.4: Morphological traits of the 10 amaranth accessions at harvest

Accession	**Digital biomass [cm³]**	**Greenness Index**	**Height [mm]**	**Hue [°]**	**Leaf area [cm²]**
AM1901	1714163.00^{d}	0.27^{c}	840.15^{e}	93.95^{b}	17541.73^{cd}
AM1902	1417940.00^{c}	0.27^{c}	735.09^{c}	91.64^{b}	18436.30^{d}
AM1903	1546600.00^{cd}	0.24^{bc}	670.00^{b}	96.06^{bc}	16574.30^{c}
AM1904	892980.00^{b}	0.23^{b}	657.14^{b}	97.56^{bc}	15086.30^{b}
AM1905	1918080.00^{e}	0.18^{a}	744.47^{cd}	98.92^{c}	23486.69^{f}
AM1906	1550193.33^{cd}	0.22^{ab}	659.93^{b}	94.70^{bc}	21663.23^{e}
AM1907	2629126.67^{g}	0.23^{b}	786.49^{d}	93.15^{b}	32780.81^{i}
AM1908	2400606.67^{f}	0.30^{d}	745.95^{cd}	111.84^{d}	28359.03^{h}
AM1909	415641.00^{a}	0.18^{a}	614.94^{a}	86.81^{a}	10170.81^{a}
AM1910	1990726.67^{e}	0.23^{b}	828.65^{e}	101.55^{c}	24978.00^{g}
LSD (5%)	**189226.00**	**0.04**	**42.00**	**6.70**	**1185.00**

Values are presented as Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* ($P \leq 0.05$) *different. LSD = Least Significant difference at 5% level of significance.*

The hue angle of the leaves was measured as degrees hue, with values ranging from 0 to 360^0 for the different colors of the visible spectrum. Most accessions had statistically similar hue values. However, Accession AM1908 (*A. dubius*) had significantly higher hue value (111.8°, more green), while Accession AM1909 (*A. blitum*) which had a hue of 86.8° (more yellow). The total leaf area was measured by the scanners and presented in cm^2 (Table 3.4). Wide variations of 3D-leaf areas were observed among the genotypes, with the leaf area of Accession AM1907 (*A. cruentus*) – 32781 cm^2, being about three times higher than that of accession AM1909 (*A. blitum*) – 10171 cm^2.

3.3.2 Nutritional traits

The nutritional traits were analyzed and expressed in fresh weight basis. The means from the analysis of variance for the nutritional traits showed significant difference (Table 3.5 and Table 3.6) among the ten amaranth accessions evaluated.

Table 3.5: Oxalate, carotenoids and vitamin C in accessions per 100 g fresh weight of leaves

Accession	Oxalate (mg)	Carotenoids (mg)					Vit c (mg)
		Violax	***Neox.***	***Lutein***	***α-Car***	***β-Car***	
AM1901	421.61^{g}	5.50^{c}	3.85^{cd}	10.64^{bcd}	0.21^{a}	4.40^{cd}	133.53^{ef}
AM1902	393.31^{fg}	4.03^{b}	2.98^{ab}	9.74^{abc}	0.23^{ab}	3.45^{ab}	127.91^{def}
AM1903	280.13^{cd}	3.12^{a}	2.83^{a}	9.19^{a}	0.26^{abcd}	3.54^{ab}	113.32^{cd}
AM1904	327.08^{e}	5.45^{c}	3.70^{cd}	10.72^{cd}	0.32^{d}	4.82^{d}	121.37^{de}
AM1905	308.14^{de}	4.24^{b}	4.21^{d}	10.84^{cd}	0.30^{cd}	3.41^{a}	91.15^{b}
AM1906	205.92^{a}	5.64^{c}	3.52^{bc}	9.58^{abc}	0.28^{bcd}	4.01^{bc}	140.88^{f}
AM1907	269.56^{bcd}	4.24^{b}	2.96^{ab}	9.43^{ab}	0.24^{abc}	3.36^{a}	105.87^{c}
AM1908	369.11^{f}	8.43^{e}	6.20^{f}	15.30^{e}	0.77^{f}	6.63^{e}	127.93^{def}
AM1909	232.57^{ab}	5.49^{c}	3.99^{cd}	11.87^{d}	0.80^{f}	6.41^{e}	69.31^{a}
AM1910	241.09^{abc}	7.03^{d}	4.82^{e}	11.74^{d}	0.57^{e}	4.88^{d}	127.02^{def}
LSD (5%)	39.52	0.78	0.55	1.15	0.07	0.55	14.20

Values represent Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD = Least Significant difference at 5% level. Violax – violaxanthis, Neox – neoxanthin, α-Car – alpha carotene, β-Car – beta carotene*

The oxalate contents ranged between 205 mg/100 g in accession AM1906 (*A. cruentus*) and 421 mg/100 g in AM1901 (*A. hypochondriacus*). Among the accessions with low oxalate levels were AM1909 (*A. blitum*) and AM1910 (*A. dubius*). Five predominant carotenoids were determined in all the accessions including; violaxanthin, neoxanthin, lutein, alpha-carotenes and beta-carotene. Alpha-carotene was detected in lowest amounts compared to other carotenoids, ranging between 0.21 – 0.80 mg/100 g, while lutein amounts were comparably high at 9.2-15.3 mg/100 g. Accession AM1908 (*A. dubius*) showed relatively high content of most carotenoids while AM1903 (*A. hypochondriacus*) showed comparably lower amounts of all the carotenoids. The vitamin C content of the leaves ranged from a low of 69.3 mg/100 g to a high of 140.8 mg/100 g in AM1909 (*A. blitum*) and AM1906 (*A. cruentus*), respectively.

Table 3.6: Mineral, phenolic acids and flavonoid levels in the accessions per 100 g fresh weight of leaves

Accession	**Calcium (mg)**	**Iron (mg)**	**Zinc (mg)**	**Phenolic acids (mg)**		**Flavonoids (mg)**	
				Chlorogenic	***Caffeic***	***Quercetin***	***Kaempf***
AM1901	410.39^{bc}	3.72^{bc}	0.47^{ab}	6.26^{cd}	7.64^{de}	27.55^{ef}	2.37^{cde}
AM1902	378.30^{ab}	3.37^{ab}	0.41^{a}	8.90^{ef}	7.31^{cd}	29.87^{fg}	3.47^{g}
AM1903	335.85^{a}	4.06^{c}	0.46^{a}	8.26^{e}	5.72^{a}	22.03^{c}	3.28^{fg}
AM1904	474.33^{cd}	4.02^{c}	0.68^{c}	10.01^{g}	9.36^{f}	31.87^{g}	2.12^{bc}
AM1905	373.09^{ab}	3.99^{c}	0.47^{ab}	6.80^{d}	7.01^{cd}	38.86^{h}	2.41^{de}
AM1906	424.31^{bc}	3.78^{bc}	0.68^{c}	9.56^{fg}	9.46^{f}	18.92^{b}	2.55^{e}
AM1907	424.07^{bc}	3.29^{ab}	0.47^{ab}	5.53^{c}	6.80^{bcd}	39.02^{h}	3.13^{f}
AM1908	555.50^{e}	4.05^{c}	0.81^{d}	1.08^{a}	8.25^{e}	23.68^{cd}	1.94^{b}
AM1909	513.91^{de}	3.11^{a}	0.57^{b}	4.66^{b}	6.04^{ab}	6.48^{a}	0.87^{a}
AM1910	544.65^{e}	3.20^{a}	0.67^{c}	0.80^{a}	6.61^{bc}	26.10^{de}	2.19^{bcd}
LSD (5%)	**59.30**	**0.47**	**0.09**	**0.81**	**0.82**	**2.85**	**0.26**

Values represent Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD = Least Significant difference at 5% level.*

The highest content of calcium, iron and zinc were detected in Accession AM1908 (*A. dubius*). The amount of calcium in this accession was however not significantly different from AM1909 (*A. blitum*) and AM1910 (*A. dubius*). Accessions AM1902 (*A.*

hypochondriacus) and AM1903 (*A. hypochondriacus*) contained significantly lower calcium and zinc, while AM1909 (*A. blitum*) and AM1910 (*A. dubius*) was significantly low in iron contents (Table 3.6). Two phenolic acids; chlorogenic acid, caffeic acid; and two flavonoids; quercetin and kaempferol; were detected, with significant ($P \leq 0.05$) differences in their contents among the ten amaranth accessions. Accession AM1908 (*A. dubius*) and AM1910 (*A. dubius*) had notably low contents of chlorogenic acid of 1.08 mg/100 g and 0.80 mg/100 g respectively compared to accession AM1904 (*A. hypochondriacus*) which had 10.01 mg/100 g (Table 3.6).

3.3.3 Association between morphological and Nutritional traits

All correlation coefficients were derived between the morphological traits at harvest (Table 3.4) and nutritional traits analyzed (Table 3.5 and Table 3.6). Six traits showed significant ($P \leq 0.05$) correlations (Table 3.7).

A positive and significant ($P \leq 0.05$) association was observed between hue and three carotenoids; violaxanthin ($r = 0.71$), neonxanthin ($r = 0.77$) and lutein ($r = 0.71$) as well as hue with zinc content of leaves ($r = 0.71$). Greenness of leaves was also positively and significantly associated with the amounts of oxalates ($r = 0.70$) and vitamin C ($r = 0.70$). Moderate associations were also observed in other traits including digital biomass with chlorogenic acid, quercetin and kaempferol; plant height with oxalate, chlorogenic acid and quercetin; hue with β-carotene, vitamin C, calcium, iron and chlorogenic acid; leaf area with quercetin and chlorogenic acid (Table 3.7).

Table 3.7: Correlation coefficients between morphological traits and nutritional traits

	Digital Biomass	Greenness	Height	Hue	Leaf Area
Oxalates	0.15	0.70*	0.43	0.22	-0.03
Violaxanthin	0.19	0.37	0.22	0.71*	0.26
Neoxanthin	0.27	0.28	0.23	0.77*	0.29
Lutein	0.15	0.34	0.11	0.71*	0.18
α-Carotene	-0.17	-0.05	-0.21	0.35	-0.07
β-Carotene	-0.26	0.15	-0.21	0.39	-0.20
Vitamin C	0.36	0.70*	0.38	0.48	0.27
Calcium	-0.01	0.12	0.06	0.49	0.14
Iron	0.12	0.24	-0.18	0.48	0.00
Zinc	0.04	0.19	-0.17	0.71*	0.17
Chlorogenic Acid	-0.43	-0.19	-0.48	-0.54	-0.43
Caffeic Acid	-0.02	0.27	-0.12	0.31	0.08
Quercetin	0.60	0.13	0.55	0.15	0.59
Kaempferol	0.46	0.34	0.29	-0.04	0.35

*Values are presented as correlation coefficients, r, at 95% confidence level. * represents significance.*

3.4 Discussion

Nutritional phenotyping of plants can be very useful in selection of nutrient rich genotypes, and this can be advantageous to both breeders and consumers. Across continents, amaranth species have evolved with distinct genotypic and phenotypic characteristics, displaying variation in morphology, nutrients and bioactive compounds. The results on components of amaranth accessions in this study corroborate the results reported for green (Sarker, Hossain, & Oba, 2020) and red morph *Amaranthus* leafy vegetables (Sarker & Oba, 2019c). The analysis of variance for both morphological and nutritional traits showed highly significant differences among the accessions, indicating the wide genotypic and phenotypic variability between and within amaranth species, and varieties. The morpho-nutritional and bioactive compounds in green amaranth (Rashad & Sarker, 2020) and *A. hypochondriacus* (Sarker, 2020a) were reported to have significant differences. The accessions used in this study were all breeding lines, and even though some are of species considered as grain amaranths, such as *A. hypochondriacus* and *A. cruentus*; they are actually multipurpose accessions as their leaves are also consumed as vegetables. Other species used were *A. dubius* and *A. blitum*, which are among the most commonly vegetable species consumed in East Africa (Ochieng *et al*., 2019).

3.4.1 Morphological traits

The daily changes of yield characteristics such as biomass, height and leaf area can be affected by genotype and plant nutrition status. These characteristics captured by Phenospex scanners could also be affected by environmental factors such as wind speed. Under outdoor environment, high wind speed could cause slight bending of plants and this could lead to variations in height measurements. The amaranth accessions in this study were grown under the same conditions. Therefore, differences in morphological traits witnessed in this study imply that differences in genetic makeup was responsible for differences in growth speed, rates of nutrient uptake and metabolism. This could also be due to difference in root traits and hydraulic characteristics (Vadez, 2014). Plant height has been shown to correlate with plant biomass in other crops such as wheat (Schirrmann *et al*., 2016); and can also be used to estimate growth rate of plants (Zhou *et al*., 2019). Leaf color is regarded as the immediate indicator of plant performance. The greenness of leaves in a specific environment is determined by genotype-specific properties such as chlorophyll content, plant health, age and leaf morphological characteristics such as thickness and surface structure (Walter *et al*., 2015). Other factors such as plant nutrition and environmental stresses may also affect the greenness of leaves. Analysis of leaf color characteristics such greenness and hue in the present study could also be affected by environmental factors, especially light. Literature has shown that amaranth redness/greenness (a*) and chroma are affected by environmental factors, such as drought (Sarker & Oba, 2018d), salinity (Sarker & Oba, 2018f). Monitoring color changes of plant leaves can be useful in assessing plant nutrition status.

Among the 10 accessions evaluated in this study, accessions AM1908 (*A. dubius*) and AM1910 (*A. dubius*) had the highest ranking in most of the morphological traits. These two accessions are from same species and share many physical characteristics, except for visible purple coloration on the leaves of AM1908. Accession AM1909 (*A. blitum*) recorded low values in all the morphological characteristics. This is because it is determinate, low branching, has pale green small leaves and has a short/medium height. Yield associated traits such as leaf size, leaf color type and intensity of greenness are useful in selection for breeding, as enhancements of these traits also improves marketability and consumer preference (Dinssa *et al*., 2019). Biomass increment over time is one of the most important morphological parameter determining crop performance and yield (Pandey *et al*., 2018). Both plant height and leaf area determine vegetable yield (Tejaswini *et al*., 2017). Accessions AM1907, AM1908 and AM1910 had significantly higher leaf area values, showing that they had larger leaves or have vigorous vegetative growth, or both. These specific accessions also had significantly higher digital biomasses in the respective order, and can therefore be regarded as those accessions with the highest vegetable yields of the ten accessions studied. The high foliage yield was obtained from vegetable amaranth (Sarker, Islam, & Oba, 2018). This finding is also in agreement with the report by Dinssa *et al*. (2020) that AM38/Madiira 2 (AM1907) has high leaf yield (Dinssa *et al*., 2020). Broader vegetable leaves can also be expected to increase light interception, which could result in increased production of photosynthates.

All the amaranth accessions examined in this study primarily had green leaves, in comparison with other varieties with purple to red leaf colors, mostly from the *tricolor* species. The results, however, showed that the intensity of the green color of the leaves was different between the amaranth accessions, which is likely associated with differences in chlorophyll content in the leaves. The greenness of the leaves is one of the preference traits considered by most farmers and consumers (Oduwaye *et al.*, 2019). In this study, the hue angle values ranged between 86^0-111^0, which all fall under the green range of the visible color spectrum, where 86^0 is nearer to yellow, and 111^0 nearer to dark green. The high hue angle of AM1908 (*A. dubius*) is probably due to the presence of purplish coloration in the leaf veins. Hue is a qualitative attribute of color related to traditional expressions and is used to define color differences with reference to grey color with the same lightness (Comert *et al.*, 2020).

3.4.2 Nutritional traits

Indigenous leafy vegetables are an important source of essential vitamins, minerals and bioactive compounds, while they are often low in calories, fats and sugars (Sarker, Oba, *et al.*, 2020; Sarker & Oba, 2019b, 2020c). In this study, concentrations of the various nutritional compounds were found to be largely influenced by the variety/accession (Sarker *et al.*, 2016, 2017; Sarker *et al.*, 2018b). Amaranth is one of the vegetables reported to contain relatively high amounts of the antinutrient oxalate. However, the oxalate levels found in 100 g of leaves in this study (205.9 - 421.6 mg) are at least 10 times lower than the threshold that is immediately dangerous to life or health (IDLH) of 5 grams per day (CDC, 1994). The major negative effect of oxalate is binding of divalent cations such as calcium and iron and forming insoluble complexes, hence reducing the absorption and bioavailability of these minerals. High levels of vitamin C of up to 140.8 mg/100 g were detected in this study. The high vitamin C (184.77 mg/100 g) was also previously obtained from vegetable amaranth VA14 (Sarker *et al.*, 2018a). The vitamin C, however, may not be accessible in similar levels during consumption as most of it is lost during post-harvest handling processes and cooking. Vitamin C and oxalate values were similar to those reported by Tejaswini *et al*., (2017) of 67-172 mg/100 g and 251-656 mg/100 g of vitamin and oxalate respectively, in genotypes of *A. tricolor*. Five carotenoids were identified in all the amaranth accessions in this study, and their concentrations were comparable with an earlier report that green leafy vegetables contain lutein, beta carotene, neoxanthin, violaxanthin and alpha carotenes in decreasing order of abundance (Yuan *et al*., 2015). Besides their functions in plants as accessory pigments and as photoprotectors, carotenoids are also essential in the human body. Apart from being good sources of provitamin A, they also possess antioxidant activity (Sarker & Oba, 2020a), which helps lower the risks of long-term degenerative diseases (Birol *et al*., 2015). Other studies have shown that beta carotene and lutein play an important role in eye health and can be used as supplements to reduce the risk of glaucoma and age related macular degeneration (Chew *et al*., 2013). Carotenoid content and profiles are therefore one of the quality traits of plants, especially vegetables that may have direct influence on crop productivity, nutritional quality and health promoting properties (Yuan *et al*., 2015). Mineral contents of the various amaranth accessions differed significantly, indicating different capacities of absorption and accumulation of the minerals by the various accessions growing in similar conditions. The present findings corroborate the results reported for amaranth vegetable (Sarker *et al*., 2015a) and *A. lividus* (Chakrabarty *et al*., 2018). Accession AM1908 (*A. dubius*) contained high levels of all the minerals determined, that is, calcium iron and zinc. Quercetin was the most abundant of the two flavonoids that were detected, while kaempferol was the least in terms of abundance. Similarly, 3 flavonoids were reported in the leaves of *A. tricolor* (Sarker & Oba, 2018a), 9 flavonoids in drought-tolerant vegetable amaranth (Sarker, 2020b; Sarker & Oba, 2020b), salt-tolerant vegetable amaranth (Sarker *et al*., 2020) and *A gangeticus* (Sarker & Oba, 2020d). Accession AM1904 (*A. hypochondriacus*) and AM1907 (*A. cruentus*) were notably high in the flavonoids; while AM1909 (*A. blitum*) was the lowest in content of flavonoids. The flavonoid values in this study were higher than those reported for the same flavonoids in red color *A. tricolor* and green *A. lividus* by Sarker and Oba (2019), who also reported a high correlation between flavonoids and antioxidant activity (Sarker & Oba, 2019a). Two phenolic acids (chlorogenic acid and caffeic acid) were detected in the samples, with a relatively similar abundance. Accessions AM1904 and AM1906 had the highest levels of the two phenolic acids while AM1908 and AM1910 had the lowest amounts. Similarly, abundant chlorogenic acid and caffeic acid were also reported in *Amaranthus* leafy vegetables (Sarker & Oba, 2018d). From the nutritional contents of the ten amaranth accessions, AM1908 had comparably high amounts of nutrients. However, besides being rich in most of the nutrients and bioactive compounds, it was, however, among the ones with the highest levels of anti-nutrient, oxalate.

3.4.3 Association between morphological and nutritional traits

Selection of vegetables based on physical characteristics is a popular practice used by both farmers and consumers. Farmers mostly consider yield associated characteristics such as biomass, plant height and leaf area as desirable traits (Ndinya *et al*., 2020), while consumers mostly consider appearance in terms of freshness, and leaf color, which is also an important attribute as this is believed to be tied to the nutrient content. Oxalate and vitamin C content in amaranth are reported to be positively correlated (Wang *et al*., 2018), and this study showed that the concentration of both compounds is associated with leaf greenness. The level of greenness has also been shown to affect sensory attributes in vegetables. A study by Adeka *et al*., (2019) reported that greener color of the leaf blade is negatively correlated with bitterness in spider plant (*Gynandropsis gynandra*), which could mean that the greenness of the leaves is also related to other compounds which were not determined in this work. Greenness of most plant leaves relates to the nitrogen content, and the nitrates have been shown to have some association with oxalate contents (Solberg *et al*., 2015). There is a close relation between the oxalate pathway and the ascorbic acid pathway. High levels of carotenoids, calcium and iron have also been reported in dark green accessions of amaranth compared to light green accessions (Dinssa *et al*., 2018). This study also found significant associations between hue with carotenoids including violaxanthin, neoxanthin and lutein ($P \leq 0.05$). These three are also some of the most abundant carotenoids in green leafy vegetables. Hue of the leaves of plants can be attributed to the color compound which may be present in the leaves, such as the carotenoids, betalains and anthocyanins. Zinc also showed positive correlation with hue values showing that accumulation of color compound may be related to mineral accumulation. Currently, high micronutrient contents and dark green leaf color are some of the priority traits breeders are working on in amaranth; as these were among the traits found to be most considered by seed companies in Kenya and Tanzania (Ochieng *et al*., 2019).

3.5 Conclusion

The ten amaranth accessions analyzed in this study were rich in nutrients, regardless of the species. Though some species were bred specifically for grain production, i.e., *A. hypochondriacus* and *A. cruentus*, their leaves can still be used as nutritious vegetables. The study showed that morphological traits differ among amaranth accessions, leading to differences in yield. Nutritional differences were also observed, with AM1908 (*A. dubius),* generally showing higher nutrient contents than other accessions. Significant correlations were observed between greenness with oxalate and vitamin C contents, as well as between hue and carotenoids, while other morphological contents were not significantly related to the studied nutritional components. Color of the leaves can therefore be used as a breeding trait indicator of association with some nutritional attributes.

CHAPTER FOUR

NUTRIENT ACCUMULATION AND TRANSCRIPTION OF SELECTED BIOSYNTHESIS GENES IN RESPONSE TO WATER STRESS IN LEAF AMARANTH (*AMARANTHUS SPP)*

Summary

Amaranth is a hardy leafy vegetable, with most of its species able to grow wildly under harsh environmental conditions. It is an important vegetable among many African communities, including those in East Africa. However, whether and how water stress affects its nutritional profile is still unclear. The objective of this study was therefore to determine the effect of water deficit stress on accumulation of selected nutrients, metabolites and on transcription of their specific biosynthesis genes in leaf amaranth. Ten accessions of amaranth were grown in a greenhouse at temperatures between 25°C to 27°C. Half of the plants were subjected to drought stress conditions from the third week after transplanting, while the other half were watered normally as a control for three days. Putative genes involved in the biosynthetic pathways of oxalates, vitamin C and carotenoids were identified and their expression levels compared under well-watered and water deficit stress conditions in the amaranth plants. Nutritional analysis of the leaves was also done for oxalates, vitamin C, carotenoids, flavonoids, phenolic acids, calcium, iron and zinc. There was no significant ($P \leq 0.05$) change in calcium, iron and zinc content due to the stress, while biochemical components significantly ($P \leq 0.05$) increased. Correlation analysis showed low correlation coefficients between expression of most genes and the concentration of respective biochemical components. However, GDP-L-galactose phosphorylase (GGP/VTC2) gene was up-regulated in all the accessions and showed significant association with vitamin C content. There was also significant correlation between lycopene beta cyclase (LCY) gene with total carotenoids. All genes of same pathways showed significant correlations, and the gene-gene vs associated components showed significant partial correlations. In conclusion, water deficit stress increased the accumulation of

carotenoids, flavonoids and phenolic acids as well as vitamin C. It also resulted in changes in gene expression patterns. While a single gene in a pathway did not strongly affect a particular component, association between several genes affects the synthesis pathway.

4.1 Introduction

Amaranth vegetable (*Amaranthus spp.*) is one of the African leafy vegetables from the family *Amaranthaceae*. Amaranth shares some phenotypic traits with other plants of the sub-family, *Chenopodeaceae*, which include beets, Swiss chard and spinach. The vegetable has been shown to be one of the most tolerant to abiotic stress among the African indigenous vegetables. It has high genetic diversity as well as phenotypic plasticity (Rastogi & Shukla, 2013), and well adapted to a wide range of environmental conditions. Because it undergoes C4-cycle of photosynthesis, amaranth can sustain high photosynthetic activity and water use efficiency under high temperatures and high radiation intensity, making it an ideal crop that can withstand abiotic stress conditions under climate change (Kulakow & Hauptli, 2018).

Drought stress can be defined as moderate loss of water that results in closure of stomata, hence limiting transpiration and gas exchange. In severe cases, drought stress can lead to disruption in biosynthesis of metabolites, photosynthesis and cell membrane stability, which may be lethal to the plant (Hussain *et al*., 2019). This type of stress can largely affect physiological processes in the plant as the plant responds through acclimatization or resistance (Olowolaju *et al*., 2018). Amaranth cultivars exhibit morphological, physiological as well as biochemical changes when subjected to water stress (Sarker & Oba, 2018c). They grow well under heat and drought stress, and are also fairly tolerant to a variety of unfavorable abiotic conditions, including salinity, acidity, or alkalinity, making them well suited to subsistence agriculture (Sogbohossou *et al*., 2015). The plant also has a tap root system which enables it to reach for water in deeper parts of the soil. Coupled with high nutritional value, the adaptability characteristics makes amaranth a potential crop for significant impact on the problem of malnutrition (Achigan-Dako *et al*., 2014). Studies on transcriptional factors that relate to biosynthetic genes may help in better understanding of the regulatory machinery that influences metabolite accumulation in plants (Ye *et al*., 2015). Genes related to drought stress are expressed differently in order to regulate plant physiological processes during such stress conditions. The biosynthetic pathways of important metabolites in amaranth such as oxalate, vitamin C and beta carotene could be affected, either causing reduction or increase in certain components; hence affecting the nutritional properties of the vegetable. A study on the effect of heat stress in *Amaranthus hybridus* showed that the stress not only affects growth parameters such as biomass, but also negatively affects important phytochemical attributes of the plant (Olowolaju *et al*., 2018).

Biosynthetic pathways of some plant metabolites such as oxalates have not been well explained. However, some genes that are responsible for the metabolic processes have been documented (Cai *et al*., 2018). The study by Cai *et. al*., (2018) on spinach also showed that oxalate accumulation in the vegetables is regulated by a complex regulatory mechanism which varies in different varieties. Biosynthesis of oxalate has been postulated to relate to various pathways including photorespiration, tri-carbocyclic-acid (TCA) cycle via isocitrate which is converted to glyoxylate by isocitrate lyase. Third is through photosynthetic pathways via ascorbate peroxidase enzyme (APX) and oxaloacetate (Cai *et al*., 2018; Igamberdiev & Eprintsev, 2016). Oxidation of glyoxylate by the enzyme glycolate oxidase (GLO) has been reported as evidence of oxalate accumulation (Igamberdiev & Eprintsev, 2016; Yu *et al*., 2010). The glyoxylate is produced in plants from photorespiration and isocitrate pathways; the latter being the dominant in oxalate biosynthesis (Miyagi *et al*., 2013) as photorespiration is reduced in C4 plants and C3-C4 intermediates.

Vitamin C biosynthesis takes place through various pathways. However, the L-galactose or the Smirnov-Wheeler pathway is the most important pathways for ascorbate biosynthesis in plants (Shiri & Zebarjadi, 2018). The pathway consists of Guanosine diphosphate (GDP)-D-mannose and contains L-galactose arrangement. Several transformation, gene expression and mapping studies have suggested that the concentration of ascorbic acid in plants is majorly regulated by enzyme GDP-L-galactose phosphorylase/ Vitamin C defective 2 (GGP/VTC2) and GDP-D-mannose 3',5'-epimerase (GME) (Laing *et al*., 2017; Laing *et al*., 2015; Yoshimura *et al*., 2014). The GGP gene, also known as VTC2, has been found to be a key enzyme in the biosynthetic pathway of L-ascorbic acid. It is responsible for conversion of GDP-L-galactose to L-galactose-1-P in the first step of the Smirnov-Wheeler pathway. It is also used in different plants for increasing vitamin C content (Shiri & Zebarjadi, 2018). Increase in expression of GGP in plants can therefore indicate a significant increase in the concentration of ascorbate in tissues.

Carotenoids are a group of natural pigments largely produced by plants, algae, phototropic bacteria and some mycetes (Enfissi *et al*., 2017). Biosynthesis of carotenoids begins with condensation of two molecules of geranylgeranyl pyrophosphate (GGPP) by the enzyme phytoene synthase (PSY) to form phytoene. This is considered as the rate-limiting step of carotenoid biosynthesis (Sun *et al.*, 2018). The phytoene then undergoes sequential dehydrogenation processes yielding lycopene (Enfissi *et al.,* 2017). The biosynthetic pathway then branches after formation of lycopene. Lycopene-ε-cyclase (LCYE) acts on one end leading to the formation of α-carotene, while Lycopene-β-cyclase (LCYB) works on the other end to form β-carotene (Zhang *et al*., 2021). This makes LCYB emblematic in the formation of beta carotene. Carotenoids act as accessory pigments to maximize light harvesting efficiency of photosystems (Schweiggert & Carle, 2017) among other functions. Besides their functions in plants, carotenoids are also essential in the human diet owing to their nutrition and health benefits. Apart from being good source of provitamin A, they also possess antioxidant activity, which helps lower the risk of long-term degenerative diseases. Research interests on these pigments has led to the identification and characterization of genes and enzymes involved in their biosynthetic and catabolic core reactions (Nisar *et al*., 2015). Study results point to the fact that the enzymes involved in carotenoid metabolism are encoded by nuclear genes, synthesized in the cytosol, translocated within plastids, and sorted to specific organelle sub-domains depending on plastid type and morphology (Sun *et al*., 2018).

The aim of this study was to determine the effect of drought stress on nutrients, metabolites and on transcription of their selected biosynthesis genes in leaf amaranth accessions.

4.2 Materials and Methods

4.2.1 Plant Material and crop establishment

Ten accessions of amaranth were used in this study.

Table 4.1: Amaranth Accessions

Acc. No.	Entry Name	Species	Seed Color	VI No.
AM1901	Abuku amaranth 5	*A hypochondriacus*	Brown	None
AM1902	RW-AM-16-ES13-6	*A. hypochondriacus*	Yellow/Brown	None
AM1903	AH-TL-Sel	*A. hypochondriacus*	Yellow/Brown	AVAM1605
AM1904	Abuku amaranth 6	*A. hypochondriacus*	Brown	None
AM1905	Madiira 1	*A. cruentus*	Black	VI060290
AM1906	SSCFS-002	*A. cruentus*	Creamy/White	None
AM1907	Madiira 2	*A. cruentus*	Black	VI060470
AM1908	UG-AM-23	*A. dubius*	Black	VI050448
AM1909	Blitum-Kenya	*A. blitum*	Black	VI050997
AM1910	A-GARE	*A. dubius*	Black	VI062426

The seeds of seven accessions were obtained from WorldVeg-Arusha while three were obtained from WorldVeg-Taiwan. The study was conducted in a greenhouse at World Vegetable Center, located in Shanhua, Taiwan (latitude 23°8'3" N, longitude

120°17'18" E). The greenhouse was controlled at temperatures of between 25°C to 27°C. Thirty seeds of each of the ten different amaranth accessions were planted in trays, with a medium composed of perlite, vermiculite and peat moss (in a 3:1:1 v/v ratio). Germination trays were kept under semi-controlled greenhouse conditions at the World Vegetable Center in Shanhua, Taiwan.

Two weeks after sowing, 20 plants per accession were transplanted into 4” pots in a greenhouse in a completely randomized design (CRD). Half of the plants were subjected to drought stress from the third week after transplanting, the other half were grown as a control. The field capacity of soil in representative pots was measured gravimetrically, by measuring the soil volumetric water content (IAEA, 2008). Prior to the stress treatment, sample pots containing the soil media with plants were saturated with water and let to drain overnight to determine the weight at 100% field capacity. Watering of the experimental plants was withheld for three days, during which the pots were weighed to determine soil water content evolution. In three days, the field capacity was below 30%, this was also evident by moderate to severe plant wilting. The control plants were watered to capacity throughout the experiment. Samples for nutritional analysis and gene expression analysis were then collected.

4.2.2 Nutrition and metabolites analysis

Leaves of the plants were harvested at five weeks after planting and after the water stress treatment. Laboratory analysis of (anti)nutrient including dry matter, Calcium, Iron, Zinc, vitamin C, Carotenoids, flavonoids and oxalate was done.

***Determination of oxalate content*:** Oxalate content of the samples was determined by Libert’s HPLC method of oxalate analysis (Libert, 1981) with modifications according to Yu *et al*., (2010), as described in 3.2.4.

***Determination of carotenoids*:** Extraction was done according to Rodriguez (2001). Separation and identification of carotenoids was performed on a HPLC system (Waters 2695, Milford, MA, USA) equipped with an auto-sampler, a photodiode array detector (Waters 996) monitoring at wavelengths between 210 - 700 nm as described in 3.2.4.

***Determination of vitamin C*:** The vitamin C content was determined by UV spectroscopy based on coupling 2,4-dinitrophenylhydrazine (DNPH) with ketonic groups of dehydroascorbic acid through the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP), as described by Hanson *et al.*, (2004). The method is described in 3.2.4

Determination of minerals: Mineral contents (Ca, Fe, Zn) were analyzed by strong acid digestion followed by atomic absorption spectrophotometry (AAS) (AOAC, 2016), as described in 3.2.4.

Determination of flavonoids: After methanol extraction, flavonoids were separated by LC-MS and quantified according to Zhang *et al*.,(2016), as described in 3.2.4.

4.2.3 Gene expression study

Selection of target genes, reference genes and primer design

Target enzymes were identified from the various biosynthetic pathways of oxalates, ascorbic acid and beta carotene. These included Glycolate oxidase (GLO) and Ascorbate peroxidase (APX) for the oxalate biosynthesis; GDP-L-galactose phosphorylase (GPP/VTC 2) and GDP-D-mannose 3',5'-epimerase (GME) for the vitamin C biosynthesis/ L-galactose pathway; Phytoene synthase (PSY) and Lycopene beta cyclase (LCY) for the beta carotene biosynthesis. Their gene sequences for *Amaranthus hypochondriacus* were then retrieved from the phytozome portal (https://phytozome.jgi.doe.gov/pz/portal.html#).

Recommended candidate reference genes, Actin (*AhyACT*), was selected based on literature as the housekeeping gene (Vera Hernández *et al*., 2018). The reference gene is known to be required for basal cell functions in all cells across all conditions. The genes have a stable expression regardless of cell type, cell cycle stage or tissue environment (Dheda *et al*., 2004; Kozera & Rapacz, 2013; Van Acker *et al*., 2019).

Table 4.2: Selected genes and primer sequences

Gene Name	Symbol	Locus in Phytozome		Sequences (5' to 3')
Ascorbate peroxidase	*Am*APX	AHYPO_015530	L R	TTATGCTTCGGATCCTGCTC CACCTCTTTGTGGCCATTCT
Glycolate oxidase	*Am*GLO	AHYPO_016961	L R	AGACTTGGTCGAAGGGAGGT CTCCAGCTTCTGCTGCTCTT
Phytoene synthase	*Am*PSY	AHYPO_008590	L R	ATGTTAGCAAACCCGACAGG AGCCTCACCGAGCAAACTTA
Lycopene beta cyclase	*Am*LCY	AHYPO_000004	L R	TCGTCCATGACGAATCAAAA CCAAATGAGAGTCCCTCCAA
GDP-L-galactose phosphorylase	*Am*VTC 2	AHYPO_006370	L R	CAGGCTTTGACTGTCGATGA CTGGGCAACAAAACCGTAGT
GDP-D-mannose 3',5'-epimerase	*Am*GME	AHYPO_009645	L R	TATGGTCCTTTCGGAACCTG CAGCCATTTCGTTCATGCTA
Beta-Actin	*Am*ACT7	AHYPO_019889	L R	CTGGGTTTGCTGGAGATGAT GCTCACAATACCGTGCTCAA

Based on the gene sequence obtained from transcriptome assembly database available on Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html#), primers were designed using Primer3 Input; Version 0.4.0 software (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) under default parameters. The designed primers (Reverse and Forward) for both target genes and reference genes were synthesized at Genomics, Taiwan.

Sample collection, RNA isolation and quality controls

The sample collection was done when the plants were at five weeks, and after the water-deficit stress treatment. The second leaf, was picked using forceps and placed into a clean labeled plastic vial and then kept in a precooled cooler box. The plant tissues were harvested in triplicate for treatment and control of the different varieties.

About 100 g of each sample were ground to a fine powder with pestle and mortar under liquid nitrogen for RNA isolation. The total RNA was extracted using the TRIzol reagent (Invitrogen, USA) according to manufacturer's instructions. Binding, washing and elution of the RNA was done using Invitrogen™ PureLink™ RNA Mini Kit. The extracted mRNA was then kept at -80^0c awaiting transcription. The quality of the RNA samples was determined by agarose gel electrophoresis.

cDNA Synthesis

First-strand cDNA was synthesized with 5 µg of total RNA in a final reaction volume of 20 µL, using qScript® cDNA Synthesis Kit (Quantabio, Beverly Inc.) and according to the manufacturer's instructions, in a Peltier thermocycler (DNA Engine, BIO-RAD) programmed as follows: 1 cycle at 22ºC for 5 min, 1 cycle at 42ºC for 30 min and 1cycle at 85ºC for 5 min. The cDNA was stored at -20ºC. The quality of the RNA samples was determined by agarose gel electrophoresis.

RT-qPCR analysis

The RT-qPCR mixture contained 2 µL of undiluted cDNA, 10 µL of 2× QuantiNova^{TM} SYBR Green PCR Master Mix (QIAGEN), 1.2 µL of each gene primer pair and 6.8 µL of Nuclease free water in a final volume of 20 µL. RT-qPCRs with no template controls were also performed for each primer pair, using nuclease free water in place of cDNA. The Real-Time PCR reactions were performed employing the Rotor-Gene Q Series software, Version 2.1.0.9. All the RT-qPCRs were performed under the following conditions: hold for 2 min at 95°C; 45 cycles of 30 sec at 95°C, 45 sec at 50ºC and 45 sec at 72ºC; and a final 5 min hold at 72ºC in 72-well optical reaction tubes. Three biological replicates of each sample were used for the Real-Time PCR analysis.

4.2.4 Data Analysis

The data for the nutritional/ biochemical components was analyzed and T-Test used to determine the significance in the accumulation of the various components. The expression level of genes in each reaction was determined by the cycle threshold Ct, the cycle at which fluorescence from the reaction exceeds a set crossing point. To analyze the expression variation of the candidate genes, the Excel-based methods were used using the $2^{-\Delta\Delta}$ Ct method. The fold changes were calculated in excel and T-Test was done to determine the differences between the control and water stressed samples. The correlation coefficients were computed using Pearson Correlation coefficients, and the significance of the correlations tested ($\mathrm{P} \leq 0.05$).

4.3 Results

4.3.1 Nutritional Traits

The results of the nutrient contents are presented as mean and standard deviations of ten amaranth accessions. All the results are presented on dry weight basis, to eliminate any differences that may have been caused by dehydration.

There was a significant decrease in oxalates due to the drought stress (Figure 4.1). The amounts of vitamin C were, however, significantly ($P \leq 0.05$) increased in the water stressed samples compared to the control. There was also a slight reduction the amounts of calcium, which was not statistically significant($P \leq 0.05$).

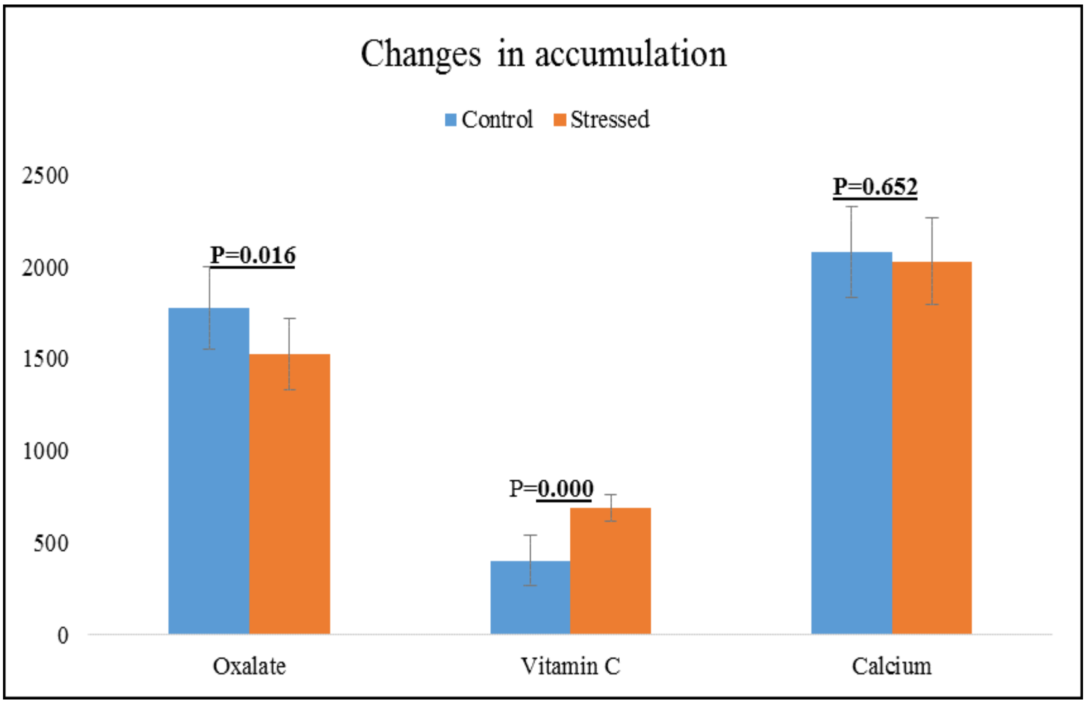

Figure 4.1: Changes in accumulation of Oxalates, Vitamin C and Calcium in mg/100 g DW due to water stress

There was a general reduction in iron and zinc contents due to drought stress (Figure 4.2). However, this change was not significant ($P \leq 0.05$).

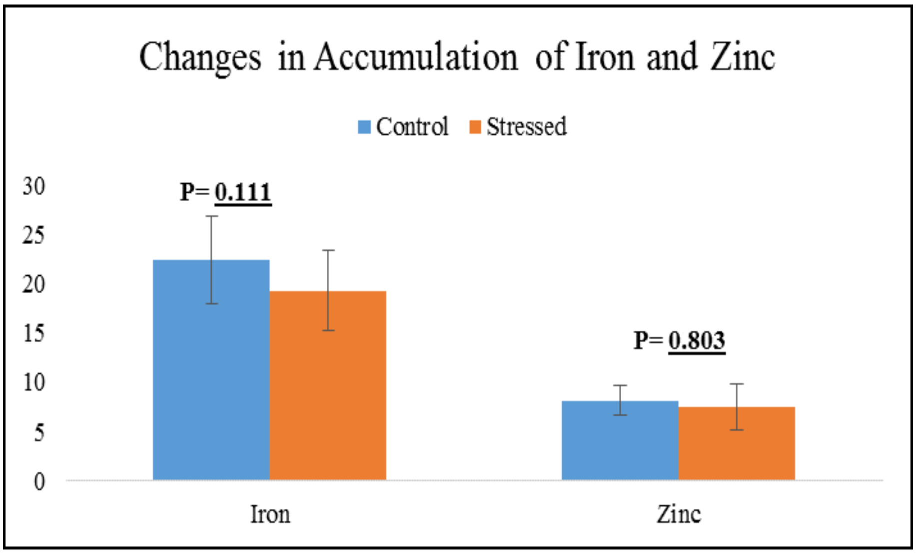

Figure 4.2: Accumulation of Iron and Zinc in mg/ 100 g DW

Five carotenoids were detected in the samples; violaxanthin, neoxanthin, lutein alpha-carotenes and beta-carotene (Figure 4.3).

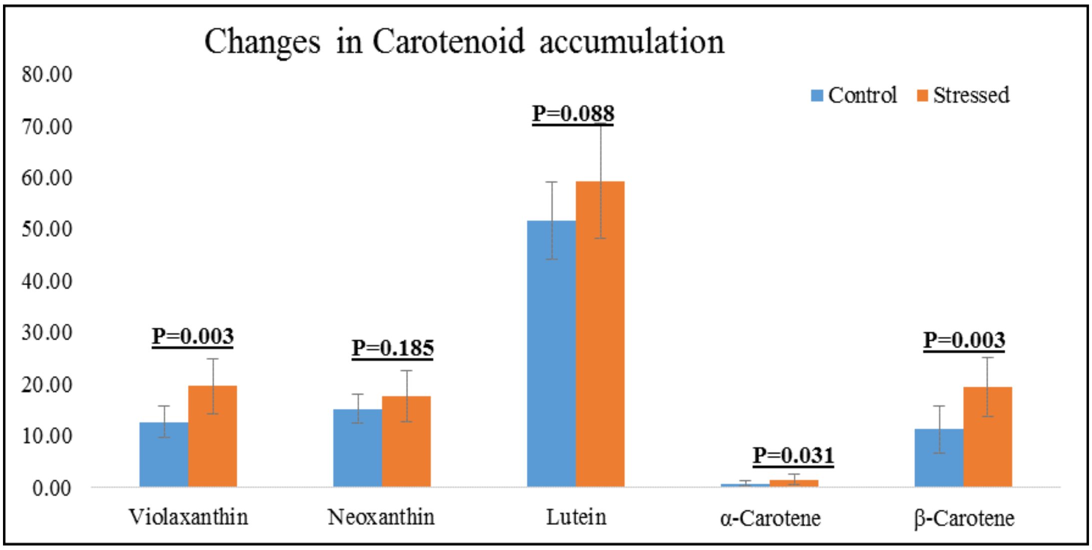

Figure 4.3: Changes in accumulation of carotenoids in mg/100 g DW

Lutein was the most abundant while alpha carotene was in the lowest among the carotenoids. There was significant (5% level) increase in violaxanthin, α-carotene, β-carotene; while the increase in neoxanthin and lutein was not significant.

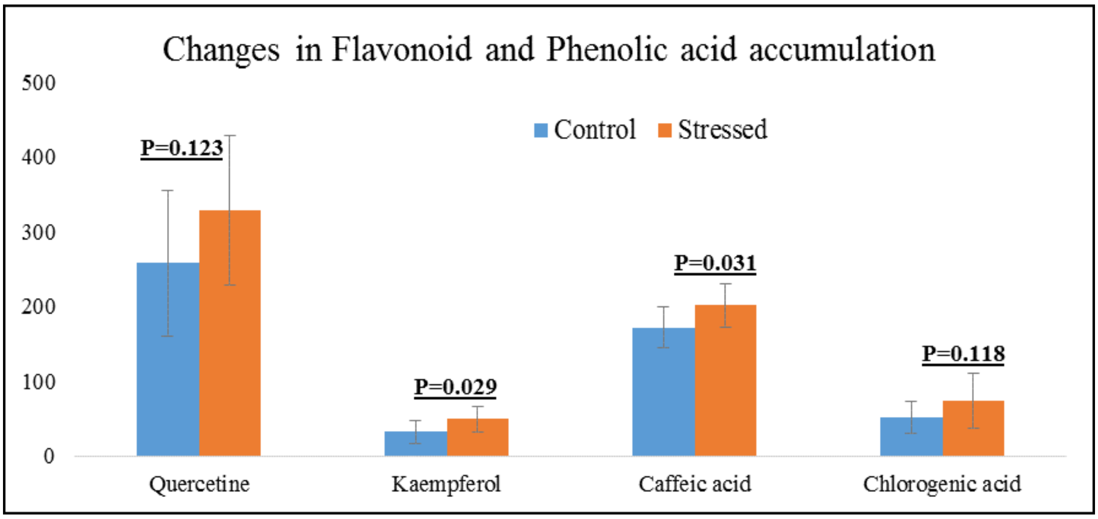

Figure 4.4: Changes in accumulation of Flavonoids and Phenolic acids in µmole/ 100 g DW

Quercetin and caffeic acid were the most abundant flavonoid and phenolic acids respectively (Figure 4.4). All the flavonoids and phenolic acids increased with stress. However, quercetin and chlorogenic acid did not change significantly ($P \leq 0.05$).

4.3.2 cDNA Quality

Agarose Gel electrophoresis was carried out to determine the quality of the cDNA against 100-1000 bp DNA marker. The amplicon lengths for all the cDNA samples were about 200 pb (Figure 4.5). Of the 60 triplicates, two samples, Accession-2-Control-Replication-1 (C2-1) and Accession-5-Control-Replication-1 (C5-1) did not amplify fully and were visualized as smear (Figure 4.5).

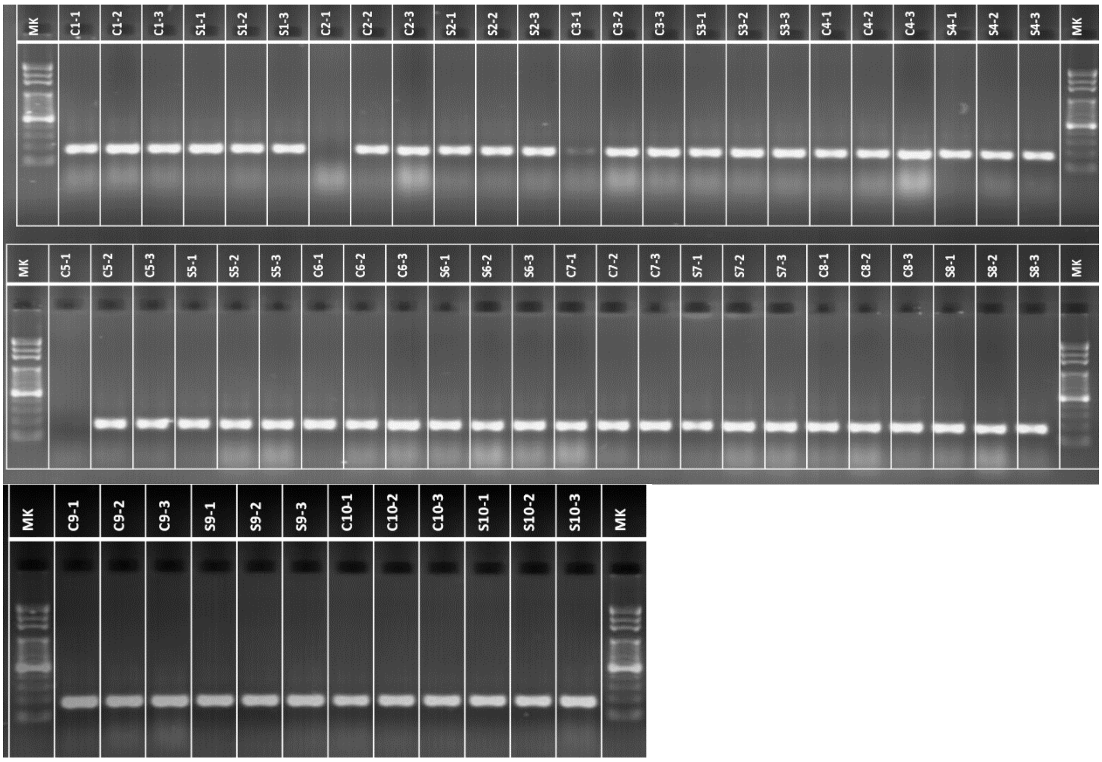

Figure 4.5: Amplification profile of cDNA of the samples by Gel Electrophoresis of the 10 accessions under control (C) and water stress (S) in 3 replications.

4.3.3 Changes in Biosynthetic Gene expressions and Biochemical Accumulation

The expression patterns are presented in Table 4.3. Significant up-regulations and down-regulations of the genes in the various samples were observed, while changes in some genes remained insignificant.

Table 4.3: Gene expressions between Control and Drought stress

Sample	GLO	APX	VTC2	GME	PSY	LCY
AM1901	1.69*	-1.95*	1.30*	-9.80*	-7.13*	-1.74*
AM1902	4.51*	-5.07*	2.06*	-13.01*	-11.59*	-1.88*
AM1903	3.72*	-2.49*	6.31*	-4.10*	-2.67*	-1.46 ns
AM1904	1.64 ns	-2.53 ns	1.70*	-1.24 ns	1.79*	1.66 ns
AM1905	-1.57*	-1.86 ns	2.55*	-6.39*	-1.61*	-1.43 ns
AM1906	-1.62*	-2.03*	2.63*	-2.50 ns	-2.10*	-0.63 ns
AM1907	-3.67*	-2.32*	3.21*	-3.39 ns	-2.29 ns	-1.32 ns
AM1908	-1.73 ns	-1.60 ns	12.02*	1.69 ns	-0.48 ns	2.06*
AM1909	-1.95 ns	-1.60 ns	12.79*	0.17 ns	-1.57 ns	1.37 ns
AM1910	-2.74*	-1.79*	3.34*	-1.82 ns	-1.34*	1.19 ns

**= significant; ns= not significant; – (-ve) = downregulation*

VTC2 upregulated in all the accessions. Glycolate oxidase (GLO) was up-regulated in all the accessions of the *A. hypochondriacus* species, while it was slightly down regulated in the other accessions. Ascorbate peroxidase (APX) was down regulated in all the amaranth accessions. However, this change was not significant in four accessions. GDP-L-galactose phosphorylase (GGP/VTC2) was up regulated in all accessions, and mostly in accessions AM1908 and AM1909, which showed 12-fold up regulations. GDP-D-mannose 3',5'-epimerase (GME) was down regulated in all accessions except accession 8 which showed very little effect. Phytoene synthase (PSY) and Lycopene beta cyclase (LCY), were down regulated in most accessions, accession AM1904 showed up regulation of both genes, while accession AM1908, AM1909 and AM1910 showed up regulation of LCY.

The percentage change in oxalates, vitamin C and carotenoids is presented in Table 4. Oxalates were significantly reduced due to water stress, except for three accessions, where the reduction was not significant ($P \leq 0.05$).

Table 4.4: Percentage (%) change in biochemical components

Sample	Oxalate	Vitamin C	Beta carotene	Total carotenoids
AM1901	-0.18ns	74.04*	47.76*	24.84*
AM1902	-20.73*	63.68*	39.39*	23.47*
AM1903	-12.94*	155.10*	77.68*	26.54*
AM1904	-4.74ns	55.73*	28.09*	28.00*
AM1905	-19.21*	35.06*	30.77*	19.11*
AM1906	-15.85*	23.32*	8.79ns	14.01*
AM1907	-23.03*	44.95*	65.82*	17.60*
AM1908	-4.20ns	79.80*	85.66*	31.71*
AM1909	-14.25*	224.04*	81.76*	43.56*
AM1910	-15.25*	31.77*	66.50*	24.43*

**= significant; ns= not significant; – (-ve) = reduction*

There were significant ($P \leq 0.05$) increases in vitamin C, beta carotene and total carotenoids in all the amaranth accessions due to drought stress.

4.3.4 Metabolite accumulation associations with gene expression

A correlation study was done between the differences in (anti)nutrients in the various accessions versus the fold changes in transcription of genes. Correlation coefficients between genes and corresponding metabolite was determined as well as their P-Values. VTC2 gene showed a significant correlation with vitamin C levels; as well as LCY gene with total carotenoids.

Table 4.5: Correlation between Genes and Components

Items	Correlation Coefficient	P value (0.05)
Genes Vs Nutritional Components Correlations		
GLO & Oxalate	-0.26	0.48
APX & Oxalate	0.25	0.49
VTC2 & Vitamin C	0.71*	0.02
GME & Vitamin C	0.14	0.69
PSY & Beta Carotene	-0.32	0.36
LCY & Beta Carotene	0.41	0.24
PSY & Total Carotenes	0.16	0.63
LCY & Total Carotenes	0.6*	0.05
Genes Vs Genes of same pathways Correlations		
GLO & APX	-0.63*	0.02
VTC2 & GME	0.63*	0.05
PSY & LCY	0.67*	0.03
Genes: Gene Vs Component Partial correlations		
GLO,APX - Oxalate	-0.71*	0.03
GME,VTC2 - Vitamin C	0.76*	0.01
PSY, LCY - Beta carotene	0.7*	0.03
PSY, LCY - Total carotenes	0.73*	0.02

*Values are presented as correlation coefficients, r, and P-Values at 95% confidence level. * represents significance.*

Significant correlations were also seen between genes of the same pathways (Table 4.5). A further partial correlation study between two genes of the same pathway with the corresponding component showed significance in all cases.

4.4 Discussion

Water stress affects plants in different ways, and the expression of genes involved in metabolite biosynthesis is known to vary in stress conditions depending on species. The hypothesis in this study was that nutrient accumulation and biosynthesis genes that contribute to the formation of nutritional and antinutritional components such as vitamin C, carotenoids and oxalates during water stress conditions in amaranth would help elucidate the molecular basis for their vegetable nutritional quality. During instances of drought, the first response by many plants is the closure of stomata. Metabolic adjustment is the second step in plant adaptation to drought and is critical in maintaining the water status and physiological activity of plant cells, especially during relatively short-term drought (Krasensky & Jonak, 2012), causing difference in accumulation of biochemical products.

In the present study, accumulation of oxalates was found to be suppressed by drought stress. This could be due to reduces biosynthesis or increased degradation of oxalates. Other bioactive compounds such as ascorbic acid, carotenoids, flavonoids and phenolic acids were found to be induced by drought stress. Minerals were also reduced due to water stress. However, the reduction in calcium, iron and zinc were not significant. Drought stress not only affects soil water availability, which is directly involved in mineral nutrient uptake by plants, but also depresses acropetal translocation of these mineral nutrients due to lowered transpiration pull (Stagnari *et al*., 2016).

The results of nutrient accumulation in this study corroborates those of Sarker and Oba (2018d), who reported increase in iron, vitamin C, total carotenoids, flavonoids and phenolic acids with severity of water stress in *A. tricolor*. The increase in antioxidative compounds in water stressed plants can be attributed to the adjustments to counteract the oxidative stress brought about by drought stress. Regulation of compounds with anti-oxidant activity such as ascorbic acid has been found to relate to abiotic stresses;

which normally causes oxidative stress. Hence ascorbate as an antioxidant is increased to detoxify the reactive oxygen species (Fenech *et al*., 2019).

Carotenoids play important roles within the plant as accessory pigments to maximize light harvesting efficiency of photosystems (Schweiggert & Carle, 2017). Carotenoids also act as photo-protectors, shielding against photo-oxidation of the cell constituents and detoxifying the triplet state chlorophyll molecules and the highly reactive oxygen species (ROS) produced by photosynthesis (Hashimoto *et al*., 2016). Carotenoids, abscisic acid (ABA) and strigolactones (SLs) pathways share biosynthetic intermediaries. These are key phytohormones regulating plant development and environmental stress responses (Al-Babili & Bouwmeester, 2015; Hou *et al*., 2016). In a study on *Achillea pachycephala*, phenolic flavonoids and their biosynthesis genes in the leaves were upregulated under drought stress (Gharibi *et al*., 2019). Increase of the expression levels of flavonoid genes and up-regulation of leaf flavonoids in *Triticum aestivum* have also been reported (Ma *et al*., 2015).

Gene expression analysis of the accessions indicated that some transcripts responded to drought stress in a systematic manner, while others did not show a clear trend. The transcripts responded to the stress by down-regulation or up-regulation, while others did not show significant change. In this study, APX gene was seen to be down regulated by drought stress in amaranth. Contradicting results were reported in a study on Arabidopsis, that showed that APX gene was upregulated under drought stress (Liu *et al*., 2019). That study also pointed out that overexpression of APX in Arabidopsis positively regulates drought tolerance by regulating the stomata aperture.

A unidirectional response trend was clear for VTC2 in this study. Higher leaf vitamin C coincided with induced VTC2 and GME expression. The major regulatory control point for ascorbic acid biosynthesis is exerted by GGP/ VTC2 (Laing *et al*., 2015). This gene can also be used to increase vitamin C content of other plants and also for resistance to environmental stresses (Shiri & Zebarjadi, 2018). VTC2/GGP which converts GDP-galactose to galactose-1-phosphate is the key enzyme in the ascorbate pathway, and has been shown to control ascorbic acid in many plant species (Li *et al*., 2016; Zhang *et al*., 2015). Transformations with GGP result in very significant increases in tissue ascorbate concentrations. Co-expression of GGP (VTC2) and GME results in strong synergistic increase in ascorbate concentrations (Laing *et al*., 2015). Aside from the biosynthetic genes, ascorbic acid levels are also regulated by environmental factors such as light, temperature, salt and drought (Fenech *et al.*, 2019; Laing *et al.*, 2015; Li *et al.*, 2016) Overexpression of genes involved in ascorbic acid metabolism such as GGP leads to an increase in resistance to abiotic stress by regulating the generation of ascorbic acid in plants (Liu *et al*., 2019).

During drought stress, LCY has been reported to be downregulated, channeling precursors towards beta-carotenoids, leading to enhanced apocarotenoids which improve drought and salinity tolerance (Dhami & Cazzonelli, 2020). This present study also reports a downregulation of LCY in most accessions, with enhancement of beta carotene in all accessions. However, the correlation between the two was not significant. The downregulation of APX in many accessions, coincided with the reduction in amounts of oxalates in the stressed samples, even though the correlation was not significant.

The correlation between genes of the same pathways were all significant, as well as the partial correlations between genes of the same pathways with the corresponding components. Correlation between GLO and APX as well as the partial correlation between these genes and oxalates was negative. This trend could mean that the regulation of components may not be significantly affected by a single gene, but several genes in the pathway. For instance, GME is involved in the formation of GDP-L-galactose which is all used in production of ascorbic acid. However, by itself, it has little effect on ascorbate concentrations in plant tissues (Bulley & Laing, 2016). Another study also reported that overexpression of GME alone did not cause significant increase in the ascorbate content of Arabidopsis plant (Yoshimura *et al*., 2014). The results of the present study suggests that VTC2 and GME can be reliable gene markers of vitamin C concentrations in vegetable leaves. In another study on carrot taproots, drought stress was reported to increase beta-carotene levels, which correlated with expression levels of PSY and LCY genes (Zhang *et al*., 2021).

4.5 Conclusion

Accumulation of most biochemical components in amaranth were found to increase with drought stress, especially secondary metabolites including carotenoids, flavonoids and phenolic acids. Transcription of most genes studied were, however, not significantly correlated to the respective biochemical components. But significant positive and negative correlations were observed between genes of the same pathway as well as partially between the genes and associated metabolites. The conclusion of this study, therefore, is that a single gene in the pathway may not strongly affect a particular component, but association between several genes affects the synthesis pathway.

CHAPTER FIVE

PREFERENCE, UTILIZATION PRACTICES AND CHALLENGES TO CONSUMPTION OF VEGETABLE AMARANTH IN KENYA AND TANZANIA

Summary

African leafy vegetables such as amaranth, have been utilized since time immemorial, both as food and medicine. These vegetables grew naturally in most rural environments, but currently most of them are cultivated for both home consumption and for sale. The aim of this study was to identify the most preferred amaranth species, cooking and utilization practices, as well as the beliefs and attitudes that encourage or discourage use of this vegetable. The study was carried out in seven counties of Kenya, and in three regions in Tanzania. Twenty Focus Group Discussions (FGD) with members of the community and twenty Key Informant Interviews (KII) with agricultural and nutrition officers were conducted in the study areas to obtain information on preferred varieties; sources of amaranth vegetables; common cooking methods; alternative uses; beliefs and taboos surrounding amaranth consumption; and the challenges experienced in production and consumption. The findings of the study showed that amaranth is one of the most commonly consumed indigenous vegetable in Kenya and Tanzania. The preference for varieties and cooking habits differs depending on the community and individuals. *Amaranthus dubius* and *A. blitum* were most common in Kenya; while *A. dubius* and *A. hypochondriacus* were most common in Tanzania. Most people consumed these vegetables because they were affordable and available or because of circumstance of lacking other foods. Regarding cooking, final taste was mostly considered rather than nutritional attribute. Several alternative uses of amaranth such as uses as medicine and livestock feed were also reported, as well as some beliefs and taboos surrounding the vegetable. Training on nutritional attributes and promotion of food preparation practices that ensure maximum nutrient benefits from amaranth is needed at the community level to realize the nutritional

importance of the vegetables. Hands-on training and demonstrations were the most preferred modes of passing information.

5.1 Introduction

The amaranth vegetable (*Amaranthus* spp.) is one of the most commonly consumed African Indigenous Vegetables (AIVs). However, these vegetables have faced many years of neglect (Abukutsa-Onyango, 2011); with young consumers and urban dwellers equating the vegetables with traditional lifestyles (Matenge *et al*., 2012). All over the world, there has been notable changes in food consumption patterns, referred to as nutrition transition, as people adapt to new socio-economic and environmental changes (Blas *et al*., 2019; Dlamini & Viljoen, 2020). This could either result in decrease or increase in consumption of certain foods such as the traditional vegetables. Demand and consumption of African indigenous vegetables such as amaranth is currently on the rise (Karanja *et al*., 2012; Krause *et al*., 2019) owing to recent research on the indigenous vegetables, which has shown that the vegetables are rich in vitamins, minerals, bioactive compounds and are also easy to produce and cook (Lin *et al*., 2011). Despite the growing demand, there has been minimal improvement in the nutrition and health of consumers as micronutrient deficiencies (hidden hunger) continues to cause poor motor and cognitive development and even lead to death (Biesalski, 2013).

Nutritional benefits from vegetables amaranth could depend on several factors, including species selected (Nyonje *et al*., 2014), production practices and cooking method used. Certain beliefs and negative attitudes have over the years led to neglect of amaranth vegetable. Among such attitudes was consideration of the vegetable as a weed and a poor man's food. Certain attitudes may also exist that lead to increased consumption of this vegetables, for example, spider plant (*Cleome gynandra*) is believed to be a blood booster in some areas of Kenya. There are many amaranth varieties with morphological and genetic variability, which is observed in plant characteristics such as inflorescence type, seed color, precocity, leaf color, nutritional contents, resistance to pests and diseases (Akaneme & Ani, 2013; Erum *et al*., 2012); some of which also serve as basis for selection by consumers.

The consumption of indigenous vegetables is greatly influenced by cultural backgrounds, hence some types and varieties are associated only with specific communities (Croft *et al*., 2014). Most rural communities in parts of Kenya and Tanzania are believed to be the biggest consumers of African indigenous vegetables including amaranth. A study conducted in Kenya indicated a higher consumption intensity of leafy AIVs in rural dwellers compared to urban dwellers with a mean of four and two times a week, respectively (Gido *et al*., 2017b). However, preferences may vary as to types and varieties of different amaranth consumed, which could be determined by cultural and social factors. A study carried out in Tanzania showed that strong cultural food beliefs and taboos still exist among communities, which strongly influence attitudes towards traditional vegetables in general (Kansiime *et al*., 2018). It is however not clear which beliefs are these and whether they negatively or positively affect consumption of amaranth.

Utilization methods of this vegetable could also differ, as well as cooking methods, which may affect the nutritional attributes of the amaranth either positively or negatively. Different communities prefer different species of the vegetables, but specific species for the different regions is not known. Factors including unfamiliar taste, small leaf sizes, low shelf life and seasonal availability were reported to contribute to low consumption of indigenous vegetables including amaranth in various populations in Kenya (Mncwango *et al*., 2020).

Cooking vegetables improve edibility, and also induce significant changes in physical characteristics, chemical composition, biological characteristics and bioavailability of vegetable nutritional components. Most leafy vegetables like amaranth are cooked prior to consumption based on convenience and taste preferences rather than retention of nutrients and health promoting compounds (Hossain *et al*., 2017). Cooking vegetables destroys microorganisms and reduces anti-nutrients, thereby increasing the safety; it also enhances digestibility of food and bioavailability of nutrients. Cooking methods and duration of cooking of amaranth vegetables, as well as added ingredients varies widely in communities. In Western Kenya for instance, vegetable amaranth is mostly cooked in combination with other vegetables including spider plant (*Cleome gynandra*) and nightshade (*Solanum scabru /vilosum*), and the cooking process involves boiling for about 40 minutes then frying in onions and oil with optional addition of milk or cream (Musotsi *et al*., 2017). Musotsi (2019) recommended that the best practices for vegetable preparation methods to preserve micronutrients should involve rapid cooking methods such as stir frying or steaming rather than boiling and intensive frying methods (Musotsi *et al*., 2019). The recipes may be different in different regions of East Africa. However, this information about the different recipes is not documented and therefore this study could help determine the various amaranth based food practices in different regions of Kenya and Tanzania, where amaranth consumption is considered common.

The objective of this study was to assess consumer awareness, attitudes and utilization practices of amaranth in Kenya and Tanzania. The study is guided by the research questions;

i. Do consumers have different preferences for different amaranth?
ii. What are the utilization practices of amaranth?
iii. What challenges, beliefs or attitudes increase or decrease amaranth utilization?

The information from this study can be used for promotion strategies and develop behavior change communication strategies to increase amaranth consumption and promote better nutrition practices.

5.2 Methodology

5.2.1 Study Areas

A total of ten study target sites including seven counties in Kenya and three regions in

Tanzania were purposively selected (Figure 5.1), based on the leaf amaranth production volumes as documented by Horticultural Crops Directorate (HCD) in Kenya (HCD., 2016) and by different research reports in Tanzania (Ochieng *et al*., 2019; URT, 2017). The high production areas also translate to high consumption areas. The counties in Kenya included Kiambu, Bungoma, Vihiga, Nyamira, Bomet, Kilifi and Kwale; while the regions in Tanzania included Kilimanjaro, Dar-es-Salaam and Mwanza. Two sub-counties/districts were then selected purposively from the counties on the basis of their utilization of amaranth.

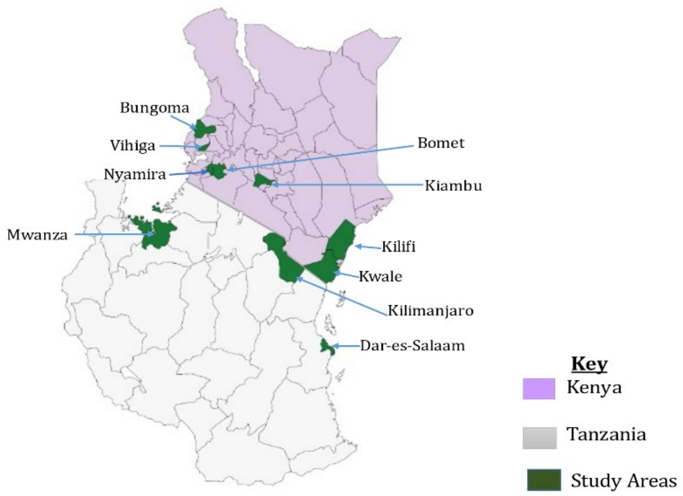

Figure 5.1: Study Areas

5.2.2 Study Participants

The study collected qualitative data from 20 focus group discussions (FGDs) and 20 Key Informant Interviews (KIIs) in the ten target sites with two FGD and two KII conducted in each site. The FGD each study site included 8-12 community members comprising female household members from vegetable amaranth growing areas. Female members were preferred since they are more familiar with food preparation and utilization at household level. Recruitment of FGD participants was done purposively, with the help of county and regional officers in the areas. Key informants were purposively selected, comprising agricultural and nutrition officers, who have first-hand knowledge of utilization practices in the various areas.

Participants for FGD and KII were female/ male farmers who grow and consume African indigenous vegetables, female/male agriculture and nutrition officers, female members of households, over the age of 18 from households that cook and consume AIVs including amaranth, and consent to participate in the study.

5.2.3 Ethical considerations

Ethical clearance was sought from Jomo Kenyatta University of Agriculture and Technology Ethical Review Committee and from the WorldVeg Institutional Biosafety and Research Ethics Committee (IBREC). A research permit was also sought from the National Commission for Science, Technology and Innovation (NACOSTI). Permission was also sought from various local government authorities before undertaking the survey. Written informed consent was obtained from each participant who took part in FGD and KII. All documents including participant information sheets, consent forms, FGD and KII guides, were translated to Kiswahili. During the FGD, consent was provided by project member to all participants upon the study information; all participants were provided with a detailed study information sheet, and thereafter requested to sign a consent form. Confidentiality of their data was assured and that the data was to be solely used for the study. Participation in the study was purely voluntary and all respondents were at liberty to withdraw at any time.

5.2.4 Data collection and analysis

Data was collected by qualitative method following set guidelines for Focus Group Discussions and Key Informant Interviews (Dzino-Silajdzic, 2018; van Eeuwijk & Angehrn, 2017; World Bank, 2020). A total of 14 FGDs were conducted in seven counties of Kenya, and 6 FGDs in three regions of Tanzania. A total of 20 Key informant interviews were also conducted in Kenya and Tanzania. Data collection was done between October and November 2020 in Kenya; and March – April 2021 in Tanzania. For both the FGDs and KIIs, the interviews were conducted at the locations and times that were convenient to the interviewees; with some FGDs being conducted at volunteer farmers' homes; while some, including all KIIs were conducted at the subcounty and district offices. The interviews were facilitated by one researcher at any given time, and supported by others for note taking and administration.

Upon administration of the informed consent, discussions and interview were facilitated using pre-written FGD and KII guides (Appendix IV). The information collected included indigenous vegetables consumed and their sources, methods of preparation, utilization, possible preservation practices and the beliefs around amaranth. Both methods took between 30 minutes and 1.5 hours per study. All interviews were audio recorded, complemented by written short notes.

Qualitative data from the FGDs and KIIs was transcribed and translated, then coded to variable categories. Common themes were then established and clustered in a pattern order to clarify variables predicting concepts. The themes were in line with the study objectives. Inferences were then made from the data under each theme and conclusions drawn.

5.3 Results and Discussion

5.3.1 Preferred Species and their sources

Several African indigenous vegetables were reported to be consumed in the various regions of Kenya and Tanzania. From the results of the survey, consumption differs with regions, counties and even communities in the different parts of East Africa. The common AIVs include amaranth (*Amaranthus spp*.), nightshade (*Solanum scabrum/vilosum*), spider plant (*Cleome gynandra*), cowpea leaves (*Vigna anguiculata*), jute mallow (*Corchus olitorius*), slender leaf (*Crotalaria brevidens*), pumpkin leaves (*Cucurbita moschata*), Ethiopian kale (*Brassica carinata*) vine spinach (*Basella alba*) bitter lettuce (*Launaea cornuta*), moringa (*moringa oleifera*), *Asystasia gangetica,* wandering jew (*Tradescantia zebrina*) and black jack (*Bidens* *pilosa*).

Amaranth was among the most preferred vegetables in most areas where the study was done. In about 90% of the FGDs, amaranth was mentioned among the three preferred indigenous vegetable, and was also the number one most preferred in all areas of Tanzania as well as in coastal and central counties of Kenya (Kilifi, Kwale and Kiambu). High preference for Amaranth in other areas of Africa have been reported (Omotayo *et al*., 2020). It can be suggested that it may indeed be one of the most common in Africa. This high preference for amaranth compared to other AIVs has been attributed to its taste and availability of numerous species everywhere (Mncwango *et al*., 2020). About seven major species of amaranth were reported to be known *A. dubius A. blitum, A. hypochondriacus, A. lividus, A. cruentus*, red amaranth (*A. hybridus*) and A. *spinosus*. However, some of these species are not used as food.

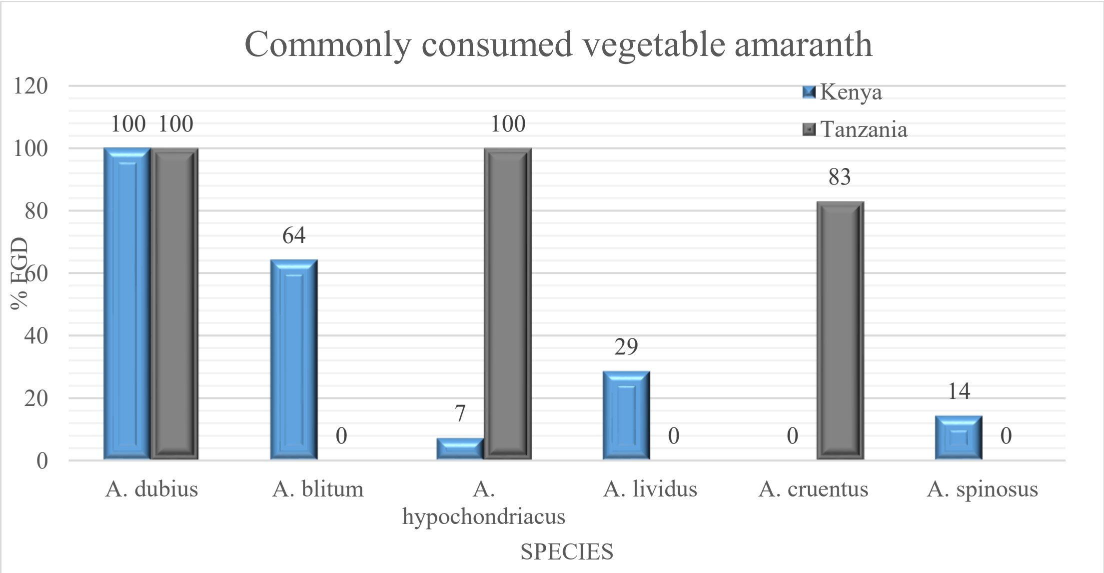

Figure 5.2: Common amaranth vegetables in Kenya and Tanzania

The respondents were familiar with different amaranth species. However, some species are more preferred for use as food, owing to their taste. The most preferred species of amaranth in Kenya were *A. dubius* and *A. blitum*, while *A. dubius* and *A. hypochondriacus* were most commonly used in Tanzania (Figure 5.2).

Most preferred as vegetables		**Less preferred as vegetables**
		
A. dubius	*A. hypochondriacus*	*A. spinosus* (thorny amaranth)
		
A. cruentus (Madiira 1)	*A. blitum*	*A. hybridus* (red amaranth)

Figure 5.3: Pictures amaranth species identified by respondents

Local taxonomy for amaranth differed widely, as some species had more than one name. Aside from the local names, leaf size and color of stem and leaves are other common characteristics that are used to differentiate the different amaranth species (Figure 5.Figure 5.2). In parts of Western Kenya and areas near the rift valley, the most preferred species have local name which are in the local languages, such as; *A. dubius*

(Mchicha – Swahili, Terere – Kikuyu, Ododo – Luo, Tsimboga – Luhya, Emboga – Kisii, Chelwanda – Kalenjin, Mchicha bwasi – Tanzania); *A. hypochondriacus* (Soisoi – Luo, Dodo – Kisii, Mchicha lishe – Tanzania); and *A. blitum* (Logatsi – Mijikenda, Livogoi – Luhya, Emboga nyerere – Kisii, Mborochik – Kalenjin, Mchicha pwene – Tanzania). *A. lividus* was also among those reported to be consumed in Bomet and Nyamira counties. However, it is not planted and only harvested from the wild. An *A. cruentus* variety, Madiira 1, also has a growing preference in Tanzania. This is a newly released variety with peculiar morphology, such as long curly leaves which has given it the nickname *mchicha bangi* as it is likened to the *cannabis* plant.

Other species of amaranth which were known but not considered for eating were the *A. spinosus*, which was not preferred because of its thorns. This brings out the regional difference in preference for species, as the *A. spinosus* has been reported to be among the most preferred species in South Africa (Mncwango *et al*., 2020). *A. hybridus* (red amaranth), is also not preferred because of its deep purple soup that it produces when cooked. The red amaranth was also reported to cause stomach upset in some people. In Nyamira County of Kenya, the red amaranth was also associated with certain beliefs which hinders its consumption. "*That red one even if you find in your farm, you should pluck and throw it away*" said and FGD respondent in Nyamira. These two amaranth species are therefore not planted and only grow as weeds. In certain areas such as Mwanza region of Tanzania, these two species were reported to have some medicinal uses.

In all the areas, the main sources of amaranth were household cultivation and buying from local markets (Table 5.2). More farmers are venturing in the production of amaranth because it needs less inputs, has short growth period, fairly resistant to drought, attacked by fewer pests, and the market demand is rising. This shows an improving trend from earlier reports which indicated that the vegetables are semi-cultivated and are mostly collected from the wild (Chipungahelo, 2015; Van Rensburg *et al*., 2015). In all the discussions in both Kenya and Tanzania, it was reported that at least 30% of community members grow amaranth, either for sale, home consumption or both. Commercial production is also practiced by some individuals in the different communities, and the varieties for this are based on preferences. In central and coastal Kenya, *A. dubius* is grown in large scale, while in Western Kenya, *A. blitum* is common for commercial production. In Tanzania, *A. hypochondriacus* is the most common for large scale production. It is planted and uprooted after 21 days for sale in markets. During rainy seasons, people rarely buy the vegetables as they are available in plenty even appearing as weeds in their farms. During such seasons, the vegetables can also be obtained from neighbors for free. Physical appearance including tenderness and freshness are the main attributes considered by consumers when buying the vegetables, while a few consider pesticide use.

5.3.2 Preparation and consumption

Amaranth, like other green leafy vegetables, is cooked before consumption. Cooking is done to improve palatability, texture, taste, eliminate potential pathogens and to neutralize poisonous or irritating components (Odendo *et al*., 2020). The general procedure before cooking involves destalking, washing, draining and chopping which is optional. The cooking methods for amaranth vary with consumers, and the most common cooking practices as reported by the respondents includes boiling then frying; boiling only; frying/sauteing only; steaming; and fermenting (Table 5.1). From this study, it was clearly noted that a great number of respondents considered taste of the cooked rather than nutrition quality, yet cooking is known to cause significant changes in nutrition of vegetables.

Table 5.1: Common amaranth cooking methods in Kenya and Tanzania

Recipe name	Method	Areas where practiced
Boiling only	Wash vegetables, layer the cooking pot with banana leaves to avoid burning of the vegetables, arrange the unchopped vegetables sprinkling a little salt between the layers, boil without turning (for about 1 hour) until the water dries up to prevent steam from escaping.	Bungoma, Vihiga, Bomet
	Old recipe: no washing, boiled for about one hour, add salt to taste.	Nyamira
	Boil in water for 20 minutes, add salt to taste (milk or cream can also be added)	Nyamira, Bomet, Kilifi, Kwale
Boiling then frying	Boil for 10-20 minutes, drain the water, and then fry in oil, onions and tomatoes. Cook while stirring for about 10 minutes.	Bungoma, Vihiga, Nyamira, Bomet, Kwale, Kilifi, Mwanza
Steaming	Wash vegetables and place in sufuria, add chopped onions, tomatoes and salt and steam for 10-15 minutes	Kwale, Kilimanjaro, Dar-es-Salaam, Mwanza
Frying/ sauteing	Fry onions and tomatoes in oil, add chopped amaranth and cook while stirring for about 10 minutes	Kiambu, Bungoma, Vihiga, Bomet, Kilifi, Kwale, Kilimanjaro, Mwanza
	Wash, dry in the sun to drain water, then fry in oil, onions and tomatoes.	Nyamira, Bomet, Kwale
"Fermenting"	Boil for 20 minutes, add salt to taste. Heat once a day while adding milk each time for 2 to 3 days (mostly done in combination with other vegetables)	Bungoma
Mixed with other vegetables	Wash all the vegetables, boil together for about 30 minutes, fry in oil, onions and tomatoes. (Cowpeas, spiderplant, nightshade)	Bungoma, Nyamira, Bomet, Kilifi, Kwale, Kilimanjaro
Amaranth with Coconut oil	Place in *sufuria*, add coconut milk and tiny wild cherry tomatoes, cook for 15 minutes and serve.	Kilifi
Gunjamato	Boil amaranth, add maize flour to make a mixture of ugali and vegetable	Kwale
With bhajia	Mix chopped amaranth leaves with gram flour, make into *bhajia* dough and fry	Kwale, Kilimanjaro

Amaranth vegetables were prepared either on their own or in combination with other vegetables; such as cowpeas, spider-plant, bitter lettuce, nightshade; and other foods such as beans, meat, small fish (*Omena*). The reasons given for mixing with other vegetables were to reduce bitterness of other vegetables such as spider-plant, to improve texture in cowpeas, to improve flavor of nightshade. In other foods, the mixing was done to save the time for cooking. In some cases, mixing was also done with more than one vegetable to increase the amount during low vegetable seasons. "*In dry seasons, one may have to harvest all the available types and mix together to get enough portion for the whole family*", said a key informant in Kilimanjaro, Tanzania. Added ingredients varied with regions and included oil, onions, tomatoes, carrots, coconut cream/milk, cow milk/cream, peanut paste, African lye, and salt. Amaranth vegetables are mostly eaten with maize meal (*ugali*), rice, bananas and potatoes in both Kenya and Tanzania. Generally, about 30% of the population eat amaranth as a main dish, while most eat as side dish.

Consumption of amaranth and other indigenous vegetables was reported to be on the rise in Kenya and Tanzania. A study done in South Africa reported a contrary situation, indicating that consumption and usage of amaranth is on the decline in the country (Maseko *et al*., 2017). The respondents noted that the younger people in their household prefer other foods over the vegetables. A similar report was given in a study conducted in part of Kenya, where mothers reported that their young ones consume very low amounts of amaranth and other traditional vegetables in their diet (Gewa *et al*., 2019). Another study also reports that the rural elderly were more likely to accept and consume amaranth and other indigenous vegetables due to their experience in AIV preparation, cooking, consumption and other uses (Ayanwale *et al*., 2016; Gido *et al*., 2017a). Even though most people consume amaranth frequently, not for its nutritional attributes, but because it is cheap, available, or because of circumstance related to poverty; there has been notable change in cooking from the traditional methods. The traditional methods involved long boiling time extending to hours, coupled with fermentation for several days (Musotsi *et al*., 2005; Musotsi *et al*., 2017; Wafula *et al*., 2016; Wakhanu *et al.*, 2016). It was noted by the key informants that training and advise on cooking are taken seriously and there has been changes in attitudes. "*We have been advising our clients, the old, those with children and HIV patients to incorporate the amaranth in their foods*" said a nutrition key informant in Nyamira County. However, some individuals still prefer the old cooking habits. "*My mother's vegetable was much better than my wife's, the old methods were very good and that is why I don't have ulcers. I beseech people to go back to the old cooking methods*" said an FGD respondent in Bungoma.

Table 5.2: summary of responses from survey in Kenya and Tanzania

Cnty/ Rgn		**KM**	**BG**	**VG**	**NY**	**BT**	**KF**	**KL**	**KR**	**DR**	**MZ**	**%**
Sources	Grown	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
	Bought	✓	✓	⊗	✓	✓	✓	✓	✓	✓	✓	90
	Found wild	✓	✓	✓	✓	✓	✓	✓	⊗	⊗	⊗	70
Amaranth as main ingredient		✓	⊗	⊗	⊗	✓	✓	✓	✓	✓	✓	70
Amaranth as minor ingredient		✓	✓	✓	✓	✓	✓	✓	⊗	⊗	⊗	70
Preservation		⊗	⊗	⊗	✓	⊗	⊗	⊗	⊗	✓	⊗	20
Other uses	Medicinal	✓	✓	✓	✓	✓	✓	✓	⊗	⊗	✓	80
	Animal feed	✓	✓	⊗	⊗	⊗	⊗	⊗	✓	✓	✓	50
Beliefs	Adds Blood	✓	⊗	⊗	⊗	⊗	✓	✓	✓	✓	✓	60
	Good for eyes	⊗	⊗	⊗	⊗	⊗	⊗	⊗	✓	✓	✓	30
	Sign of poverty	⊗	⊗	⊗	⊗	✓	⊗	✓	⊗	⊗	✓	30
	Causes stomach upset	✓	✓	✓	⊗	✓	⊗	⊗	⊗	⊗	⊗	40
Taboos		⊗	⊗	⊗	✓	✓	✓	⊗	⊗	⊗	⊗	30
Preferred mode of information												
Neighbors		⊗	⊗	✓	⊗	⊗	✓	⊗	✓	✓	⊗	40
Demonstrations		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
Face-to-face training		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100
Media (Radio, TV, Social)		✓	✓	⊗	⊗	⊗	⊗	⊗	⊗	✓	⊗	30

KM – Kaimbu, BG – Bungoma, VG – Vihiga, NY – Nyamira, BT – Bomet, KF – Kilifi, KL – Kwale, KR – Kilimanjaro, DR – Dar-es-Salaam, MZ – Mwanza

5.3.3 Medicinal and Alternative uses of amaranth

Amaranth, and all the other indigenous leafy vegetables are believed to have nutritional and medicinal properties. Majority of respondents believed that the rise in consumption of amaranth in their regions have greatly contributed to fewer cases of some illnesses such as eye problems and cases of anemia. "*Nowadays there are no drugs (supplements) we are given for blood issues, we eat vegetables*" said a respondent in Kilifi. This could be due to its richness in iron and beta-carotene among other micronutrients (Ashraf *et al*., 2018). This corroborates with the results of a study in Tanzania, which associated the consumption of indigenous vegetables with micronutrient adequacy, and that the absence of indigenous vegetables in an area can be an indication of micronutrient deficiencies especially in women of child bearing age (Conti *et al*., 2012). Amaranth seeds can be used in porridge flour. This is mainly for the dual-purpose types that also produce edible grains. In some communities, lactating mothers are advised to boil amaranth and drink the soup, to boost their blood. In some cases, raw amaranth leaves were blended in to juice or pounded to get the extract. Such, the juice was also used as an appetizer. In Kilifi County, the amaranth was mixed with jute mallow (teleza) and taken to relieve constipation. This can be attributed to the high contents of soluble and insoluble fibre in amaranth (Ogwu, 2020; Sarker & Oba, 2019c). In Bomet county, amaranth is used as a traditional detoxifier; the leaves are boiled with pepper and the soup taken hot, it causes a lot of sweating which removes toxins; "*You just boil it with chilies, drink the soup and sit under a tree. Sweat will just be flowing and it cleans your body*" said an FGD respondent in Bomet. The sweat induction and fever reduction by amaranth has been reported to be due to its soporific and febrifuge effects (Olusanya *et al*., 2021). In the same region of Bomet, Mborochik (*A. blitum*) is mixed with another herb and the extract used as relief for menstrual pain.

Ash from burnt amaranth stalk was reported to stop bleeding. Thorny amaranth was also used as medicine to cure mouth wounds in children and ulcers, as reported in one

FGD in Mwanza, Tanzania. The whole plant is harvested, dried and burned to char (not to white ash). This is then ground to powder, mixed with a little salt and is licked, without mixing with water. Other studies have reported wound healing and anti-inflammatory properties in amaranth (Moyo *et al*., 2018; Vithya & Jayshree, 2017). *A. dubius* has been reported to control inflammation (Tufts *et al*., 2015), while *A. spinosus* was reported to control oedema (Olajide *et al*., 2004). Additional finding in this study was that red amaranth can be charred and ground to cure coughs and tonsils. The charred powder from red amaranth can also be used to cure external wounds. Red amaranth was used to make *ugoro* (tobacco snuff) – charred, mixed with wood ash and dissolved in water. This was then mixed with ground tobacco to make it stronger/bitter.

Apart from their use as food and medicine, amaranth leaves, grains and stalk also have other non-food uses. Excess produce, especially during rainy seasons can be used as animal feed (cattle and chicken), to make organic manure and the stalks can also be used for firewood. Although farmers feed amaranth to animals solely to reduce loses, use of the leaves and grains of amaranth have been reported to lead to better health and production in several farm animals (Manyelo *et al*., 2020).

5.3.4 Beliefs and taboos

Some positive beliefs contribute to increased consumption. For instance, amaranth is believed to 'add blood' in the body, contribute to good eyesight, eliminate marasmus in children, boost milk production for breast feeding mothers and helps in cleaning the kidney. "*We rarely have low HB cases here because we encourage the consumption of amaranth, we rarely even give iron supplements*" said a nutrition key informant in Nyamira county. "*If you eat a lot of amaranth, there's no day you will be told you don't have enough blood*" said a respondent in Kilifi. Despite its availability and nutritional attributes, amaranth vegetable still remains underexploited, and this can partly be attributed to certain negative beliefs about the consumption of the vegetable or use of some species.

Amaranth is a food for the poor. While amaranth is may be considered as worth a super food (Olusanya *et al*., 2021), the vegetable being poor mans' food as reported during the study is an indication that the information on the nutritional importance of the vegetable is still needed, "*we still have a hard time convincing people in the rural areas that these vegetables are better than exotic, the urban people have quite changed*" said a key informant in Vihiga. At a focus group discussion in Kwale County in Kenya, the community members stated they eat a lot of amaranth vegetables because they are poor and cannot afford more precious foods. "*Many eat amaranth because they are poor, if you give me a lot of money now, I will not run to buy amaranth, you do not need money to get amaranth. Very few eat it for nutritional benefits*" said a respondent in Kwale County. In some FGDs in Tanzania, it was also reported that eating amaranth as the main dish is a sign of poverty, and the rich can only use it as a side dish. Other studies have reported that a few members of the community associated the vegetables with low class people and poor households (Chacha & Laswai, 2020). Most respondents reported that they would not freely serve amaranth meal to visitors, at it can show that they lacked proper food to serve. "*If you serve amaranth vegetables only to a visitor, you will be seen as if you don't have anything*" said respondents in Mwanza. In a study on knowledge of indigenous vegetables in three counties in Kenya it was reported that most respondents disagreed that the vegetables are poor people's food, food for older generation or old fashioned food (Ntawuruhunga *et al*., 2020). The study also reported high positive attitudes towards AIVs in Busia, Nyamira and Machakos counties of Kenya.

The first sprouts of amaranth at the start of rainy season are believed to cause stomach upset in some individual. This was reported in 5 FGDs in Kenya. While this was not reported in Tanzania, a case of red amaranth causing bloody diarrhea was mentioned. In western Kenya, there is some skepticism about growing and consuming the grain/dual purpose types of amaranths, e.g., *A. hypochondriacus*. This is because when this type was introduced in the region, seeds were given to HIV/AIDS patients.

Therefore, the grain/dual purpose amaranths are still being stigmatized. Red amaranth (*A. hybridus*) is not consumed in certain parts of Kenya, because it is believed to causes fights/wrangles in homes when cooked in the home (*mboga ya fitina/ troublesome vegetable*). This belief was very evident in the two FGDs that were conducted in Nyamira county in Kenya. "*That vegetable, if you just cook and eat in your house, troubles and quarrels will just start and you will not even understand why*" said a respondent in Nyamira. The key informants also confirmed that this belief exists, but no one really understands how the vegetable is related to household wrangles.

Some taboos related to harvesting of amaranth were also reported. For instance, it is a taboo for a woman who gave birth, and the baby came out legs first, to harvest the vegetables as the vegetables will dry up. Another one was that if a baby develops upper teeth first before the lower teeth, then the mother cannot harvest vegetables as they will dry up mysteriously. Certain members of the community are not allowed to harvest vegetables, as they were believed to have some curse that destroys vegetable farms. It was also believed that some harvesting containers affect growth of the vegetables – the vegetables will not grow well if harvested vegetables are put in a tray (*uteo*) or wrapped in a cloth. "*Since the tray is for threshing, if you use it for harvesting, you are chasing away the vegetables*" said an FGD respondent in Kilifi. It was also believed that vegetables should not be harvested by pinching – a knife was to be used to cut off the tender stems. It was also believed that allowing different people in the vegetables garden can cause drying – one should harvest his/her own vegetables, even if they are for somebody else to use.

5.3.5 Preservation

In most study areas, preservation of amaranth was not practiced. The major reason given by the respondents for not preserving is that the vegetable is readily available in most seasons of the year. In Nyamira County in Kenya, the respondents reported that a few people dried the vegetables, solely to send to their relatives living in overseas countries. The absence or limited practice of preservation of the vegetable leads to a lot of wastage due to postharvest losses especially during seasons of vegetable abundance. In a few areas, there are recent training on preservation; "*We have a project where we are trying to build capacities on vegetable preservation. Currently very few are doing preservation*" said a key informant in Bungoma. For most vegetable traders, the usual practice, which has also been reported in other studies, is sprinkling of water to maintain the freshness as long as possible (Gogo *et al*., 2018). The lack of preservation further leads to low consumption of the vegetables in dry seasons, when the vegetables availability gets very low and the prices high.

5.3.6 Challenges and Ways of improving production and consumption of amaranth vegetables

Most of the challenges put across during this study were production related, with a few consumption-related challenges (Table 5.3). These challenges were mentioned in almost all the FGDs and KIIs, an indication that these challenges cut across all the counties and regions in Kenya and Tanzania.

Majority of the respondents identified lack of quality seed of desirable varieties as one of the main challenges restricting cultivation of amaranth. In Vihiga County, Kenya, several respondents noted that the seed of *A. blitum*, which is the most preferred in the region, is very scarce and expensive when found, "*I think we lost touch with seed producers and multipliers, so this specie is slowly disappearing; but it is the one that is most preferred by our farmers*" said a key informant in Vihiga, Kenya. This is aggravated by the fact that this vegetable is mostly harvested by uprooting, and very rarely left to produce seeds.

Table 5.3: Challenges and possible solutions

Challenge	Solutions suggested by respondents
Lack of quality seed of preferred varieties	Researchers to work with seed companies on seed availability and qualities such as productivity, pest resistance, flood and drought resistance
Lack of markets in seasons of abundance	Improve market structure to ease marketing Train on vegetable preservation and value addition
Low vegetable volumes in dry seasons	Train on water conservation and kitchen gardening
Pests and diseases	Training on pest control and safe pesticide use
Amaranth causes stomach upset in some people	Training on proper preparation and cooking
Vegetable safety concerns	Training on good agricultural practices and safe pesticide use
Little information on nutritional value and nutrient conservation in the vegetable	Return Agri-nutrition department in local governments

In Dar-Es-Salaam in Tanzania, it was also noted that not many seed companies have taken up the production and supply of indigenous vegetable seeds including amaranth. Lack of market during rainy seasons when the vegetables are abundant, which is coupled with very low prices during these seasons is the reason why some farmers have been demoralized and have abandoned the production of these vegetables. Training on reduction of these losses is important in dealing with this problem. The challenge of pests and diseases especially in dry seasons has caused some farmers to use a lot of pesticides on these vegetables, and in most instances, the postharvest interval is not adhered to. The farmers therefore require training on how to produce these vegetables organically and on proper use of pesticides. Lack of water for some people during dry seasons affects both production and consumption. Farmers either have to stop production or g*et al*ternative water sources. The production is also affected by pests. Prices of vegetables then go up which affects availability and affordability, leading to reduced consumption. Despite the effect of drought on the vegetables, it was noted that the vegetables would still be available in dry seasons. Similar findings have also been reported in other parts of Africa (Dlamini & Viljoen, 2020; Omotayo *et al*., 2020), insinuating the drought tolerance of the vegetables. Other consumption related challenges were stomach upsets for some people caused by red amaranth hence hindering its consumption; "*this red one if you eat a lot, you'll really have diarrhea*" said a respondent in Kiambu County. Some respondents also reported that they got stomach upsets when they consumed a lot of the vegetables at the beginning of the rainy seasons. Some people around urban centers produced the vegetables using dirty water, creating fear of consumption among consumers in these areas. "*When your vegetables have large leaves, buyers can suspect you have done sewage farming, even when you haven't*" said a key informant in Kiambu County. Most key informants in Kenyan counties, reported that changes in local government structures had destabilized most nutrition related extension work. It was further observed that in some cases, crucial departments which synergized agriculture and community nutrition were removed, and this had created a big challenge in agri-nutrition information transfer. "*As crops department, we used to work with nutritionists, but the county governments came with different priorities, and our nutritionists were taken to health department; we hoped to have home economics, but they are busy with other areas in meat and fisheries*" said agricultural key informant in Kenya.

Besides these challenges, preference for exotic vegetables caused underproduction and underutilization of amaranth vegetables. This was coupled with social changes that led to loss of traditional food knowledge, as well as the transition of the dietary lifestyles to fast foods especially in younger generation, who preferred sugary, fatty and salty tastes of snacks and fast foods (Mayekiso *et al*., 2017; Olusanya *et al*., 2021; Taleni & Goduka, 2013). Novel preparation methods that encourage consumption of amaranth based foods is therefore recommended.

Training on the nutritional importance of the vegetable amaranths was the most requested information in all the areas where the study was carried out. "*Some individuals harvest the vegetables and leave them in the sun, not knowing the effect of that on the nutrients*" said a key informant in Dar-es-Salaam, Tanzania. Face to face trainings coupled with demonstrations were reported as the most preferred modes of receiving information in all the FGSs and KIIs.

5.4 Conclusion

From this study, preference for indigenous vegetables was found to differ with region as well as individuals. Amaranth was shown to be a common vegetable in Kenya and Tanzania. Though many species of amaranth are known to people, *A. dubius* and *A. blitum* were the most common for vegetable consumption in Kenya, while *A. dubius* and *A. hypochondriacus* were the most common in Tanzania. Red amaranth and the spiny amaranth were less preferred in all the regions. The preference for species is mostly based on taste and availability. Consumption of amaranth vegetables was generally reported to be increasing, with many people growing it both for sale and home consumption. Some people also still wait for it to self-replicate as a weed, while others buy it from the markets. Cooking methods varied; and included boiling and frying. Most people considered taste rather than nutrition quality when cooking, hence some methods led to reduced nutritional value. Most people also consumed the amaranth because it was cheap, available, or because of circumstances, and would not eat the vegetable if they had a better option. The major constraints to utilization were low availability in dry seasons, pests, lack of quality seed in some places and low shelf life. The study pointed out some cooking practices that destroy nutrients as well as some negative beliefs and attitudes in a few areas. Training is needed on nutritional importance and conservation of the vegetables.

CHAPTER SIX

ENHANCING THE NUTRITIONAL QUALITY OF AMARANTH THROUGH SPECIFIC FOOD PREPARATION METHODS

Summary

Food preparation methods applied to African traditional vegetables vary greatly depending on preferences of various consumers. The amaranth vegetable is one of the most commonly consumed vegetable, with high nutritional quality. The bioaccessibility of some minerals such as iron is, however, low since it is non-heme, and is also bound by anti-nutrients such as oxalates. This study aimed at evaluating the nutrient retention of amaranth vegetable dishes prepared using selected Kenyan traditional recipes, and to enhance the iron bioavailability of amaranth dishes using food preparation methods. Nutrient retentions of amaranth prepared by three common food methods were analyzed. In-vitro iron bioavailability of amaranth dishes with or without bioavailability enhancers as well as an amaranth meal incorporating a common maize meal staple food was also studied. The nutrient retentions of the various dishes used in this study was fairly high with at least 85% retention of minerals and an increase of up to 45% in three carotenoids. The increase in carotenoids could be due to cooking and also use of oil in cooking. It can be concluded that incorporating vitamin C, adding an iron rich vegetable and boiling of the vegetable significantly improves the iron bioavailability and hence the iron uptake by the body. Incorporating lemon juice enhanced dialysable iron of the selected recipe by up to 66%. There was no significant $(P \leq 0.05)$ effect by the amaranth components on the iron bioavailability of ugali. These methods could therefore be incorporated into household recipes to increase micronutrient intake.

6.1 Introduction

The genus *Amaranthus* consists of many species, which are often considered as pseudo-cereals in Europe and America, but are mostly grown as vegetables in Africa (Achigan-Dako *et al*., 2014). It is considered as one of the African indigenous vegetables. The edible parts of the plants range from seeds, leaves and tender shoots. It is one of the most commonly consumed African indigenous vegetables in Kenya, East Africa and other parts of Africa (Kansiime *et al*., 2018). It is a cheap source of micronutrients that can contribute to reduced cases of micronutrient malnutrition. Apart from being a rich source of most micronutrients, amaranth is also a source of phytochemicals that are useful to the human body. In recent years, its production has risen from that of a subsistence crop, to a commercial crop, finding itself on the shelves of most supermarkets in urban areas. In some case, supply cannot match demand (Cernansky, 2015). Its tender leaves and stem are used in many countries in Africa in the form of infusions, salads, sauces, soups; singly or mixed with other vegetables (Achigan-Dako *et al*., 2014).

This vegetable is, however, not consumed raw. It goes through food preparation methods which vary among consumers based on convenience and taste preferences rather than nutrient retention (Hossain *et al*., 2017). Preparation of these vegetables is done in different ways, according to the traditional recipes and culinary traditions of different communities (Musotsi, 2017). The general preparation process for African Indigenous vegetables involve sorting, destalking, washing and sometimes cutting (Musotsi *et al*., 2017), followed by varied cooking durations with unclear effects on the nutritional quality. These cooking methods induce a series of changes in physical properties, chemical properties and enzymatic modifications in various foods (Rothwell *et al*., 2015), affecting concentration and bioavailability of nutrients. Findings by different researchers on phytochemical and biological changes during cooking have been inconsistent and sometimes contradictory (Zhao *et al*., 2019). In order to maximize the nutritional benefits from amaranth vegetables, it is important to subject them to a cooking method that results in optimal nutrient retention and bioavailability (Habwe, 2012).

Despite the notable increase in consumption of indigenous vegetables including amaranth, micronutrient malnutrition still remains a public health problem in several parts of Africa, the most affected being children and women of child bearing age. In Kenya, 27.2% of women in reproductive age have anemia (Global Nutrition Report, 2019). In some areas of Kenya 76% of children have been reported to have been anemic at least at one point since birth (Kao *et al*., 2019). This hidden hunger could be linked to the high rates of morbidity and mortality among children and women especially in rural areas.

Though the common approach for combating iron deficiency anemia comprises supplementation and food fortification, diet modification and proper food preparation methods would be a cheaper and more sustainable approach. Modification of the diet may be done to improve nutritional value of common dishes and iron bioavailability. These may be through providing dietary information to include iron rich foods and improve cooking skills (Lion *et al*., 2018). Amaranth vegetable as a good source of dietary iron, can contribute to addressing the challenge of iron deficiency anemia. However, the bioavailability of the iron in amaranth is low due to its non-heme nature, as well as the presence of anti-nutrients such as oxalates which bind iron. This results in decreased absorption of most of the iron contained in the vegetable in the upper gut. Bioavailability of iron from a food source in the body is usually influenced by enhancers and inhibitors (Kapil, 2017). It has been noted that iron absorption in the upper gut can range from 1% to 40%, and this can be doubled with certain cooking practices or by changing the composition of the meal such as by addition of other vegetables and fruits containing ascorbic acid (Kapil, 2017).

The aim of this study was to evaluate the nutrient retention of amaranth vegetable dishes prepared using some traditional recipes, and also to enhance the iron bioavailability of amaranth dishes using food preparation methods.

6.2 Materials and Methods

6.2.1 Experimental design and treatments

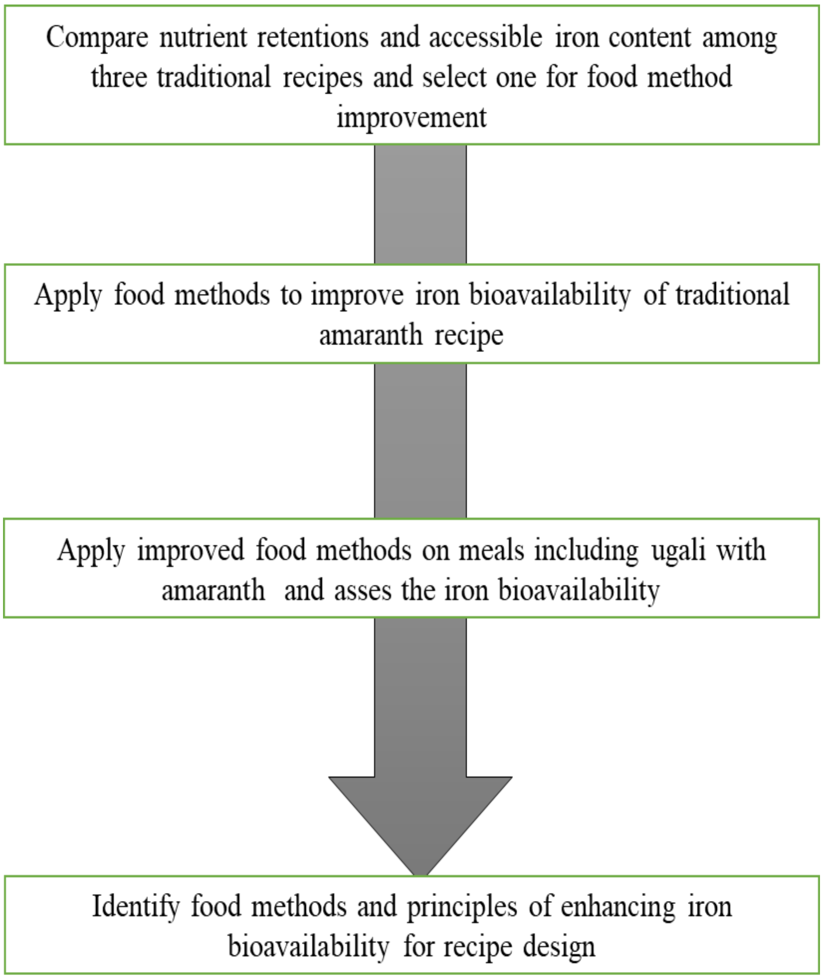

Figure 6.1: Summary of the study flow

The study was carried out sequentially in three stages, involving different treatments at each stage. In the first stage, three traditional cooking methods were selected based on literature (Faber et al., 2010; Musotsi et al., 2005; Musotsi et al., 2017; Oluoch et al., 2012) . Freshly harvested leaves were prepared using three (3) traditional recipes and fresh leaves (uncooked) as well as a mixture of the common ingredients used as control. The effect of cooking on retention of vitamins, minerals and oxalates, as well as the effect of cooking on iron bioavailability was determined. Thereafter, one of the selected recipes was chosen for improvement through use of iron bioavailability enhancers including high ascorbic acid ingredients (Blanco-Rojo & Vaquero, 2019). The effect of boiling as well as addition of iron rich vegetables to amaranth iron bioavailability was also studied. At the third stage, the iron bioavailability of the traditional and improved cooking methods was analyzed in combination with a common staple, maize meal "ugali" (thick porridge).

6.2.2 Experimental material

This study was carried out using three varieties of amaranth; Madiira 1(*A. cruentus*), Madiira 2 (*A. cruentus*) and AH-TL-Sel (*A. hypochondriacus*). These are improved varieties that were developed by World Vegetable Centre and released in Kenya and Tanzania. The vegetables were grown in the open field in Shanhua, Taiwan. Planting was done uniformly in trays and later transplanted into the open field after three weeks. Later the leaves were harvested uniformly at six weeks after planting, just before flowering. The edible parts (leaves and tender stems) were then separated and the vegetables from the three varieties mixed in the ratio 1:1:1. Other ingredients used in recipe preparation including onions, tomatoes, lemons and soybean oil were obtained from local supermarket in Shanhua, Taiwan. Moringa leaves were obtained from the World Vegetable Center in Taiwan, while maize flour was obtained from Kenya.

6.2.3 Chemicals

All reagents used were of analytical reagent (AR) grades. They included pepsin (P-7000, from porcine stomach mucosa), hydrochloric acid, pancreatin (P-1750, from porcine pancreas), bile extract (B-8631, porcine), Sodium hydrogen carbonate, sodium hydroxide, trichloroacetic acid (TCA), 4,7-diphenyl-1,10-phenanthroline disulfonic acid, hydroxylammoniumchloride, sodium acetate, H_2SO_4, standard oxalic acid, 2,4-dinitrophenylhydrazine (DNPH), 2,6-dichlorophenolindophenol (DCPIP), metaphosphoric acid thiourea standard L-(+)-ascorbic acid, Acetone, ethyl ether, methanol, potassium hydroxide, hexane, Tetrahydrofuran (THF). The chemicals were purchased from Merck and Sigma–Aldrich Co. (St. Louis, MO, USA).

6.2.4 Preparation of samples

The fresh vegetables were destalked to separate edible portions comprising leaves and tender stems. All the vegetables used in the different experiments were washed with water then rinsed with distilled water. Onions and tomatoes were chopped into small pieces which were used for frying the vegetables. All the cooking was done in stainless steel pans over uniform heat on an electric cooker. The recipes in the study are shown in Table 6.1.

Table 6.1: Recipes used in the study

Sample	Description/ Preparation process
Experiment 1: Nutrient retention and iron bioavailability among recipes	
Amaranth Raw	These were freshly harvested amaranth leaves which were used as control reference in the study
Mixed ingredients	This was the uncooked control consisting of a mixture of amaranth leaves, onions, tomatoes, and oil mixed together but with no cooking. The ratios of ingredients were the same as that used for the cooked samples. The mixture was blended to ensure homogeneity
Recipe 1	Approximately 200 g of fresh whole leaves (not chopped) were placed in 200 g of boiling distilled water in a cooking pan. This was covered and let to boil for 10 minutes, stirring every 3 minutes. The boiled vegetables were then stir-fried without straining in 10 g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes.
Recipe 2	Approximately 200 g of fresh vegetables were chopped to medium size (about 1cm wide) and then placed in 200 g of boiling distilled water in a cooking pan. This was covered and let to boil for 10 minutes, stirring every 3 minutes. The boiled vegetables were then stir-fried without straining in 10g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes
Recipe 3	Approximately 200 g of fresh vegetables were chopped to medium size (about 1cm wide) and stir-fried in 10 g non-fortified soybean oil, 25 g of chopped red bulb onions and 25 g chopped tomatoes for five minutes. This was without boiling.
Experiment 2: Enhancing iron dialysability	
Improved Recipe 1 with more tomato	This was prepared as the Recipe 1, with double the quantity of tomatoes (50 g of chopped tomatoes). Components of tomatoes including vitamin C and lycopene have been reported to have positive effects on iron bioaccessibility (Garcia-Casal, 2006; Singh *et al*., 2016).
Improved Recipe 1 with Lemon Juice	This was prepared as Recipe 1, with one tablespoon (10 mL) of freshly squeezed lemon juice added. Lemon juice has been shown to increase iron bioavailability in foods (Singh *et al*., 2016), and this is attributed to its ascorbic acid as well as other components
Samples for boiling effect experiment	Two samples of 100 g fresh amaranth leaves were boiled in 200 mL distilled water for five minutes. After boiling, excess water was discarded in one sample. This was also done with another set of samples, with a boiling time of 15 minutes. Fresh amaranth leaves were used as control.
Samples for inclusion of moringa	Three different samples of 100 g of amaranth, 100 g of fresh moringa leaves, and 100 g of a mixture of amaranth leaves and moringa leaves (50:50); were boiled separately in 200 mL distilled water for 5 minutes. The remaining water was not discarded. Dialysable iron was determined in these samples together with fresh amaranth and fresh moringa leaves to determine the effect of adding moringa, a high iron vegetable, on dialysable iron of an amaranth dish.
Experiment 3: dialysability of iron in amaranth meal combination	
Amaranth meals	Recipe 1 (Traditional recipe) and improved Recipe 1 with lemon were prepared. "*Ugali*" (thick porridge) was prepared using iron fortified maize flour from Kenya (Jogoo Maize Flour). Dialysable iron was determined for the two recipes, ugali, and a combination of ugali with each of the dishes in a ratio of 1:1.

6.2.5 Nutrient Retention Analysis

Determination was done for the nutritional components of the amaranth recipes as well as a mixture of all the ingredients (uncooked), and this was compared with the fresh amaranth sample. Components analyzed included oxalates, vitamin C, minerals (Ca, Fe and Zn) and carotenoids (violaxanthin, lutein, α-carotene and β-carotene)

Determination of Oxalates

Determination of oxalates was done by HPLC (Libert, 1981) with modifications suggested by Yu *et al*, (2002) as described in 3.2.4.

Determination of Vitamin C

The vitamin C content was determined based on coupling 2,4-dinitrophenylhydrazine (DNPH) with ketonic groups of dehydroascorbic acid through the oxidation of ascorbic acid by 2,6-dichlorophenolindophenol (DCPIP), (Hanson *et al*., 2004) as described in 3.2.4.

Determination of Carotenoids

Determination of carotenoids was done by HPLC (Rodriguez, 2001) as described in 3.2.4.

Determination of Minerals

Minerals were determined by strong acid digestion method and atomic absorption spectrophotometer (AAS) (AOAC, 2016) as described in 3.2.4.

6.2.6 Analysis of Iron Bioaccessibility

In-vitro iron dialysability assay

Dialysability of iron was determined using in vitro dialysability method (Luten *et al*., 1996) with simulated peptic and pancreatic digestion. A pepsin solution was prepared by dissolving 16 g of pepsin (P-7000, from porcine stomach mucosa) in 100 mL of 0.1 M HCl. The pancreatin solution contained 4 g of pancreatin (P-1750, from porcine pancreas) and 25 g of bile extract (B-8631, porcine) with 1000 mL of 0.1 M $NaHCO_3$.

The sample dry matter content was adjusted to 5% using distilled water, then homogenized. The pH of homogenized sample was adjusted to pH 2.0 with 6 M HCl, then 20 g was weighed into 125 mL conical flasks in three replications. Distilled water was also weighed in the same manner to act as blank.

Peptic digestion - To 20 g of weighed samples, 0.75 mL pepsin solution was added. The mixture was covered well using parafilm and incubated at 37°C for 2 h with shaking.

Titratable acidity - To one replication of each digested sample, a titration was performed in which 20 mL of gastric digest was mixed with 5 mL of pancreatin-bile suspension and the amount of 0.5 M NaOH needed for this mixture to achieve a pH of 7 ± 0.05 was determined.

Pancreatic digestion - Segment of dialysis tubing (6-8 cm) was soaked in distilled water for about 30 minutes. A solution of 0.5 M $NaHCO_3$, being equivalent to the volume of 0.5 M NaOH needed for the pancreatic digestion (titratable acidity), was made up to 25 mL with distilled water. These solutions were transferred into the dialysis tubes, tied on both sides, then placed into the conical flask containing gastric digest and incubated for 30 minutes at 37^0C. After 30 minutes, 5 mL of pancreatin-bile mixture was added and the incubation continued for another 2 hours. The dialysis tubes were then removed, washed in distilled water and the contents weighed. To 5 mL of dialysate, 2.5 mL of protein precipitant containing 10% TCA and 10% HCl in distilled water was added, heated in boiling water bath for 10 minutes and centrifuged at 10000 rpm for 5 minutes.

To determine the dialysable iron, 3 mL of the supernatant was reacted with 2 mL of Bathophenanthroline reagent, containing 0.025% of 4,7-diphenyl-1,10-phenanthroline disulfonic acid and 10% hydroxylammoniumchloride in 2 M sodium acetate. The mixture was let to stand for 15 minutes before reading at OD 535 nm. Iron standard was used for calibration.

6.2.7 Data analysis

The data collected was subjected to Analysis of Variance (ANOVA) using Genstat statistical software. Separation of means for the various treatments was done using Duncan's Multiple Range Test (DMRT).

6.3 Results

6.3.1 Nutrient and oxalate retention of the amaranth dishes

The nutrient contents of the various recipes were calculated on dry weight basis. The change in nutrient content was expressed as percentage of the mixed ingredients in raw form.

Table 6.2: Amounts of oxalates and Vitamin C in the recipes per 100 g DW

SAMPLE	OXALATE (mg)	VITAMIN C (mg)
Amaranth raw	2943[c]	524.20[c]
Mixed Ingredients	1720[b]	244.93[b]
Recipe 1	1592[a]	146.45[a]
Recipe 2	1605[a]	172.98[a]
Recipe 3	1677[b]	173.99[a]
LSD	60.68	46.11

Values are presented as Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD= Least Significant difference at 5% level of significance*

There were significant $(P \leq 0.05)$ differences in the oxalate contents of the various samples. The level of oxalates was lower in the cooked samples compared to the raw samples, with the lowest amounts detected in Recipe 1 (Table 6.2). Compared to the uncooked mixed ingredient recipe, cooking reduced the oxalate content. Recipe 3, where there was no boiling, retained the highest oxalate content.

There was a reduction in vitamin C content in all the recipes. The amounts in the three recipes were, however, not significantly $(P \leq 0.05)$ different. The vitamin C in the mixed ingredients was much lower than in fresh amaranth. This may be due to the loss of the vitamin through oxidation.

Four carotenoids were detected in the samples including violaxanthin, lutein, alpha carotene and beta carotene (Table 6.3). The different carotenoids were affected differently by the different cooking methods.

Table 6.3: Carotenoids content in the recipes in 100 g DW

SAMPLE	**Violaxanthin (mg)**	**Lutein (mg)**	**α–Carotene (mg)**	**β –Carotene (mg)**
Amaranth raw	8.37^{c}	47.64^{c}	2.39^{a}	22.65^{c}
Mixed Ingredients	4.06^{b}	23.52^{a}	1.93^{a}	12.65^{a}
Recipe 1	0.00^{a}	33.51^{b}	2.35^{a}	18.20^{b}
Recipe 2	0.00^{a}	34.29^{b}	2.38^{a}	18.13^{b}
Recipe 3	3.30^{b}	31.75^{b}	2.60^{a}	16.77^{b}
LSD	0.93	3.96	0.62	2.03

*Values are presented as Mean, **n = 3.** Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD= Least Significant difference at 5% level of significance*

Violaxanthin was completely destroyed in the recipes that involved boiling, while the contents of the other carotenoids significantly increased in the three recipes when compared to the mixed ingredients in raw form.

There were no significant ($P \leq 0.05$) differences the amounts of the various minerals among the three cooked recipes (Table 6.4). The calcium content was slightly higher in all the recipes compared to the uncooked mixed ingredients, while iron and zinc contents were lower.

Table 6.4: Mineral contents of recipes in 100 g DW

SAMPLE	CALCIUM (mg)	IRON (mg)	ZINC (mg)
Amaranth raw	2403.09^{c}	30.15^{b}	4.42^{b}
Mixed Ingredients	1218.69^{a}	14.72^{a}	2.36^{a}
Recipe 1	1322.81^{ab}	13.82^{a}	2.01^{a}
Recipe 2	1418.10^{b}	12.59^{a}	2.12^{a}
Recipe 3	1309.35^{ab}	13.91^{a}	2.30^{a}
LSD	141.10	4.00	0.43

*Values are presented as Mean, **n = 3.** Means within the same column with different superscripts were significantly* ($P \leq 0.05$) *different. LSD= Least Significant difference at 5% level of significance*

However, these differences were not significant. Recipe 3 showed the lowest losses of iron and zinc.

6.3.2 Iron Bioaccessibility in the recipes

The bioaccessible iron was calculated as the dialysable iron percentage of the total iron in each sample.

$$\%\ Bioaccessibility = \frac{Dialyzable\ iron}{Total\ iron} \times 100\%$$

Table 6.5: Percentage Iron Bioaccessibility of the dishes

SAMPLE	% Bioaccessibility
Amaranth raw	11.02^{a}
Mixed Ingredients	17.53^{b}
Recipe 1	21.92^{bc}
Recipe 2	24.22^{c}
Recipe 3	21.59^{bc}
LSD	5.89

Values are presented as Mean, ***n = 3.*** *Means within the same column with different superscripts were significantly* $(P \leq 0.05)$ *different. LSD= Least Significant difference at 5% level of significance*

The three preparation methods/recipes had higher iron dialysability compared to the raw sample. There were however no significant differences in iron dialysability among the dishes (Table 6.5).

6.3.3 Enhancement of dialysable iron in amaranth

To improve dialysable iron and improve iron delivery from amaranth meals, further experiments were carried out to identify the effect of preparation methods and some ingredients in enhancing iron dialysability in amaranth dishes. Recipe 1 was chosen to act as control in the enhancement study. This recipe was chosen randomly since there was no significant difference in the bioaccesssible iron among the three recipes. The recipe also had significantly low amount of oxalates.

The methods that were considered for enhancement in this study were inclusion of high ascorbic acid ingredients as ascorbic acid enhances iron bioavailability; boiling (Blanco-Rojo & Vaquero, 2019; Nomkong *et al*., 2019; Singh *et al*., 2016); and cooking the amaranth in combination with high iron vegetables as a way of increasing the total iron content.

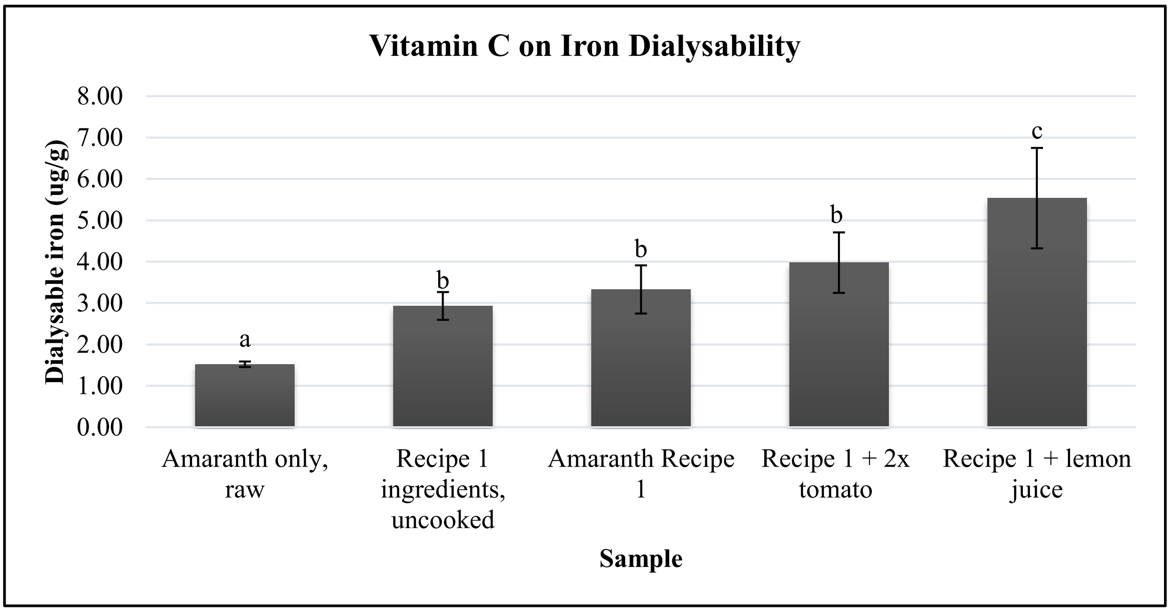

Figure 6.2: Effect of addition of Vitamin C rich ingredients on dialysable Iron of Amaranth recipes

Values are presented as Mean, ***n = 3.*** *Means with different superscripts were significantly* $(P \leq 0.05)$ *different.*

The first experiment involved the addition of lemon juice, which is a source of vitamin C into the dish. Recipe 1 from the first experiment was chosen for this improvement. One recipe was made by doubling the amount of tomatoes used in the Recipe1 (Recipe 1+2X tomato), while another recipe was made by adding 10 mL fresh lemon juice to the Recipe 1 (Recipe 1 + lemon juice), and comparing against the original Recipe 1.

The addition of lemon juice, a source of vitamin C had a positive effect on iron dialysability (Figure 6.2). Increase in the dialysable iron due to doubling the tomato content was, however, not statistically significant $(P \leq 0.05)$ compared to the control recipe (Recipe 1). Adding lemon juice significantly $(P \leq 0.05)$ enhanced dialysable iron of the traditional recipe (Recipe 1), compared to the raw amaranth. Lemon juice enhanced the dialysable iron by over 200% in comparison to the raw amaranth leaves, while the increase was 66% higher than the traditional recipe.

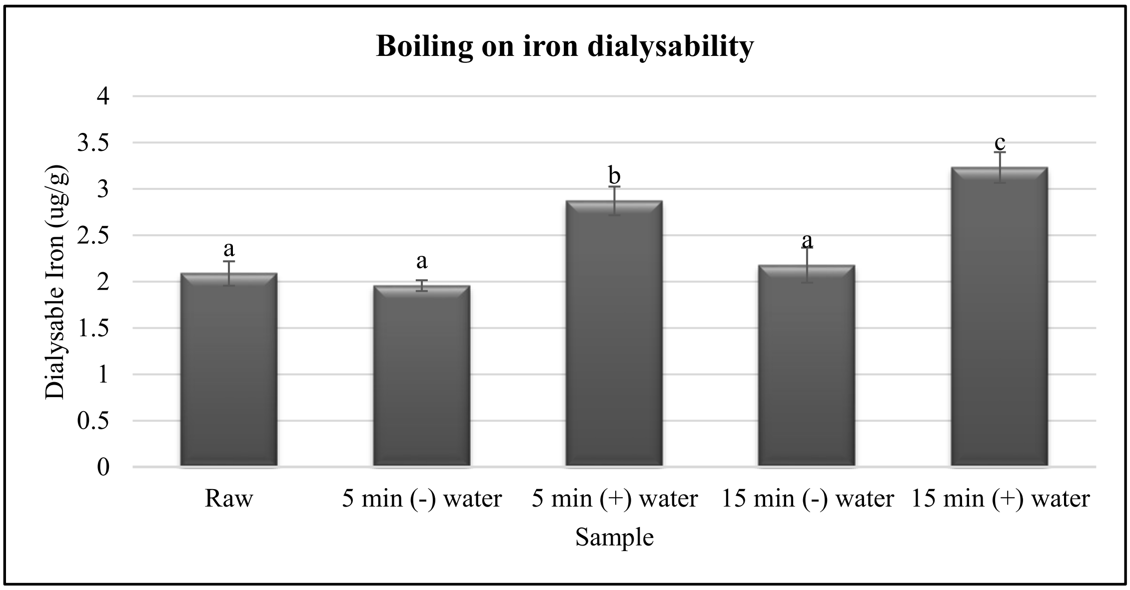

Figure 6.3: Effect of boiling and removal of excess water on iron dialysability of Amaranth

Values are presented as Mean, $\boldsymbol{n = 3}$. *Means with different superscripts were significantly* $(P \leq 0.05)$ *different.*

The effect of boiling on the iron dialysability of amaranth was then determined. Studies on other vegetables have shown that boiling can enhance bioavailability of iron (Nomkong *et al.*, 2019). While boiling led to significant increase in dialysable iron, discarding excess water reduced the dialysable iron content. This could be attributed to the leaching of iron in the discarded water. Longer boiling (15 minutes) times enhanced dialysable iron compared to shorter boiling time (5 minutes). There was no significant difference in dialysable iron in the raw amaranth compared to the boiled amaranth with discarded water (Figure 6.3)

Addition of moringa leaves also enhanced the dialysable iron of the amaranth. This could be partly because moringa has higher dialysable iron, and also because it contains higher amounts of vitamin C, which is an enhancer (Gopalakrishnan *et al*., 2016; Shija *et al*., 2019).

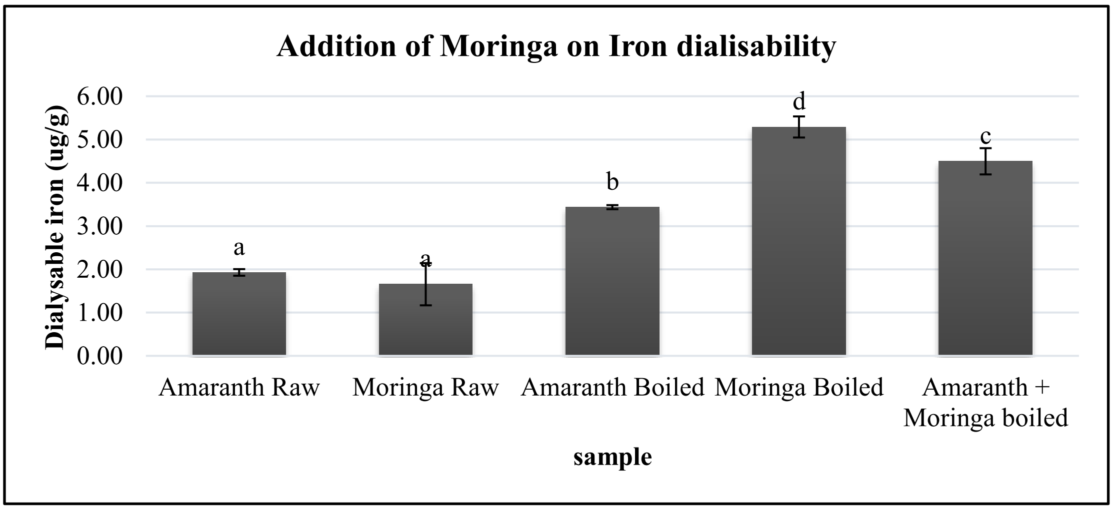

Figure 6.4: Effect of including Moringa leaves on iron dialysability of Amaranth dish

Values are presented as Mean, ***n = 3.*** *Means with different superscripts were significantly* $(P \leq 0.05)$ *different.*

The quantity of dialysable iron when the traditional recipe (Recipe 1) and improved recipe (Recipe 1 one with lemon juice) is combined with a common staple, Maize meal/Thick porridge (*"ugali"*) was then determined. These were mixed in the ration of 1:1. This was to evaluate if the biochemical components of the cooked amaranth had any effect on the dialysable iron of the maize meal, which was iron fortified.

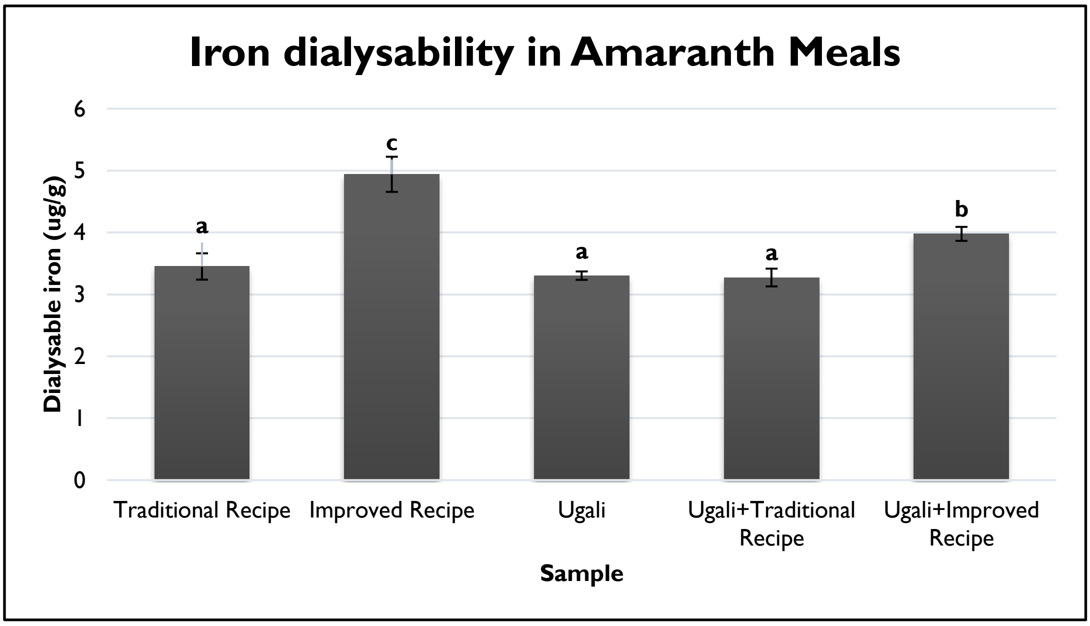

Figure 6.5: Iron dialysability in traditional recipe and improved recipe in combination with maize meal

Values are presented as Mean, ***n = 3.*** *Means with different superscripts were significantly* $(P \leq 0.05)$ *different.*

The improved recipe with lemon juice was used for this study in comparison with Recipe 1, and each was mixed with *"ugali"*. Meal combination with improved recipe had significantly higher dialysable iron, at 21% more than the meal combination with the traditional recipe.

6.4 Discussion

African traditional vegetables form an important part of most diets in rural parts of Africa. Low consumption of micronutrient rich foods including green leafy vegetables has been reported to be associated with the incidence of mineral deficiencies. The minerals whose deficiency is of utmost public health concerns in East Africa are iron (Fe), calcium (Ca) and zinc (Zn) (Singh & Prasad, 2018). Despite the content of micronutrients in these vegetables, the preparation methods can greatly affect their bioavailability in the human body. Some anti-nutrients present in these vegetables such as oxalates may also bind to minerals hence reducing their bioavailability.

Oxalate, a major anti-nutrient in amaranth vegetables is not known to be heat sensitive; and its main reduction strategy could be by boiling and discarding of boiling water (Akhtar *et al*., 2011). This method can, however, be counter-productive as most minerals and vitamins are also lost in the process. Recipe1 and Recipe 2 in this study, both of which involved boiling, showed statistically significant reduction in the oxalate content (Table 6.2). Reduction of oxalates due to cooking of vegetables has also been reported in other studies (Akhtar *et al*., 2011; Bayengue *et al*., 2017). The amount of oxalate in these two recipes were not significantly different, showing that there was no effect of chopping on the oxalate contents. The main effect of concern from oxalate is the interference with bioaccessibility of minerals including iron and calcium in the body. Even though many people can endure normal amounts of oxalate rich foods, people with certain conditions such as enteric hyperoxaluria need to lower their oxalate intake (Popova & Mihaylova, 2019). In sensitive people, even small amounts of oxalate can cause burning in the eyes, ears, mouth and throat; while large amounts may cause abdominal pain, muscle weakness, nausea and diarrhea (Natesh *et al*., 2017).

During cooking, some nutritional components are reduced, which are mainly the vitamin components. For instance, a study conducted by Traore *et.al*., showed that boiling amaranth for 30 minutes completely destroyed the beta carotene in the vegetables (Traoré *et al*., 2017). All the recipes used in this study led to significant changes in the nutritional attributes of the dishes.

In this study, combination of size reduction or lack of it (chopping) with or without boiling (Table 6.2 and 6.3) retained statistically similar content of vitamin C, Iron and Zinc. This would have been due to the effect of the mixed ingredients which are known to contain these nutrients

Vitamin C was significantly reduced in the recipes compared to the raw ingredients by up to 40%. The retention of vitamin C in recipes of this study was, however, much higher than those reported in other indigenous vegetable dishes where 70% and 93% losses were reported in African nightshade and spider plant, respectively (Musotsi *et al*., 2019). Vitamin C is easily lost during cooking since it is water soluble and temperature sensitive so it is easily degraded during cooking. Vitamin C cannot be synthesized by the body and therefore must be obtained from the diet (Singh & Prasad, 2018), vegetables forming part of the dietary sources. The vitamin is a cofactor in numerous physiological reactions such as collagen gene expression, peptide hormone activation, carnitine synthesis, and it is also an effective antioxidant (Lee *et al*., 2018).

Carotenoids are essential in the human diet owing to their nutrition and health benefits. Apart from being a good source of pro-vitamin A, they also possess antioxidant activity, which helps lower the risks of long-term degenerative diseases. Their bioavailability can however be modulated using dietary factors such as mechanical disintegration, enzymatic maceration of matrix compounds, the addition of lipids, and thermal treatments (Schweiggert & Carle, 2017). In this study, the amounts of carotenoids were much lower in the recipes compared to that in the fresh amaranth sample. However, compared to the mixed ingredients in raw form, three carotenoids, lutein, α-carotene and β-carotene, were found to be enhanced by cooking, while violaxanthin, was not detected in recipes that included boiling. There was no significant ($P \leq 0.05$) difference in the carotenoid contents between the chopped and un-chopped samples. Cooking of vegetables has been reported to promote breakdown of the cellulose structures of the plant cell thereby releasing carotenoids. It also denatures carotenoid-protein complexes hence improving the extractability of the carotenoids in the cooked food (Miglio *et al*., 2008). It is also assumed that this enhanced extractability is associated with enhanced bioavailability of these compounds (Lee *et al*., 2018). Addition of lipids has also been shown to have a positive effect on carotenoid bioavailability (Schweiggert & Carle, 2017). The use of oil in preparing the recipes in this study could have contributed to the release of more carotenoids from the matrix. Cooking was shown to enhance the carotenoid content in amaranth vegetables. There are also studies that show negative effect of cooking on carotenoids (Bureau *et al*., 2015; Zhang *et al.*, 2020). The conflicting results could be due to differences in the starting materials as well as methodologies (Cilla *et al*., 2018), such as differences in the cooking time. The possible reason for decrease in violaxanthin in some recipes include the high instability of this xanthophyl during boiling, the activation of neoxanthin synthase enzyme transforming violaxanthin to neoxanthin, activation of xanthophyl cycle where violaxanthin is converted to zeaxanthin (Saga *et al*., 2010). Cleaving of violaxanthin onto abscisic acid, or the conversion of violaxanthin to abscisic acid by the action of carotenoid-cleavage dioxygenase may also occur (Pasaporte *et al*., 2014).

Minerals in vegetables such as calcium, iron and zinc are quite stable and not affected much by cooking. The iron and zinc contents of the three recipes in this study were not significantly different from that of the uncooked ingredients. There was, however, some increase in calcium content compared to the raw ingredients, which was significant in Recipe 2 (Table 6.4). This increase can be attributed to release of complexes which are normally formed between calcium and other compounds such as oxalates. Similar results of slight increase in calcium contents on various species of amaranth and other leafy vegetables have also been reported (Amalraj & Pius, 2014).

In-vitro assays have been used to estimate the bioaccessible nutrients from different food products. These assays mimic the digestion process, with the various enzymes as well as the conditions of the digestive tract control. Dialysis tubing is used to determine the amount of iron released from the food matrix, hence recorded as the dialysable iron. In most foods especially those of plant origin such as amaranth vegetables, the iron concentration may not indicate its bioaccessibility (Amagloh *et al*., 2017). In this study (Table 6.4), the iron content of the raw amaranth was much higher than that in the recipes. However, the percent dialysable iron of the recipes was, however, higher than that of the raw amaranth (Table 6.5). One of the factors that can affect the bioaccessibility of this vegetable iron is their non-heme nature. The non-heme iron mostly exists in complexes, which can be degraded in the gastrointestinal tract during digestion owing to the action of pepsin and hydrochloric acid. Once released from food components, most non-heme iron is present in the ferric form (Fe^{3+}), with low solubility and bioavailability (Han, 2011). Another factor that is known to reduce the bioaccessibility of iron in amaranth leaves is oxalate which binds divalent minerals such as iron hence reducing their bioaccessibility. During cooking, iron is released from the complexes, and some ingredients such as tomatoes used in cooking may also enhance the bioaccessibility of the iron (Nomkong *et al*., 2019). In this study, cooking involving boiling and heating during frying may have enhanced the bioaccessibility of the iron. On the other hand, cooking itself softens the food matrix, releasing bound components, and also alters inherent mineral absorption inhibitors such as soluble dietary fiber thus improving the bioaccessibility of nutrients generally (Platel & Srinivasan, 2015).

This study clearly shows that food preparation methods such as boiling of the vegetables may be used to improve the bioaccessible iron significantly. Inclusion of ingredients that are rich in ascorbic acid may result in higher amounts of bioaccessible iron. This is because these vitamin C rich dietary components are capable of reducing the ferric iron to bioaccessible ferrous iron (Blanco-Rojo & Vaquero, 2019). The enhancing effect of vitamin C on mineral bioaccessibility has also been observed in other studies (Singh & Prasad, 2018). In this study, lemon juice was used as a source of vitamin C, and it resulted in significant improvement in the bioaccessible iron. Other organic acids in the lemon juice such as citric acid could also be responsible for the enhancement of iron dialysability. These acids are reported to have the ability of chelating iron to form soluble complexes. They also have the ability to lower pH, which increases the solubilization of iron from the food (Rodriguez-Ramiro *et al*., 2019)

Inclusion of ingredients which are rich in iron also proved to be beneficial in improving the dialysable iron content. As most indigenous vegetables have also been shown to be good sources of iron, including them as ingredients could also result in higher iron content in the dish, hence higher bioaccessible iron. *Moringa oleifera* leaves in this study were used to determine if the anti-nutritional effects of oxalate in amaranth can be masked by including more iron in the dish. Moringa leaves have previously been reported to be rich in iron. Suzana *et al* reported values of 14.6 mg/100 g leaf extract, this being up to four times higher than spinach (Suzana *et al*., 2017); while Yang reported values of 9.2 mg/100 g fresh leaves (Yang *et al*., 2006). Other iron rich vegetables can also be included in amaranth recipes to increase the total iron in the vegetable dishes. Kruger *et al*., reported that combining amaranth with spider plant (80:20 ratio) enhanced iron bioavailability of the dish from 9.7% to 25%, while combination of amaranth with cowpea leaves (80:20 ratio) slightly enhanced iron bioavailability from 9.7% to 10.1% (Kruger *et al*., 2015).

Combination of amaranth recipe with *"ugali"* (50:50) did not result in any significant difference in dialysable iron compared to the Recipe1 alone. In most parts of East Africa, amaranth dishes are eaten together with maize meal *"ugali",* which is either fortified or unfortified. This study evaluated meal interactions which would affect iron bioavailability and showed that components in amaranth did not have any inhibitory effects on the iron bioavailability of the iron-fortified maize meal.

6.5 Conclusion

The amaranth dishes prepared using the three different recipes all showed high nutrient retentions. Food based methods such as incorporating high vitamin C foods and high iron foods as ingredients in vegetable preparation significantly enhanced the dialysable iron in the amaranth-based recipes, which translated to improvement in the bioavailable iron in the recipes. Use of such methods can be applied in increasing micronutrient intake and reduction of prevalence of micronutrient malnutrition.

CHAPTER SEVEN

NUTRITIONALLY IMPROVED AMARANTH BASED RECIPES AND ASSESSMENT OF THEIR CONSUMER ACCEPTABILITY

Summary

Development of appropriate recipes act as very strong message delivery channel and can be a useful approach in increasing consumption of indigenous vegetables. A number of people within the population are not familiar with proper ways of preparing some of the indigenous vegetables and this affects acceptability and nutrient delivery from these vegetables. The objective of this study was to develop amaranth based recipes which would deliver high amounts of nutrients to consumers and also improve the consumption through diversity. About 16 amaranth based recipes were developed under five nutritional themes. Part of the recipes were adapted from an exploratory amaranth utilization survey carried out in Kenya and Tanzania, while some were improved with insight from a laboratory iron bioavailability enhancement in chapter 6 of this study. Suggestions on the improvements were also made based on nutrition and consumption related factors that may affect nutrient contents of amaranth dishes. A recipe book with detailed preparation steps of the various recipes was prepared for dissemination to the wider community at large. Ten representative recipes were subjected to sensory evaluation in two locations; Dar-es-Salaam, Tanzania and in JKUAT, Kenya. The evaluation showed a high acceptability of the recipes across the region, gender, and ages.

7.1 Introduction

African indigenous vegetables including amaranth vegetables have been consumed in most African household since time immemorial. These vegetables have been shown to have several agronomic advantages such as ease of growth and adaptability to wide environmental conditions. With increased research on the nutritional and health properties of the amaranth vegetable, its consumption has been on an increasing trend, with many people in both rural and urban areas consuming the vegetables. Despite the increase, vegetable intake is still low, with the average vegetable intake in East Africa being recorded as ranging about 98 g /day (Kalmpourtzidou *et al*., 2020). While there is no specific guideline for vegetable intake alone, this value is much lower than the WHO/FAO minimum recommended intake of 400 g/day for fruits and vegetables (WHO/FAO, 2003). There is therefore need to improve vegetable intake in order to achieve the health benefits that are associated with the vegetables.

In most traditional cooking methods of African indigenous vegetable, the taste of the vegetable has been prioritized more than its nutritional attributes. Some of these methods included washing vegetables after chopping, drying vegetables in the sun after harvest, prolonged boiling and extended cooking coupled with fermentation. These practices destroy and greatly reduce some important nutrients and bioactive compounds in the vegetables, rendering the vegetables less nutritious and of very low importance to the body. Such practices have also partly contributed to the neglect and improper mindset that the vegetable is for the poor who cannot afford good nutritious food. The amaranth vegetable has been shown to be rich in micronutrients such as iron. However, iron deficiency anemia still remains a public health problem, even in the areas where this vegetable is consumed. The iron in this vegetable is mostly bound in complexes, which reduce its bioavailability. Some antinutrients present in the vegetables such as oxalate also reduce the bioavailability of the iron. Cooking practices as well as inclusion of some ingredients such as milk which contains casein can further inhibit the absorption of this iron by the body (Blanco-Rojo & Vaquero, 2019).

Some vegetables are bitter in taste, among the toughest in texture and generally have poor flavor properties compared to other foods. These properties predisposes these vegetables to low acceptance (Poelman *et al*., 2017). Among some individuals who prefer stronger tastants, their preference for amaranth is low because of its mild taste. Consumption of amaranth among such individuals can be improved by introducing ingredients which can give the vegetables a stronger flavor. On the other hand, individuals who are sensitive to stronger or bitter tastes tend to consume less of other indigenous vegetables. Mixing of leafy vegetables therefore provides taste and flavor that are often more acceptable as compared to homogenous vegetables (van Stokkom *et al*., 2019). Amaranth can be mixed with other vegetables to mask the bitter taste and improve the overall consumption of vegetables. This mixing can also be done to tenderize the texture of other vegetables such as cowpea leaves, or even to supplement nutrients in other foods such as meat. This mixing can provide diversity in cooking and also increase amaranth consumption. This measure of providing choice and variety can greatly improve vegetable preference (Parizel *et al*., 2017). Amaranth can also be used as a minor ingredient in many other foods, including street foods which have been considered to be high in calories and low in micronutrients, to supplement the nutrients.

The aim of this work was to develop nutritious amaranth-based recipes and assess their consumer acceptability through sensory evaluation.

7.2 Methodology

7.2.1 Development of Recipes

This was done through adoption of existing amaranth-based recipes which were reported in an amaranth utilization survey carried out in Kenya and Tanzania. Five thematic areas were suggested, which would help increase the consumption of amaranth as well as the nutrient delivery from amaranth dishes. The themes included high nutrient amaranth dishes; improving iron intake and bioavailability; enhancing flavor; amaranth as an ingredient in other foods; and enhancing street food with amaranth. Nutrition and utilization related factors on these themes were identified and improvements suggested in each case. Recipes were then made with incorporating these improvements (Table 7.1). Some suggestions especially on improving iron bioavailability were informed by a laboratory study on amaranth meals (Chapter 6).

7.2.2 Sensory evaluation of Recipes

The sensory evaluation was carried out in two locations; Dar-es-Salaam, Tanzania and in JKUAT, Kenya.

Sample Preparation

Ten amaranth-based dishes from the recipe book were prepared for sensory evaluation. These were:

1. A1 - Stir fried amaranth with onion
2. A2 - Amaranth with carrots
3. A3 - Amaranth with lemon juice
4. A4 - Amaranth with spider plant
5. A5 - Amaranth with ginger and garlic
6. A6 - Amaranth with green pepper
7. A7 - Amaranth in meat stew
8. A8 - Amaranth with peanut
9. A9 - Amaranth with coconut
10. A10 - Amaranth salad

The recipes were prepared as described in the amaranth recipe book (Appendix VII)

Evaluation of Samples

The sensory analysis was performed using a preference Hedonic scale. The sensory panel in consisted of 53 and 46 untrained/ consumer panelists in Kenya and Tanzania respectively. The 10 recipes were grouped into two and were then administered to the panelists at two different times. The 1st set of 5 recipes was administered at mid-morning and the 2nd set at late afternoon. The properties evaluated were appearance, aroma, texture, taste/flavor and general acceptability. A hedonic scale from 1 to 5 was used for evaluation of the sensory attributes as well as the general acceptability of the recipes, with 1 indicating "Dislike very much", 3 for "Neither like nor Dislike" while

5 indicated “Like very much”.

During the evaluation, each participant was briefed about the study and what was expected of them. The participants were informed that their participation was completely voluntary and that they would not receive any compensation for participating in the study. They were then provided with a sensory evaluation form upon their consent. The participants were asked to fill in their demographic information on the form including age and gender, as well as the number of times they consumed amaranth in the previous two weeks. Each participant was asked to rate the sensory characteristics and overall acceptability of the amaranth recipes by filling out score sheets. Water was provided for all participants, and they were asked to rinse their palates between tasting of the different samples.

Participants evaluated each recipe by considering how much they liked or disliked the different characteristics of each recipe as well as the overall acceptability by scoring on a scale of 1 to 5. The participants were requested to refrain from discussing their opinions, but give their comments about the recipes which they recorded on the forms.

7.2.3 Data Analysis

The data collected from the sensory evaluation was subjected to statistical analysis using SPSS software. Descriptive data was generated from the analysis. Significance tests were also done using Analysis of Variance (ANOVA) and T-tests.

7.3 Results

7.3.1 Developed Recipes

Table 7.1: Thematic framework for amaranth recipe development

Theme	Nutrition and utilization related factors	Proposed improvements	Recipes
1. High nutrient dishes	Some factors that lead to loss of nutrients: – poor handling – prolonged cooking	– Shorter cooking time – Include ingredients to add to the nutrients and bioactive compounds	– Stir fried Amaranth – Amaranth with colored pepper – Amaranth with carrots – Steamed amaranth
2. Improved iron intake and bioavailability	Low iron bioavailability due to non-heme iron and presence of other compound e.g. oxalates.	– Boiling to improve iron bioavailability – Adding vitamin C rich ingredients to improve bioavailability of iron – Cooking together with other iron rich leafy vegetables to increase the total iron content	– Sauteed amaranth – Amaranth with lemon juice – Amaranth mixed with high iron vegetable (Moringa/ spiderplant)
3. Enhancing taste and flavor	Mild flavor and taste affect acceptability. Milk is used to enhance flavor, which could affect mineral bioavailability.	– Added ingredients to improve flavor e.g. groundnut paste, ghee – Improving taste through addition of pepper and other spices	– Amaranth with coconut cream – Amaranth with groundnut paste – Amaranth with spices
4. Amaranth as ingredient in other foods	➢ Tender ➢ Mild taste ➢ High nutrient value	– Use amaranth to improve tenderness of other vegetables and to reduce bitter taste in crotalaria and spider-plant – Amaranth can improve the nutritional content of other dishes.	– Amaranth with cowpea leaves – Amaranth with spiderplant – Amaranth with meat
5. Enriching Street foods with amaranth	Most street food are high fat and high carbohydrates, considered unhealthy.	– Adding blanched or dried amaranth into salads. – Incorporating amaranth leaves in foods such as samosa (dumplings) as a minor ingredient.	– Amaranth in Kachumbari – Amaranth in Samosa – Amaranth in Bhajia

A total of 16 amaranth vegetable-based recipes were prepared and compiled into a recipe book (Appendix VII).

7.3.2 Sensory Evaluation/ Consumer acceptability

The ratio of males to females in the evaluation done in Kenya was almost equal, while there were more females who participated in the evaluation done in Tanzania. The panelists in Kenya were also dominated by the young generation (20-30 years), while those in Tanzania was dominated by the middle aged (30-60 years).

Table 7.2: Demographic characteristics of the panelists (%)

	Kenya	**Tanzania**
Gender of Panelists		
Male	50.9	34.8
Female	49.1	65.2
Age of Panelists		
Less than 20 years	32.1	0
20-30 years	54.7	13.0
31-40 years	9.4	50.0
41-50 years	3.8	19.6
51-60 years	0	17.4
Over 60 years	0	0

Kenya, n = 53; Tanzania, n = 46

More than 50% of participants in Kenya and Tanzania generally liked all the sensory attributes moderately to very much.

The acceptance of the recipes was high. Over 50% of respondents from Kenya and over 70% of the respondents in Tanzania reported that they can frequently consume the amaranth-based recipes. The frequency of consumption of amaranth was reported to be higher in Tanzania than in Kenya, with over 60% of respondents reporting to consume amaranth more than once in a week (Table 7.3)

Table 7.3: Percentage responding to general acceptability of the recipes and frequency of amaranth consumption

	Kenya	Tanzania
General acceptability of the recipes		
I would eat this only if I were forced to	10.0	0.2
I would hardly eat this	16.2	4.1
I would eat this if available but would not go out of my way	24.7	23.3
I can frequently eat this	25.7	42.4
I can eat this food in every opportunity I have	23.4	30.0
Frequency of eating amaranth in the past two weeks		
Not a single time	39.6	8.7
Once in two weeks	18.9	13.0
Once in a week	9.4	13.0
More than once a week	30.2	54.3
Every day	1.9	10.9

Data presented as average percentages. Kenya, n = 53; Tanzania, n = 46

About 26% of participants in Kenya reported low acceptability, compared to 4% of participants from Tanzania. Despite this, the general acceptability of the recipes was still considerably very high.

The sensory ratings for the individual recipes were significantly different for both the evaluation in Kenya and in Tanzania.

Table 7.4: Hedonic rating of the sensory characteristics of the recipes

Sample	Appearance	Aroma	Texture	Taste/flavor	Overall acceptability
Kenya					
A1	3.4^{b}	3.4^{b}	3.4^{b}	3.1^{bc}	3.2^{b}
A2	3.9^{cd}	3.7^{bc}	3.6^{b}	3.5^{cd}	3.5^{bc}
A3	3.9^{cd}	3.6^{bc}	3.7^{b}	3.5^{cd}	3.5^{bc}
A4	2.8^{a}	2.6^{a}	2.4^{a}	1.8^{a}	2.0^{a}
A5	3.6^{bc}	3.7^{bc}	3.4^{b}	3.0^{b}	3.1^{b}
A6	4.3^{d}	3.7^{bc}	3.6^{b}	3.6^{cd}	3.5^{bc}
A7	4.2^{d}	4.4^{d}	4.4^{c}	4.6^{e}	4.3^{d}
A8	3.4^{b}	3.8^{c}	3.6^{b}	3.7^{d}	3.5^{bc}
A9	3.6^{bc}	3.6^{bc}	3.7^{b}	3.6^{d}	3.5^{bc}
A10	4.1^{d}	3.9^{c}	3.8^{b}	3.8^{d}	3.7^{c}
Tanzania					
A1	3.8^{bc}	3.8^{bcd}	3.5^{a}	3.7^{bc}	4.2^{cd}
A2	3.5^{ab}	3.5^{ab}	3.3^{a}	3.4^{ab}	3.8^{ab}
A3	3.4^{ab}	3.3^{a}	3.5^{a}	3.2^{a}	3.7^{a}
A4	3.3^{a}	3.6^{abc}	3.5^{a}	3.3^{ab}	3.6^{a}
A5	3.6^{ab}	3.7^{abc}	3.7^{ab}	3.5^{ab}	3.9^{abc}
A6	3.5^{ab}	3.5^{ab}	3.7^{ab}	3.6^{ab}	3.7^{a}
A7	4.3^{d}	4.1^{d}	4.2^{c}	4.1^{cd}	4.2^{cd}
A8	4.4^{d}	4.2^{d}	4.2^{c}	4.4^{d}	4.3^{d}
A9	4.1^{cd}	3.9^{cd}	4.0^{bc}	4.1^{cd}	4.1^{bcd}
A10	4.1^{cd}	4.2^{d}	4.1^{c}	4.2^{cd}	4.3^{d}

Means with same superscript within each column are not significantly different. For the sensory attributes a 5-point Hedonic scale was used (5=like very much, 1=dislike very much)

In both Kenya and Tanzania evaluations, recipes A7, A8 and A10 were ranked highest, while recipe A4 ranked lowest in most attributes. From the Kenyan participants, the rating of nine recipes were all above average (3), with recipe A7 scoring a value corresponding to like moderately. In the Tanzanian side, all the ten recipes scored above average, with four of the recipes' scores tending towards the 'like very much' score.

Table 7.5: Participant characteristics vs sensory ratings

Sample	Appearance	Aroma	Texture	Taste/ flavor	General acceptability	Freq. of eating
Country						
Kenya	3.7	3.6	3.6	3.4	3.4	2.3
Tanzania	3.8	3.8	3.8	3.8	4.0	3.5
P Value	*0.444*	*0.172*	*0.077*	*0.001*	*0.001*	*0.001*
Gender of Panelists						
Male	3.7	3.6	3.6	3.6	3.6	2.7
Female	3.8	3.8	3.7	3.6	3.7	2.9
P value	*0.166*	*0.261*	*0.331*	*0.814*	*0.534*	*0.517*
Age of Panelists						
< 20 years	3.7^{a}	3.6^{a}	3.6^{a}	3.4^{a}	3.4^{a}	1.9^{a}
20-30 years	3.7^{a}	3.7^{a}	3.6^{a}	3.3^{a}	3.4^{a}	2.6^{ab}
31-40 years	3.8^{a}	3.7^{a}	3.7^{a}	3.7^{b}	3.9^{b}	3.3^{bc}
41-50 years	3.8^{a}	3.9^{a}	3.9^{a}	3.8^{b}	4.0^{b}	3.8^{c}
51-60 years	3.9^{a}	3.9^{a}	3.6^{a}	3.8^{b}	4.1^{b}	$3.3b^{c}$
P Value	*0.929*	*0.458*	*0.516*	*0.009*	*0.001*	*0.001*

Means with different superscript within each column, and P<0.05 show significant difference.

Nationality of participants did not significantly affect their preference on appearance, aroma and texture of the amaranth recipes, while taste preference, general acceptability and the frequency of consumption were significantly different between the two countries. There was also no significant effect of gender on the rating of the amaranth recipes. There was no significant effect of the age of participants on their rating of appearance, aroma and texture, while the taste rating, general acceptability and frequency of amaranth consumption differed significantly among the age groups, with the older generations giving higher rating on taste and general acceptability as well as higher frequency of amaranth consumption.

7.4 Discussion

Acceptability of indigenous vegetables such as amaranth has remained low, despite some improvement in consumption of these foods over the years. A recipe-based approach encompassing dietary diversification, nutrient retention and availability of practicable recipes provides an effective method of improving consumption of amaranth and ultimately eliminating malnutrition cases. Taste, preparation method and nutrient content are the main factors that influence acceptability of vegetables. Most traditional recipes entail preparation methods that may destroy the nutritional attributes, or inclusion of some ingredients that may further bind some nutrients in the vegetables. Altering vegetable preparation methods while retaining optimum nutrients is an easy way of influencing sensory attributes, and this can help increase vegetable intake for better nutrition.

In a bid to increase vegetables acceptance, combining vegetables to mask or minimize bitterness, enhance taste and provide variety of texture has been suggested as a promising strategy to increase vegetable acceptance and consumption (van Stokkom *et al*., 2019). Addition of ingredients of different colors, creates colorfulness and color contrasts, which positively impacts visual attractiveness of the vegetables (Paakki *et al*., 2019). The recipe A10 registered a high score in appearance, which could be attributed to the different colors of the ingredients (raw tomatoes, raw onions, blanched amaranth leaves) that were used in the recipe.

Inclusion of other ingredients such as herbs and spices may also enhance taste and boost the nutrition value of vegetable. In a study to assess the impact of exposure to herbs and spices added to vegetables, it was reported that as much as it may not rapidly increase intake, repeat exposures to the vegetables with herbs and spices can eventually increase the consumption (Fritts *et al*., 2019).

Food perception by humans is multisensory, with taste, smell, sight and touch being the most important in vegetable meals. Five taste modalities; sweet, sour, bitter, salty, umami; have been reported in vegetables, with the intensities being affected by preparation methods (van Stokkom *et al*., 2016). Low consumption of African indigenous vegetables may also be affected by taste perception of individuals. In this study, recipe A4, which was combined with spiderplant scored lowest because of the perceived bitter taste of this vegetable, despite some individuals actually liking the recipe. Certain individuals are more sensitive to bitter compounds because they possess the phenotype, 6-npropylthiouraccil: PROP taster status or genotype TAS2R38 (Sandell *et al*., 2014; Shen *et al*., 2016). The effects of taste enhancement has also been reported to be dependent on the tastant and tastant concentration, and may not necessarily lead to increased acceptance of a particular food (van Stokkom *et al*., 2018).

The respondents from Tanzania gave significantly higher ratings for taste, general acceptability and frequency of amaranth consumption. It was also that ratings were also significantly different for the different age groups. This finding corroborates with findings that demographic factors greatly influence taste of vegetables (Shen *et al*., 2016).

7.5 Conclusion

The general acceptability of the formulated recipes was fairly high. Aside from the five nutritional themes which the recipes are based on, they also involved a generally short cooking time of just about ten minutes to prevent nutrient loss. Taste, general acceptability and frequency of consuming amaranth was seen to be related origin and age of the participants, while gender did not significantly affect the acceptability. We therefore conclude that the suggestions would be acceptable to many and will contribute to improved vegetable consumption and reduced malnutrition.

CHAPTER EIGHT

GENERAL CONCLUSIONS AND RECOMMENDATION

8.1 Conclusions

Morphological traits differed greatly among amaranth accessions, and this could be due to their genotypic and phenotypic make up. Nutritional differences were also observed, and accessions of *Amaranthus dubius* species showed higher nutrient content generally than other species. Significant correlations were observed between greenness with oxalate and vitamin C content, as well as between hue with carotenoids, while other morphological traits were not significantly related to the nutritional components.

Accumulation of nutrients changed when the plants were subjected to drought stress. Most phytochemicals with anti-oxidant properties including carotenoids, ascorbic acid, flavonoids and phenolic acids were found to increase with water stress. A single gene in the biosynthetic pathway did not strongly affect a particular trait, but association between several genes affected the synthesis pathway. However, changes in transcription of GGP/VTC2 gene can be used to predict accumulation changes of ascorbic acid, as these were found to significantly correlate.

Amaranth vegetable was found to commonly consumed vegetable in Kenya and Tanzania, with preference for species differing with regions and individuals. The high preference for amaranth by both producers and consumers was due to its ease of availability, adaptability to the environment, cheapness to acquire, ease of cooking and good taste among other reasons. Cooking methods of amaranth differed, and most were traditional cooking methods. Cooking practices were mostly aimed at enhancing taste rather than nutritional value, with some cooking methods leading to loss of nutrients.

Cooking affected nutrients in amaranth differently. Vitamin C was significantly reduced by cooking, while carotenoids were enhanced. Mineral nutrients were shown to be least affected by cooking methods. Iron bioavailability was shown to be enhanced by cooking. Food based methods such as incorporating high vitamin C foods and high iron foods as ingredients in vegetable preparation showed significant impact on enhancing the dialyzable iron, which points to improvement in the bioavailable iron in from the dishes. Incorporating these methods by consumers and food handlers could help in increasing micronutrient intake and reducing micronutrient malnutrition through enhancing nutrient bioavailability.

Sixteen (16) amaranth-based recipes that were developed and provided a variety of nutritious amaranth-based foods, had high consumer acceptance in the sensory evaluation, which suggests a high willingness by consumers to adopt these recipes. These recipes are therefore recommended for promotional activities that would ensure uptake by consumers.

To curb micronutrient malnutrition, we must diversify our diets and include more vegetables, especially leafy vegetables. We must eradicate negative attitudes about these vegetables and adopt better cooking practices of vegetables for better nutrition and wellbeing.

8.2 Recommendations

8.2.1 General recommendations

- Colour of the leaves can therefore be used by breeders as a breeding trait indicator of nutritional attributes. Hue can also be used as an indicator for carotenoid content, and provitamin A carotenoids.
- The study recommends consumption of amaranth, even those that have been grown under water deficit stress, as amaranth dishes are generally of high nutritional value.
- Availability and adoption of recipes that ensure high nutrients and higher bioavailability is recommended to consumers, as well as training to the

community on the nutritional importance of amaranth and other indigenous vegetables by extension officers and researchers.

- There should be training to the community on the nutritional importance of amaranth and other indigenous vegetables by extension officers and researchers.

8.2.2 Recommendations for Future work

- Further investigation should be conducted on how soil nutrients and water status affect morphological phenotypes and nutrients in amaranth.
- Further research should also be conducted to assess how water stress affects biosynthetic pathways of other metabolites in amaranth such as betalains, flavonoids and phenolic acids.
- Further research should also be conducted involving *in-vitro* simulations and *in-vivo* trials on the nutrient uptake from amaranth vegetables by the body.

REFERENCES

Aboagye, R. G., Seidu, A., Ahinkorah, B. O., Arthur-holmes, F., Cadri, A., Dadzie, L. K., Hagan, J. E., & Eyawo, O. (2021). Dietary Diversity and Undernutrition in Children Aged 6–23 Months in Sub-Saharan Africa. *Nutrients*, *13*(3431), 1–22. https://doi.org/10.3390/ nu13103431

Abukutsa-Onyango, M. O. (2011). *Researching African Indigenous Fruits and Vegetables – Why?* https://knowledge.cta.int/en/Dossiers/Commodities/Fruits/Feature-articles/Researching-African-Indigenous-Fruits-and-Vegetables-Why.html

Abukutsa Onyango, 2010. (2010). *Strategic repositioning of African indigenous vegetables in the Horticultural sector* (Issue June).

Achigan-Dako, E. G., Sogbohossou, O. E. D., & Maundu, P. (2014). Current knowledge on Amaranthus spp.: Research avenues for improved nutritional value and yield in leafy amaranths in sub-Saharan Africa. *Euphytica*, *197*(3), 303–317. https://doi.org/10.1007/s10681-014-1081-9

Adeka, R., Lukhoba, C., Odhiambo, J., & Maundu, P. (2019). Morphological Traits as Indicators of Bitterness in Traditional Vegetables : The Case of Spider Plant (Gynandropsis gynandra) in Kenya. *Asian Journal of Research in Botany*, *2*(3), 1–15.

Adhikary, D., Khatri-Chhetri, U., & Slaski, J. (2020). Amaranth: An Ancient and High-Quality Wholesome Crop. In V. Y. Waisundara (Ed.), *Nutritional Value of Amaranth*. IntechOpen. https://doi.org/10.5772/intechopen.88093

Afari-Sefa, V., Rajendran, S., Kessy, R. F., Karanja, D. K., Musebe, R., Samali, S., & Makaranga, M. (2016). Impact of nutritional perceptions of traditional African vegetables on farm household production decisions: A case study of smallholders in Tanzania. *Experimental Agriculture*, *52*(2), 300–313. https://doi.org/10.1017/S0014479715000101

Agbaire, P. O. (2012). *Levels of anti-nutritional factors in some common leafy edible vegetables of southern Nigeria*. *3*(May), 99–101.

Ahinkorah, B. O., Amadu, I., Seidu, A. A., Okyere, J., Duku, E., Hagan, J. E., Budu, E., Archer, A. G., & Yaya, S. (2021). Prevalence and factors associated with the triple burden of malnutrition among mother-child pairs in sub-saharan africa. *Nutrients*, *13*(6), 1–13. https://doi.org/10.3390/nu13062050

Akaneme, F. I., & Ani, G. O. (2013). Morphological assessment of genetic variability among accessions of Amaranthus hybridus. *Wolrd Appl. Sci. J.*, *28*(4), 568–577.

Akhtar, M. S., Israr, B., Bhatty, N., & Ali, A. (2011). Effect of cooking on soluble and insoluble oxalate contents in selected Pakistani vegetables and beans. *International Journal of Food Properties*, *14*(1), 241–249. https://doi.org/10.1080/10942910903326056

Akin-Idowu, P., Ademoyegun, O., Olagunju, Y., Aduloju, A., & Adebo, G. (2017). *Phytochemical Content and Antioxidant Activity of Five Grain Amaranth Species*. *5*, 249–255.

Akter, S., Netzel, M., Tinggi, U., Fletcher, M., Osborne, S., & Sultanbawa, Y. (2020). Interactions Between Phytochemicals and Minerals in Terminalia ferdinandiana and Implications for Mineral Bioavailability. *Frontiers in Nutrition*, *7*(December), 1–10. https://doi.org/10.3389/fnut.2020.598219

Al-Babili, S., & Bouwmeester, H. J. (2015). Strigolactones, a novel carotenoid-derived plant hormone. *Annual Review of Plant Biology*, *66*(January), 161–186. https://doi.org/10.1146/annurev-arplant-043014-114759

Amagloh, F. K., Atuna, R. A., Mcbride, R., Carey, E. E., & Christides, T. (2017). Nutrient and Total Polyphenol Contents of Dark Green Leafy Vegetables, and Estimation of Their Iron Bioaccessibility Using the In Vitro Digestion/Caco-2 Cell Model. *Foods*, *6*(54), 1–12. https://doi.org/10.3390/foods6070054

Amalraj, A., & Pius, A. (2014). Bioavailability of calcium and its absorption inhibitors in raw and cooked green leafy vegetables commonly consumed in India – An invitro study. In *Food Chemistry*. Elsevier Ltd. https://doi.org/10.1016/j.foodchem.2014.08.031

AOAC. (2016). *Official Methods of Analysis 20th Edition* (20th ed.). Association of Official Analytical Chemists International.

Arasaretnam, S., Kiruthika, A., & Mahendran, T. (2018). Nutritional and mineral composition of selected green leafy vegetables. *Ceylon Journal of Science*, *47*(1), 35. https://doi.org/10.4038/cjs.v47i1.7484

Ashraf, M. Y., Ashraf, M., & Ozturk, M. (2018). Underutilized Vegetables: A Tool to address Nutritional Issues, Poverty Reduction and Food Security. In *Global Perspectives on Underutilized Crops*. Springer, Cham. https://doi.org/10.1007/978-3-319-77776-4_1

Ayanwale, A. B., Amusan, C. A., Adeyemo, A., & Oyedele, D. J. (2016). Analysis of Household Demand for Underutilized Indigenous Vegetables Analysis of Household Demand for Underutilized Indigenous Vegetables. *International Journal of Vegetable Science*, *22*(6), 570–577. https://doi.org/10.1080/19315260.2015.1103350

Bayengue, B., Suzanne, S., Mathieu, N., Bertin, S. E., Calvin, M. J., Ngane, N., Annie, R., & Clergé, T. (2017). *Effects of Cooking Time on Some Antinutrients Contents and in vitro Digestibility of Leaves Proteins of Gnetum spp Email address : To cite this article : 6*(2), 99–104. https://doi.org/10.11648/j.ijnfs.20170602.16

Beaulah, A., Rajamanickam, C., & Swaminathan, V. (2020). Nutritive Values and Importance of Tropical Green Leafy Vegetables in Human Diet – A Review. *International Journal of Current Microbiology and Applied Sciences*, *9*(9), 656–669. https://doi.org/10.20546/ijcmas.2020.909.083

Beswa, D., Dlamini, N. R., Siwela, M., Amonsou, E. O., & Kolanisi, U. (2016). Effect of Amaranth addition on the nutritional composition and consumer acceptability of extruded provitamin A-biofortified maize snacks. *Food Science and Technology (Campinas)*, *36*(1), 30–39. https://doi.org/10.1590/1678-457X.6813

Biesalski, H. K. (2013). *Hidden Hunger. (Der verborgene Hunger)*. Springer Spektum.

Birol, E., Meenakshi, J., Oparinde, A., Perez, S., & Tomlins, K. (2015). Developing country consumers' acceptance of biofortified foods: a synthesis. *Food Security*, *7*, 555–568.

Blanco-Rojo, R., & Vaquero, M. P. (2019). Iron bioavailability from food fortification to precision nutrition. A review. *Innovative Food Science and Emerging Technologies*, *51*(April 2018), 126–138. https://doi.org/10.1016/j.ifset.2018.04.015

Blas, A., Garrido, A., Unver, O., & Willaarts, B. (2019). A comparison of the Mediterranean diet and current food consumption patterns in Spain from a nutritional and water perspective. *Science of the Total Environment*, *664*(February), 1020–1029. https://doi.org/10.1016/j.scitotenv.2019.02.111

Bugbee, B. (2016). Toward an optimal spectral quality for plant growth and development: The importance of radiation capture. *Acta Horticulturae*, *1134*, 1–12. https://doi.org/10.17660/ActaHortic.2016.1134.1

Bulley, S. M., & Laing, W. (2016). Ascorbic Acid-Related Genes. In R. Testolin, H. Huang, & A. Ferguson (Eds.), *The Kiwifruit Genome, Compendium of Plant Genomes,* (pp. 163–177). Springer International Publishing Switzerland. https://doi.org/10.1007/978-3-319-32274-2_13

Bureau, S., Mouhoubi, S., Touloumet, L., Garcia, C., Moreau, F., Bédouet, V., & Renard, C. M. G. C. (2015). Are folates, carotenoids and vitamin C affected by cooking? Four domestic procedures are compared on a large diversity of frozen vegetables. *LWT - Food Science and Technology*, *64*(2), 735–741. https://doi.org/https://doi.org/10.1016/j.lwt.2015.06.016

Cai, X., Ge, C., Xu, C., Xiaoli, W., Wang, S., & Quanhua, W. (2018). Expression

Analysis of Oxalate Metabolic Pathway Genes Reveals Oxalate Regulation Patterns in Spinach. *Molecules*, *23*(1286), 1–15. https://doi.org/10.3390/molecules23061286

Cardoso, C., Afonso, C., Lourenço, H., Costa, S., & Nunes, M. L. (2015). Bioaccessibility assessment methodologies and their consequences for the risk-benefit evaluation of food. *Trends in Food Science and Technology*, *41*(1), 5–23. https://doi.org/10.1016/j.tifs.2014.08.008

Castanheira, I. (2016). The effect of cooking methods on the mineral content of quinoa (Chenopodium quinoa), amaranth (Amaranthus sp.) and buckwheat (Fagopyrum esculentum). *Journal of Food Composition and Analysis*. https://doi.org/10.1016/j.jfca.2016.02.006

CDC, C.-D.-C.-P. (1994). *Oxalic acid: Immediately Dangerous to Life or Health Concentrations (IDLH)*. The National Institute for Occupational Safety and Health (NIOSH).

Cernansky, R. (2015). The rise of Africa's super vegetables. *Nature*, *522*(7555), 146–148.

Chacha, J. S., & Laswai, H. S. (2020). Traditional Practices and Consumer Habits regarding Consumption of Underutilised Vegetables in Kilimanjaro and Morogoro Regions, Tanzania. *International Journal of Food Science*, *2020*. https://doi.org/10.1155/2020/3529434

Chakrabarty, T., Sarker, U., Hasan, M., & Rahman, M. (2018). Variability in mineral compositions , yield and yield contributing traits of stem amaranth (Amaranthus lividus). *Genetica*, *50*(3), 995–1010. https://doi.org/10.2298/GENSR1803995C

Chen, D., Neumann, K., Friedel, S., Kilian, B., Chen, M., Altmann, T., & Klukas, C. (2014). Dissecting the phenotypic components of crop plant growth and drought responses based on high-throughput image analysis. *Plant Cell*, *26*, 4636–4655.

Chew, E. Y., Clemons, T. E., SanGiovanni, J. P., Danis, R., Ferris, F. L., Elman, M.,

& Al., E. (2013). Lutein, zeaxanthin and omega-3 fatty acids for age-related macular degeneration: The Age-Related Eye Disease Study 2 (AREDS2) randomized clinical trial. *Journal of the American Medical Association*, *309*(19), 2005–2015.

Chidozie Ogwu, M. (2020). Value of Amaranthus [L.] Species in Nigeria . *Nutritional Value of Amaranth*, *January*. https://doi.org/10.5772/intechopen.86990

Chipungahelo, M. S. (2015). Knowledge sharing strategies on traditional vegetables for supporting food security in Kilosa district, Tanzania. *Library Review*, *64*(3), 229–247. https://doi.org/10.1108/LR-10-2014-0105

Chukwuebuka, E., & Chinenye, I. J. (2015). *Biological Functions and Anti-nutritional Effects of Phytochemicals in Living System*. *10*(2), 10–19. https://doi.org/10.9790/3008-10231019

Cilla, A., Bosch, L., Barberá, R., & Alegría, A. (2018). Effect of processing on the bioaccessibility of bioactive compounds – A review focusing on carotenoids, minerals, ascorbic acid, tocopherols and polyphenols. *Journal of Food Composition and Analysis*, *68*, 3–15. https://doi.org/10.1016/j.jfca.2017.01.009

Comert, E. D., Mogol, B. A., & Gokmen, V. (2020). Relationship between color and antioxidant capacity of fruits and vegetables. *Current Research in Food Science*, *2*, 1–10. https://doi.org/10.1016/j.crfs.2019.11.001

Conti, M. V., De Giuseppe, R., Monti, M. C., Mkindi, A. G., Mshanga, N. H., Ceppi, S., Msuya, J., & Cena, H. (2012). Indigenous vegetables: a sustainable approach to improve micronutrient adequacy in Tanzanian women of childbearing age. *European Journal of Nutrition*. https://doi.org/10.1038/s41430-021-00865-x.

Costa, E. M. R., Damasceno-Silva, K. J., Rocha, M. M., Medeiros, A. M., & Filho, C. J. A. (2013). Genetic divergence among African cowpea lines based on morphoagronomic traits. *Genetics and Molecular Research*, *12*(4), 6773–6781. https://doi.org/10.4238/2013.December.16.3

Croft, M. M., Marshall, M. I., Odendo, M., Ndinya, C., Ondego, N. N., Obura, P., Hallett, S. G., Croft, M. M., Marshall, M. I., Odendo, M., Ndinya, C., Ondego, N., Obura, P., Formal, S. G. H., & Seed, I. (2018). Formal and Informal Seed Systems in Kenya : Supporting Indigenous Vegetable Seed Quality Formal and Informal Seed Systems in Kenya : Supporting Indigenous Vegetable Seed Quality. *The Journal of Development Studies*, *54*(4), 758–775. https://doi.org/10.1080/00220388.2017.1308487

Croft, M. M., Marshall, M. I., & Weller, S. C. (2014). Consumers' preference for quality in three African indigenous vegetables in Western Kenya. *Journal of Agricultural Economics and Development*, *3*(5), 67–077. http://academeresearchjournals.org/journal/jaed

Das Choudhury, S., Samal, A., & Awada, T. (2019). Leveraging image analysis for high-throughput plant phenotyping. *Frontiers in Plant Science*, *10*(April), 1–8. https://doi.org/10.3389/fpls.2019.00508

Das, S. (2012). Systematics and taxonomic delimitation of vegetable, grain and weed amaranths: a morphological and biochemical approach. *Genet Resour Crop Evol*, *59*(2), 289–303.

Das, Saubhik. (2016). Amaranthus: A Promising Crop of Future. In *Amaranthus: A Promising Crop of Future*. https://doi.org/10.1007/978-981-10-1469-7

Demidchik, V. V, Shashko, A. Y., Bandarenka, U. Y., & Smolikova, G. N. (2020). Plant Phenomics : Fundamental Bases , Software and Hardware Platforms , and Machine Learning. *Russian Journal of Pla*, *67*(3), 397–412. https://doi.org/10.1134/S1021443720030061

Dhami, N., & Cazzonelli, C. I. (2020). Environmental impacts on carotenoid metabolism in leaves. *Plant Growth Regulation*, *0123456789*. https://doi.org/10.1007/s10725-020-00661-w

Dheda, K., Huggett, J. F., Bustin, S. A., Johnson, M. A., Rook, G., & Zumla, A. (2004).

Validation of housekeeping genes for normalizing RNA expression in real-time PCR. *BioTechniques*, *37*, 112–119. https://doi.org/10.1016/j.ymeth.2010.01.003

Dias, A. N., Araújo, Lara, S. B., Miranda, L. S., Pires, S. I. C., Pires, C. V., & Halboth, N. V. (2012). Influence of color on acceptance and identification of flavor of foods by adults. *Food Science and Technology*, *32*(2), 296–301. https://doi.org/10.1590/S0101-20612012005000059

Dinssa, F., Nyabinda, N., Byrnes, D., Ndinya, C., Merchant, E., & Simon, J. (2020). Performances of Vegetable Amaranth Entries in Yield and Nutrient Contents in Tanzania and Kenya, and Variety Release. *Journal of Medicinally Active Plants*, *9*(3), 181.

Dinssa, F.F., Yang, R., Ledesma, D. R., Mbwambo, O., & Hanson, P. (2018). Effect of leaf harvest on grain yield and nutrient content of diverse amaranth. *Scientia Horticulturae*, *236*, 146–157. https://doi.org/10.1016/j.scienta.2018.03.028

Dinssa, F.F, Hanson, P., Dubois, T., Tenkouano, A., Stoilova, T., Hughes, J. d'A, & Keatinge, J. D. . (2016). AVRDC - The World Vegetable Center ' s women-oriented improvement and development strategy for traditional African veget .... AVRDC – The World Vegetable Center ' s women-oriented improvement and development strategy for traditional African vegetables in. *European Journal of Horticultural Science*, *81*(2), 91–105. https://doi.org/10.17660/eJHS.2016/81.2.3

Dinssa, Fekadu Fufa, Hanson, P., Ledesma, D. R., Minja, R., Mbwambo, O., Tilya, M. S., & Stoilova, T. (2019). Yield of Vegetable Amaranth in Diverse Tanzanian Production Environments. *HortTechnology*, *29*(4), 516–527. https://doi.org/10.21273/HORTTECH04374-19

Dlamini, V. V, & Viljoen, A. T. (2020). Investigating Knowledge on Indigenous Green Leafy Vegetables Amongst Rural Women in Eswatini. *Journal of Consumer Sciences*, *5*(Special Edition, Food and nutrition challenges in South Africa), 52–66.

Dzino-Silajdzic, V. (2018). *Focus Group Discussions, Practical guide*. Catholic Relief Services.

Ebert, A. W. (2020). The Role of Vegetable Genetic Resources in Nutrition Security and Vegetable Breeding. *Plants*, *9*(736), 1–20. https://doi.org/doi:10.3390/plants9060736

Ebert, A. W., Wu, T., & Wang, S. (2011). *International Cooperators' Guide: Vegetable amaranth (Amaranthus L.)*. 1–9.

Enfissi, E. M. A., Nogueira, M., Bramley, P. M., & Fraser, P. D. (2017). The regulation of carotenoid formation in tomato fruit. *Plant Journal*, *89*(4), 774–788. https://doi.org/10.1111/tpj.13428

Erum, S., Naeemullah, M., Masood, S., Qayyum, A., & Rabbani, M. A. (2012). Genetic divergence in Amaranthus collected from Pakistan. *Journal of Animal & Plant Science*, *22*(3), 653–658.

Faber, M., Oelofse, a, Van Jaarsveld, P. J., Wenhold, F. a M., & Jansen van Rensburg, W. S. (2010). African leafy vegetables consumed by households in the Limpopo and KwaZulu-Natal provinces in South Africa. *South African Journal of Clinical Nutrition*, *23*(1), 30–38.

FAO, IFAD, UNICEF, WFP, & WHO. (2020). *The State of Food Security and Nutrition in the World 2020. Transforming food systems for affordable healthy diets*. FAO. https://doi.org/10.4060/ca9692en

Fekadu, H. (2013). Effect of Traditional Processing Methods on Nutritional Composition and Anti-nutritional Factors of Anchote (Coccinia Abyssinica (lam.) Cogn) Tubers Grown in Western Ethiopia. *Journal of Food Processing & Technology*, *04*(07). https://doi.org/10.4172/2157-7110.1000249

Fenech, M., Amaya, I., Valpuesta, V., & Botella, M. A. (2019). Vitamin C content in fruits: Biosynthesis and regulation. *Frontiers in Plant Science*, *9*(January), 1–21. https://doi.org/10.3389/fpls.2018.02006

Fritts, J. R., Bermudez, M. A., Hargrove, R. L., Alla, L., Fort, C., Liang, Q., Cravener, T. L., Rolls, B. J., D'Adamo, C. R., Hayes, J. E., & Keller, K. L. (2019). Using Herbs and Spices to Increase Vegetable Intake Among Rural Adolescents. *Journal of Nutrition Education and Behavior*, *51*(7), 806-816.e1. https://doi.org/10.1016/j.jneb.2019.04.016

Garcia-Casal, N. (2006). Carotenoids increase iron absorption from cereal based food in the humans. *Nutr Res*, *26*, 340–344.

Gerrano, A. S., Rensburg, W. S. J., & Adebola, P. O. (2014). Agro-morphological variability of Amaranthus genotypes in South Africa. *Acta Horticulturae*, *11*(1035), 183–187.

Gewa, C. A., Onyango, A. C., Angano, F. O., Stabile, B., Komwa, M., Thomas, P., & Krall, J. (2019). Mothers' beliefs about indigenous and traditional food affordability, availability and taste are significant predictors of indigenous and traditional food consumption among mothers and young children in rural Kenya. *Public Health Nutrition*, *22*(16), 2950–2961. https://doi.org/10.1017/S1368980019001848

Gharibi, S., Sayed Tabatabaei, B. E., Saeidi, G., Talebi, M., & Matkowski, A. (2019). The effect of drought stress on polyphenolic compounds and expression of flavonoid biosynthesis related genes in Achillea pachycephala Rech.f. *Phytochemistry*, *162*(March), 90–98. https://doi.org/10.1016/j.phytochem.2019.03.004

Gido, E. O., Ayuya, O. I., Owuor, G., & Bokelmann, W. (2017a). Consumer Acceptance of Leafy African Indigenous Vegetables: Comparison Between Rural and Urban Dwellers. *International Journal of Vegetable Science*, *23*(4), 346–361. https://doi.org/10.1080/19315260.2017.1293758

Gido, E. O., Ayuya, O. I., Owuor, G., & Bokelmann, W. (2017b). Consumption intensity of leafy African indigenous vegetables: towards enhancing nutritional security in rural and urban dwellers in Kenya. *Agricultural and Food Economics*, *5*(1). https://doi.org/10.1186/s40100-017-0082-0

Global Nutrition Report. (2019). *Country overview: Malnutrition burden*. Global Nutrition Report. https://globalnutritionreport.org/resources/nutrition-profiles/africa/eastern-africa/kenya/

Global Nutrition Report. (2020). Action on equity to end malnutrition. In *Global Nutrition Report* (Issue June). http://www.segeplan.gob.gt/2.0/index.php?option=com_content&view=article&id=472&Itemid=472

Gogo, E. O., Opiyo, A., Ulrichs, C., & Huyskens-Keil, S. (2018). Loss of African Indigenous Leafy Vegetables along the Supply Chain. *International Journal of Vegetable Science*, *24*(4), 361–382. https://doi.org/10.1080/19315260.2017.1421595

Gopalakrishnan, L., Doriya, K., & Kumar, D. S. (2016). Moringa oleifera: A review on nutritive importance and its medicinal application. *Food Science and Human Wellness*, *5*(2), 49–56. https://doi.org/10.1016/j.fshw.2016.04.001

Habwe, F. (2012). *Food Preparation and Processing Methods on Nutrient Retention and Accessibility in Selected Indigenous Vegetables from East Africa*. *January*.

Han, O. (2011). Molecular mechanism of intestinal iron absorption. *Metallomics*, *3*(2), 103–109.

Hanson, P. M., Yang, R. Y., Wu, J., Chen, J. T., Ledesma, D., Tsou, S. C. S., & Lee, T. C. (2004). Variation for antioxidant activity and antioxidants in tomato. *Journal of the American Society for Horticultural Science*, *129*(5), 704–711. https://doi.org/10.21273/jashs.129.5.0704

Hashimoto, H., Uragami, C., & Cogdell, R. J. (2016). Carotenoids and Photosynthesis. In *Stange, C. (ed.) Carotenoids in Nature: Biosynthesis, Regulation and Function. Subcellular biochemistry (79)* (pp. 111–139). Springer International

Publishing Switzerland. https://doi.org/10.1007/978-3-319-39126-7_4

Hatfield, J. L., & Prueger, J. H. (2010). Value of using different vegetative indices to quantify agricultural crop characteristics at different growth stages under varying management practices. *Remote Sensing*, *2*(2), 562–578. https://doi.org/10.3390/rs2020562

HCD. (2016). Validated Report 2015-2016. *Directorate of Horticultural Crops*, *Validated report*, 52–53. https://www.agricultureauthority.go.ke/wp-content/uploads/2016/09/Horticulture-2015_2016-Validated-Report3.pdf

Hiscock, L., Bothma, C., Hugo, A., Van Biljon, A., & Van Rensburg, W. S. J. (2018). Overall liking and sensory profiling of boiled Amaranthus leaves using the check-all-that-apply question. *CYTA - Journal of Food*, *16*(1), 822–830. https://doi.org/10.1080/19476337.2018.1464521

Hossain, A., Khatun, M. A., Islam, M., & Huque, R. (2017). Enhancement of antioxidant quality of green leafy vegetables upon different cooking method. *Preventive Nutrition and Food Science*, *22*(3), 216–222. https://doi.org/10.3746/pnf.2017.22.3.216

Hou, X., Rivers, J., León, P., McQuinn, R. P., & Pogson, B. J. (2016). Synthesis and Function of Apocarotenoid Signals in Plants. *Trends in Plant Science*, *21*(9), 792–803. https://doi.org/10.1016/j.tplants.2016.06.001

Hurrell, R., & Egli, I. (2010). Iron bioavailability and dietary reference values. *American Journal of Clinical Nutrition*, *91*(5), 1461S-1467S. https://doi.org/10.3945/ajcn.2010.28674F

Hussain, S., Hussain, S., Qadir, T., Khaliq, A., Ashraf, U., Parveen, A., Saqib, M., & Rafiq, M. (2019). Drought stress in plants: An overview on implications, tolerance mechanisms and agronomic mitigation strategies. *Plant Science Today*, *6*(4), 389–402. https://doi.org/10.14719/pst.2019.6.4.578 ISSN:

IAEA. (2008). *Field Estimation of Soil Water Content: A Practical Guide to Methods,* *Instrumentation and Sensor Technology*. International Atomic Energy Agency.

Igamberdiev, A. U., & Eprintsev, A. T. (2016). Organic acids: The pools of fixed carbon involved in redox regulation and energy balance in higher plants. *Frontiers in Plant Science*, *7*(2016JULY), 1–15. https://doi.org/10.3389/fpls.2016.01042

Ishiekwene, I., Dada, T., Odoko, J., & Nwose, E. (2019). Promoting African indigenous vegetables and its medical nutrition properties: A mini-narrative review based on Ukwani communities of Delta State Nigeria. *Integrative Food, Nutrition and Metabolism*, *6*(4), 1–6. https://doi.org/10.15761/ifnm.1000256

Jafari, S. M., & McClements, D. J. (2017). Nanotechnology Approaches for Increasing Nutrient Bioavailability. In *Advances in Food and Nutrition Research* (1st ed., Vol. 81). Elsevier Inc. https://doi.org/10.1016/bs.afnr.2016.12.008

Jimoh, M. O., Afolayan, A. J., & Lewu, F. B. (2018). Suitability of Amaranthus species for alleviating human dietary deficiencies. *South African Journal of Botany*, *115*, 65–73. https://doi.org/10.1016/j.sajb.2018.01.004

Johannsen, W. (2014). The genotype conception of heredity. *Int. J. Epidemiol.*, *43*, 989–1000.

Kachiguma, N. A., Mwase, W., Maliro, M., & Damaliphetsa, A. (2015). Chemical and Mineral Composition of Amaranth (Amaranthus L.) Species Collected From Central Malawi. *Journal of Food Research*, *4*(4), 92. https://doi.org/10.5539/jfr.v4n4p92

Kadoshnikov, S. I., Kadoshnikova, I. G., Kulikov, Y. A., & Martirosyan, D. M. (2008). Researches of Fractional Composition of Protein of Amaranth. *Current Nutrition & Food Science*, *4*(3), 196–205. https://doi.org/10.2174/157340108785133347

Kalmpourtzidou, A., Eilander, A., & Talsma, E. F. (2020). Global vegetable intake and supply compared to recommendations: A systematic review. *Nutrients*, *12*(6), 22–29. https://doi.org/10.3390/nu12061558

Kamble, V. S., & Jadhav, V. D. (2013). Traditional Leafy Vegetables : A Future Herbal Medicine. *International Journal of Agricultural and Food Science*, *3*(2), 56–58.

Kamenwa, R. (2017). *State of Nutrition in*. *April*.

Kansiime, M. K., Ochieng, J., Kessy, R., Karanja, D., Romney, D., & Afari-Sefa, V. (2018). Changing knowledge and perceptions of African indigenous vegetables: the role of community-based nutritional outreach. *Development in Practice*, *28*(4), 480–493. https://doi.org/10.1080/09614524.2018.1449814

Kao, J., Mutuku, F., Martin, S., Lee, J., Mwandi, J., Mukoko, D., Malhotra, I., King, C. H., & LaBeaud, A. D. (2019). Early childhood anemia in a birth cohort in coastal Kenya: Links to infection and nutrition. *American Journal of Tropical Medicine and Hygiene*, *101*(1), 242–252. https://doi.org/10.4269/ajtmh.17-0688

Kapil, R. (2017). Bioavailability & absorption of Iron and Anemia. *Indian Journal of Community Health*, *29*(4), 453–457.

Karanja, D., Okoko, N., Kiptarus, E., Okong, P., Samali, S., Katunzi, A., Mtwaenzi, H., Mwakitwange, F., Afari-sefa, V., Musebe, R., Kimani, M., & Kimenye, L. (2012). *Promoting farmer led seed enterprises of African indigenous vegetables to boost household incomes and nutrition in Kenya and Tanzania.* 1–14.

Kariuki, L. W., Lambert, C., Purwestri, R. C., Maundu, P., & Biesalski, H. K. (2017). Role of food taboos in energy, macro and micronutrient intake of pregnant women in western Kenya. *Nutrition and Food Science*, *47*(6), 795–807. https://doi.org/10.1108/NFS-09-2016-0146

Khamis, A. G., Mwanri, A. W., Ntwenya, J. E., & Kreppel, K. (2019). The influence of dietary diversity on the nutritional status of children between 6 and 23 months of age in Tanzania. *BMC Pediatrics*, *19*(1), 1–9. https://doi.org/10.1186/s12887-019-1897-5

Khanam, U. K. S., & Oba, S. (2013). Bioactive substances in leaves of two amaranth species, Amaranthus tricolor and A. hypochondriacus. *Canadian Journal of Plant Science*, *93*(1), 47–58. https://doi.org/10.4141/CJPS2012-117

Kozera, B., & Rapacz, M. (2013). Reference genes in real-time PCR. *Journal of Applied Genetics*, *54*(4), 391–406. https://doi.org/10.1007/s13353-013-0173-x

Krasensky, J., & Jonak, C. (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *Journal of Experimental Botany*, *63*(4), 1593–1608. https://doi.org/10.1093/jxb/err460

Krause, H., Faße, A., & Grote, U. (2019). Welfare and food security effects of commercializing African indigenous vegetables in Kenya. *Cogent Food & Agriculture*, *5*(1). https://doi.org/10.1080/23311932.2019.1700031

Kruger, J., Mongwaketse, T., Faber, M., van der Hoeven, M., & Smuts, C. M. (2015). Potential contribution of African green leafy vegetables and maize porridge composite meals to iron and zinc nutrition. *Nutrition*, *31*(9), 1117–1123. https://doi.org/10.1016/j.nut.2015.04.010

Kulakow, P. A., & Hauptli, H. (2018). Genetic Characterization of Amaranth. In *Amaranth Biology, Chemistry, and Technology* (pp. 9–22). CRC Press, Taylor & Francis Group 1994.

Laing, W. A., Martínez-Sánchez, M., Wright, M. A., Bulley, S. M., Brewster, D., Dare, A. P., Rassam, M., Wang, D., Storey, R., Macknight, R. C., & Hellens, R. P. (2015). An upstream open reading frame is essential for feedback regulation of ascorbate biosynthesis in arabidopsis. *Plant Cell*, *27*(3), 772–786. https://doi.org/10.1105/tpc.114.133777

Laing, W., Norling, C., Brewster, D., Wright, M., & Bulley, S. (2017). *Ascorbate Concentration In Arabidopsis thaliana And Expression Of Ascorbate Related Genes Using RNAseq In Response To Light And The Diurnal Cycle*. https://doi.org/10.1101/138008

Lee, S., Choi, Y., Jeong, H. S., Lee, J., & Sung, J. (2018). Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. *Food Science and Biotechnology*, *27*(2), 333–342. https://doi.org/10.1007/s10068-017-0281-1

Li, D., Li, C., Yao, Y., Li, M., & Liu, L. (2020). Modern imaging techniques in plant nutrition analysis: A review. *Computers and Electronics in Agriculture*, *174*(April), 105459. https://doi.org/10.1016/j.compag.2020.105459

Li, H., Huang, W., Wang, G. L., Wu, Z. J., & Zhuang, J. (2016). Expression profile analysis of ascorbic acid-related genes in response to temperature stress in the tea plant, Camellia sinensis (L.) O. Kuntze. *Genetics and Molecular Research*, *15*(4), 1–10. https://doi.org/10.4238/gmr.15048756

Li, P., Zhang, S., Zhang, S., Li, F., Zhang, H., Cheng, F., Wu, J., Wang, X., & Sun, R. (2015). Carotenoid biosynthetic genes in Brassica rapa: Comparative genomic analysis, phylogenetic analysis, and expression profiling. *BMC Genomics*, *16*(1), 1–11. https://doi.org/10.1186/s12864-015-1655-5

Libert, B. (1981). Rapid determination of oxalate acid by reverse-phase high performance liquid chromatography. *Journal of Chromatography*, *210*, 540–543.

Lin, J.-T., Liu, S.-C., Shen, Y.-C., & Yang, D.-J. (2011). Comparison of various preparation methods for determination of organic acids in fruit vinegars with a simple ion-exclusion liquid chromatography. *Food Analytical Methods*, *4*(4), 2243–2254.

Lion, R., Arulogun, O., Titiloye, M., Shaver, D., Jain, A., Godwin, B., Sidibe, M., Adejumo, M., Rosseel, Y., & Schmidt, P. (2018). The effect of the "Follow in My Green Food Steps" programme on cooking behaviours for improved iron intake : a quasi-experimental randomized community study. *International Journal of Behavioral Nutrition and Physical Activity*, *15*(79), 1–14.

Liu, H., Bruning, B., Garnett, T., & Berger, B. (2020). Hyperspectral imaging and 3D technologies for plant phenotyping: From satellite to close-range sensing.

Computers and Electronics in Agriculture, *175*(July), 105621. https://doi.org/10.1016/j.compag.2020.105621

Liu, J. X., Feng, K., Duan, A. Q., Li, H., Yang, Q. Q., Xu, Z. S., & Xiong, A. S. (2019). Isolation, purification and characterization of an ascorbate peroxidase from celery and overexpression of the AgAPX1 gene enhanced ascorbate content and drought tolerance in Arabidopsis. *BMC Plant Biology*, *19*(1), 488. https://doi.org/10.1186/s12870-019-2095-1

Liu, X. X., Zhou, K., Hu, Y., Jin, R., Lu, L., Jin, W., & Yong, X. (2015). Oxalate synthesis in leaves is associated with root uptake of nitrate and its assimilation in spinach (Spinacia oleracea L .) plants. *Journal of Science of Food and Agriculture*, *95*, 2105–2116. https://doi.org/10.1002/jsfa.6926

Luten, J., Crews, H., & Flynn, A. (1996). Interlaboratory trial on the determination of the In Vitro iron dialysability from food. *Journal of the Science of Food and Agriculture*, *72*(4), 415–424. https://doi.org/10.1002/(SICI)1097-0010(199612)72:4%3C415::AID-JSFA675%3E3.0.CO;2-X

Ma, X., Xin, Z., Wang, Z., Yang, Q., Guo, S., Guo, X., Cao, L., & Lin, T. (2015). Identification and comparative analysis of differentially expressed miRNAs in leaves of two wheat (Triticum aestivum L.) genotypes during dehydration stress. *BMC Plant Biology*, *15*(1), 1–15. https://doi.org/10.1186/s12870-015-0413-9

Makobo, N. D., Shoko, M. D., & Mtaita, T. A. (2010). *Nutrient Content of Amaranth (Amaranthus cruentus L .) Under Different Processing and Preservation Methods*. *6*(6), 639–643.

Mampholo, B. M., Sivakumar, D., & Thompson, A. K. (2016). Maintaining overall quality of fresh traditional leafy vegetables of Southern Africa during the postharvest chain. *Food Reviews International*, *32*(4), 400–416. https://doi.org/10.1080/87559129.2015.1094817

Manyelo, T. G., Sebola, N. A., van Rensburg, E. J., & Mabelebele, M. (2020). The probable use of genus amaranthus as feed material for monogastric animals. *Animals*, *10*(9), 1–17. https://doi.org/10.3390/ani10091504

Martinez-Lopez, A., Millan-Linares, M. C., Rodriguez-Martin, N. M., Millan, F., & Montserrat-de la Paz, S. (2020). Nutraceutical value of kiwicha (Amaranthus caudatus L.). *Journal of Functional Foods*, *65*, 103735. https://doi.org/https://doi.org/10.1016/j.jff.2019.103735

Maseko, I., Id, T. M., & Tesfay, S. (2017). *African Leafy Vegetables : A Review of Status , Production and Utilization in South Africa*. 1–16. https://doi.org/10.3390/su10010016

Matenge, S. T. P., Merwe, D. Van Der, Beer, H. De, Bosman, M. J. C., & Kruger, A. (2012). Consumers' beliefs on indigenous and traditional foods and acceptance of products made with cow pea leaves. *African Journal of Agricultural Research*, *7*(14), 2236–2242. https://doi.org/10.5897/AJAR10.858

Mayekiso, A., Taruvinga, A., & Mushunje, A. (2017). Perceptions and determinants of smallholder farmers' participation in the production of indigenous leafy vegetables: The case of Coffee Bay, Eastern Cape province of South Africa. *African Journal of Science, Technology, Innovation and Development*, *9*(3), 281–287. https://doi.org/10.1080/20421338.2017.1322748

Mbwambo, O., Abukutsa Onyango, M. O., Dinssa, F. F., & Ojiewo, C. (2015). Performances of elite amaranth genotypes in grain and leaf yields in Northern Tanzania. *Journal of Horticulture and Forestry*, *7*(2), 16–23. https://doi.org/10.5897/JHF2014.0377

Mepba, H. D., Eboh, L., & Banigo, D. E. B. (2007). Effects of processing treatments on the nutritive composition and consumer acceptance of some Nigerian edible leafy vegetables. *African Journal of Agriculture Nutrition and Development*, *7*(1), 1–18. https://doi.org/10.2331/suisan.35.791

Miglio, C., Chiavaro, E., Visconti, A., Fogliano, V., & Pellegrini, N. (2008). Effects of different cooking methods on nutritional and physicochemical characteristics of selected vegetables. *Journal of Agricultural and Food Chemistry*, *56*(1), 139–147. https://doi.org/10.1021/jf072304b

Miyagi, A., Uchimiya, M., Kawai-Yamada, M., & Uchimiya, H. (2013). An antagonist treatment in combination with tracer experiments revealed isocitrate pathway dominant to oxalate biosynthesis in Rumex obtusifolius L. *Metabolomics*, *9*, 590–598. https://doi.org/10.1007/s11306-012-0486-5

Mncwango, N. C., Mavengahama, S., Ntuli, N. R., & Jaarsveld, C. M. V. A. N. (2020). Diversity , consumption dynamics and ethnomedical claims of traditional leafy vegetables consumed by a rural community in the KwaMbonambi area , northern KwaZulu-Natal , South Africa. *Biodiversitas*, *21*(3), 1201–1207. https://doi.org/10.13057/biodiv/d210347

Mnzava, N. M., Dearing, J. A., Guarino, L., & Chweya, J. A. (1999). Bibliography of the genetic resources of traditional African vegetables. In H. de Koeijer (Ed.), *International Plant Genetic Resources Institute* (Vol. 2).

Mokganya, M. G., & Tshisikhawe, M. P. (2019). Medicinal uses of selected wild edible vegetables consumed by Vhavenda of the Vhembe District Municipality, South Africa. *South African Journal of Botany*, *122*, 184–188. https://doi.org/10.1016/j.sajb.2018.09.029

Moyo, S. M., Mavumengwana, V., & Kayitesi, E. (2018). Effects of cooking and drying on phenolic compounds and antioxidant activity of African green leafy vegetables. *Food Reviews International*, *34*(3), 248–264. https://doi.org/10.1080/87559129.2017.1289384

Musotsi, A., Makokha, A., Abukutsa-Onyango, M., & Kilonzi, S. M. (2019). Quantitative Changes of Ascorbic acid and Beta carotene in African nightshade (S olanum nigrum) and Spider plant (Cleome gynandra) due to traditional cooking methods used in western Kenya. *Journal of Food and Nutritional*

Sciences Research, *1*(1), 51–63.

Musotsi, A.A, Sigot, A. ., & Abukutsa-Onyango, M. . (2005). African Indigenous Vegetables Recipe Documentation and their role in food security. *Proceedings of the Third Horticulture Workshop OnSustainable Horticultural Production in the Tropics,26th -29th November 2003.*, 105-111Musotsi, A. ., Sigot, A. ., Abukutsa-Onya.

Musotsi, Ann Aswani. (2017). Meal attitudes and practices influencing consumption of African Indigenous Vegetables in Kenya. *African Journal for Horticultural Sciences*, *12*, 1–7.

Musotsi, Ann Aswani, Abukutsa-Onyango, M., & Makokha, A. (2017). Changing food consumption habits: A Case of African Indigenous Vegetables for Food and Nutrition Security in Kakamega County, Western Kenya. *African Journal of Horticultural Sciences*, *12*, 30–39. http://www.hakenya.net/ajhs/index.php/ajhs/article/view/183

Namrata, Gautam, N., & Shiv, K. (2021). Fortification of Pasta with Amaranth Leaf Powder and Defatted Soy Flour: Effects on Physical, Cooking and Sensory Characteristics. *Journal of Advanced Scientific Research*, *12*(3), 86–95. http://www.sciensage.info/journal/1359303580JASR_3006121.pdf

Nana, F. W., Hilou, A., Millogo, J. F., & Nacoulma, O. G. (2012). Phytochemical composition, antioxidant and xanthine oxidase inhibitory activities of Amaranthus cruentus L. and Amaranthus hybridus L. Extracts. *Pharmaceuticals*, *5*(6), 613–628. https://doi.org/10.3390/ph5060613

Nardo, A. E., Suárez, S., Quiroga, A. V., & Añón, M. C. (2020). Amaranth as a Source of Antihypertensive Peptides. *Frontiers in Plant Science*, *11*(September), 1–15. https://doi.org/10.3389/fpls.2020.578631

Natesh, N. H., Abbey, L., & Asiedu, S. (2017). An Overview of Nutritional and Antinutritional Factors in Green Leafy Vegetables. *Horticulture International*

Journal, *1*(2), 1–9. https://doi.org/10.15406/hij.2017.01.00011

Ndinya, C., Onyango, E., Dinssa, F., Odendo, M., & Simon, J. (2020). Participatory variety selection of three african leafy vegetables in Western Kenya. *Journal of Medicinally Active Plants*, *9*(3), 145. https://doi.org/10.7275/mkq0-3p85

Nisar, N., Li, L., Lu, S., Khin, N. C., & Pogson, B. J. (2015). Carotenoid metabolism in plants. *Molecular Plant*, *8*(1), 68–82. https://doi.org/10.1016/j.molp.2014.12.007

Niveyro, S. L., Mortensen, A. G., Fomsgaard, I. S., & Salvo, A. (2013). Differences among five amaranth varieties (Amaranthus spp.) regarding secondary metabolites and foliar herbivory by chewing insects in the field. *Arthropod-Plant Interactions*, *7*(2), 235–245. https://doi.org/10.1007/s11829-012-9219-y

Nomkong, R. F., Ejoh, R. A., Dibanda, R. F., & Gabriel, M. N. (2019). Bioavailability of Iron and Related Components in Cooked Green Leafy Vegetables Consumed in Cameroon. *Food and Nutrition Sciences*, *10*(9), 1096–1111. https://doi.org/10.4236/fns.2019.109079

Ntawuruhunga, D., Affognon, H. D., Fiaboe, K. K. M., & Abukutsa-onyango, M. O. (2020). Farmers ' knowledge , attitudes and practices (KAP) on production of African indigenous vegetables in Kenya. *International Journal of Tropical Insect Science*. https://doi.org//10.1007/s42690-019-00085-8

Nyonje, W. A., Makokha, A. O., & Abukutsa-Onyango, M. O. (2014). Anti-Nutrient, Phytochemical and Antiradical Evaluation of 10 Amaranth (Amaranthus spp.) Varieties Before and After Flowering. *Journal of Agricultural Science*, *6*(4), 68. https://doi.org/10.5539/jas.v6n4p68

Ochieng, J., Afari-sefa, V., Karanja, D., & Kessy, R. (2017). How promoting consumption of traditional African vegetables affects household nutrition security in Tanzania. *Renewable Agriculture and Food Systems*, *33*(2), 105–115. https://doi.org/10.1017/S1742170516000508

Ochieng, J., Schreinemachers, P., Ogada, M., Dinssa, F. F., Barnos, W., & Mndiga, H. (2019). Adoption of improved amaranth varieties and good agricultural practices in East Africa. *Land Use Policy*, *83*(August 2018), 187–194. https://doi.org/10.1016/j.landusepol.2019.02.002

Odendo, M., Ndinya-omboko, C., Merchant, E. V, & Minyatta-onyango, E. (2020). Do Preferences for Attributes of African Indigenous Vegetables Recipes Vary between Men and Women ? A case from Western Kenya. *Journal of Medicinally Active Plants*, *9*(3), 126–132.

Oduwaye, O. A., Ayo-Vaughan, M. A., Porbeni, J. B. O., & Oyelakin, O. O. (2019). Genetic diversity in Amaranth (Amaranthus spp .) based on phenotypic and RAPD markers. *Nig. J. Biotech*, *36*(1), 62–68. https://doi.org/10.4314/njb.v36i1.9

Olaide, M., Jide, A., & Bayo, F. (2018). South African Journal of Botany Suitability of Amaranthus species for alleviating human dietary de fi ciencies. *South African Journal of Botany*, *115*, 65–73. https://doi.org/10.1016/j.sajb.2018.01.004

Olajide, O. A., Ogunleye, B. R., & Erinle, T. O. (2004). Anti-inflammatory Properties of Amaranthus spinosus Leaf Extract . *Pharmaceutical Biology*, *42*(7), 521–525. https://doi.org/10.3109/13880200490893285

Olawoye, B. T., & Gbadamosi, S. O. (2017). Effect of different treatments on in vitro protein digestibility , antinutrients , antioxidant properties and mineral composition of Amaranthus viridis seed. *Cogent Food & Agriculture*, *94*(1), 1–14. https://doi.org/10.1080/23311932.2017.1296402

Olowolaju, E. D., Okunlola, G. O., Adejumo, A. M., & Adelusi, A. A. (2018). Effects of Prior Heat Stress on the Growth and Phytochemical Contents Accumulation of Amaranthus hybridus (Linn.). *Notulae Scientia Biologicae*, *10*(2), 240–244. https://doi.org/10.15835/nsb10210270

Oluoch, M. O., Habwe, F. O., Ngegba, J. B., & Koskei, K. R. (2012). Food Preparation and Processing Methods on Nutrient Retention and Accessibility in Selected Indigenous Vegetables from East Africa. *Journal of Medicinally Active Plants*, *October 2017*.

Olusanya, R., Unathi, K., Nomali, N., & Chinsamy, M. (2021). Underutilization Versus Nutritional-Nutraceutical Potential of the Amaranthus Food Plant: A Mini-Review. *Applied Sciences*, *11*(15), 6879. https://doi.org/10.3390/app11156879

Omotayo, A. O., Ndhlovu, P. T., Tshwene, S. C., & Aremu, A. O. (2020). Utilization pattern of indigenous and naturalized plants among some selected rural households of North West Province, South Africa. *Plants*, *9*(8), 1–15. https://doi.org/10.3390/plants9080953

Onyango, C. M., Harbinson, J., Imungi, J. K., & Onwonga, R. N. (2012). Effect of Nitrogen Source , Crop Maturity Stage and Storage Conditions on Phenolics and Oxalate Contents in Vegetable Amaranth (Amaranthus hypochondriacus). *Journal of Agricultural Science*, *4*(7), 219–230. https://doi.org/10.5539/jas.v4n7p219

Paakki, M., Sandell, M., & Hopia, A. (2019). Visual attractiveness depends on colorfulness and color contrasts in mixed salads. *Food Quality and Preference*, *76*, 81–90. https://doi.org/10.1016/J.FOODQUAL.2019.04.004

Palavecino, P. M., Penci, M. C., Calderón-Domínguez, G., & Ribotta, P. D. (2016). Chemical composition and physical properties of sorghum flour prepared from different sorghum hybrids grown in Argentina. *Starch/Staerke*, *68*(11–12), 1055–1064. https://doi.org/10.1002/star.201600111

Pandey, R., Meena, M., Paul, V., Jain, N., Chinnusamy, V., & Rathod, G. (2018). *Visual image based phenoyping of biomass and growth in plants under field conditions Visual image based phenoyping of biomass and growth in plants under field conditions* (Issue February 2019).

Parizel, O., Labouré, H., Marsset-Baglieri, A., Fromentin, G., & Sulmont-Rossé, C. (2017). Providing choice and/or variety during a meal: Impact on vegetable liking and intake. *Appetite*, *108*, 391–398. https://doi.org/10.1016/j.appet.2016.10.027

Pasaporte, M. S., Rabaya, F. J. R., Toleco, M. M., & Flores, D. M. (2014). Xanthophyll content of selected vegetables commonly consumed in the Philippines and the effect of boiling. *Food Chemistry*, *158*(3297), 35–40. https://doi.org/10.1016/j.foodchem.2014.02.090

Penniston, K. L. (2014). *Dietary oxalate and calcium oxalate stones : a theoretical or real concern ?* https://doi.org/10.1007/978-1-4614-9575-8

Peter, K., & Gandhi, P. (2017). Rediscovering the therapeutic potential of Amaranthus species : A review. *Egyptian Journal of Basic and Applied Sciences*. https://doi.org/10.1016/j.ejbas.2017.05.001

Platel, K., & Srinivasan, K. (2015). Bioavailability of micronutrients from plant foods: An update. *Critical Reviews in Food Science and Nutrition*, *56*(10), 1608–1619. https://doi.org/10.1080/10408398.2013.781011

Platel, K., & Srinivasan, K. (2016). *Bioavailability of Micronutrients from Plant Foods : An Update Bioavailability of Micronutrients from Plant Foods : An Update*. *8398*. https://doi.org/10.1080/10408398.2013.781011

Poelman, A. A. M., Delahunty, C. M., & de Graaf, C. (2017). Vegetables and other core food groups: A comparison of key flavour and texture properties. *Food Quality and Preference*, *56*, 1–7. https://doi.org/10.1016/j.foodqual.2016.09.004

Popova, A., & Mihaylova, D. (2019). Antinutrients in Plant-based Foods: A Review. *The Open Biotechnology Journal*, *13*(1), 68–76. https://doi.org/10.2174/1874070701913010068

Punchay, K., Inta, A., Tiansawat, P., Balslev, H., & Wangpakapattanawong, P. (2020). Nutrient and mineral compositions of wild leafy vegetables of the karen and lawa communities in thailand. *Foods*, *9*(12). https://doi.org/10.3390/foods9121748

Radek, M., & Savage, G. P. (2009). Oxalates in some Indian green leafy vegetables. *International Journal of Food Sciences and Nutrition*, *7486*(2008), 246–260. https://doi.org/10.1080/09637480701791176

Rashad, M. M. I., & Sarker, U. (2020). Genetic variations in yield and yield contributing traits of green amaranth. *Genetika*, *52*(1), 393–407. https://doi.org/doi.10.2298/GENSR2001393R

Rastogi, A., & Shukla, S. (2013). Amaranth: A New Millennium Crop of Nutraceutical Values. *Critical Reviews in Food Science and Nutrition*, *53*(2), 109–125. https://doi.org/10.1080/10408398.2010.517876

Rodriguez-Ramiro, I., Dell'Aquila, C., Ward, J. L., Neal, A. L., Bruggraber, S. F. A., Shewry, P. R., & Fairweather-Tait, S. (2019). Estimation of the iron bioavailability in green vegetables using an in vitro digestion/Caco-2 cell model. *Food Chemistry*, *301*(July), 125292. https://doi.org/10.1016/j.foodchem.2019.125292

Rodriguez, D. (2001). A Guide to Carotenoid Analysis in Foods. In *Life Sciences*.

Rothwell, J. A., Medina-Remón, A., Pérez-Jiménez, J., Neveu, V., Knaze, V., Slimani, N., & Al., E. (2015). Effects of food processing on polyphenol contents: A systematic analysis using phenol-explorer data. *Molecular Nutrition & Food Research*, *59*(1), 160–170.

Saga, G., Giorgetti, A., Fufezan, C., Giacometti, G. M., Bassi, R., & Morosinotto, T. (2010). Mutation analysis of violaxanthin de-epoxidase identifies substrate-binding sites and residues involved in catalysis. *Journal of Biological Chemistry*, *285*(31), 23763–23770. https://doi.org/10.1074/jbc.M110.115097

Samtiya, M., Aluko, R. E., & Dhewa, T. (2020). Plant food anti-nutritional factors and their reduction strategies: an overview. *Food Production, Processing and Nutrition*, *2*(1), 1–14. https://doi.org/10.1186/s43014-020-0020-5

Sánchez-López, F., Robles-Olvera, V. J., Hidalgo-Morales, M., & Tsopmo, A. (2020).

Characterization of Amaranthus hypochondriacus seed protein fractions, and their antioxidant activity after hydrolysis with lactic acid bacteria. *Journal of Cereal Science*, *95*, 103075. https://doi.org/https://doi.org/10.1016/j.jcs.2020.103075

Sandell, M., Hoppu, U., Mikkilä, V., Mononen, N., Kähönen, M., Männistö, S., Rönnemaa, T., Viikari, J., Lehtimäki, T., & Raitakari, O. T. (2014). Genetic variation in the hTAS2R38 taste receptor and food consumption among Finnish adults. *Genes and Nutrition*, *9*(6), 1–8. https://doi.org/10.1007/s12263-014-0433-3

Sarker, U. (2020a). Nutritional and bioactive constituents and scavenging capacity of radicals in Amaranthus hypochondriacus. *Scientific Reports*, *0123456789*, 1–10. https://doi.org/10.1038/s41598-020-71714-3

Sarker, U. (2020b). Phenolic profiles and antioxidant activities in selected drought - tolerant leafy vegetable amaranth. *Scientific Reports*, *0123456789*, 1–11. https://doi.org/10.1038/s41598-020-71727-y

Sarker, U., Hossain, M. N., Iqbal, M. A., & Oba, S. (2020). Bioactive Components and Radical Scavenging Activity in Selected Advance Lines of Salt-Tolerant Vegetable Amaranth. *Frontiers in Nutrition*, *7*(November), 1–15. https://doi.org/10.3389/fnut.2020.587257

Sarker, U., Hossain, M., & Oba, S. (2020). Nutritional and antioxidant components and antioxidant capacity in green morph Amaranthus leafy vegetable. *Scientific Reports*, 1–10. https://doi.org/10.1038/s41598-020-57687-3

Sarker, U., Islam, G., Rabbani, G., & Oba, S. (2015a). Genotype variability in composition of antioxidant vitamins and minerals in vegetable amaranth. *Genetika*, *47*(1), 85–96. https://doi.org/10.2298/GENSR1501085S

Sarker, U., Islam, M. T., & Oba, S. (2019). Salinity stress accelerates nutrients, dietary fiber, minerals, phytochemicals and antioxidant activity in Amaranthus tricolor leaves. *PLoS ONE*, *13*(11), 1–18. https://doi.org/10.1371/journal.pone.0206388

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2015b). Variability, heritability and genetic association in vegetable amaranth (Amaranthus tricolor L.). *Spanish Journal of Agricultural Research*, *13*(2), 1–8. https://doi.org/10.5424/sjar/2015132-6843

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2016). Genetic variation and interrelationships among antioxidant,quality, and agronomic traits in vegetable amaranth. *Turkish Journal of Agriculture and Forestry*, *40*, 526–535. https://doi.org/10.3906/tar-1405-83

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2017). Genotypic diversity in vegetable amaranth for antioxidant, nutrient and agronomic traits. *Indian Journal of Genetics and Plant Breeding*, *77*(1), 173–176. https://doi.org/10.5958/0975-6906.2017.00025.6

Sarker, U., Islam, M. T., Rabbani, M. G., & Oba, S. (2018a). Variability in total antioxidant capacity, antioxidant leaf pigments and foliage yield of vegetable amaranth. *Journal of Integrative Agriculture*, *17*(5), 1145–1153. https://doi.org/10.1016/S2095-3119(17)61778-7

Sarker, U., Islam, T., & Oba, S. (2018). Antioxidant leaf pigments and variability in vegetable amaranth. *Genetica*, *50*(1), 209–220. https://doi.org/10.2298/GENSR1801209S

Sarker, U., Islam, T., Rabbani, G., & Oba, S. (2018b). Phenotypic divergence in vegetable amaranth for total antioxidant capacity , antioxidant profile , dietary fiber , nutritional and agronomic traits. *Acta Agriculturae Scandinavica, Section B- Soil & Plant Science*, *68*(1), 67–76. https://doi.org/10.1080/09064710.2017.1367029

Sarker, U., & Oba, S. (2018a). Augmentation of leaf color parameters, pigments, vitamins, phenolic acids, flavonoids and antioxidant activity in selected

Amaranthus tricolor under salinity stress. *Scientific Reports*, *8*(1), 12349. https://doi.org/10.1038/s41598-018-30897-6

Sarker, U., & Oba, S. (2018b). Catalase, superoxide dismutase and ascorbate-glutathione cycle enzymes confer drought tolerance of Amaranthus tricolor. *Scientific Reports*, *8*(1), 1–12. https://doi.org/10.1038/s41598-018-34944-0

Sarker, U., & Oba, S. (2018c). Drought Stress Effects on Growth, ROS Markers, Compatible Solutes, Phenolics, Flavonoids, and Antioxidant Activity in Amaranthus tricolor. *Applied Biochemistry and Biotechnology*, *186*(4), 999–1016. https://doi.org/10.1007/s12010-018-2784-5

Sarker, U., & Oba, S. (2018d). Drought stress enhances nutritional and bioactive compounds, phenolic acids and antioxidant capacity of Amaranthus leafy vegetable. *BMC Plant Biology*, *18*(1). https://doi.org/10.1186/s12870-018-1484-1

Sarker, U., & Oba, S. (2018e). Response of nutrients, minerals, antioxidant leaf pigments, vitamins, polyphenol, flavonoid and antioxidant activity in selected vegetable amaranth under four soil water content. *Food Chemistry*, *252*, 72–83. https://doi.org/10.1016/j.foodchem.2018.01.097

Sarker, U., & Oba, S. (2018f). Salinity stress enhances color parameters , bioactive leaf pigments , vitamins , polyphenols , flavonoids and antioxidant activity in selected Amaranthus leafy vegetables. *Journal of the Science of Food and Agriculture*, *99*, 2275–2284. https://doi.org/10.1002/jsfa.9423

Sarker, U., & Oba, S. (2019a). Antioxidant constituents of three selected red and green color Amaranthus leafy vegetable. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-52033-8

Sarker, U., & Oba, S. (2019b). Nutraceuticals , antioxidant pigments , and phytochemicals in the leaves of Amaranthus spinosus and Amaranthus viridis weedy species. *Scientific Reports*, *9*(20413), 1–11.

https://doi.org/10.1038/s41598-019-50977-5

Sarker, U., & Oba, S. (2019c). Protein , dietary fiber , minerals , antioxidant pigments and phytochemicals , and antioxidant activity in selected red morph Amaranthus leafy vegetable. *PLoS ONE*, *4*(12), 1–16. https://doi.org/doi.10.1371/journal.pone.0222517

Sarker, U., & Oba, S. (2020a). Leaf pigmentation , its profiles and radical scavenging activity in selected Amaranthus tricolor leafy vegetables. *Scientific Reports*, 1–10. https://doi.org/10.1038/s41598-020-66376-0

Sarker, U., & Oba, S. (2020b). Nutraceuticals, phytochemicals, and radical quenching ability of selected drought- tolerant advance lines of vegetable amaranth. *BMC Plant Biology*, *20:564*, 1–16. https://doi.org/10.1186/s12870-020-02780-y

Sarker, U., & Oba, S. (2020c). Nutrients , minerals , pigments , phytochemicals , and radical scavenging activity in Amaranthus blitum leafy vegetables. *Scientific Reports*, *10:3868*, 1–10. https://doi.org/10.1038/s41598-020-59848-w

Sarker, U., & Oba, S. (2020d). Polyphenol and flavonoid profiles and radical scavenging activity in leafy vegetable Amaranthus gangeticus. *BMC Plant Biology*, *20*(1), 1–12. https://doi.org/10.1186/s12870-020-02700-0

Sarker, U., & Oba, S. (2020e). The Response of Salinity Stress-Induced A. tricolor to Growth, Anatomy, Physiology, Non-Enzymatic and Enzymatic Antioxidants. *Frontiers in Plant Science*, *11*(October), 1–14. https://doi.org/10.3389/fpls.2020.559876

Sarker, U., Oba, S., & Daramy, M. A. (2020). Nutrients , minerals , antioxidant pigments and phytochemicals , and antioxidant capacity of the leaves of stem amaranth. *Scientific Reports*, 1–9. https://doi.org/10.1038/s41598-020-60252-7

Savage, G., & Klunklin, W. (2018). Oxalates are Found in Many Different European and Asian Foods - Effects of Cooking and Processing. *Journal of Food Research*, *7*(3), 76. https://doi.org/10.5539/jfr.v7n3p76

Schippers, R. R. (2000). African Indigenous Vegetables, An Overview of the Cultivated Species. In *Natural Resources International Limited,*.

Schirrmann, M., Hamdorf, A., Garz, A., Ustyuzhanin, A., & Dammer, K.-H. (2016). Estimating wheat biomass by combining image clustering with crop height. *Computers and Electronics in Agriculture*, *121*, 374–384. https://doi.org/10.1016/j.compag.2016.01.007.

Schröter, D., Neugart, S., Schreiner, M., Grune, T., Rohn, S., & Ott, C. (2019). Amaranth's 2-caffeoylisocitric acid—an anti-inflammatory caffeic acid derivative that impairs NF-κB signaling in LPS-Challenged RAW 264.7 Macrophages. *Nutrients*, *11*(3). https://doi.org/10.3390/nu11030571

Schweiggert, R. M., & Carle, R. (2017). Carotenoid deposition in plant and animal foods and its impact on bioavailability. *Critical Reviews in Food Science and Nutrition*, *57*(9), 1807–1830. https://doi.org/10.1080/10408398.2015.1012756

Seminario, A., Song, L., Zulet, A., Nguyen, H. T., González, E. M., & Larrainzar, E. (2017). Drought Stress Causes a Reduction in the Biosynthesis of Ascorbic Acid in Soybean Plants. *Frontiers in Plant Science*, *8*(June), 1–10. https://doi.org/10.3389/fpls.2017.01042

Shah, L. R., Afroza, B., Khan, S. H., & Habib, M. (2018). Morphological characterization of Amaranthus spp . under temperate environment using NBPGR descriptor. *Journal of Pharmacognosy and Phytochemistry*, *7*(1), 2716–2718.

Shen, Y., Kennedy, O. B., & Methven, L. (2016). Exploring the effects of genotypical and phenotypical variations in bitter taste sensitivity on perception, liking and intake of brassica vegetables in the UK. *Food Quality and Preference*, *50*, 71–81. https://doi.org/10.1016/j.foodqual.2016.01.005

Shija, A. E., Massaga, J. J., Rumisha, S. F., Oriyo, N. M., & Kilima, S. P. (2019). Effect of Moringa Oleifera leaf powder supplementation on reducing anemia in children below two years in Kisarawe. *Food Science & Nutrition*, *7*(April), 2584–

2594. https://doi.org/10.1002/fsn3.1110

Shiri, S., & Zebarjadi, A. (2018). Isolation and characterization of GDP-L-galactose phosphorylase gene of vitamin C biosynthesis pathway from kiwi. *Journal of Applied Biotechnology Reports*, *5*(2), 59–63. https://doi.org/10.29252/jabr.05.02.04

Shkembi, B., & Huppertz, T. (2022). Calcium absorption from food products: Food matrix effects. *Nutrients*, *14*(1), 1–31. https://doi.org/10.3390/nu14010180

Singh, A., Bains, K., & Kaur, H. (2016). Effect of inclusion of key foods on in vitro iron bioaccessibility in composite meals. *Journal of Food Science and Technology*, *53*(4), 2033–2039. https://doi.org/10.1007/s13197-015-2154-z

Singh, P., & Prasad, S. (2018). Determination of ascorbic acid and its influence on the bioavailability of iron, zinc and calcium in Fijian food samples. *Microchemical Journal*, *139*(2017), 119–124. https://doi.org/10.1016/j.microc.2018.02.019

Smirnoff, N. (2018). Ascorbic acid metabolism and functions: A comparison of plants and mammals. *Free Radical Biology and Medicine*, *122*(November 2017), 116–129. https://doi.org/10.1016/j.freeradbiomed.2018.03.033

Sogbohossou, O. E. D., Achigan-Dako, E. G., Assogba Komlan, F., & Ahanchede, A. (2015). Diversity and Differential Utilization of Amaranthus spp. along the Urban-Rural Continuum of Southern Benin. *Economic Botany*, *69*(1), 9–25. https://doi.org/10.1007/s12231-014-9294-3

Solberg, S. O., Yndgaard, F., & Axelsson, J. (2015). Nitrate and oxalate in germplasm collections of spinach and other leafy vegetables. *Emirates Journal of Food and Agriculture*, *27*(9), 698–705. https://doi.org/10.9755/ejfa.2015-04-050

Stagnari, F., Galieni, A., & Pisante, M. (2016). Drought stress effects on crop quality. In *Water Stress and Crop Plants: A Sustainable Approach* (First Edit, Vol. 2, Issue February 2018, pp. 375–392). John Wiley & Sons. https://doi.org/10.1002/9781119054450.ch23

Stetter, M. G., & Schmid, K. J. (2016). Phylogenetic relationships and genome size evolution within the genus Amaranthus indicate the ancestors of an ancient crop. *BioRxiv Preprint*, *109*, 80–92. https://doi.org/10.1101/085472

Sun, T., Yuan, H., Cao, H., Yazdani, M., Tadmor, Y., & Li, L. (2018). Carotenoid Metabolism in Plants: The Role of Plastids. *Molecular Plant*, *11*(1), 58–74. https://doi.org/10.1016/j.molp.2017.09.010

Suresh, S., Chung, J. W., Cho, G. T., Sung, J. S., Park, J. H., Gwag, J. G., & Baek, H. J. (2014). Analysis of molecular genetic diversity and population structure in Amaranthus germplasm using SSR markers. *Plant Biosystems*, *148*(4), 635–644. https://doi.org/10.1080/11263504.2013.788095

Suzana, D., Suyatna, F. D., Andrajati, R., Sari, S. P., & Mun, A. (2017). Effect of Moringa oleifera Leaves Extract Against Hematology and Blood Biochemical Value of Patients with Iron Deficiency Anemia. *Journal of Yougn Pharmacists*, *9*(1), 79–84.

Taleni, V., & Goduka, N. (2013). Perceptions and Use of Indigenous Leafy Vegetables (ILVs) for Nutritional Value: A Case Study in Mantusini Community, Eastern Cape Province, South Africa. *International Conference on Food and Agricultural Sciences*, *55*(2013), 127–131. https://doi.org/10.7763/IPCBEE.2013

Tang, Y., & Tsao, R. (2017). Phytochemicals in quinoa and amaranth grains and their antioxidant, anti-inflammatory, and potential health beneficial effects: a review. *Molecular Nutrition and Food Research*, *61*(7), 1–16. https://doi.org/10.1002/mnfr.201600767

TDHS. (2016). Tanzania Demorgraphic and Health Survey Indicator Survey (TDHS-MIS) 2015-2016. *Dar Es Salaam, Tanzania, and Rockville, Maryland, USA: MoHCDGEC, MoH, NBS, OCGS, and ICF*, *1*(1), 1–630.

Tejaswini, N., Saidaiah, P., K, R. R., & Ramesh, T. (2017). Evaluation of vegetable amaranth (Amaranthus tricolor . L) genotypes for yield and yield attributing traits. *Journal of Pharmacognosy and Phytochemistry*, *6*(6), 2572–2578.

Thakur, A., Sharma, V., & Thakur, A. (2019). An overview of anti-nutritional factors in food. *International Journal of Chemical Studies*, *7*(1), 2472–2479. https://www.researchgate.net/publication/336983167

Thapa, R., & Blair, M. W. (2018). Morphological Assessment of Cultivated and Wild Amaranth Species Diversity. *Agronomy*, *8*(272), 1–13. https://doi.org/10.3390/agronomy8110272

Thompson, B., & Meerman, J. (2014). Towards long-term nutrition security: the role of agriculture in dietary diversity. In B. Thompson & L. Amoroso (Eds.), *Improving diets and nutrition: food-based approaches.* (p. 246). Food and Agriculture Organization of the United Nations. https://doi.org/10.1079/9781780642994.0246

TNNS. (2018). Tanzania National Nutrition Survey using SMART Methodology (TNNS). In *Dar es Salaam, Tanzania: MoHCDGEC, MoH, TFNC, NBS, OCGS, and UNICEF* (Issue June).

Traoré, K., Parkouda, C., Savadogo, A., Ba/Hama, F., Kamga, R., & Traoré, Y. (2017). Effect of processing methods on the nutritional content of three traditional vegetables leaves: Amaranth, black nightshade and jute mallow. *Food Science and Nutrition*, *5*(6), 1139–1144. https://doi.org/10.1002/fsn3.504

Tsutsumi, N., Tohya, M., Nakashima, T., & Ueno, O. (2017). Variations in structural , biochemical , and physiological traits of photosynthesis and resource use efficiency in Amaranthus species (NAD-ME-type. *Plant Production Science*, *1008*, 1–13. https://doi.org/10.1080/1343943X.2017.1320948

Tuazon-Nartea, J., & Savage, G. (2013). Investigation of Oxalate Levels in Sorrel Plant Parts and Sorrel-Based Products. *Food and Nutrition Sciences*, *04*(08), 838–843. https://doi.org/10.4236/fns.2013.48109

Tufts, H. R., Harris, C. S., Bukania, Z. N., & Johns, T. (2015). Antioxidant and Anti-

Inflammatory Activities of Kenyan Leafy Green Vegetables, Wild Fruits, and Medicinal Plants with Potential Relevance for Kwashiorkor. *Evidence-Based Complementary and Alternative Medicine*, *2015*. https://doi.org/10.1155/2015/807158

URT. (2017). *2016/17 Annual Agriculture Sample Survey*. 88. https://nbs.go.tz/nbs/takwimu/Agriculture/2016_17_AASS_ report.pdf

USAID. (2017). Kenya: Nutrition Profile. In *United States Agency for International Development* (Issue June 2017). https://www.usaid.gov/sites/default/files/documents/1864/Kenya-Nutrition-Profile-Mar2018-508.pdf

Uusiku, N. P., Oelofse, A., Duodu, K. G., Bester, M. J., & Faber, M. (2010). Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. *Journal of Food Composition and Analysis*, *23*(6), 499–509. https://doi.org/10.1016/j.jfca.2010.05.002

Vadez, V. (2014). Root hydraulics: The forgotten side of roots in drought adaptation. *Field Crops Research*, *165*, 15–24. https://doi.org/10.1016/j.fcr.2014.03.017

Vadez, V., Kholová, J., Hummel, G., Zhokhavets, U., Gupta, S. K., & Hash, C. T. (2015). LeasyScan : a novel concept combining 3D imaging and lysimetry for high-throughput phenotyping of traits controlling plant water budget. *Journal of Experimental Botany*, *66*(18), 5581–5593. https://doi.org/10.1093/jxb/erv251

Van Acker, S. I., Van Acker, Z. P., Haagdorens, M., Pintelon, I., Koppen, C., & Zakaria, N. (2019). Selecting Appropriate Reference Genes for Quantitative Real-Time Polymerase Chain Reaction Studies in Isolated and Cultured Ocular Surface Epithelia. *Scientific Reports*, *9*(1), 1–11. https://doi.org/10.1038/s41598-019-56054-1

van Eeuwijk, P., & Angehrn, Z. (2017). *How to … Conduct a Focus Group Discussion (FGD) Methodological Manual* (Amena Briet (ed.)). Swiss Tropical and Health

Institute.

Van Rensburg, W. S. J., Zulu, N. L., Gerano, A. S., & Adebola, P. O. (2015). Seed production of African leafy vegetables: Some experiences. *Acta Horticulturae*, *1102*, 121–125. https://doi.org/10.17660/ActaHortic.2015.1102.14

van Stokkom, V. L., de Graaf, C., Wang, S., van Kooten, O., & Stieger, M. (2019). Combinations of vegetables can be more accepted than individual vegetables. *Food Quality and Preference*, *72*(October 2018), 147–158. https://doi.org/10.1016/j.foodqual.2018.10.009

van Stokkom, V. L., Teo, P. S., Mars, M., de Graaf, C., van Kooten, O., & Stieger, M. (2016). Taste intensities of ten vegetables commonly consumed in the Netherlands. *Food Research International*, *87*, 34–41. https://doi.org/10.1016/j.foodres.2016.06.016

van Stokkom, Vera L., de Graaf, C., van Kooten, O., & Stieger, M. (2018). Effect of Taste Enhancement on Consumer Acceptance of Pureed Cucumber and Green Capsicum. *Journal of Food Science*, *83*(10), 2578–2585. https://doi.org/10.1111/1750-3841.14325

van Zonneveld, M., Kindt, R., Solberg, S., N'Danikou, S., & Dawson, I. K. (2021). Diversity and conservation of traditional African vegetables: Priorities for action. *Diversity and Distributions*, *27*(2), 216–232. https://doi.org/10.1111/ddi.13188

Venskutonis, P. R., & Kraujalis, P. (2013). Nutritional Components of Amaranth Seeds and Vegetables: A Review on Composition, Properties, and Uses. *Comprehensive Reviews in Food Science and Food Safety*, *12*(4), 381–412. https://doi.org/10.1111/1541-4337.12021

Vera Hernández, F. P., Martínez Núñez, M., Ruiz Rivas, M., Vázquez Portillo, R. E., Bibbins Martínez, M. D., Luna Suárez, S., & Rosas Cárdenas, F. de F. (2018). Reference genes for RT-qPCR normalisation in different tissues, developmental stages and stress conditions of amaranth. *Plant Biology*, *20*(4), 713–721.

https://doi.org/10.1111/plb.12725

Vithya, S., & Jayshree, N. (2017). Phytochemical Analysis and in Vitro Anticancer Study of Ethanolic Extract of Leaves of Amaranthus Cruentus Linn. Against Colon Cancer Cell Line (Hct-116). *World Journal of Pharmacy and Pharmaceutical Sciences*, *6*(5), 1644–1658. https://doi.org/10.20959/wjpps20175-9230

Wafula, E. N., Franz, C. M. A. P., Rohn, S., Huch, M., & Mathara, J.M.M,. Trierweiler, B. (2016). Fermentation of African Indigenous Leafy Vegetables to Lower Post-Harvest Losses , Maintain Quality and Increase Product Safety. *Afr. J. Hort. Sci.*, *9*(May), 1–13.

Wakhanu, J. A., Kimiywe, J., & Hudson, N. (2016). Oxalate Levels in Selected African Indigenous Vegetable Recipes from the Lake Victoria Basin, Kenya. *International Journal of Agriculture and Environmental Research*, *01*(02), 88–105. www.ijaer.in

Walter, A., Liebisch, F., & Hund, A. (2015). Plant phenotyping: From bean weighing to image analysis. *Plant Methods*, *11*(1). https://doi.org/10.1186/s13007-015-0056-8

Wang, S. tai, & Ebert, A. (2012). Breeding of leafy amaranth for adaptation to climate change. *Regional Symposium on High Value Vegetables in Southeast Asia: Production, Supply and Demand (SEAVEG2012)*, *January*, 24–26. http://amis.afa.gov.tw/v-asp/top-v.asp

Wang, X., Cai, X., Xu, C., Zhao, Q., Ge, C., Dai, S., & Wang, Q. hua. (2018). Diversity of nitrate, oxalate, vitamin C and carotenoid contents in different spinach accessions and their correlation with various morphological traits. *Journal of Horticultural Science and Biotechnology*, *93*(4), 409–415. https://doi.org/10.1080/14620316.2017.1404438

WHO/FAO. (2003). Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO / FAO Expert Consultation. In *World Health Organization*.

Wong, K. V. (2017). Amaranth Grain and Greens for Health Benefits. *Nutrition and Food Science International Journal*, *2*(2), 1–3. https://doi.org/10.19080/NFSIJ.2016.02.555584

World Bank. (2020). *Understanding People ' s Perspectives on Identification : A Qualitative Research Toolkit* (Issue July). World Bank.

Yadav, S. K., & Sehgal, S. (2003). Effect of domestic processing and cooking methods on total, HCL extractable iron and in vitro availability of iron in Bathua and fenugreek leaves. *Nutrition and Health*, *17*(1), 61–63. https://doi.org/10.1177/026010600301700107

Yang, R., Chang, L., Hsu, J., Weng, B. B. C., Palada, C., Chadha, M. L., & Levasseur, V. (2006). *Nutritional and Functional Properties of Moringa Leaves − From Germplasm , to Plant , to Food , to Health*. 1–9.

Ye, J., Hu, T., Yang, C., Li, H., Yang, M., Ijaz, R., Ye, Z., & Zhang, Y. (2015). Transcriptome profiling of tomato fruit development reveals transcription factors associated with ascorbic acid, carotenoid and flavonoid biosynthesis. *PLoS ONE*, *10*(7), 1–25. https://doi.org/10.1371/journal.pone.0130885

Yoshimura, K., Nakane, T., Kume, S., Shiomi, Y., Maruta, T., Ishikawa, T., & Shigeoka, S. (2014). Transient expression analysis revealed the importance of VTC2 expression level in light/dark regulation of ascorbate biosynthesis in Arabidopsis. *Bioscience, Biotechnology and Biochemistry*, *78*(1), 60–66. https://doi.org/10.1080/09168451.2014.877831

Yu, L., Jiang, J. Z., Zhang, C., Jiang, L. R., Ye, N. H., Lu, Y. S., & Al., E. (2010). Glyoxylate rather than ascorbate is an efficient precursor for oxalate biosynthesis in rice. *Journal of Experimental Botany*, *61*, 1625–1634. https://doi.org/10.1093/jxb/erq028

Yu, Le, Peng, X., Yang, C., Liu, Y., & Fan, Y. (2002). Determination of Oxalic Acid in Plant Tissue and Root Exudate by Reversed Phase High Performance Liquid Chromatography. *Chinese Journal of Analytical Chemistry*, *30*, 1119–1122.

Yuan, H., Zhang, J., Nageswaran, D., & Li, L. (2015). Carotenoid metabolism and regulation in horticultural crops. *Horticulture Research*, *2*(July). https://doi.org/10.1038/hortres.2015.36

Zhang, G.-Y., Liu, R.-R., Zhang, C.-Q., Tang, K.-X., Sun, M.-F., Yan, G.-H., & Liu, Q.-Q. (2015). Manipulation of the rice L-galactose pathway: evaluation of the effects of transgene overexpression on ascorbate accumulation and abiotic stress tolerance. *PLoS ONE*, *10:e012587*.

Zhang, R., Wang, Y., Li, T., Tan, G., Tao, J., Su, X., & Xu, Z. (2021). Effects of simulated drought stress on carotenoid contents and expression of related genes in carrot taproots. *Protoplasma*, *258*, 379–390. https://doi.org/10.1007/s00709-020-01570-5

Zhang, S., Ji, J., Zhang, S., Guan, C., & Wang, G. (2020). Effects of three cooking methods on content changes and absorption efficiencies of carotenoids in maize. *Food Funct.*, *11*(1), 944–954. https://doi.org/10.1039/C9FO02622C

Zhang, X., Zhou, X., Liu, X., Li, X., & Whang, W. (2016). Development and Application of an HPLC-UV Procedure to Determine Multiple Flavonoids and Phenolics in Acanthopanax Leaf Extracts. *Journal of Chromatographic Science*, *54*(4), 574–582. https://doi.org/10.1093/chromsci/bmv189

Zhao, Chao, Liu, Y., Lai, S., Cao, H., Guan, Y., San Cheang, W., Liu, B., Zhao, K., Miao, S., Riviere, C., Capanoglu, E., & Xiao, J. (2019). Effects of domestic cooking process on the chemical and biological properties of dietary phytochemicals. *Trends in Food Science and Technology*, *85*(December 2017), 55–66. https://doi.org/10.1016/j.tifs.2019.01.004

Zhao, Chunjiang, Zhang, Y., Du, J., Guo, X., Wen, W., Gu, S., Wang, J., Fan, J., & Jimenez-berni, J. A. (2019). Crop Phenomics : Current Status and Perspectives.

Frontiers in Plant Science, *10*(June), 1–16. https://doi.org/10.3389/fpls.2019.00714

Zhou, J., Fu, X., Zhou, S., Zhou, J., Ye, H., & Nguyen, H. T. (2019). Automated segmentation of soybean plants from 3D point cloud using machine learning. *Computers and Electronics in Agriculture*, *162*(December 2018), 143–153. https://doi.org/10.1016/j.compag.2019.04.014

APPENDICES

Appendix I: Research Permits

THIS IS TO CERTIFY THAT:
MISS. WINNIE AKINYI NYONJE
of JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY, 0-200 Nairobi,has been permitted to conduct research in *All Counties*

on the topic: *MOLECULAR MARKER BASED CHARACTERIZATION OF NUTRITIONAL AND BIOACTIVE TRAITS OF VEGETABLE AMARANTH VARIETIES FOR IMPROVED BIOAVAILABILITY AND ACCEPTABILITY*

for the period ending:
30th April,2020

Permit No : NACOSTI/P/19/42041/29429
Date Of Issue : 30th April,2019
Fee Recieved :Ksh 2000

Applicant's Signature

Director General
National Commission for Science, Technology & Innovation

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REPUBLIC OF KENYA

National Commission for Science, Technology and Innovation

RESEARCH LICENSE

Serial No.A 24423

CONDITIONS: see back page

Appendix II: Research Licence

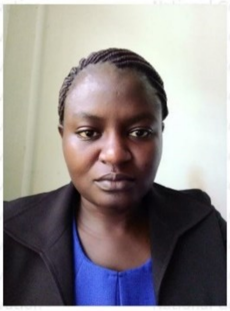
REPUBLIC OF KENYA

NATIONAL COMMISSION FOR
SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **721211**

Date of Issue: **29/September/2020**

RESEARCH LICENSE



This is to Certify that Miss.. Winnie Akinyi Nyonje of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Bomet, Bungoma, Kiambu, Kilifi, Kwale, Nyamira, Vihiga on the topic: Molecular Marker Based Characterization of Nutritional and Bioactive traits of Vegetable Amaranth Varieties for Improved Bioavailability and Acceptability for the period ending : 29/September/2021.

License No: **NACOSTI/P/20/6923**

721211

Applicant Identification Number

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Appendix III: Participant Information Sheet

Consumer Survey on Amaranth Vegetable Utilization Practices

Introduction

We are a team of researchers from Jomo Kenyatta University of Agriculture and Technology (JKUAT) in collaboration with the World Vegetable Centre (WordVeg/AVRDC). We are carrying out research on the utilization and consumption practices of amaranth vegetables across parts of Kenya and Tanzania. This is part of a PhD research project conducted by Winnie Nyonje at JKUAT. This research seeks to study Amaranth food preparation methods and recipes and develop a behavior change strategy for improved nutrition in East Africa.

Procedure: If you accept to participate in this research, we will conduct a focused group discussion, where we will be discussing about amaranth vegetable utilization in this area. The data collected will be used provide information on the various cooking methods and utilization practices of amaranth vegetables in East Africa.

Benefits: The benefits of your participation in this study include contributing to knowledge on amaranth production and consumption practices in East Africa, as well as any ways to improve its consumption. Your knowledge, concerns and aspirations will be compiled and shared with other researchers, educators and decision makers to determine next steps in improving food and nutrition status in the communities. There are no monetary benefits.

Risks/discomfort: No known risk is expected from participating in this research. We would like to ask you, however, not to tell us anything that is sensitive and might damage your reputation, financial standing or present any unacceptable risk to you.

Confidentiality: Your study data will be handled as confidentially as possible. If results of this study are published or presented, individual names, quotes, photographs and other personally identifiable information will not be used unless you give explicit permission for this.

Rights: Your participation in this research is completely voluntary. You are free to

decline to take part in the study and you can also decide to withdraw at any moment without a reason. If you choose to decline or withdraw from the interview, there will be no penalty to you or loss of benefits to which you are otherwise entitled.
This study is conducted by Winnie Nyonje, from the department of Human Nutrition Sciences, JKUAT. For any questions, contact Winnie Nyonje on +254723363401, e-mail: winnienyonje@yahoo.com

For further concerns, contact:
Prof. Mary Abukutsa – study supervisor
Jomo Kenyatta University of Agriculture and Technology (JKUAT)
P.O. Box 62000-00200, Nairobi.

This research is approved by the Jomo Kenyatta University of Agriculture and Technology (JKUAT) ethical review board and WorldVeg Institutional Biosafety and Research Ethics Committee (IBREC).

Appendix IV: Consent Form

Consumer Survey on Amaranth Vegetable Utilization Practices

I understand the purpose of the research and the language that is being used for the interview. I therefore voluntarily and freely agree to participate. I understand that the information I give will be purely for the purposes of this research. My questions and concerns have been addressed well and I am free to withdraw from the interview at any point without a reason and there will be no consequences.

Participant's Name ……………………………………………………………………

Signature ………………………………   Date ……………………….

Should you have any question or problems contact Winnie Nyonje:

Department, Human Nutrition Sciences, JKUAT
Phone: +254723363401
E-mail: winnienyonje@yahoo.com
Or

Prof. Mary Abukutsa
Jomo Kenyatta University of Agriculture and Technology (JKUAT)
P.O. Box 62000-00200, Nairobi.

Appendix V: Guidelines for Focus Group Discussion and Key Informant Interview on Amaranth Utilization Practices

FGD Guide for Amaranth Vegetable Utilization Practices
Country ……………. County/ Region ………… Sub-county/ District …………

Discussion questions

1. Which African indigenous vegetables (AIVs) produced and consumed in this area and how do they compare to other vegetable?
2. What are the common varieties of amaranth grown and consumed in this area? Are there varieties that are more preferred or disliked and why?
3. Where do people in this area obtain amaranth for consumption, and how are the amaranth vegetable prepared and cooked?
4. What do people think about the consumption of amaranth and other AIVs vegetables in this area? Are there beliefs and taboos associated with the vegetables? If so, which ones.
5. What is the general perception of people in this area about preservation of amaranth vegetables and other indigenous vegetables?
6. What are the constraints/challenges affecting the production and utilization of amaranth in the community?
7. How can production and consumption of amaranth vegetables be improved at the community and household level; and what kinds of information would prompt you to include more amaranth and other indigenous vegetables in your meals?
8. How do you prefer to receive information about amaranth? (Such as demonstrations by experts, discussion with neighbors, through training, printed materials, radio, TV, text messages, social media, etc.)

KII Guide for Amaranth Vegetable Utilization Practices
Date of Interview…………….. Country…………….. County/ Region …………….

Time Start: ……………..…………… Time stop: ……………………..…………

Interviewer Name …………………………………………………………………

Name of Respondent ……………………… Position of Respondent………………..

Organization ………………………………………………………………………

Interview Questions

1. Which indigenous vegetables are mostly produced in this area?
2. Are there indigenous vegetables that are more preferred than others? Which ones and why?
3. Are there different varieties/types of amaranth vegetables produced in the area? What characteristics are used to differentiate them?
4. What has been the production and consumption trends of amaranth vegetables and other indigenous vegetables in the area, increasing or decreasing and why?
5. Are they produced mainly for sale or home consumption?
6. How are the amaranth vegetables utilized? (as vegetables, medicine or other uses)
7. Please describe the general cooking method of vegetable amaranth in the area
8. Do the community members preserve amaranth and other vegetables? If yes what are the methods of preservation
9. What are the constraints/challenges to production and consumption of amaranth vegetables in the area?
10. Are there any beliefs or taboos related to vegetables amaranth in this area? If yes, explain
11. How can the production of amaranth and other indigenous vegetables be improved in your community?

Appendix VI: Sensory Evaluation Questionnaire for Amaranth Recipes

SENSORY EVALUATION QUESTIONNAIRE FOR AMARANTH RECIPES

Participation in the Taste Panel Trial is Voluntary

RECORDING SHEET

Date:..........................Taster Name(optional):....................Gender: M[ ]......F[ ]

Age group (Please tick) :<20:...20-30:........30-40:..40-50:............50-60 ......>60 ...

You are provided with coded cooked Vegetable Amaranth. Please score the samples according to the scale provided below by filling in the table against each sample attributes.

Dislike very much……………1

Dislike moderately………….2

Neither like nor dislike…….3

Like moderately…………….4

Like very much……………...5

For general acceptability

I can eat this food in every opportunity I have………………………………5

I can frequently eat this……………………………………………………..…4

I would eat this if available but would not go out of my way……………….3

I would hardly ever eat this…………………….……………………………2

I would eat this only if I were forced to…………………………….………… 1

Sample	Appearance	Aroma	Taste/Flavor	Texture	General Acceptability
A1					
A2					
A3					
A4					

A5					
A6					
A7					
A8					
A9					
A10					

In the last two weeks, about how often have you eaten amaranth vegetables? (Tick where applicable)

- Not a single time...............
- once in two weeks......
- Once a week ....
- More than once a week...........
- Every day......................

General comments (Any ingredient you feel should have been added or removed: Recipe is overcooked or undercooked etc): ....

....

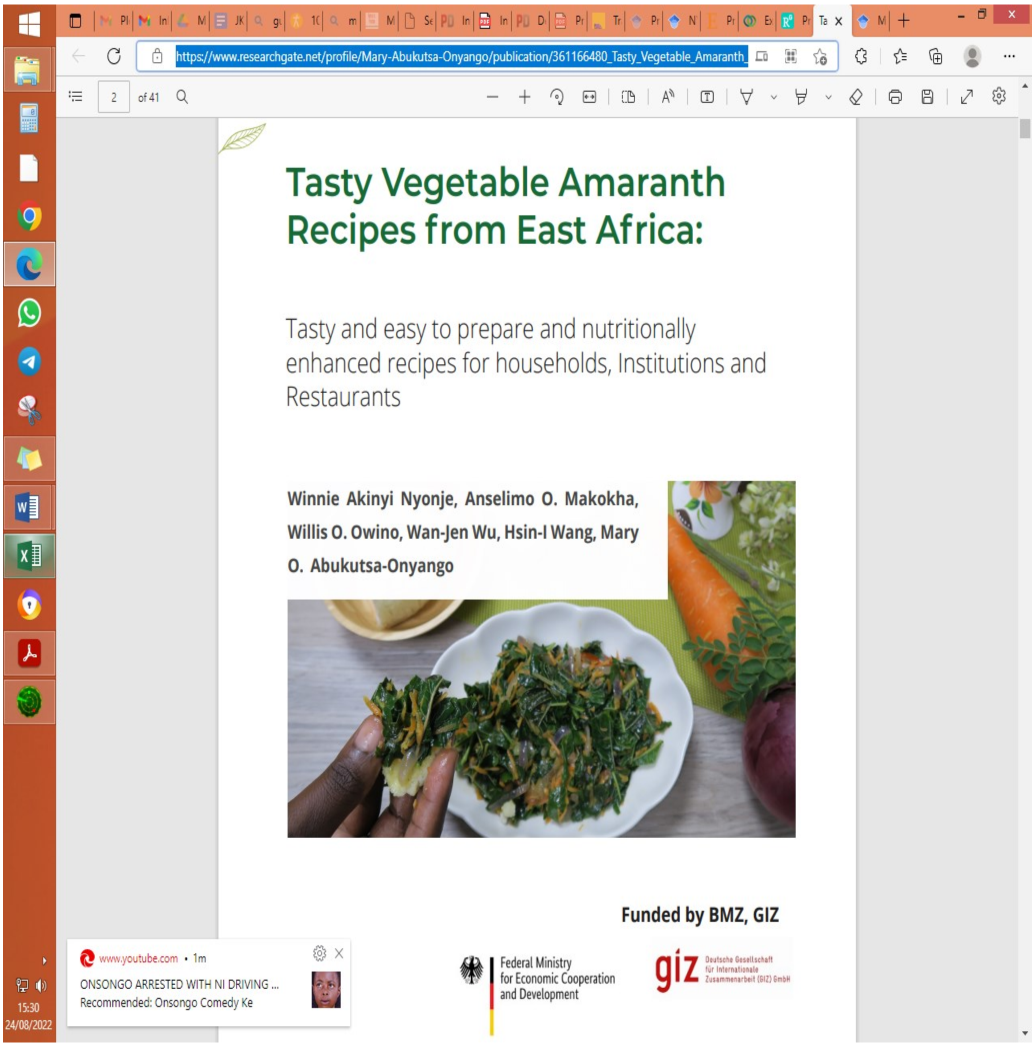
....

....

Thank you for your participation in our taste panel trial. Your contribution is very much appreciated

Appendix VII: Recipe book

Nyonje, W. A., Makokha, A. O., Owino, W. O., Wu, W. J., Wang, H. I., & Abukutsa-Onyango, M. O. (2021). Tasty Vegetable amaranth recipes from East Africa: Tasty and easy to prepare and nutritionally enhanced recipes for households, institutions and Restaurants. *Jomo Kenyatta University of Agriculture and Technology available at (JKUAT*.https://scholar.google.com/citations?user=bqLNUf4AAAAJ&hl=en&oi=sra



Follow the link to access the full text Tasty-Vegetable-Amaranth-Recipes-from-East-Africa.pdf (researchgate.net) https://scholar.google.com/citations?user=bqLNUf4AAAAJ&hl=en&oi=sra