SHELF LIFE STABILITY AND NUTRIENT CONTENT OF VEGETABLE AMARANTH

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Shelf Life Stability and Nutrient Content of Vegetable Amaranth

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DECLARATION

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

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DEDICATION

I dedicate this thesis to my husband Job Ngugi Murimi for his love and care and my daughters, Kayla Murugi Murimi and Kelsy Gathoni Murimi for setting high achievement standards on me. Thanking my mother Pamella Nyaura, my father, Dr. Simon Peter Othieno Nyaura, my brother, Edward Nyaura and my sister Alister Nyaura for walking the journey with me. My appreciation also goes to my grandmother Grace Nyanjom, my aunties Beatrice Makawiti, Joan Nyanjom, Julie Nyanjom, Elizabeth Nyanjom, Judy Nyanjom, my uncles Martin Nyanjom, Wilson Nyanjom, Mike Nyanjom, my Late uncle Jim Nyanjom and my mother in law Emily Ngugi for their support and commitment during my studies. You are my greatest inspiration and encouragement. I would like to encourage all my cousins and close relatives namely: Jasper, Paul, Maria, Maggie, Nancy, Grace, Jabari, Purity, Griffin, Nicole, Tess, Biko, Lynn, Ryan Munene, Gitonga, Munene, John Paul, Emily, Kayla, Julio, Kelsy, Alanna, Alisa, Aldwin, Atieno and Naino to aim for the sky just like I have done.

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ABSTRACT

Poor storage and handling conditions causes over 30% losses in leafy vegetables after harvesting. To increase the shelf life of the vegetables during storage and handling, more efforts are being put forward as there are research gaps in knowledge and technology on the storage techniques of the vegetables. The main objective of the study was to find out the physico-chemical changes of vegetable amaranth, with the main focus on the variety *Amaranthus dubius* stored under low temperature as well as modified atmospheric packaging. The harvested leaves were initially stored in modified atmosphere bags and placed in the cold room at 5 °C, 10 °C, 15 °C and 25 °C and a relative humidity of 75% with 25 °C acting as the control. The vegetable was analysed to determine the Vitamin C, β -carotene, moisture content, respiration rates and visual appeal changes. The Vitamin C content of the fresh leaf amaranth was 41 mg/100g. The loss of ascorbic acid was much greater in samples stored at higher temperatures as compared to the samples stored at lower temperatures. The leaves stored at 25 °C lost Vitamin C content to 8 mg/100g after 4 days of storage whereas the leaves stored at 5 °C lost the Vitamin C content to 8 mg/100g after 23 days of storage. The study also found that control sample lost 88% of ascorbic acid in 4 days as compared to 55% loss at 5 °C after 23 days. Storage temperature significantly ($P<0.05$) influenced the amount of Vitamin C retained in the vegetables. The content of β -carotene in the fresh leaf amaranth vegetable was 61.4 µg/g. It was deduced that the loss in carotene was slightly greater at room temperature (25 °C) and decreased with decreasing storage temperatures. At the end of the storage, the retentions of Beta carotene were 6.86, 19.6, 26.14 and 31.53 µg/g at 25 °C, 15 °C, and 10 °C respectively. Storage temperature significantly ($P<0.05$) influenced the amount of β-carotene retained in the vegetables. Storing the vegetables for longer time at room temperature (25 °C) rapidly increases the respiration rates of the vegetables with leaves stored at 25 °C experiencing the highest respiration rate as compared to storage temperatures of 5 °C, 10 °C, 15 °C. These results indicate that the shelf life and marketing period of amaranth vegetable can be prolonged by maintaining quality attributes and external appearance. Better retentions of vitamin C and β-carotene can be achieved by storage at modified atmospheric storage at 5 °C. Farmers and supermarkets should use 5 °C and relative humidity of 75% for storage of amaranth leaves for better retention of vitamins, colour and visual appeal.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Harvested Traditional Leafy vegetables (TLVs) are living tissues with continuing metabolism after harvest (Mampholo *et al.,* 2016). They are subject to respiration, water loss and cell softening throughout the postharvest system. The storage life of a product varies with species, variety, pre and post-harvest conditions. There is scope to control storage life through postharvest management of the quality of the vegetables (Mitchell, 2012).

The inherent potential of the TLVs can be harnessed through post-harvest management to reduce incidence of diet related diseases, provide essential micro-nutrients and fibre, alleviation of malnutrition, and provide high anti-oxidant content (James, 2017). The TLVs provide new income opportunities that could be the engine to drive economic growth (Senyolo *et al.,* 2018). The consumption of traditional leafy vegetables has a positive effect on the health of human beings (Randhawa *et al.,* 2015). Daily consumption of traditional vegetables in diets improve vision, health of digestive system and reduce the risk of stroke, heart disease and chronic diseases (Boeing *et al.,* 2012).

Vegetable amaranth is a popular traditional leafy vegetable crop grown and consumed in many parts of Kenya (Onyango *et al.,* 2008). The leaves are among the most nutritious leafy vegetables, rich in protein, vitamins and mineral salts (Onyango *et al.,* 2008). Their fast growth and great biomass make them some of the most high-yielding vegetables, meaning more leaves for consumption and selling (James, 2017). Some varieties of leafy amaranth are used as leafy vegetables, with *Amaranthus hypochondriachus* (L.), *A. tricolor* (L.), *A. hybridus* (L.), *Amaranthus dubius* and *A. Blitum* (L.) being the most popular (Randhawa *et al.,* 2015). Amaranth has a great amount of genetic diversity, phenotypic plasticity, and is extremely adaptable to adverse growing conditions, resists heat and drought, has no major disease problem, and is among the easiest of plants to grow in agriculturally marginal

lands (Shukla and Rastogi, 2013).

Lack of access to land and financing, insufficient irrigation water, dysfunctional seed system, low prices and technical skills on amaranth production and storage are some of the constraints facing producers while consumers are grappling with high prices, poor quality, inconsistent supply of vegetables whose safety cannot be guaranteed. High perishability and low storage capacity in the fresh form are the main constraint to increased production, marketing and consumption of traditional leafy vegetables (Onyango *et al.*, 2007). This makes farmers to sell them soon after harvest (Kimiywe, 2015). The freshness of leafy vegetables can be extended for limited periods by storage at modified atmospheric packing and low temperatures, correct humidity and good sanitation (FAO, 2004). It was against this background that the study sought to determine the shelf life stability and nutrient content of vegetable *Amaranthus dubius* when stored in modified atmospheric shelf life bags, at different storage temperatures.

1.2 Statement of the Problem

Inappropriate post-harvest storage technologies and seasonality of production and supply of traditional leafy vegetables have resulted into high losses of these vegetables (Parffitt *et al.,* 2010). Lack of storage contributes to qualitative and quantitative losses of vegetables (Kader, 2005). Qualitative losses such as loss of caloric and nutritive value, loss of acceptability by consumers, and loss of edibility are more difficult to measure than quantitative losses of fresh fruits and vegetables. While reduction of quantitative losses is a higher priority than qualitative losses, consumer dissatisfaction with produce quality results in a greater percentage of the total postharvest losses. Current knowledge can be applied to improve the handling systems especially packaging and cold chain maintenance of vegetables to assure their quality and safety (Kader, 2005).

Modified atmosphere packaging bag is a useful storage system that significantly increase the shelf life of vegetables (Villanueva *et al.,* 2005). The MAP bag results to changes in the composition of the atmosphere in the packaging bag due to the dynamic interaction between the metabolic processes of the packaged product, in

which O_2 is consumed and CO_2 is generated. The aim of the system is to balance these two gases in such a way that constant levels are reached in the packaging bag and that equilibrium levels are as favorable as possible to preserve the product (Patiño and Herrera, 2018).

MAP, when combined with proper postharvest handling procedures and temperature control management, can have a positive impact on the quality and shelf life of vegetables The increasingly global nature of food production and the increased emphasis on reducing chemical preservatives have put the spotlight on storage methods (Kader, 2003). Yet until now, there have been very few current resources available to increase the shelf life of the vegetable.

1.3 Justification

In the last two to three decades, there have been major changes in food consumption habits, including a significant increase in the consumption of fresh vegetables due to health concerns. These changes have created the need for the development and application of adequate technologies to preserve these perishable vegetables (Kader, 2005).

Leafy vegetables especially amaranth grow and mature very fast therefore the leaves can be harvested within two weeks after sowing, while grains mature in 45-75 days (Nyonje, 2015). Amaranth, is a highly nutritious leafy vegetable (Ebert, 2014). Benefits of amaranth can be attained if availability and storage techniques are optimal (Achigan-Dako *et al*., 2014). This research presents an opportunity to research on storage methods that increases shelf life of the amaranth vegetable. There is need for more evidence on the impact of storage on the nutritional quality of vegetables exposed to different post-harvest and value addition technologies (Soliva, 2003). There is also need for reliable and sustainable storage systems of vegetables to reduce food and nutrition insecurity. There is therefore need to determine the shelf life and nutrient stability of amaranth stored at specific temperatures and modified atmosphere storage, through more efficient storage methods and adoption of modern technologies thus will increase vegetable supply.

1.4 Objectives

1.4.1 Main Objectives

To determine the shelf life and nutrient stability of vegetable amaranth (*Amaranthus dubius*) stored at specific temperatures and modified atmosphere storage.

1.4.2 Specific Objectives

i. To determine the change in vitamin C and β-carotene of *Amaranthus dubius* under modified atmosphere storage.
ii. To determine the respiration rate and visual appeal of *Amaranthus dubius* under modified atmosphere storage.

1.5 Hypothesis

1. There is no significant change in vitamin C and β-carotene in *Amaranthus dubius* leaves when stored at different temperatures.
2. There is no significant difference in respiration and visual appeal changes in amaranth leaves stored in modified atmosphere and at four different temperatures.

CHAPTER TWO

LITERATURE REVIEW

2.1 Post-Harvest Losses of Vegetables

Vegetable loss refers to quantity and quality, in which the economic value of produce is degraded (Kiaya, 2014).The Food and Agriculture Organisation estimates that one third of the vegetable produced globally for human consumption is lost or wasted along the supply chain. Losses are even higher in Africa, and have a negative effect on food security, nutrition and economic stability (Kader, 2004). Quality losses include those that affect the nutrient/caloric composition, the acceptability, and the edibility of the amaranth vegetable. These losses are generally more common in developed countries (Kader, 2002). Quantity losses refer to those that result in the loss of the amount of the vegetable. Loss of quantity is more common in developing countries (Lee and Kader, 2000). In Sub-Saharan Africa as much as 50% of fruits and vegetables, 40% of roots and tubers and 20% of cereals, legumes and pulses are lost before they even hit the market. In recent years problems with safety have also contributed to post-harvest losses of the vegetable. Consumer dissatisfaction with vegetable produce quality results in a greater percentage of the total postharvest losses (Kader, 2004).

Poor food handling, including poor storage and sanitation, may also result in food losses (Kader, 2002). Food safety standards and practices have been put in place in Kenya but not all are feasible for adoption by small farmers and traders. More must be done to help people who fall into these groups if the country is serious about tackling vegetable losses.

2.2 Potential of TLVs in addressing food security in Kenya

In the past 20 years, very little progress has been achieved in reducing food insecurity, child malnutrition and hunger in Africa (De and Whiteside, 2003). Under-nutrition and micronutrients deficiencies are widespread and affect mainly women and children (Salam *et al*., 2013). To address these problems, increased consumption of traditional leafy vegetables is promoted as sources of both

macronutrients and micronutrients. Widely promoted African leafy vegetables include *Amaranthus* spp., a taxonomic group cultivated worldwide ((Achigan-Dako *et al*., 2014).

Food security is also the most important pillar of the big four agenda in Kenya. World Food Summit adopted the following definition: "food security, at the individual, household, national, regional and global levels is achieved when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life" (Berry *et al*., 2015). The emphasis is on consumption and access of vegetables by vulnerable people. To attain food security, there are clear-cut measures and interventions that the government should come-up with to prevent vegetable losses. These include modern post-harvest storage facilities, among others.

2.3 Traditional leafy vegetables (TLVs) in Kenya

In Kenya, there are about 200 traditional plant species used as traditional leafy vegetables (Onyango *et al.*, 2008). Of these, only a few have been fully domesticated by Kenyan communities, while more are semi-domesticated and the majority are collected from the wild. The most commonly consumed TLVs in Kenya include the *Amaranthus* spp. (Pig weed), *Vigna unguiculata* (Cowpea leaves), *Solanum nigrum* (Black nightshade), *Cleome gynandra* (Cat's whiskers), *Cucurbita* spp. (Pumpkin leaves) and *Corchorus* spp. (Jute), (Muthoni and Nyamongo, 2010). These vegetables are rich sources of vitamin C, proteins, fibre and minerals potassium, phosphorus, calcium and zinc (Uusiku *et al.*, 2010). These vegetables are either purchased or home-grown for personal consumption. In addition, these vegetables also contain high contents of phytochemicals such as phenolic compounds (including flavonoids), which also possess strong antioxidant properties and have been implicated in the prevention of cancer, arteriosclerosis and diabetes (Nyonje, 2015). These leafy vegetables are especially valuable in the diets of children and women (Nyonje, 2015).

Amaranthus is a good addition for diets based on cereals and tubers as it is rich in lysine (Valcárcel and Lannes, 2012). Amaranthus is a good addition for diets based

on cereals and tubers as it is rich in lysine (Chillo *et al.*, 2008). In the recent past like other TLVs, this vegetable was sold only in informal markets, but now is sold in green grocers and supermarkets (Onyango, 2008). Nevertheless, the vegetable, once harvested the vegetable has a very short shelf-life (Pinela and Ferreira, 2017).

2.4 Varieties, utilization and nutrition composition of amaranth

2.4.1 Varieties of Amaranth

Amaranthus, known as amaranth or pigweed, is an annual plant distributed worldwide in warm, humid regions (Ebert *et al*., 2011). Approximately 60 species are recognized with inflorescences and foliage ranging from purple and red to gold (Pinto, 2018). Although several species are often considered weeds, people in Kenya and around the world value amaranths as leaf vegetables, cereals, and ornamentals (Achigan-Dako *et al*., 2014). Amaranths are botanically distinguished by their small chaffy flowers, arranged in dense, green or red, monoecious or dioecious inflorescences, with zero to five perianth segments and two or three styles and stigmata, and by their dry membranous, indehiscent, one-seeded fruit (Muriuki *et al*., 2014).

Amaranth is the collective name for the all the domesticated species of the genus *Amaranthus* (family *Amaranthaceae*). It is one of the oldest food crops in the Kenya with evidence of cultivation dating back to over 7000 years in Puebla, Mexico (Winnie, 2015). Although virtually unlisted in formal agricultural statistics, it may be the most widely grown crop in the tropics (National Research Council, 1984). This is can be as a result of the ability of the genus to grow under a wide range of climatic conditions coupled with its competitive ability which permits cultivation with minimum management (Shukla *et al*., 2006).

The most popular leafy species are *A. tricolor*, *A. hybridus* and *A. blitum*. *Amaranthus hypochondriacus* is considered to be used for both grain and vegetable purposes. Among the varieties commonly found in Kenya are *A. dubius* and *Amaranthus hybridus*, which is grown as vegetables while *Amaranthus cruentus* is grown as grain and *A. hypochondriacus* is dual purpose. *Amaranthus caudatus* grows

quickly from seed and produce tassels as long as the human arm (Achigan-Dako *et al*., 2014). There is also a variety with green tassels. Another variety is the *A. hypochondriacus* 'pygmy torch'. This plant is rather small and is soon swallowed up by other members of the tropical borders. Although the foliage often starts out with an element of purple, it becomes less so as the plants grow. The leaves are particularly colorful and would make a useful addition to the colour palette available to the gardener (Nyonje, 2015). Figure 2.1 displays images of various amaranth species.

Amaranthus dubius spp. is utilized as food in diverse geographical regions. The nutritional quality of *Amaranthus dubius* leaves is better as compared to the other leafy vegetables (Wani *et al*., 2013). Muriuki (2014) reported that calcium (336.47mg/100g) and iron (18.64mg/100g) content were significantly ($p<0.05$) higher in *Amaranthus dubius* than in the other species.

Physiological as well as genetic and nutritional studies have revealed their potential economic value (Abukutsa, 2010). With respect to its use in agriculture, its importance arises from its high rate of productivity (Onyango, 2008). As a rapidly growing crop, the large amounts of protein in both seed and leaf with high lysine, the high overall nutritional value, and the water use efficiency for the C4 photosynthetic pathway. Amaranths are important in the culture, diet, and agricultural economy of the people (Adamson, 2011). Genetic, ethno-botanical, and agronomic research needs to be undertaken to develop amaranths as an important food plant in modern agriculture. While vegetable and grain types can be differentiated, often both the grain and leaves are used (Achigan-Dako *et al*., 2014). The vegetable types are generally smooth leafed, with an indeterminate growth habit, which produces succulent axillary growth. The floral buds arise directly in the leafy axils. Grain types have a main stem axis that terminates in an apical large branched inflorescence (Onyango, 2010). *Amaranthus* spp. are utilized for food in diverse geographical areas. The consumption of vegetable amaranth helps balance vitamin and mineral intake and serves as an inexpensive rich source of protein and dietary fiber (Gupta *et al.*, 2005)

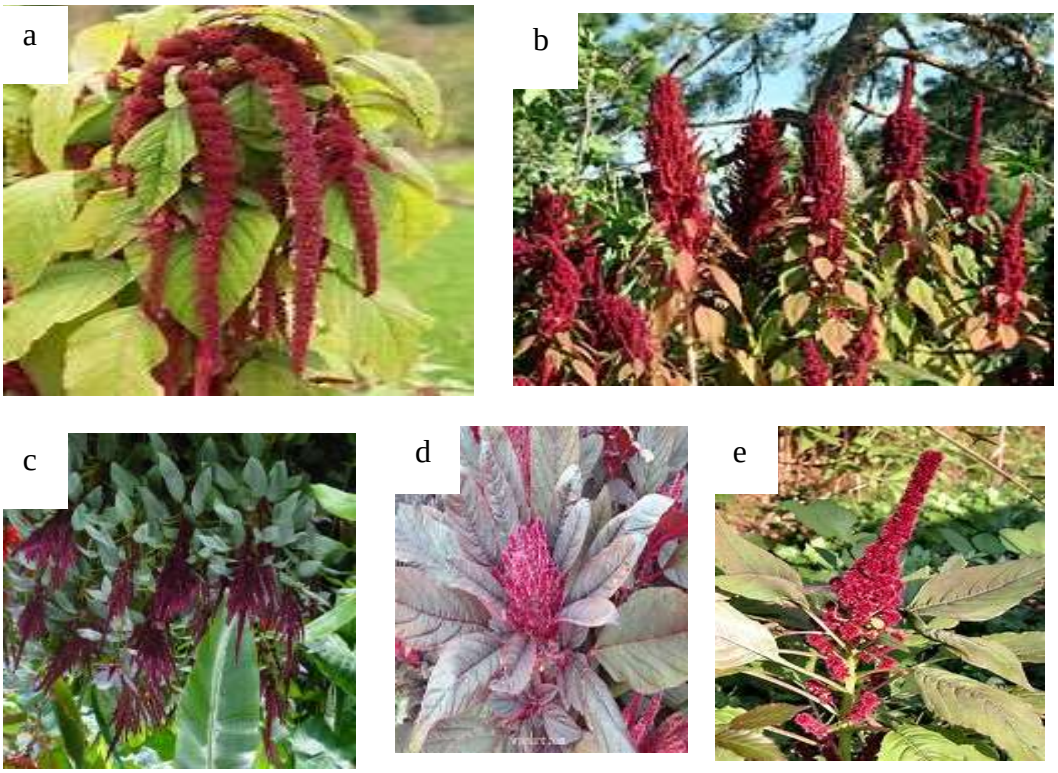

Figure 2.1: Different vegetable amaranth species (a) Amaranthus hypochondriacus, (b) Amaranthus caudatus, (c) Amaranthus dubius, (d) Amaranthus hybridus, and (e) Amaranthus Cruentus.

Photo by Carl Lewis on *https://www.tropicalpermaculture.com/amaranth-plant.html*

2.4.2 Utilization of Amaranth

In Kenya, leafy amaranth is cooked in unspecified amount of water for 7 – 9 minutes followed by discarding the cooking water and then frying the vegetables in a small quantity of oil and is eaten as a side dish with *ugali* (Nyonje, 2015). The vegetable is also cooked in a mixture with beans, maize and bananas and/or potato and mashed to form a heavy mixture referred to as *kienyeji* (Swahili). The cooked leaves (not mixed with other foods) have been reported to be rich in calcium, iron and vitamins A and C (Onyango, 2010). Amaranth leaves should however be washed them in fresh water before cutting. When possible steam or stew rather than boiling or blanching. The water from steaming should be used in cooking to make soup or sauce. Generally,

long cooking or keeping vegetables hot for a long time has a destructive effect on the vitamins (Funke, 2011).

2.4.3 Nutrition composition of Amaranth

Amaranthus is a good supplement for diets based on cereals and tubers as it is rich in lysine (Jansen *et al.,* 2004). Table 2.1 shows the nutrient content of amaranth leaves and other major vegetables.

Table 2.1: Comparison of proximate composition and energy value of amaranth leaves and grain with other TLVs (Muriuki, 2014)

Food Ingredients	β-carotene mg/100 g	Vitamin C mg/100 g	Vitamin B1 mg/100 g	Vitamin B2 mg/100 g	Niacin mg/100 g
Finger Millet	4.17 ±0.04	1.00 ±0.00	0.31 ±0.30	0.08 ±0.02	4.29 ±0.03
Amaranth grain	0.07 ±0.01	4.50 ±0.01	0.07 ±0.00	0.30 ±0.10	1.20 ±0.01
Pigeon pea	0.05 ±0.03	4.80 ±0.00	0.72 ±0.08	0.14 ±0.33	2.90 ±0.10
Field bean	0.55 ±0.01	9.05 ±0.20	0.37 ±0.16	0.12 ±0.00	2.30 ±0.04
Groundnut	0.03 ±0.05	1.00 ±0.07	1.60 ±0.40	0.14 ±0.01	17.7 ±0.08
Pumpkin seed	0.04 ±0.14	1.00 ±0.03	0.37 ±0.01	0.83 ±0.01	3.12 ±0.14
Sunflower seed	0.00 ±0.00	1.00 ±0.01	1.05 ±0.20	0.27 ±0.04	3.59 ±0.11
Pumpkin	1.27 ±0.07	3.70 ±0.00	0.06 ±0.00	0.04 ±0.00	0.50 ±0.31
Butternut	5.08 ±0.01	3.80 ±0.04	0.05 ±0.00	0.02 ±0.20	0.50 ±0.00
Sweet potato	0.69 ±0.02	3.16 ±0.06	0.10 ±0.03	0.06 ±0.03	0.60 ±0.05
Drumstick leaves	11.10 ±0.12	234.01 ±0.45	0.08 ±0.00	0.04 ±0.01	0.78 ±0.04
Pumpkin leaves	2.66 ±0.04	14.03 ±0.10	0.08 ±0.12	0.06 ±0.01	0.32 ±0.01
Amaranth leaves	4.29 ±0.20	62.93 ±0.03	0.42 ±0.09	0.44 ±0.03	0.70 ±0.00

In addition to being a major source of minerals and vitamins amaranth also contributes to the intakes of other phytochemical such as phenolic compounds (Jiménez and Grusak, 2017) and isothiocyanates poses strong antioxidant properties, and have been implicated in the prevention and suppression of diseases such as cancer, arteriosclerosis ageing (Bengmark *et al.,* 2009) and currently the management

of human immune-deficiency virus (Devasagayam *et al.,* 2004).Earlier studies have established the abundance of antioxidants in Amaranthus leaves (Nyonje, 2015). Antioxidants, phenolic compounds act as free radical scavengers and act to prevent diseases which follow free radical mechanism in human (Devasagayam *et al.*, 2004). Traditional vegetable amaranth is recommended as a good food with medicinal properties for patients with constipation, fever, hemorrhage, anaemia or kidney complaints, young children, lactating mothers (Grubben, 2004).

2.5 Postharvest Handling and factors influencing shelf life of amaranth

2.5.1 Postharvest Handling

Post-harvest begins at the moment of separation of the amaranth vegetable from the plant that produced it to by a deliberate human act with an intention of starting it on its way to the table (Goswami &Mangaraj, 2011).

Amaranth vegetable produce is highly perishable with some estimates suggesting a postharvest loss of 30 – 50% in vegetables (Buzby *et al.,* 2014) . Most studies on post-harvest technology have so far concentrated on grains and other durable products, which are stored dry and a substantial technology has been developed to deal with these problems (Atanda *et al.*, 2011). The losses occur due to in-effective postharvest management as well as lack of adequate processing and marketing. Vegetable losses have several negative impacts on farmer's income, consumer prices and nutritional quality of the produce (Weinberger & Lumpkin, 2007).

Today, enormous volumes of quality horticultural crops produced in technologically advanced countries are made available to millions of people through improved post-harvest handling (Atanda *et al.*, 2011). Thus, historically and by necessity, post-harvest technology is part of the normal development processes in agriculture. These handling procedures are not fully recognized in less developed countries where agriculture may be characterized as disjointed. Production is not linked with marketing. With perishable crops like vegetables, storage, packaging, transport and handling technologies are practically non-existent (Mbuk *et al.,* 2011).

It is, therefore, important that postharvest procedures should be given much attention as production practices, and there must be a mutual undertaking, in the stages from planting until the vegetable reaches the consuming public, between the growers and those who will handle the vegetable after harvest.

MAP can be applied in the post-harvest handling to extend the shelf life of the vegetables (Artés and Allende, 2015).

2.5.2 Factors influencing shelf life of amaranth

Quality cannot be increased nor improved after harvest, but can only be maintained (Prusky, 2011). Delays between harvest and consumption or processing of leafy vegetables can result in direct losses due to respiration, water loss and decay, and indirect losses such as those of nutritional quality and flavor (FAO, 2004). These losses would result into a short shelf-life for *Amaranthus dubius* whose market value is mainly determined by their physical appearance.

Shelf life is the recommended time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected conditions of distribution, storage and display (Giannakourou and Taoukis, 2003). For *Amaranthus dubius*, shelf life is charged by wilting and physical appearance, discoloration and decay all reduce the shelf life of the vegetable.

Temperature is usually the most vital environmental factor limiting shelf life of fresh fruits, leafy vegetables and herbs (Del *et al.,* 2009). Temperature management especially cool storage extends shelf life and maintain quality of fresh fruits and leaf vegetables (Lee and Kader, 2000).

Deterioration of vegetables can also result from physiological breakdown due to water loss, natural ripening processes, temperature injury, physical damage, or invasion by microorganisms (Wang, 2010). All these factors can interact, and all are influenced by temperature. Moisture loss may detract the vegetables from their appearance, saleable weight, and nutritional quality (Kader, 2010).

The respiration rates also strongly determine the postharvest life of vegetables. The higher the storage temperature the higher the respiration and transpiration rates and the greater the transit losses (rotting and wilting) leading to a shorter postharvest life of the vegetables (Mitchell, 2012).

Preparation and processing methods of vegetables further reduce the composition, including the nutritional value of vegetables. For example, water soluble vitamins such as the C and B vitamins leach into the cooking water at high rates during cooking and are therefore lost if the cooking water is discarded as is the case with TLVs cooking method. Fat-soluble compounds such as carotenoids may be stabilized or made more available by cooking (Garrett *et al.*, 1999).

2.5.3 Modified atmospheric packaging

Modified atmosphere storage and controlled atmosphere storage are preservation methods that would maintain the natural quality of amaranth as well as extend the storage life. They maintain the storage life of food. They both differ based on the degree of control exerted over the atmosphere composition (Jayas and Jeyamkondan, 2002). In MA storage, the gas composition is modified initially and it changes dynamically depending on the respiration rate of food product and permeability of film storage structure surrounding the food product. In CA storage, the gas atmosphere is continuously controlled throughout the storage period (Majidi *et al.,* 2014) .

The modified atmosphere packaging uses the plant products natural respiration to create a high carbon dioxide, low oxygen atmosphere that extends the life span of the stored produce. The package provides an improved storage environment for vegetables and herbs during distribution, thereby ensuring high product quality for consumers (Fonseca and Brecht, 2002).

Modified atmosphere packaging (MAP) is used to preserve the freshness of produce by controlling their biochemical metabolism for example, respiration. For a MAP system to work effectively, optimal packaging materials with proper gas permeability properties must be selected. Modified atmosphere packaging dramatically extends

the shelf life of packaged vegetable products, and in some cases MAP products do not require any further special care or treatments during distribution (Han, 2005). In most cases, however, extending shelf life and maintaining quality require a multiple-hurdle technology system. Modified atmosphere packaging (MAP) in combination with refrigeration temperatures could be used as a mild preservation technique for safety of minimally processed fruits and vegetables. However, the effect of MAP on microorganisms can vary, depending mainly on the storage conditions and the type of packaged product (Irtwange, 2006). Technology is critical for most MAP applications, because the modified atmosphere provide an un-natural gas environment that could create serious unexpected microbial problems such as the growth of anaerobic bacteria and production of microbial toxins.

2.6 Preservation of vegetables

Thermal processing is one of the most common current forms of vegetable preservation because it efficiently reduces microbial population, destroys natural enzymes and renders horticultural products more palatable (Novak, 2010). Most canned vegetables are produced under conditions of commercial sterility, and have a shelf life of 2 years or longer. Thermal processing essentially involves either heating unsterile vegetables in their final containers (canning), or heating the vegetables prior to packaging and then packaging under sterile conditions (Teixeira, 2019).

Freezing serves as a method of preservation because water activity can be lowered to a level which prevents microbial activity and reduces the rates of chemical reactions. (Barrett *et al.*, 2012). There are three basic freezing methods used commercially: freezing in air, freezing by indirect contact with the refrigerant, and freezing by direct immersion in a refrigerating medium. Prior to freezing, most vegetables are exposed to a short blanching treatment with either steam or hot water to inactivate enzymes. While the thermal exposure in frozen vegetables and fruits is relatively low, the freezing and thawing process itself results in significant tissue structure damage, depending on the rate and temperature at which each is applied. This degradation of plant tissue may allow loss of cellular integrity and interaction of

enzymes and nutrient substrates, resulting in nutrient loss in addition to deterioration of texture, colour and flavour (Barrett *et al.*, 2012)

There are relatively newer technologies which may or may not be in commercial practice. These include high-pressure processing and use of various electric methods such as microwave, pulsed electric fields and between electric fields, ohmic processing. One tremendous advantage of these advanced methods is the uniform application of pressure or electric fields to the product as a whole, rather than needing to rely on heat or freezing temperature penetration from the external surface to the inside of the container (Pereira, 2010). During pressurization there is some heating of the material, but this is generally less than if temperature was the only means of preservation. Electric field processing generates heat locally, which also minimizes the amount of heat required. Advanced processes therefore minimize the temperature (and hence the quality) gradient in the product and shorten the process time required (Chemat, 2011).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The study was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) between the months of May - August 2013. The vegetables were grown in JKUAT farm and the analysis done in the Food Science laboratory in JKUAT. After 8 weeks of growth, the leaves were harvested and stored in shelf life bags as outlined in Section 3.2.

3.2 Crop Establishment

These *Amaranthus dubius* obtained from Amiran Kenya was planted at JKUAT farm. The seeds were sown directly by drilling in furrows of about 1 cm deep. The main sources of water were rainfall and irrigation for the crops. The plants were irrigated twice a week where there was no rain. Shallow cultivation using jembes and hand pulling of weeds was carried out to keep the plot weed free.

3.3 Harvesting/ Sample Collection

After four weeks of planting, leaves were harvested at the vegetative stage. The leaves were harvested. The leaves were separated from stalks. They were packed loosely in well-ventilated field container and taken to the JKUAT, food science and technology laboratory.

3.4 Research Design and Treatments

The leaves were stored at four different temperatures of 5 °C, 10 °C, 15 °C and 25 °C in thin film modified atmospheric shelf life bags of 35µm thickness. Analysis of nutritional and physicochemical properties of the vegetables was done until the leaves decayed after which they were disposed. Each treatment was repeated three times during analysis. Average values were then computed. Figure 3.1 shows a flowchart of the various activities that was carried out during the research.

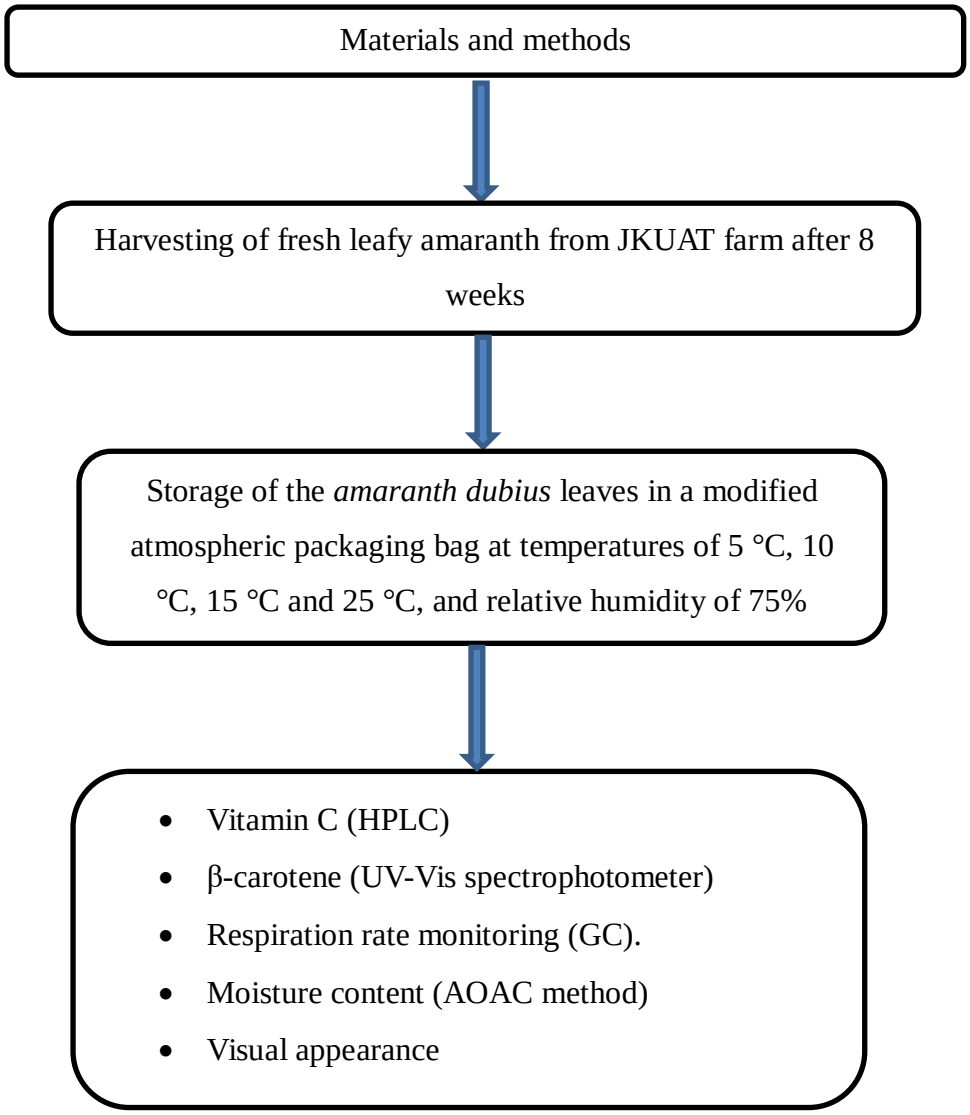

Figure 3.1: A flowchart of the various activities that was carried out during the research.

3.5 Storage of Amaranth Leaves

After harvesting samples of 500 g were placed in thin film shelf life bags obtained from Amiran Kenya. They were stored at four different temperatures. One batch was stored at 5 °C, another at 10 °C, and another at 15 °C and the other at room temperature 25 °C. The sample stored at 25 °C was the control. All batches were stored until they lost their visual appeal which was determined by the changes in the lightness of the leaves.

The packaging bags were opened and the vegetables analyzed for moisture content, ascorbic acid, β -carotene, respiration rate and colour and analyzed within one month

3.6 Nutritional Profiling

3.6.1 Determination of Vitamin C

The ascorbic acid content in the samples was determined by HPLC method (Vikram, *et al.*, 2005). About 2 g of sample was weighed and extracted with 0.8% metaphosphoric acid. This was made to 20 mL of juice. The juice was centrifuged at 10000 rpm. The supernatant was filtered and diluted with 10 mL of 0.8% metaphosphoric acid. This was passed through 0.45 µ filter and 20 µL injected into the HPLC (model C-R7A plus Shimadzu Corp., Kyoto Japan) machine. Various concentrations of ascorbic acid standards were also made to make a calibration curve. The mobile phase was 0.8% metaphosphoric acid, at 1.2 mL/min. The peak was automatically identified and quantified by comparing its retention time of the sample with the standard retention time. The amount of ascorbic acid was calculated from calibration curve of standard prepared from the equation. Typical Chromatograms for this analysis are shown in Appendix 3.

3.6.2 Determination of β -carotene

Beta carotene content was analyzed using UV Spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan).Acetone and petroleum ether extraction method was used as described by de Sá and Rodriguez-Amaya, (2004).

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

Exactly 25 mL of the extract was evaporated to dryness using rotary evaporator (Model RE.100, by Bibby Sterling Ltd, UK). The residue was dissolved with 10 ml

petroleum ether and the solution introduced into a chromatographic column. This was eluted with petroleum ether and beta carotene collected in a flask. The beta carotene elute was made to a volume of 25 mL with petroleum ether and the absorbance was read at 440 nm in a UV-Vis spectrophotometer (Shimadzu model UV – 1601 PC, Kyoto, Japan). Beta carotene standards were also prepared to make a calibration curve. The concentrations of the β -carotene standards were plotted against the peak area to obtain a straight line. The amount of β -carotene was calculated from calibration curve of standard prepared from the equation. Typical Chromatograms for this analysis are shown in Appendix II.

3.6.3 Respiration Rates Determination

Carbon dioxide was determined according to Gavril and Karaiskakis, (1999) using Gas chromatography (Models GC-8A and GC-9A, Shimadzu Corp., Kyoto, Japan) equipped with a thermal conductivity detector (GC-TDC). Each of the chromatographic columns was equilibrated with a dry ice/isopropyl alcohol bath (-80 °C) and set flow in the forward direction (from silicone oil to Porapak)

About 1 ml of the sample was injected into the sample port and flushed the loop. The loop valve was switched to the position that flushes the sample into the columns. The instrument was calibrated and another sample was injected. The check standard was injected after every injection, or at the end of a run. The concentrations of the carbon dioxide standards were plotted against the peak area to obtain a straight line. The amount of carbon dioxide was calculated from Calibration curve of standard prepared from equation. Typical Chromatograms for this analysis are shown in appendix 1.

$$\text{Rate of CO2 evolution} = (P - F) \times (V - v) \times {}^{1}/_{w} \times {}^{1}/_{T}$$

Where:

V= volume of incubation container

v = volume of leaves

w= weight of leaves

F= factor obtained from regression equation of the standard curve

P = peak area of the sample from the chromatograph

T = Incubation time in hours

3.6.4 Determination of Moisture

Moisture content was determined according to AOAC, (1995). About 5 g of fresh sample was weighed and placed in a clean dry moisture dish and the weight of the sample and dish taken. These were placed in a moisture oven and the temperatures adjusted to 105 °C. The samples were dried for 3 hours and cooled in a desiccator and weighed. The amount of moisture in the samples was calculated using the formula:

$$\% \text{ moisture} = \frac{(\text{weight before drying} - \text{weight after drying})}{\text{sample weight}} \times 100$$

3.6.5 Determination of colour and visual appearance

The colour was determined using a hunter lab colour difference meter (Minolta, Tokyo, Japan) according to Pathare *et al.,* (2013). The instrument was standardized each time with a white a black ceramic plate. The colour was measured at three different surfaces of the leaf. Results were recorded as L* values used to determine the rate of color changes of the flesh with time. Only the L* values were used as they indicate the rate of darkening or lightening of the leaf surface.

The decay of the amaranth leaves was evaluated by visual observation, noting yellowing, development of undesirable odors, and appearance of black dots and presence of mold on the leaf surface. Each bunch was checked. The appearance of dark spots on the surface of the leaf were used as decay index.

3.7 Data Analysis

The data was analyzed using Statistical Package for Social Sciences Version 20.0 (SPSS) in which the evaluation of proximate analysis of MAP storage under different temperatures was checked using analysis of variance (ANOVA) at $P < 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSIONS

4.1 Effect of modified atmospheric storage on Vitamin C

Figure 4.1 shows that Vitamin C content of *Amaranthus dubius* was retained longer at lower temperatures and depleted faster at high temperatures. The Vitamin C content of the stored leaves was significantly ($P<0.05$) affected by the storage temperature.

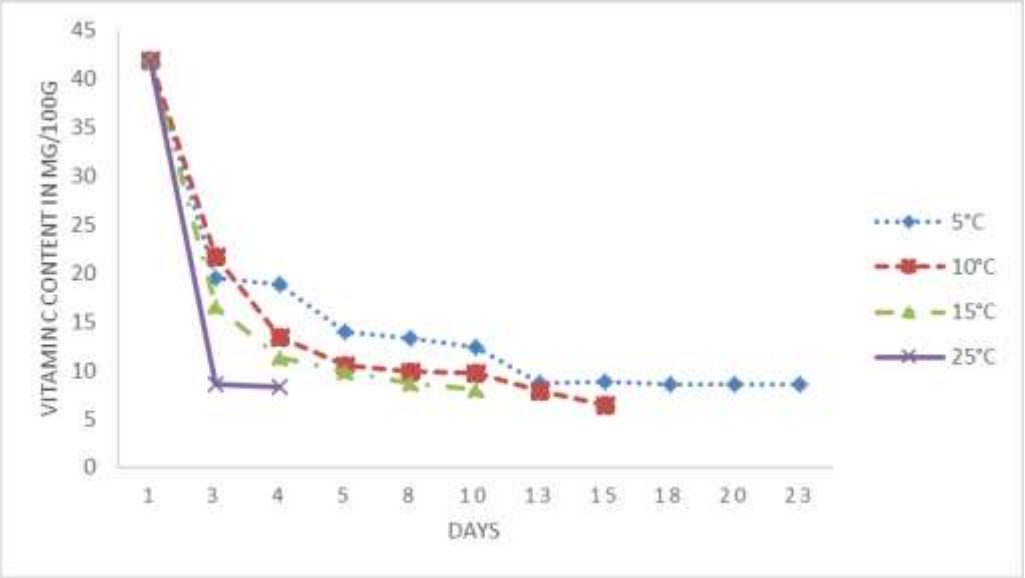

Figure 4.1: Vitamin C changes of Amaranthus dubius stored at 5 °C, 10 °C, 15 °C and 25 °C

A considerable loss of vitamin C in the four storage temperatures was noted in the first three days. Leaves stored at 25 °C experienced the fastest drop of 80% from 41.95 mg/100g to 8.3 mg/100g as compared to 50% drop at 5 °C storage temperatures. At day 3, the leaves stored at 25 °C had decayed therefore they were discarded. Between 5 to 20 days, the leaves stored at 5 °C, 10 °C and 15 °C experienced a gentle decrease in vitamin C content. The trend indicates that the most

crucial stage of increasing carbon dioxide concentration and reducing oxygen concentration within the first three days of modified atmospheric storage.

Days 10, 15 and 20 were the decay days of leaves stored at 15 °C, 10 °C and 5 °C respectively, when the leaves started yellowing and developing dark spots on the surface. At the end of storage, the retentions ranged between 8.3 mg/100 g for sample stored at 25 ºC, at day four and packaged in modified atmospheric bags and 8.5 mg/100 g for sample stored at 5 ºC. The results confirmed that storage of vegetables for three weeks at the different temperatures reduced Vitamin C content in the vegetables at different rates with high temperatures causing bigger losses. Although storage at low temperatures prolonged the amaranth shelf life compared to higher temperatures, their positive effect was negated by high incidence of Vitamin C losses evident as storage days progressed. These results were similar to the findings of Abongo *et al*. (2011) who noted a higher loss of Vitamin C at high temperatures during storage of fresh tubers in MAP.

The higher rate of ascorbic acid loss during the first days of storage as compared to the second and third days was probably due to the effect of the residual oxygen retained in the shelf life bag during the initial packaging. As storage progressed, the residual oxygen in the package decreased and therefore the rate of oxidation of ascorbic acid also decreased. Lee and Khader (2000) also noted that leafy vegetables held at 6 ^{0}C lost 10% of their ascorbic acid content in 6 days, while those held at room temperature lost 20% in only 2 days. The Required Daily Allowance for Vitamin C is 60 mg/day. Storage temperature influenced the amount of ascorbic acid retained by the *Amaranthus dubius* within the storage period. It is therefore important to choose storage temperature of 5 ºC for maximum vitamin C retention.

4.2 Effect of modified atmospheric storage on β -carotene

There was an observed change in β -carotene content of the stored amaranth leaves at different temperatures. The β -carotene content was significantly ($P<0.05$) affected by the storage temperature as indicated in Figure 4.2.

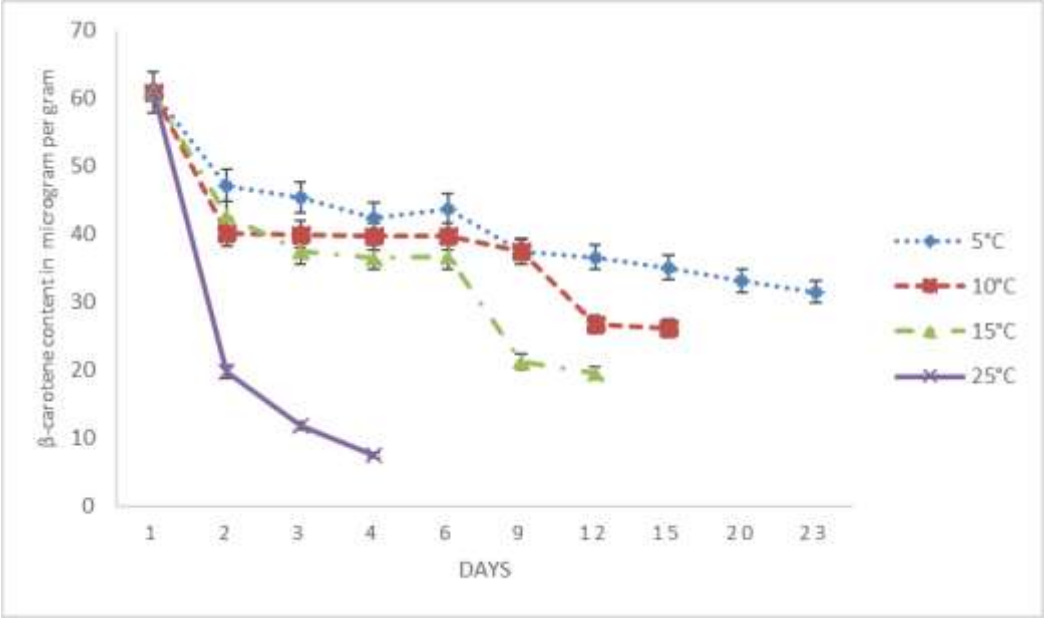

Figure 4.2: β-carotene changes of amaranth leaves stored at 5 °C, 10 °C, 15 °C and 25 °C

Storing the vegetables at room temperature (25 ℃) decreased β -carotene content of vegetables fastest as compared to storing them at storage temperatures of 15 ℃, 10 ℃ and 5 ℃. There was a significant difference among the storage temperatures. The results further revealed that storage of vegetables for three weeks significantly reduced β -carotene content for vegetables at 25 ℃ from 61.4 µg/g to 7.516 µg/g at day 4. β -carotene content for vegetables at 15 ℃ dropped from 61.4 µg/g to 19.6 µg/g at day 12. β -carotene content for vegetables at 10 ℃ dropped from 61.4 µg/g to 26.14 µg/g at day 15, whereas vegetables at 5 ℃ dropped from 61.4 µg/g to 31.53 µg/g at day 23. The stability of β-carotene in the four storage temperatures samples under modified atmosphere packaging (MAP) was different during the storage period.

Based on the study findings to enhance the shelf life of *Amaranthus dubius* the vegetable should be kept under storage facilities with low controlled temperatures as *Amaranthus dubius* tends to lose it nutritional contents when stored at higher temperatures.

Generally, the quality cannot be improved after harvest, but can only be maintained.

These losses result in a short shelf-life for vegetables whose market value is mainly determined by their physical appearance. Shelf life is the recommended time that products can be stored, during which the defined quality of a specified proportion of the goods remains acceptable under expected (or specified) conditions of distribution, storage and display (Hough, 2012). The Recommended Daily Allowance (RDA) for β -carotene is 12 µg/d. Storage temperature influenced the amount of β -carotene retained by the *Amaranthus dubius* within the storage period. It is therefore important to choose storage temperature of 5 ℃ ,10 ℃ and 15 ℃ for maximum retention of β -carotene in order to achieve the recommended daily allowance.

4.3 Effect of modified atmospheric storage on respiration rates

Figure 4.3 showed that the higher the storage temperatures the higher the rate of respiration. The amaranth stored at 25 ℃ respired at a faster rate compared to amaranth stored at 15 ℃, 10 ℃ and 5 ℃. The samples stored at 5 ℃ had the slowest respiration rate. There was a peak at every storage temperature. The peak varied among the different treatments, depending on the decay days of the *Amaranthus dubius.* The peak for he leaves stored at 25 ℃ was noted at day 4, whereas the peaks for the leaves stored at 15 ℃, 10 ℃ and 5 ℃ was noted at days 6, 12 and 15 respectively. There was a significant difference observed among the treatments.

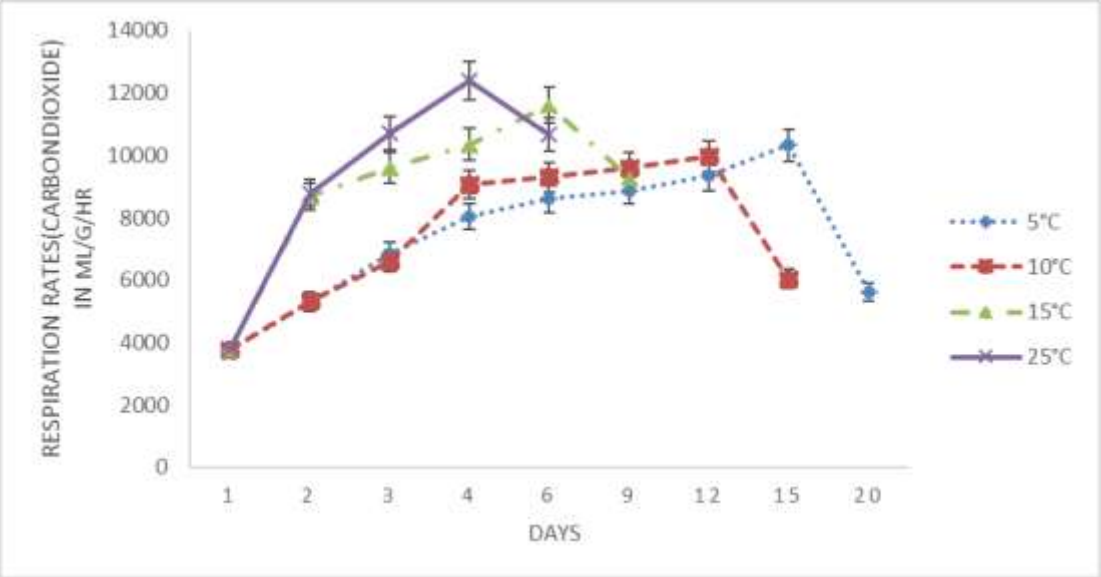

Figure 4.3: Respiration rates of amaranth vegetable stored at different temperatures

Results revealed that storing the vegetables for longer time at room temperature (25 °C) rapidly increases the respiration rates of the vegetables from 3,754 ml/g/hr. to 12,393 ml/g.hr thereafter dropped to 10,689 ml/g/hour at day 6.The control had the highest respiration rates whereas the leaves stored at 5 °C had the least respiration rates. The leaves stored at 15 °C had an increase in the respiration rates from 3,754 ml/g/hour to 11,619 ml/g/hr. then thereafter dropped to 9,284.1 ml/g/hour at day 9. The leaves stored at 10 °C had an increase in the respiration rates from 3,754 ml/g/hour to 9.959 ml/g/hr. then thereafter dropped to 6,040 ml/g/hour at day 15. The leaves stored at 5 °C had an increase in the respiration rates from 3,754 ml/g/hour to 10,333 ml/g/hr. then thereafter dropped to 5,611.8 ml/g/hour at day 20. Higher respiration rates indicate a more active metabolism and usually a faster deterioration rate. Higher respiration rates resulted in more rapid deterioration of components that determine nutritive value of the amaranth vegetable. Higher respiration rates also led to a decrease of the Vitamin C and β -carotene.

Catabolic processes such as respiration characteristically happen more quickly at higher temperatures (Hahn and Denlinger, 2007). Chemical reactions double their rate for each temperature increase of 10^{o}C because activation energy barriers become

more easily surmounted at higher temperatures (Weaver, 2014). This is the reason shelf life for leafy vegetables are generally extended by temperature control, for example by refrigeration and insulated shipping containers. The respiration rates of commodities are directly related to product temperature; the higher the temperature, the higher the respiration rate.

The respiration rate of a product strongly determines its shelf life. The higher the storage temperature the higher will be the respiration and transpiration rates and the greater the transit losses (rotting and wilting) leading to a shorter shelf life of the vegetables

4.4 Effect of modified atmospheric storage on moisture

There was a reduction in moisture content as shown in Figure 4.4. The rate of moisture content loss was affected by the treatment induced and the storage time. The leaves stored at 25 ℃ and 15 ℃ had a moisture loss of 10% after four days of storage, the leaves stored at 15 ℃ had a moisture loss of 7% whereas the leaves stored at 5 ℃ had a moisture loss of 5%.

The mean moisture content at 5 ℃ was found to be significantly different from the other storage temperatures. An increase in temperature led to an increase in the moisture loss during the storage period. The loss in moisture is usually due to respiration which are normal metabolic processes of the amaranth vegetable (Abbasi *et al.*, 2015)

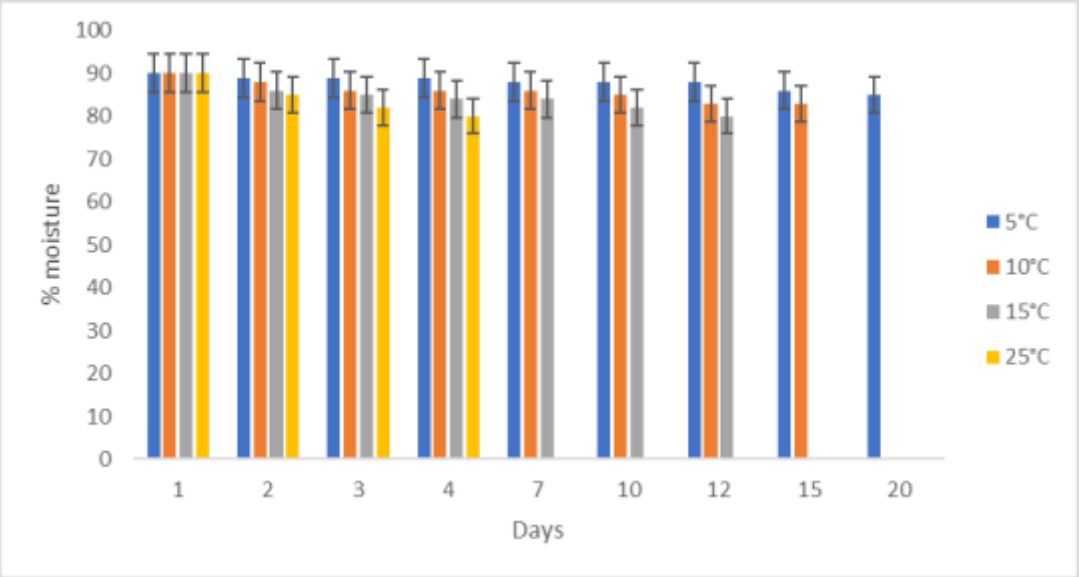

Figure 4.4: Moisture content of amaranth vegetable stored at different temperatures

4.5 Effect of modified atmospheric storage on colour changes of amaranth leaves

The colour of the product is the major quality parameter that determines consumer acceptance. MAP storage coupled with low temperatures showed a mediating effect on the rate of leaf yellowing by slowing the rate of colour degradation of the green leaves. The leaves colour was significantly ($P \leq 0.05$) different between the control MAP storage and the other samples throughout the storage duration. Colour changes are displayed in Figure 4.5 The samples stored at 25 ℃ had a L* value of 50.2 after 4 days of storage, leaves stored at stored at 15 ℃ had a L* value of 38.9 after 11 days of storage, stored at 10 ℃ had a L* value of 38.9 after 13 days of storage whereas the leaves stored at 25 ℃ had a L* value of 50.2 after four days of storage. The lowest L* value was 39.23 detected in the leaves stored at 5 ℃, while the highest L* value of 50.2 was in the leaves stored at 25 ℃ after four days of storage. The decline of the L* values seemed to be accompanied by mould development on the leaf surface. There was no significant difference in the L* value between the treatments.

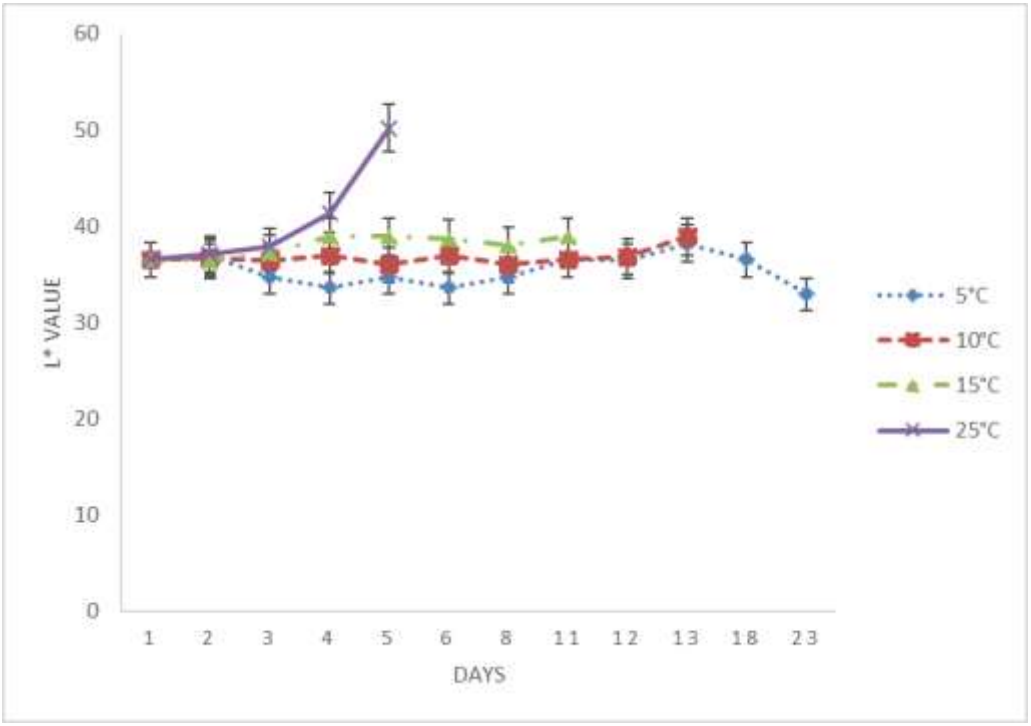

Figure 4.5: Colour changes of amaranth vegetable stored at different temperatures

4.6 Effect of modified atmospheric storage on visual appearance of amaranth leaves

Leaf yellowing was noted on the third day in the control leaves and the severity of the yellowing increased with increasing storage time as shown in Table 4.1. Leaf yellowing acts as a limiting factor apart from wilting with *Amaranthus dubius*, and it indicates that the product has reached the end of its shelf life.

Amaranth vegetables stored at 15 °C maintained acceptable visual quality for up to 12 days. However, after that time the leaf surface though green, were characterized by brownish dark spots. The amaranth stored at 10 °C maintained acceptable visual quality for up to 18 days whereas the leaves stored at 5 °C decayed at day 23. However, after that time, the leaves were characterized by mould development thus causing decay. Therefore, temperature management (especially cool storage) seems to be the most important means to extend shelf life and maintain quality of fresh fruits and vegetables that are not chilling sensitive. The leaves were characterized by

yellowing, brown staining and mould development on the leaf surface. According to (Ahmad, Siddiqui, Ahmad, and Siddiqui, 2015) Ahmad *et al.* (2015), shelf life decreases and many undesirable changes take place such as decay during postharvest. All these changes, if not governed, ultimately affect the nutrition quality. Day 15 was the decay day for amaranth leaves stored at 15 ℃. Dark irregularly distributed spots were displayed at the surface of the leaves at day 15. Day 20, leaves stored at 10 ℃ decayed. At day 20 some leaves turned yellow while others remained green but developed moulds. Day 23, leaves stored at 5 ℃ decayed. At day 23 all leaves turned black with yellow spots. Therefore, an ideal storage procedure for these vegetables should eliminate oxygen in the packaging containers to maintain their freshness (Vermeiren, 1999).

Table 4.1: Effect of modified atmospheric storage on visual appearance of amaranth leaves

TEMP	DAY 1	DAY 2	DAY 4	DAY 6	DAY 10	DAY 15	DAY 23
25 °C							
15 °C							
10 °C							
5 °C							

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion.

The Vitamin C, β-carotene, moisture content, colour and visual appeal of the leaves stored at 5 ℃ declined at a significantly lower rate as compared to the leaves stored at 15 ℃, 10 ℃ and 5 ℃. The respiration rates increased in all the storage temperatures, though this was slightly higher in the control amaranth leaves. The storage time and treatment induced had effects on the postharvest quality of the amaranth leaves.

It was therefore concluded that the modified atmospheric packaging bags increased the shelf life of the vegetables. Storage temperatures at 5 ℃ showed the most retention of Vitamin C, β-carotene, moisture content, colour and visual appeal for a longer duration of 23 days. Lower respiration rates were also attained in the storage at 5 ℃. Low temperatures of 15 ℃, 10 ℃ also increased the shelf life of the vegetable amaranth. 25 ℃ resulted to the least preservation of quality attributes of the vegetables.

5.2 Recommendations

5.2.1 Recommendations from the Study

The study recommends also that the farmers and supermarkets could store *Amaranthus dubius* at 5 ℃ since it maintains the most nutrients, colour and visual appeal over a long period of three weeks unlike at 25 ℃ which maintained the nutrients for only 3 days before decay. Creation of knowledge and awareness on the best modified atmospheric storage temperatures amongst farmers and supermarkets that have the amaranth vegetables on sale is also recommended.

5.2.2 Areas for Further Study

Based on the findings of the study, the research further recommends the following areas for further study;

i. Further research studies are necessary to determine the other nutrient quality of amaranth leaves during storage
ii. Need of advising the supermarket super intendants of the best storage conditions of the amaranth leaves in the supermarket
iii. This study utilized simple, low cost technology which should be transferred to the communities for preservation of these foods.

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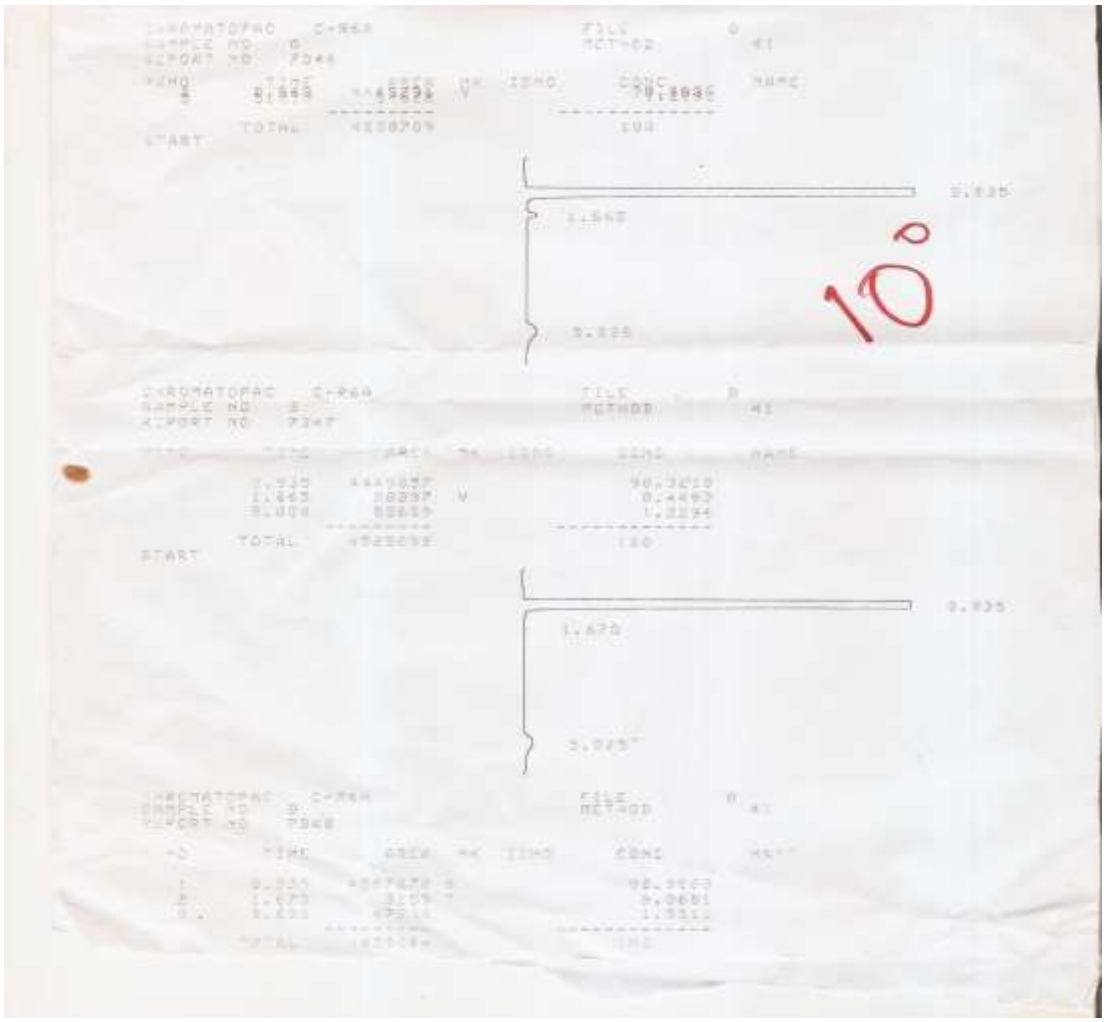
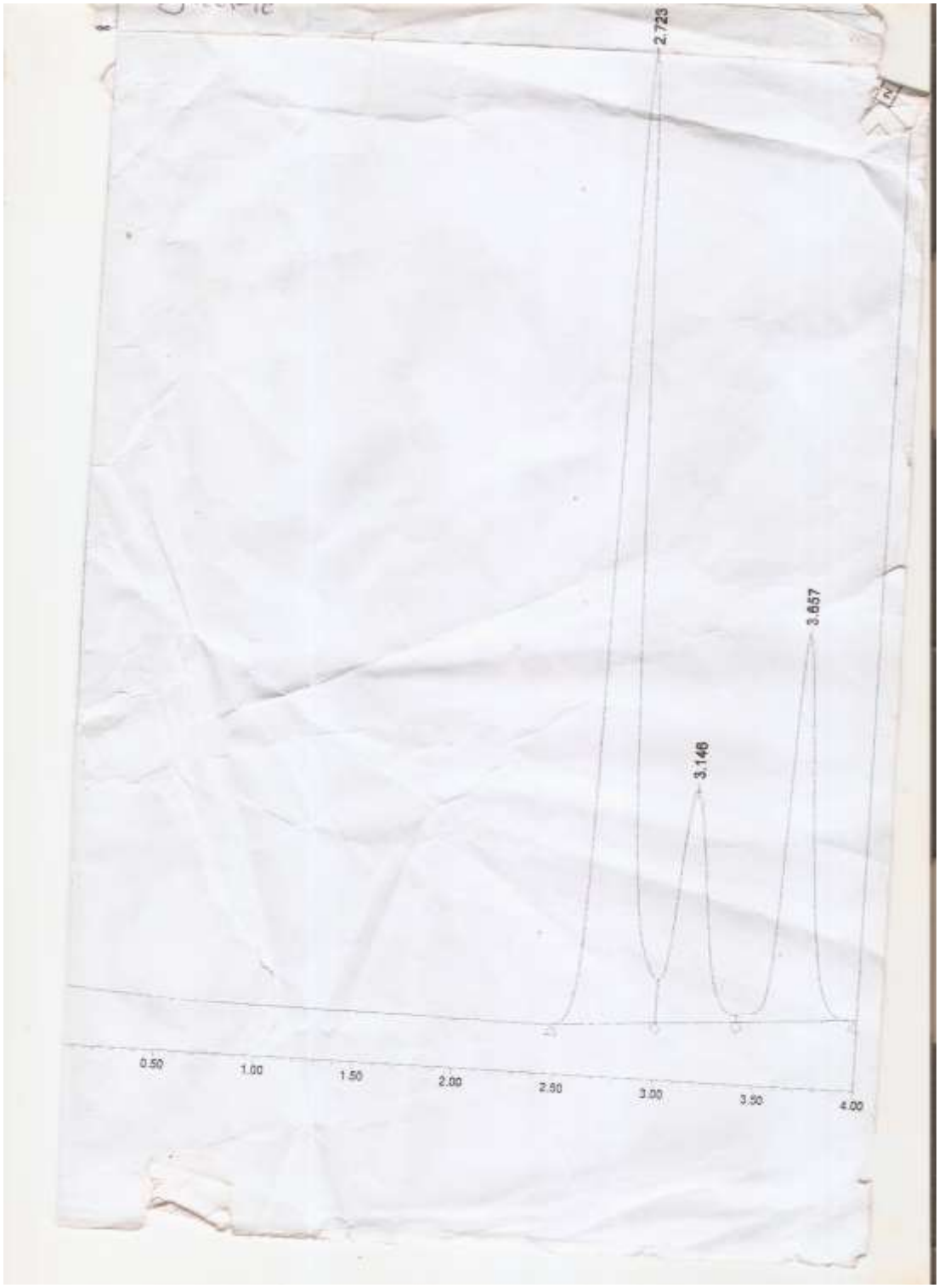
APPENDICES

Appendix I: Gas Chromatogram of carbon dioxide in the amaranth leaves during modified atmospheric storage



Appendix II: UV/visible spectrophotometer of beta carotene of amaranth leaves during modified atmospheric storage



Appendix III: HPLC determination of vitamin c in the amaranth leaves during modified atmospheric storage