

**ANALYSIS OF LYCOPENE, VITAMIN A AND BETA CAROTENE IN RED  
CACTUS (*Opuntia ficus-indica*) FRUIT IN NYERI COUNTY, KENYA**

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**DECLARATION**

I hereby declare that this is my original work and has not been presented for degree or other awards in any other university.

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**DEDICATION**

This thesis is dedicated to my loving late parents who kept encouraging me in my education even when things were tough. Second, I dedicate it to my wonderful children Rodrick and Mercy who encouraged me all through to move on and never give up. I salute you.

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**ABBREVIATIONS AND ACRONYMS**

|       |  |
|-------|--|
| ACN   | Acetonitrile                                 |
| AI    | Adequate Intake                              |
| AIDS  | Acquired Immune Deficiency Syndrome          |
| ANOVA | Analysis of Variance                         |
| ASAL  | Arid and Semi-Arid Area                      |
| BC    | Beta-Carotene                                |
| BHA   | Butylated Hydroxyl Anisole                   |
| BHT   | Butylated Hydroxyl Toluene                   |
| CEC   | Capillary Electro Chromatography             |
| CHD   | Coronary Heart Disease                       |
| CICT  | Conjunctiva Impression Cytology and Transfer |
| DGLVs | Dark Green Leafy Vegetables                  |
| DNA   | Deoxyribonucleic Acid                        |
| DRI   | Dietary References Intake                    |
| EAR   | Estimated Average Requirement                |
| FNB   | Food and Nutrition Board                     |
| FW    | Fresh Weight                                 |
| GC    | Gas Chromatography                           |
| HIV   | Human Immunodeficiency Virus                 |
| HPLC  | High Performance Liquid Chromatography       |
| IOM   | Institute of Medicine                        |
| LOD   | Limit of Detection                           |
| OCC   | Open Column Chromatography                   |
| ODS   | Octadecylsilyl silica                        |
| PG    | Propyl Gallate                               |

|        |                                |
|--------|--------------------------------|
| PSA    | Prostate Specific Antigen      |
| PTFE   | Polytetrafluoroethylene        |
| RAE    | Research Assessment Exercise   |
| RAR    | Retinoid X                     |
| RAX    | Retinoic Acid                  |
| RDA    | Recommended Dietary Allowance  |
| RPC    | Reversed Phase Chromatography  |
| RSD    | Relative Standard Deviation    |
| TBH    | Tertiary Butyl Hydroquinone    |
| THF    | Tetrahydrofuron                |
| THF    | Tetra Hydro Furan              |
| TUIL   | Tolerable Upper Intake Level   |
| UL     | Uptake Level                   |
| UV     | Ultra Violet Rays              |
| UV/vis | Ultra Violet/Visible           |
| VA     | Vitamin A                      |
| VAD    | Vitamin A Deficiency           |
| VADDs  | Vitamin A Deficiency Disorders |
| WHO    | World Health Organization      |

**ABSTRACT**

Vitamin A deficiency (VAD) has been a serious public health problem in the developing countries especially in Africa, mostly in pre-school age children and in pregnant and lactating mothers. It contributes to 7 million pregnant women and approximately 127 million of preschool-aged children and about 1-2 million deaths every year. It has been reported that about half a million new cases of blindness emerge yearly. This has for a long time been solved in addition to modern medicines by using locally available dark green leafy vegetables (DGLVs), which are believed to also contain high levels of beta-carotene(BC). Unfortunately, they are seasonal. BC is an important source of vitamin A (retinol) and its deficiency causes morbidity and mortality in children and adults. A diet rich in lycopene is related to a decreased risk of cancer of digestive track, prostate and pancreas. Vitamin A, beta-carotene and lycopene have been found in tomatoes and carrots which require a lot of water for their growth. Red cactus (*Opuntia ficus indica*) fruit grows wildly along the road and in the forest in the dry areas and it is consumed by herders. This fruit has not yet entered the market, yet it could contain substantial amounts of phytochemicals (antioxidants) such as lycopene, vitamin A and beta-carotene. It grows throughout the year in arid and a semi-arid area (ASAL) hence it can supplement DGLVs, tomatoes and carrots. The objective of this study was to determine the levels of beta-carotene, lycopene and vitamin A in Red cactus at unripe, ripe and overripe stages using highperformance liquid chromatography (HPLC). The area of study was Chaka, Thegu area of Nyeri County. The unripe (the breaker stage), the ripe (completely red) and overripe (the ripe kept for 5 days to overripe) red cactus fruits were picked with the help of a taxonomist who helped in identifying the fruit and its botanical name. The phytochemicals were extracted from the fruits and analyzed using HPLC and the data was analyzed using ANOVA. The mean of beta-carotene levels in unripe red cactus was  $0.04 \pm 0.002$  mg/100 g and increased to  $0.07 \pm 0.001$ mg/100g at the ripe stage and  $0.09 \pm 0.003$  mg/100 g when overripe. The mean lycopene levels in red cactus was  $3.26 \pm 0.12$  mg/100 g when unripe and increased to  $7.06 \pm 0.11$ mg/100g when ripe and finally to  $13.56 \pm 0.50$  mg/100 g when overripe. The mean vitamin A levels in ( $\mu$ g/100 g) in red cactus was  $1.27 \pm 0.05$   $\mu$ g/100 g when unripe and increased to  $2.14 \pm 0.03$   $\mu$ g/100 g when ripe and then decreased to  $1.47 \pm 0.06$   $\mu$ g/100 g when overripe. The results from this analysis showed that the amounts of beta-carotene, lycopene and vitamin A increased significantly ( $P < 0.001$ ) during ripening. From the results it's important that people living in the dry regions and where this fruit grow should be encouraged to eat the red cactus fruits at the ripe and overripe stage of ripening to get the maximum benefit of VA and both lycopene and beta-carotene respectively. The fruits should not be consumed at the unripe stage since their levels are low and they cannot be of much benefit. This fruit can be a supplement to other sources. The information obtained on the levels should be availed to the industries set up to produce red cactus products to improve on the utilization.

## CHAPTER ONE

### INTRODUCTION

#### 1.1 Background Information

Vitamin A (retinol), beta-carotene and lycopene are phytochemicals which occur naturally and are found in vegetables, medicinal plants, fruits, aromatic plants, leaves, roots and flowers which act as a defense system to fight against diseases. These compounds have cardio defensive effects in the body brought about by their antioxidative and anti-inflammatory activities that minimize the risk of cardiovascular disorders (Vasanthi *et al.*, 2012).

The red cactus fruit (figure 2.1) belongs to the kingdom of plantae, angiosperms and eudicots clade, the order of *caryophyllales*, *cactaceae* family, *opuntia* genus and in the species of *opuntia-ficusindica*. The binomial name of the red cactus is *Opuntia ficus-indica* (L.) Miller (Griffith, 2004). This perennial shrub when fully grown has a height of 3-5 m with cladodes that are thick and succulent and these cladodes produce flowers of either white, yellow or red when they are about 1-2 years old the colors of the fruits that they produce have colors ranging from pale green to deep red and have a taste close to that of a water melon. Their epidermis is water repellent and waxy sun reflecting (Miller, 2015). It has hard seeds which are surrounded by a fleshy portion. The shrubs which are succulent grow in arid and semi-arid climates and are drought tolerant. They originated from Mexico but are now found grown across South Africa Australia and the United States (Castellar *et al.*, 2003). The red cactus tree with fruits is represented in Figure 2.1.



**Figure 1.1: The Red Cactus Fruits with fruits at different ripening**

**Source: Self**

Red cactus fruit is green when unripe turns red when ripe and deep red when overripe. The levels of lycopene and BC in the red cactus increases with each stage of maturation but for vitamin A(retinol) the level decreases as the fruits are kept to overripe as was shown in this investigation. Red cactus fruits take about 8-10 days to ripen on the trees and to overripe they take 6-7 days. Once the fruits are picked, it takes them 6-7 days to ripen and 3-5 days to get overripe (Personal communication with the residents). This study sought to find out whether the antioxidants levels change at different maturation so as to make correct recommendation on when it is best to consume the fruit for maximum benefits. In Kenya the fruit is found in Thegu

area in Nyeri County, Doldo area in Laikipia County, Mugotio in western among other places. Cactus pear (*Opuntia ficus-indica*) is a drought resistant plant that originated in Mexico. It has flattened stem segments that are called cladodes having a moisture protein and fiber content of 92, 1-2 and 4-6% respectively (Jaramillo *et al.*, 2003).

## **1.2 Antioxidants**

An antioxidant refers to a substance which is able to prevent, delay or also remove the oxidative damage to a molecule which is targeted by inhibiting the oxidation process, (Halliwell, 2007). In plants they play the role of radical scavengers which convert radicals to species that are less reactive (Nem *et al.*, 2009). The antioxidants such as vitamin A, beta-carotene and lycopene control oxidation which can be caused by free radicals (Bjelakovic *et al.*, 2007).

### **Vitamin A**

Vitamin A acts as an antioxidant and it occurs in three major forms. These are the retinol (Vitamin A1), 3, 4-didehydroretinol (Vitamin A2), and 3-hydroxyretinol (Vitamin A3). The foods rich in vitamin A include liver, sweet potatoes, carrots, milk, egg yolks and mozzarella cheese (Baublis *et al.*, 2000). The VA is not needed daily in large quantities since it's a fat-soluble vitamin which is stored in the body in organs such as the fatty tissues and liver. Retinoid is a form of VA obtained from animal products and is utilized in our bodies without any modifications (Bohn *et al.*, 2014). Carotenoids are a form of vitamin A and the most common one is the betacarotene which is used to build the type of vitamin A used by our body (Bohn *et al.*, 2014).

### **Beta-Carotene**

The recommended dietary allowance (RDA) for vitamin A is 900 mg day for an adult male (IOM 2001). This means that an intake of 11 mg of beta-carotene would provide sufficient VA supply, assuming a conversion rate of 1:12. Beta-carotene (BC) is a pro-vitamin A which protects the body from the free radicals which cause oxidation of cells leading to chronic illnesses such as cancer and heart disease. Increasing the consumption of fruits and vegetables improve the bioavailability of BC in human serum (Nawiri *et al.*, 2013).

### **Lycopene**

Lycopene is an antioxidant which has a bright red pigment. Fruits are given the red coloration by lycopene. Such fruits include the watermelons, tomatoes, grape fruits, carrots, red bell peppers and the papayas. This antioxidant is naturally synthesized by the plants and it is a pigment which is widely and commonly used by the food industry as a food additive since it has a strong color and also it is non-toxic. This pigment is registered as E160d (Perkins-Veazie *et al.*, 2001) and it has also been approved for food use. It is characterized by many health benefits and has been increasing in demand due to its red colorant and also its anti-oxidizing chemical properties. The importance of natural food additives has gained more popularity due to the heightened use of natural rather than synthetic compounds in cosmetics, food and pharmaceuticals (Ishida *et al.*, 2001; Kong *et al.*, 2010).

Lycopene is considered to be one of the most potent antioxidants and its singleoxygen-quenching ability is double that of beta-carotene and ten times higher than that of  $\alpha$ -tocopherol (Rao and Agarwal, 2000). According to most of the

epidemiological studies done, a diet that is rich in lycopene has a lot of beneficial effects on human health (Borguini and Torres, 2009). The antioxidant property of lycopene helps in protecting the body cells from degenerative diseases by neutralizing free radicals in the body. It also prevents DNA damage in the cells and this improves cell function (Wang and Chen, 2006).

The sources of these antioxidants include tomatoes, carrots, dark green vegetables (DGLVs) among others which require the use of insecticides like karate and aldrin and a lot of water for their growth. This becomes a challenge to low income farmers and the people living in the arid and semi-arid areas where constant source of water is a problem. This can be solved by utilizing the red cactus fruit which may contain substantial amounts of lycopene, beta-carotene and vitamin A and it grows wildly in the ASAL region in all season.

The red cactus plant grows in the arid and semi-arid areas in most parts of the world and its stem store a lot of water which enable it to thrive for a long time without rain. This fruit contain essential nutrients and vitamins such as vitamin A, lycopene and beta-carotene. This study aimed at determining the levels of VA, BC and lycopene when at the unripe (breaker), ripe (completely red) and overripe stages (fruits kept for 5 days after being picked when red in color) so that accurate information on when to consume the fruit is given.

### **1.3 Problem Statement and Justification**

Vitamin A deficiency (VAD) is the leading cause of blindness in children and increases the risk of disease and death from severe infections. According to a research

done by Singh and West (2004), approximately 83 million school-aged children were said to be vitamin A deficient representing 23.4 % prevalence of VA deficiency. Abnormal conjunctiva impression cytology and impaired dark adaptation are considered valid indicators of VAD (Christian, 2002). The VAD and anaemia are considered to be major challenges among children, expecting and lactating mothers in the developing countries (Nawiri *et al.*, 2013). DGLVs contain high levels of iron and beta-carotene (BC) and therefore useful in fighting VAD and anaemia but unfortunately they are season-dependent (Nawiri *et al.*, 2013). The consumption of lycopene reduces prostate cancer by reducing the proliferation of cancer cells and inducing apoptosis (Kanagaraj *et al.*, 2007). The main sources of lycopene are tomatoes (*lycopersicon esculentum mill*) which are perishable and cannot grow without water and require the use of a lot of pesticides. BC which is a pro-vitamin A carotenoid is found in carrots, DGLVs such as spinach, cowpeas leaves (*vignaun guiculata*), *Amaranthus spp* and broccoli and all these are seasonal and are not available during the dry seasons especially for the people living in the ASAL regions (Oyugi and Nawiri, 2011).

Animal sources of VA include the liver, milk from cows and goats, butter, margarine which is eggs, meat, fish and livers which are expensive (Ramadhan 2012). Red cactus grows naturally in arid and semi-arid areas due to their stems ability to store water allowing it to survive extended periods without rain. The consumption of the fruit is very low as the benefits and the nutritional contents have not yet been explored.

The levels of beta-carotene in seedless and seeded watermelon (Perkins *et al.*, 2007) and *Solanum Indicum L.* (African nightshade) (Pollyanna *et al.*, 2009) showed an increased level of beta-carotene at different ripening stages. There is a limited data base on the levels of VA, BC and lycopene in red cactus species growing in Kenya at different stages of ripening. In order to establish the levels of these antioxidants in red cactus fruits, samples were analyzed at different stages of ripening that is when unripe (breaker), ripe and unripe in order to establish the right stage of consumption.

Lycopene content in unicorn tomatoes cultivar increased with each stage of ripening (Shiva and Jun, 2016) while retinol content decreased in different banana varieties with each ripening stage (Gerald *et al.*, 2009). For red cactus fruit to provide any beneficial use, studies at different ripening stages was necessary.

This study aimed at finding out the levels of lycopene, beta-carotene and vitamin A in red skinned cactus at different stages of ripening so that information can be made available to the people on the right stage of consuming the fruit to get maximum benefits. Thegu area in Chaka Nyeri County has cactus fruits growing wildly which can be exploited if found to have substantial amount of antioxidant.

#### **1.4 Null Hypothesis**

There is no significant difference in the amount of lycopene, beta-carotene and vitamin A levels in the red cactus (*Opuntia ficus-indica*) fruit from Thegu area in Chaka, Nyeri County at different stages of ripening.

## **1.5 Objectives**

### **1.5.1 General Objective**

To determine the levels of selected antioxidants in red cactus (*Opuntia ficus-indica*) fruits from Thegu area in Chaka, Nyeri County, at different stage of ripening.

### **1.5.2 Specific objectives**

- (i) To determine the levels of lycopene, beta-carotene and vitamin A in unripe (breaker) red cactus (*Opuntia ficus-indica*), fruits from Thegu area in Chaka, Nyeri County.
- (ii) To determine the levels of lycopene, beta-carotene and vitamin A in ripe (completely red) red cactus (*Opuntia ficus-indica*), fruits from Thegu area in Chaka, Nyeri County.
- (iii) To determine the levels of lycopene, beta-carotene and vitamin A in overripe (ripe fruits kept for 5 days) red cactus (*Opuntia ficus-indica*), fruits from Thegu area in Chaka, Nyeri County.

## **1.6 Significance of Study**

This study aimed at quantifying the antioxidants (VA, lycopene and beta-carotene) at different stages of ripening and then the information on the levels to be made available thus increasing the utilization of the fruit. There is also need to create awareness on the right stage of consumption so that to the people living in the ASAL region and the poor living in other dry regions understand the importance of allowing the fruit to mature.

Finding from this study will help in sensitizing the general public and the large scale manufacturer on the right stage of consuming the red cactus fruit which has been

underutilized for a long time. This will then educate them to utilize the red cactus fruits as a supplement to other sources of lycopene, Vitamin A and beta-carotene which will reduce the risks of cancer and vitamin A deficiency disorders (VADDs).

### **1.7 Scope and Limitation of Study**

Although there are many antioxidants, only lycopene, beta-carotene and vitamin A were analyzed from (breaker) unripe, ripe (completely red) and overripe (red fruits kept for 5 days) red cactus fruits from Thegu area in Chaka, Nyeri County. There are other varieties of cactus such as the green, yellow and purple skinned but this study focused on the red skinned cactus. The antioxidant variation in composition in different geographical areas and soil type was not investigated hence only Thegu area in Chaka, Nyeri County was sampled. Seasonal variations were not also considered. The nutrient levels in fruit and vegetable is largely influenced by among other factors cultivar or variety, climate, growth and post handling methods (Florkowski *et al.*, 2009). This study involved keeping the completely red ripe fruits to ripen naturally for 5 days.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 The Red Cactus Fruit

The red cactus plant (*Opuntia ficus-indica*) is an example of a xerophyte of about 200 to 300 species and grows mainly in semi-arid and arid zones since they are able to convert water in to biomass. This plant shows a remarkable genetic variability and hence has a high ecological adaptively. It is an all season plant found in all geographical and climatic conditions (Stintzing *et al.*, 2005). The plant can withstand draught seasons because it has a higher conversion efficiency which is higher on comparison with C3 grasses and C4 plant that have broad leaves (Snyman, 2006). When compared with C4 plants, its biomass generation per unit of water is stated to be on average 3 times higher and 5 times higher than for C3 (Guevara *et al.*, 2011). *Opuntia* cacti originated from Mexico and it is cultivated in all continents, in the hemispheres but not in Antarctica (Inglese, 2002).

##### 2.1.1 Nutritive Composition of the Cactus Fruit

Research has been done on different variety of cactus plants which revealed that isorhamnetin was contained in the purple and green skinned cactus fruits. Kaempferol was found in purple skinned, red skinned and the green skinned varieties. Ascorbic acid was found in large quantities red cactus while carotenoids were found in the yellow skinned cactus fruits (Kuti, 2004). Studies done in other countries indicated that cactus fruits are a rich source of natural antioxidants for food with the purple cactus having the most (Kuti, 2004). Quercetin has been found in all variety of cacti. Red skinned cactus has been found to have ascorbic acid (815 µg/g) while the yellow skinned cactus has the most carotenoid (23.7 µg/g) (Kuti, 2004).

Recent analysis done by Kunyaga *et al.*, 2014 from Nairobi University on *Opuntia stricta* cactus fruits indicated that the cactus pulp has about 60 mg/100 gm ascorbic acid. Minerals such as calcium, sodium, phosphorous and iron were found to be 12.8 mg/kg, 91 mg/kg, 622 mg/kg and 38 mg/kg respectively. The fruit pulp was also found to contain the following levels of sugars: 10.9 mg glucose, 6.9 mg fructose and a high level of sucrose at 18.5mg/100 g (Kunyaga *et al.*, 2014). When the cactus seeds were analyzed they were found to contain protein, oil, fiber at 4.13 %, 11.5 %, 12.3 % respectively. Beta-carotene in the *Opuntia stricta* fruit was recorded at 56 µg/100 g while the total carotenoids were found to be 289µg/100 g. The *Opuntia stricta* seed oil when analyzed was found to contain 70.0 % linoleic acid, 12.5 % palmitic acid and 12.3 % stearic acids (Kunyaga *et al.*, 2014).

Analysis done on *Opuntia ficus-indica* fruit reported 25mg/100 g to 30 mg/100 g of vitamin C, fatty acids especially the linoleic acid forms between 6-13 % while the seeds contain 3-10 % protein (Miller, 2015). The *Opuntia ficus-indica* fruit on analysis revealed high levels selenium, isorhamnetin and some flavonoids such as the kaempferol and quercetin (Kuti, 2004). This fruit has a red pulp which is mainly due to betalains at a level of 40.6 mg/100g which is mainly a combination of betanins and *indicaxanthin* (Butera *et al.*, 2002). Analysis done on red cactus fruit reported vitamin C at a level of 5.17 mg/100g, protein 1.03 %, sugars 59.4 % and carbohydrates 92.57 % (Chiteva and Wairagu, 2013).

## **2.2 Fruit Ripening Process**

Ripening is a process that makes a fruit edible. Unripe (breaker) fruits synthesize compounds such as tannins and alkaloids which help in fighting infections and cause

the fruits to taste bitter and this keeps off early eaters so as to allow the fruits to grow to maturity. The storage cells of the fruit expand as the fruit grows and they become engorged with water, organics acids, vitamins, minerals, sugars and starch. The skin color starts changing as the chlorophyll undergo degradation color changes (Grunenfelder, *et al.*, 2006). The alkaloids and tannins start to disappear and the fruit sweetens. The content of starch and acids start decreasing and the levels of sugars go up. As plants having the fruits grow, their fruits get water and nutrients from the plant and use them to create their flesh and seeds. Most growing fruits are hard and unattractive which provide protection from predators including us. As the fruit grow, the properties of the fruit change to make the fruit more attractive to potential consumers, such as animals, birds, and humans (Grierson, 2013).

The changes that take place in the fruits determine how we judge whether a fruit is ripe or not. These include external features, such as softness to the touch, and internal features, such as sweetness. Fruits also change color as they ripen due to the breakdown of a green pigment called chlorophyll, along with the creation and accumulation of other pigments responsible for red, purple, or blue hues (anthocyanin), or bright red, yellow, and orange hues (carotenoids) and other antioxidants. Cell wall break down when important cell wall polysaccharides break down and the fruit starts to get softer (Osorio and Fernie, 2013).

Ethylene is referred to as fruit-ripening hormone and every fruit has a certain level of ethylene production throughout its lifecycle. There are two types of fruits based on the response to ethylene during ripening. One is the climacteric fruits, where ripening is accompanied by a burst of ethylene. They retain more ethylene and starch which

allows ripening to continue even after they have been picked off the trees. They also absorb ethylene from the atmosphere and continue with ripening. Examples of such fruits include the avocados, melon, mangoes, tomatoes, bananas, pears, kiwis, apricots among others (Alexander, 2002). These fruits also respond to an external source of ethylene which increases their ripening rate. The other kind is the nonclimacteric fruits, in which ethylene production does not increase during ripening. Non-climacteric fruits are the fruits that ripen only on the tree. These fruits do not store their sugars as starch hence no ripening goes on after they have been picked. They produce very small amounts of ethylene and can't ripen even on addition of ethylene, they don't have increased rate of cellular respiration. Examples of such fruits include the citrus, melons, strawberries, grape fruits, pineapples, berries and cherries (Alexander, 2002).

The fruit then acquires a fruity smell because of the change in the composition of the acids and proteins. The cell walls of fruits contain polysaccharides such as pectin which are converted to a water soluble form by enzymes such as polygalacturonase and this makes the fruit soft (Xuewu *et al.*, 2008). The content of antioxidants in fruits increases as the fruit ripens (Rosat *et al.*, 2000). The stage of maturity and the variety of fruits and vegetables largely contribute to the nutritional levels of fruits (Kader 2002; Rodriguez-amaya and Kimura 2004; Bergquist *et al.*, 2007). In kales the carotenoid level increases with growth (Azevedo *et al.*, 2005). This study focused on the stage of maturity.

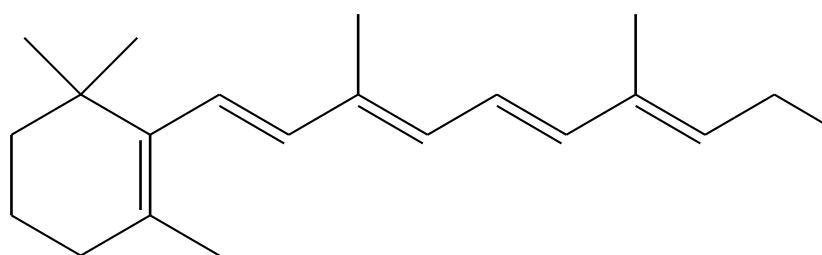
Red cactus is a climacteric fruit, meaning that it can ripen when on the tree and continue when off the tree. Red cactus assumes the ripening stages of the tomatoes

which include: The green stage: when the fruit is completely green, the breaker (unripe) stage: when the fruit is less than 10 % red, the turning stage: when the fruit is less than 30 % red, the pink stage: when the fruit is less than 60 % red, the light red stage: when the fruit is less than 90 % red and finally completely red stage when the fruit is fully red (Bashir *et al.*, 2003). All these stages were not investigated since the study aimed at establishing the right stage for maximum utilization of the fruit hence only the breaker (unripe) and the completely red stage were investigated. Xanthophylls are synthesized in the early stages of ripening while the beta-carotene and the lycopene form later in maturation. Ripening for carotenogenic fruits and vegetables is quickened by carotenogenesis whereby the chloroplasts are transformed into chromoplast and the chlorophylls decompose. Climacteric fruit can be picked when hard and matured or when just ripe. The number of days the fruits is kept to ripen or overripe depends on different fruits and climatic conditions. It takes 5 to 6 days for mangoes to ripen while sapotas take 6 to 7 days (Sudheer, 2007). When allowed to ripen naturally the ethylene hormone facilitates the ripening process by playing a physiological role.

### **2.3 Vitamin A**

Vitamin A comprises of pro-vitamin A carotenoid which are considered the dietary precursors of retinol. This mainly refers to organic nutritional compounds which are unsaturated. The intake of vitamin A (VA) in the body helps with the maintenance of the immune system, good vision, growth and development of a healthy body (Tanumihardjo, 2011). The VA helps in the growth of tooth, bones and skin and ensures correct vision and a healthy mucous membrane. Retinol (figure 2.1) is referred to as pre-formed vitamin A meaning that it can be used directly by the body.

It has molecular mass of 286 and formula  $C_{20}H_{30}O$  (National centre for biotechnology information 2004). Beta-carotenes are vitamin A precursors meaning that the body converts them to Vitamin A. The conversion of retinol is more efficient than that of beta-carotene (WebMD, 2018). The structure of VA has 20 carbon atoms and a methyl substituted cyclohexenyl ring referred to as beta-ionone ring as represented in Fig 2.2. Retinoids refers to retinol, its metabolites, and synthetic analogues that have a similar structure. Figure 2.1 shows the structure of retinol.



**Figure 2.1: Chemical structure of retinol**

**Source: (Butnariu, 2016)**

### 2.3.1 Vitamin A Deficiency

This is the lack of vitamin A in humans and is an endemic nutrition problem throughout much of the developing world mostly in children living in developing countries and approximately 127 million preschool-aged children and 7 million pregnant women are vitamin A deficient (West, 2003). The health consequences of vitamin A deficiency (VAD) include mild to severe systemic effects on innate and acquired mechanisms of host resistance to infection and growth, increased burden of infectious morbidity, mild to severe (blinding) stages of xerophthalmia, and increased risk of mortality. These consequences are defined as vitamin A deficiency disorders (VADDs). Globally, 4.4 million preschool children are said to have xerophthalmia and 6 million mothers during pregnancy suffer from night blindness (West, 2003).

This means that if vitamin A status can be improved it can go a long way in improving children health, their vision and most importantly their survival (Sommel, 2001). Those individuals lacking green and yellow vegetables and liver and fruits suffer from primary vitamin A deficiency. When babies are weaned early, they increase the risk of primary VAD (Roncone, 2006). Those who suffer from secondary VAD have impaired bile production and secretion, chronic malabsorption of lipids, have diets that have low fat and have chronic exposure to oxidants like the cigarette smoke (Roncone, 2006).

Xerophthalmia is an indicator of VAD in young children and also in pregnant women. The initial sign of this disease is night blindness where the individual is unable to see in darkness or in low light (Sommer, (2008). VAD and xerophthalmia coupled with Bitot's spots lead to low iron in the body leading to anemia (WHO 2009). The VAD leads to measles and diarrhea infections which raises severity and mortality risk before xerophthalmia attacks (WHO, 2009). In order to establish squamous metaplastic changes linked to VAD, conjunctiva impression cytology and transfer (CICT) was carried out. From this study seventy-five which formed 23.1 % of the children who were investigated were found to be normal by CICT while a total of 249 children that is 76.9 % were found to be abnormal. Two areas of study were compared and from the findings 13.2% of the total children living in Mathare held a normal CICT while those living in Kitui had 50 %, showing that in the age groups studied the children from Mathare appeared to be a lot more deficient than those living in Kitui (Munene *et al.*, 2003).

Studies done have found that breast feeding mothers have low retinol levels in breast milk (Ettyang *et al.*, 2003). VAD has also been associated with increases in susceptibility to serious infections which results to an elevated risk of child mortality and also the mortality of women who are pregnant and those lactating (Christian *et al.*, 2000). It's important to add efforts in reducing VAD such as diet fortification, diversification and supplementation (Ramakrishna and Danton-Hill, 2002). VAD is determined by signs of xerophthalmia in a person or by analyzing serum retinol levels in the blood. Levels of 20  $\mu\text{g}$  and of retinol per gram indicate VAD (Sommer and Davidson, 2002).

### **2.3.2 Chemistry of Vitamin A**

About 90 % of VA from diet is absorbed in the intestine and there is a linear relationship between the increased intake and absorption efficiency. Higher than 90 % of retinol that is stored in the body enters as retinyl esters along the absorption process of the lipid portion. Retinol is converted to 11-transretinol in the through the process of oxidation. The hydroxyl group is thus changed to form an aldehyde. The 11-transretinal then undergoes isomerization to form 11-cis-retinal which is the functional isomer of retinol that is responsible in the physiology of vision. (Ramadhan and Pryme 2012).

### **2.3.3 Vitamin A and Health**

The intake of vitamin A that is recommended together with other nutrients can be accessed in the Dietary Reference Intakes (DRIs). Food and Nutrition Board (FNB) developed the (DRIs) at the Institute of Medicine of the National Academies which

was formerly referred to as the National Academy of Science (IOM 2001). This constitutes the Recommended Dietary Allowance (RDA) which gives the recommended amount that should be taken daily enough to give the required nutrients of about (97%–98%) of healthy human beings. The recommended dietary allowances of vitamin A varies at different ages and is presented in Table 2.1.

**Table 2.1: Recommended Dietary Allowances (RDAs) for Vitamin A**

| Age          | Male        | Female      | Pregnancy   | Lactation     |
|--------------|-------------|-------------|-------------|---------------|
| 0–6 months*  | 400 mcg RAE | 400 mcg RAE |             |               |
| 7–12 months* | 500 mcg RAE | 500 mcg RAE |             |               |
| 1–3 years    | 300 mcg RAE | 300 mcg RAE |             |               |
| 4–8 years    | 400 mcg RAE | 400 mcg RAE |             |               |
| 9–13 years   | 600 mcg RAE | 600 mcg RAE |             |               |
| 14–18 years  | 900 mcg RAE | 700 mcg RAE | 750 mcg RAE | 1,200 mcg RAE |
| 19–50 years  | 900 mcg RAE | 700 mcg RAE | 770 mcg RAE | 1,300 mcg RAE |
| 51+ years    | 900 mcg RAE | 700 mcg RAE |             |               |

**Source: IOM (2001)**

\* Adequate Intake (AI), equivalent to the mean intake of vitamin A in healthy, breastfed infants.

Since the body converts all dietary sources of vitamin A into retinol, it has been reported that 1 mcg retinol which is physiologically available has been equated to 1 mcg of retinol, 24 mcg of alpha-carotene or beta-cryptoxanthin and to 12 mcg of betacarotene obtained from diet. The human body converts 2 mcg of beta-carotene from dietary supplements, to 1 mcg of retinol. The Institute of Medicine (IOM, 2001)

established that VA be stated in terms of RAE per 100 gm. The Retinol activity equivalents (RAE) are used to compare the VA activity. The conversion states that 1 µg RAE is said to be equivalent to 1 µg of retinol and that 12 µg of beta-carotene is taken to be equal to 1 µg RAE, and 24 µg of lycopene is taken to be equal 1 µg RAE.

Table 2.2 (IOM, 2001) summarizes the conversion of other antioxidants to RAE:

**Table 2.2: Conversion Factors of Antioxidants to RAE**

| <b>Nutrient</b>     | <b>µg</b> | <b>µg RAE</b> |
|---------------------|-----------|---------------|
| Lutein + zeaxanthin | 24        | 1             |
| Beta-cryptoxanthin  | 24        | 1             |
| Alpha-carotene      | 24        | 1             |

Source: IOM (2001)

The maximum daily intake in an individual which is unlikely to cause any adverse health effects is referred to as Tolerable Upper Intake Level (UL). The intake level for an adult has been set at 3,000 µg/day of the preformed vitamin A. Other levels for different ages are as shown in Table 2.3 (IOM 2001). The DRIs include levels that may reduce the risk of cardiovascular disease, osteoporosis, certain cancers, and other diseases that are diet related (Barr, 2006).

**Table 2.3: Tolerable Upper Intake Levels (ULs) for Preformed Vitamin A**

| <b>Age</b>  | <b>Male</b>               | <b>Female</b>             | <b>Pregnancy</b> | <b>Lactation</b> |
|-------------|---------------------------|---------------------------|------------------|------------------|
| 0–12 months | 600 mcg RAE<br>(2,000 IU) | 600 mcg RAE<br>(2,000 IU) |                  |                  |
| 1–3 years   | 600 mcg RAE<br>(2,000 IU) | 600 mcg RAE<br>(2,000 IU) |                  |                  |
| 4–8 years   | 900 mcg RAE<br>(3,000 IU) | 900 mcg RAE<br>(3,000 IU) |                  |                  |

|             |                              |                              |                              |                              |
|-------------|------------------------------|------------------------------|------------------------------|------------------------------|
| 9–13 years  | 1,700 mcg RAE<br>(5,667 IU)  | 1,700 mcg RAE<br>(5,667 IU)  |                              |                              |
| 14–18 years | 2,800 mcg RAE<br>(9,333 IU)  | 2,800 mcg RAE<br>(9,333 IU)  | 2,800 mcg RAE<br>(9,333 IU)  | 2,800 mcg RAE<br>(9,333 IU)  |
| 19+ years   | 3,000 mcg RAE<br>(10,000 IU) | 3,000 mcg RAE<br>(10,000 IU) | 3,000 mcg RAE<br>(10,000 IU) | 3,000 mcg RAE<br>(10,000 IU) |

**Source: IOM (2001)**

### 2.3.4 Sources of Vitamin A

These include fish oils, butter, eggs, margarine, liver and cream and they contain high levels of preformed vitamin A which is referred to as retinol (Ross *et al.*, 2010). Most dietary pro-vitamin (beta-carotene) is found in dark green leafy vegetables (DGLVs), the yellow and orange vegetable products from tomatoes, in several fruits as well as in vegetable oils (Ross *et al.*, 2010). Dairy products such as fish and liver, the fortified cereals contribute the top sources of foods having VA. Pro-vitamin A sources are the broccoli, carrots, squash and cantaloupe, (Solomon, 2006). Amaranth species provides a lot of vitamin A and other nutrients and its dietary composition stimulates the body defense mechanism which retards the progression of HIV/AIDS virus (Ng'ang'a *et al.*, 2008).

### 2.3.5 Studies Done on Vitamin A

Vitamin A was analyzed in a variety of vegetables with levels varying from 227 to 760 mg RAE/100 g respectively (Pollyanna *et al.*, 2009). The following table 2.4 gives the levels of different food staff, their RAEs and their RDAs (Ramadhan, 2012).

**Table 2.4: Dietary Sources of Vitamin A.**

| Food stuff                        | RAEs $\mu\text{g}$ | % RDA |
|-----------------------------------|--------------------|-------|
| Papaya                            | 55                 | 6     |
| Liver (beef, pork, chicken, fish) | 6500               | 722   |
| Kale                              | 681                | 76    |
| Carrots                           | 835                | 93    |
| Pumkin                            | 400                | 41    |
| Mango                             | 38                 | 4     |
| Collard greens                    | 333                | 37    |
| Broccoli                          | 800                | 89    |
| Pea                               | 38                 | 4     |
| Egg                               | 140                | 16    |
| Apricot                           | 96                 | 11    |

**Source: Ramadhan (2012)**

## 2.4 Beta-Carotene

Pro-vitamin A activity is the main function of beta-carotene known in humans. BetaCarotene (BC) intake helps to balance inadequate retinol supply in significant parts of the world (Tilman *et al.*, 2010). Beta-carotene is found in many foods and vegetables and also sold as a dietary supplement. It contributes to the orange color of many different fruits and vegetables. The rich sources of BC are the vietnamesegac (*Momordica Cochinchinensis Spreng.*) and crude palm oil. Other sources include yellow and orange fruits, such as pumpkin, cantaloupe, papayas, mangoes, orange root vegetables which include the sweet potatoes and carrots. BC in DGLVs is covered by chlorophyll (Kidmose *et al.*, 2005). The recommended daily intake of BC is in the range 2–7 mg/100 g, (Koushik *et al.*, 2006).

### 2.4.1 Beta Carotene Sources

BC is responsible for the orange color in different fruits such as mangoes, papayas, yellow and orange fruits, and in different vegetables such as orange root vegetables

such as yams and carrots, the cantaloupe and pumpkin. In the green leafy vegetables (DGLVs) such as kale, spinach, sweet gourd leaves and potato leaves, BC color is concealed by the chlorophyll (Kidmose *et al.*, 2005). BC is found in large amounts in orange and yellow fruits which include the pumpkins, mangoes, peaches, papayas and musk melon. It is also found in yams, carrots, sweet potatoes, crude palm oil and green vegetables like spinach, lettuce, cabbages, broccoli, sweet potato leaves, sweet gourd leaves and in kales (Pamplona-Roger, 2005).

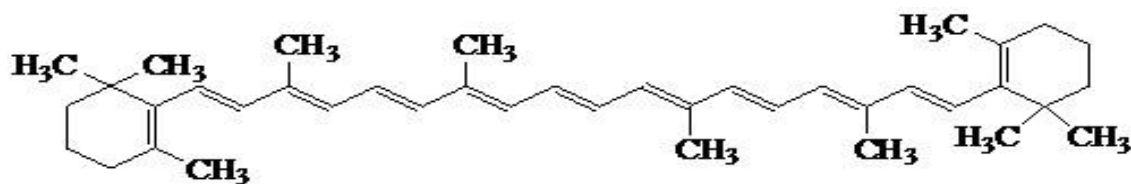
#### **2.4.2 Beta-Carotene and Health**

Since 1970s there has been interest in the role of BC due to its antioxidant properties it is an important cancer preventive agent (Cook *et al.*, 2000). BC found in diets constitutes the major precursor of vitamin A. Intact BC and BC cleavage products of retinol are found in the human body circulation after diet intake of BC. This conversion of BC in diet to retinol (vitamin A), by the use of central and exocentric cleavage, supplies the body with vitamin A (Tang *et al.*, 2012).

#### **2.4.3 Chemistry of Beta-Carotene**

Beta carotene (BC) is converted into vitamin A, an essential vitamin. It belongs to the class of carotenes which are the terpenoids commonly referred to as the isoprenoids which are manufactured biochemically from eight isoprene units resulting to a structure containing 40 carbons having a molecular mass of 536.9 g/mol and a formula of  $C_{40}H_{56}$  (Kidmose *et al.*, 2005). Beta-carotene is the most common form of carotene in plants. The structure was deduced by Karrer in 1930 (Karrer *et al.*, 1930). The BC is different from other carotenes since it has beta-rings which are found at

both ends of its molecule. Fig 2.2 represents the structure of beta-carotene which has been biosynthesized from the geranyl pyrophosphate



**Figure 2.2: Chemical Structure of Beta-Carotene**

**National Center for Biotechnology Information (2004)**

Beta-carotene undergoes tail to tail linkage that involves two C<sub>20</sub> geranyl-geranyl diphosphate molecules that combine and form C<sub>40</sub> carbon skeleton and from this molecule all other are formed (Dutta *et al.*, 2005). Beta-carotene molecule has two βionic rings which cause a cleavage of a chain molecule at –C15 and –C15 position and this result to two molecules of retinol. BC is converted to retinol through passive diffusion that occurs in the small intestinal mucosa. As this conversion is taking place no proteins are produced by enzyme 15, 15''-dioxxygenase through retinal aldehyde. The BC conversion to VA is not very complete since this form 1/6 of total retinol activity. This shows that 1mg retinol is equivalent to 6 mg BC (Ludmila and Joanna, 2018). In vitro, beta-carotene, being an antioxidant is able to quench the oxygencontaining free radicals in our body by adding the radicals to carotenoids, abstraction of hydrogen and by the electron transfer. BC also protects vitamin E from oxidation by regenerating antioxidant form of vitamin E in Lipoprotein (Krinsky, 2001).

#### 2.4.4 Studies Done on Beta-Carotene

Analysis done in the orange carrots (*Daucus carota*) reported a content of 1.906 mg/100 g while red carrots was found to have 1.187 mg/100 g. Sweet potatoes (*Ipomea Batatas*) when analysed had a level of 0.605 mg/100 g (Rich and Pulkit, 2016). The rich sources of BC in mg/100 g include: baked sweet potato 11.509, cooked carrots 8.332, dark green leafy vegetables 6.288, cooked butternut 4.570, cantaloupe melon 2.020, sweet red peppers 1.624, dried apricots 2.163, cooked Peas 1.250, cooked broccoli 0.929, (Pamplona-Roger, 2005). The beta-carotene levels of some selected vegetables (Weinberger and Msuya, 2004) are as shown in Table 2.5.

**Table 2.5: Beta-Carotene Content of Some Vegetables**

| Vegetable           | Level in mg/100 gm |
|---------------------|--------------------|
| Amaranth leaves     | 7.54               |
| Night shade         | 5.02               |
| Spider flower plant | 10.05              |
| Bitter lettuce      | 6.80               |
| Cassava leaves      | 5.43               |

**Source: Weinberger and Msuya (2004)**

#### 2.5 Lycopene

Lycopene is an antioxidant that gives the red color in fruits and vegetables. It's one of the 600 carotenoids which occur naturally (Cámara *et al.*, 2013). Tomatoes, papaya, peppers, watermelon, guava, apricots and the pink grape fruits have a deep red pigment due to lycopene and the tomato has the highest content of 85% (Arab and Steck, 2000). In human serum, about 50 % of the total carotenoids consist of lycopene with most of it found in the liver, adrenal and the prostate gland, skin, and the kidneys. Lycopene obtained from tomato products like the tomato paste has a

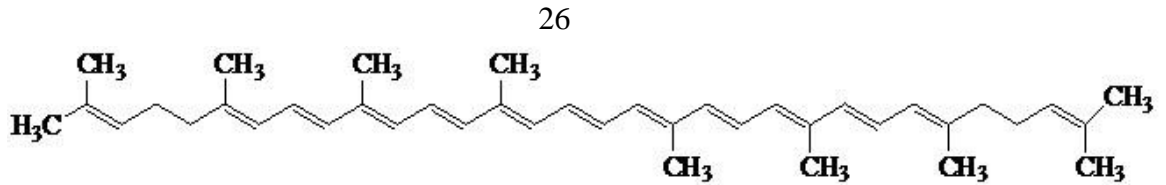
higher absorption in the body than the lycopene obtained from raw tomatoes. The gut absorbs it directly since it is a hydrocarbon and this is enabled by oils and surfactant, which help to disperse the lycopene. Lycopene is not a precursor to vitamin A hence it is not converted into vitamin A (Borguini and Torres, 2009).

### **2.5.1 Sources of Lycopene**

Lycopene is found in fruits such as papaya, guava, pink grapefruit and apricots. The color and the type of different tomatoes give different concentration. The deep red raw tomatoes have a lycopene concentration of 50 mg per 100 g while the ones that have a yellow color have a content of 5 mg/100 g (Khachik *et al.*, 2002).

### **2.5.2 Chemistry of Lycopene**

Lycopene is a natural red pigment synthesized by plants and the photosynthetic microorganisms but not by the animals. It is a very large hydrocarbon molecule with a formula of  $C_{40}H_{56}$  and a MW of 536g (Periago *et al.*, 2004). It's a an acyclic isomer of the beta-carotene carotenoid which is highly unsaturated having 11 conjugated double bonds which are linearly arranged and 2 double bonds which are unconjugated in its structure as shown in Figure 2.3. (Tapiero *et al.*, 2004) having a molecular mass of 536.9 g/mol and a formula of  $C_{40}H_{56}$ . It is considered to be a potent antioxidant since it has many conjugated double bonds it has twice beta-carotene activity that quenches singlet oxygen and 10 times the activity in vitro antioxidant of the  $\alpha$ -tocopherol but does not contain any pro-vitamin-A activity (Shi and Maguer, 2000).



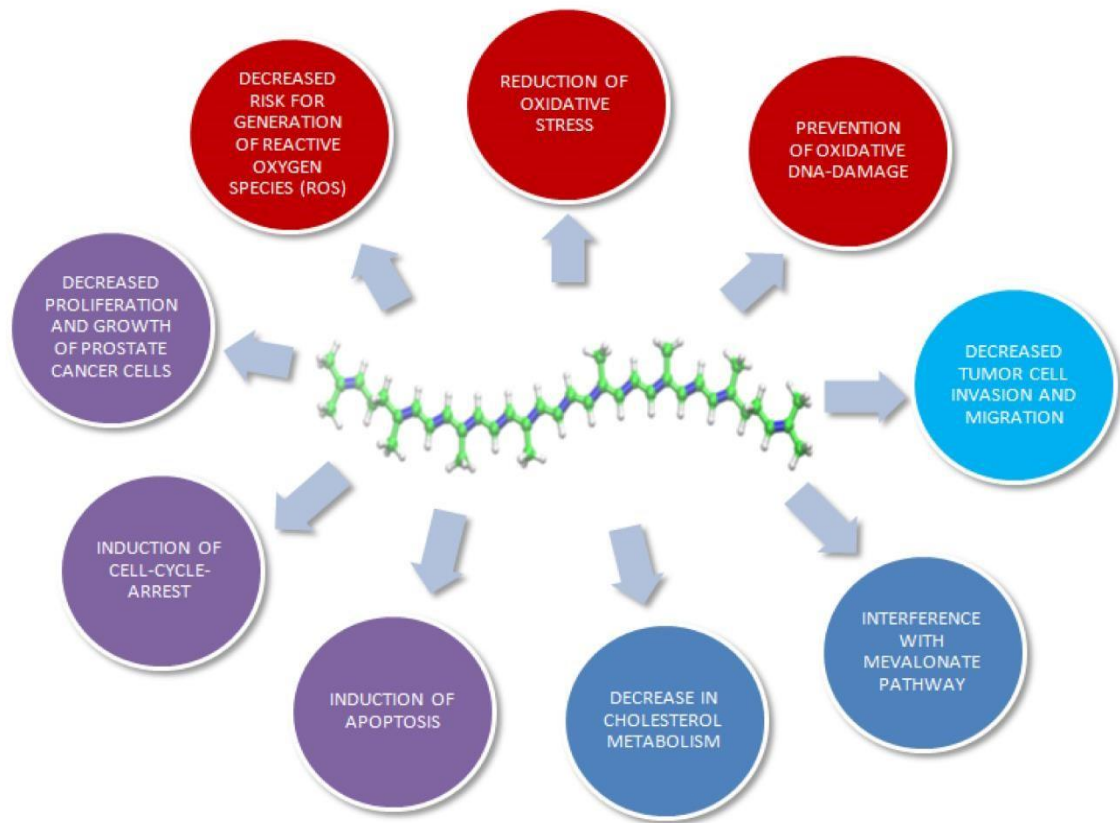
**Figure 2.3: Chemical Structure of Lycopene**

**Source: Tapiero *et al.* (2004)**

Epidemiological studies done report the consumption of diets that have a high content of lycopene reduces the infection of digestive track, the prostate and pancreatic cancer, chronic diseases such as cancer and cardiovascular diseases, the damage by the sun rays and help to manage idiopathic male fertility, the risk of (Johnson, 2000, (Riccioni *et al.*, 2007). The correct consumption of lycopene helps to reduce the damage of leukocyte and prostate tissue oxidative DNA and also leads to a decrease in prostate specific antigen (Gilenay *et al.*, 2007). Since it is non-polar, lycopene is lipophilic meaning that it is insoluble in water, and can only be dissolved in organic oils and solvents (Shi and Maguer, 2000).

### **2.5.3 Lycopene and Human Health**

An increase in the levels of lycopene status in the body leads to a lower risk of chronic disease. Figure 2.4 (Nina *et al.*, 2013) gives the summary of the role played by lycopene in the body.



**Figure 2.4: Chemical Structure of Lycopene**

**Source: Nina *et al.*, (2013)**

The regular consumption of tomatoes together with other vegetables is associated with a lower risk of cancer of the colon (Slattery *et al.*, 2000). Research carried out in people suffering from lung, breast and endometrial cancers has revealed that the intake of lycopene works better than  $\alpha$ - and beta-carotene to cause a delay in cell cycle growth progression and this limits the growth of the tumour cells. It also regulates the intercellular communication through the modulation of the irregular pathways connected with cancer (Singh and Goyal, 2008). Lycopene lowers the urinary tract symptoms mostly the benign prostatic hyperplasia, cardiovascular risk that leads to type 2 diabetes and the enlargement of the prostates and the (Ranveer *et al.*, 2013).

#### **2.5.4 Bioavailability of Lycopene**

There are several factors that can influence the bioavailability of lycopene they include the food matrix containing the lycopene, the absorption rate of the stomach and the delivery medium related to the co-ingestion of fat (Shi and Maguer, 2000). Food processing interrupts the cell walls thereby releasing the lycopene found in the fruit matrix (Su *et al.*, 2002). Bioavailability of lycopene can be increased when the physical size of the food particles is reduced (Hadley *et al.*, 2002). When tomatoes are treated with heat the release of lycopene quickened due to the weakening and the interference of lycopene–protein complexes.

The cell walls rupture and also the crystalline carotenoid aggregates are dispersed (Kun *et al.*, 2006). The rate of absorption of lycopene in the stomach and duodenum also affects its bioavailability. The activity of bile salts and pancreatic lipases assist in the digestion process. The bioavailability of lycopene is also affected by its rate of absorption in the stomach as well as in the duodenum. The dissolution and absorption of lycopene is facilitated by the lipids where by lipid droplets that contain lycopene go in to the duodenum and then transfer to the mucosal cells past passive diffusion (Riccioni *et al.*, 2008).

#### **2.5.5 Studies Done on Lycopene**

Analysis done revealed that tomato skins and extracts have more than 94% of the total carotenoids contained in the content of sample (Topal *et al.*, 2006). The daily average intake of lycopene is in the range of 6.6–10.5 mg/day for the males and for the females is in the range of 5.7–10.4 mg/day (Porrini and Riso, 2005). Water melon

(*Citrullus lanatas*) has also been found to have an average content of 4.87 mg/100g (Thurnham *et al.*, 2003), pink grape fruit 1.1 mg/100g, guava 5.2 mg/100g, tomato puree 21.8 mg/100g and papaya 1.8 mg/100g (Rao and Agwargai, 2000).

A study done on tomatoes during various stages of ripening for different cultivars showed an increase in the level of lycopene at the different stages of ripening. This was mainly attributed to the transition of chloroplast to chromoplast (Shiva and Jun, 2016). Lycopene content increased till maturity with dafnis cultivar displaying the highest increase in content of lycopene as ripening progressed. Unicorn cultivar had the highest content of 97.11 mg/100g (Shiva and Jun, 2016) at the red stage of ripening. Different levels of lycopene (mg/100g) at different stages of ripening are shown in the following Table 2.6.

**Table 2.6: Lycopene Levels of Different Tomato Cultivar During Ripening**

| Type of cultivar | Stage of ripening | Lycopene content in mg/100gm |
|------------------|-------------------|------------------------------|
| Sayran           | Breaker           | 1.65 ± 0.02                  |
|                  | Turning           | 12.26 ± 0.43                 |
|                  | Pink              | 29.13 ± 1.75                 |
|                  | Light red         | 65.07 ± 0.62                 |
|                  | Red               | 68.60 ± 2.04                 |
| Titi-Chal        | Breaker           | 1.61 ± 0.01                  |
|                  | Turning           | 9.57 ± 0.15                  |
|                  | Pink              | 40.72 ± 1.07                 |
|                  | Light red         | 72.89 ± 1.98                 |
|                  | Red               | 81.76 ± 3.15                 |
| Unicorn          | Breaker           | 1.67 ± 0.02                  |
|                  | Turning           | 9.26 ± 0.08                  |
|                  | Pink              | 34.53 ± 0.63                 |
|                  | Light red         | 63.48 ± 1.10                 |
|                  | Red               | 97.11 ± 2.23                 |

**Source: Shiva and Jun (2016)**

## 2.6 Analytical Technique

### 2.6.1 HPLC

High-performance liquid chromatography (HPLC) is a kind of column chromatography that is mostly used to identify, quantify and separate compounds that are active in biochemistry during analysis. (Martin and Guiochon, 2005). It has a column that contains stationary phase, a pump that pushes the mobile phase along the column and also a detector which indicate retention times of molecules which is proportional to the molecules under analysis, the stationary phase and the type of solvents that are in use (Liu and Lee, 2006).

High Performance Liquid Chromatography (HPLC) is an analytical technique that is used to separate compounds which are soluble in a specific solvent. This is the method that was used to determine beta-carotene, lycopene and vitamin A content due to its sensitivity and selectivity. Though it is elaborate, it's also time consuming and costly (Rodriguez-Amaya and Kimura, 2004). It uses a column which is able to hold a material used in chromatography that is the stationary phase consisting of C-18 ODS it is therefore an improved form of column chromatography. The materials used are small in size increasing the surface area to enable effective interaction of the stationary phase with the analyte molecules flowing through it.

Carotenoids have been effectively separated by the use of high performance liquid chromatography (HPLC) (Rodriguez-Amaya and Kimura, 2004). The HPLC has a reusable column which very well allows the separations to take place when carried out in controlled conditions away from air or light. The most commonly used HPLC is the reverse- Phase HPLC which has a C<sub>18</sub> column and it is compatible with the

majority of carotenoid solvents and their polarity range. It also contains a weak hydrophobic interaction with most of the analytes. The materials such as silica that are used in C<sub>18</sub> reverse-phase are accessible and they differ in carbon loading degree, the type of end capping and the structure of the bonded phase. During analysis the carotenoids are identified based on their time of retention and the absorption spectra when they are compared to those of their respective carotenoid standards (RodriguezAmaya and Kimura, 2004). During this analysis reversed phase HPLC packed with carbon 18 ODS was used as it is the most widely used form of chromatography because of its flexibility and high resolution and isocratic elution (Perry *et al.*, 2009).

### **2.6.2 Quantification of Carotenoids**

Carotenoids are quantified spectrophotometrically by either high performance liquid chromatography (HPLC) or open column chromatography (OCC). This is made possible since the carotenoids while in solution obey the Beer-Lambert law which states that absorbance is directly proportional to the concentration (Rodriguez-Amaya and Kimura, 2004). By the integration of the peak areas in the chromatograms of the HPLC the carotenoids are quantified (Perry *et al.*, 2009). However, quantification depends on availability of accurate absorption coefficients (Rodriguez-Amaya and Kimura, 2004). Other methods of extraction are gas chromatography (GC), and capillary electro chromatography (CEC) and UV/vis (Perry *et al.*, 2009).

### **2.6.3 Principles of HPLC Method**

The sample is injected automatically using an auto-sampler AS 6.1L which is designed to inject up to 10 ml in one injection. It is able to handle in 10 ml vials 30

samples and 768 samples in well designed plates. Auto-sampling is preferred to manual injection because of high accuracy and precision. It contains an injection valve which has a loop sample with several modes of injection (Perry *et al.*, 2009). The sample under analysis is introduced in very small volumes into a stream of the mobile phase and it undergoes retardation using particular chemical or physical interactions with the stationary phase as it criss crosses the entire length of the column. The nature of stationary phase and the composition of the mobile phase nature of the analyte bring about the quantity of retardation (Skoog *et al.*, 2007).

The columns that are used are straight and are short in length to ensure that the peaks do not tail by preventing excess pressure drop and pockets of air. They are made of stainless steel tube consisting of the material used during the separation. Rigid solid materials which contain silica are used in the column because they are able to resist high pressure during the elution. To maintain a neutral medium, the mobile phase is packed with counter ions that can be exchanged.

To detect when an analyte under analysis has gone over the column, detectors such as fluorescence, the UV/ Visible absorption and the refractive index are used. What comes out of the detector is noted as a chromatogram which means a series of peaks with each peak signifying a particular compound from what passes through the detector and then the absorption of the UV light in the UV/Vis detector used.

## CHAPTER THREE

### MATERIALS AND METHODS

#### 3.1 Study Area

The study area which is located in central Kenya Nyeri County, borders Nyandarua County to the West, Kirinyaga County to the East, Laikipia County to the North, Murang'a County to the South and Meru County to the North East. Chaka area is temperate and warm and it is located at 1756m above sea level. Chaka has significant rainfall with 1030 mm/ 40.6 inch annually and precipitation that occur even when the month is the driest. The temperature is about 17.4 °C | 63.2 °F.



**Figure 3.1:** Map of Thegu Area in Chaka Nyeri County

**Source:** Google earth

#### 3.2 Research Design

This study was by experimental design. Samples were picked randomly, prepared and analyzed using high performance liquid chromatography. Data obtained was analyzed using ANOVA and the T-test was carried out to compare the means of the lycopene, beta-carotene and Vitamin A in the unripe, ripe, and overripe red cactus fruits.

### 3.3 Sample Collection and Sample Preparation

Red cactus fruits were picked randomly at the beginning of March 2016 from Thegu area in Chaka, Nyeri County with the help of a taxonomist in two categories; green (breaker stage) and ripe (completely red). The ripe red cactus fruits were kept for about 5 days to overripe. The formula  $n_0 = Z^2 pq/e^2$  (Israel, 1992) was used to determine the sample size. The  $n_0$  referred to the sample size, the value for Z is found in statistical tables which contain the area under the normal curve  $n_0 = Z^2 pq/e^2$  and it was taken as 1.96.  $Z^2$  is the abscissa of the normal curve that cuts off an area  $\alpha$  at the tails ( $1 - \alpha$ ) and it equals the desired confidence level in this case 95%, assuming precision of  $\pm 5$  as the maximum variability, e is the desired level of precision taken as 0.05, p refers to the estimated proportion of an attribute that is present in the population and it was taken as 0.5, and q is 1-p which equals to 0.5 (Israel, 1992) Where  $n_0 = (1.96)^2(0.5)(0.5)/(0.05)^2 = 385$ . It was divided by five to reduce the sample to 77 fruits per sampling site and this was sampled 3 times in each sample site. One extra fruit was picked to take care of any small or genetically bad fruit that would get spoiled before analysis to make a total of 78 for easier sampling.

Trees were randomly and purposefully selected at a distance of about 1km from each site in Thegu area Chaka in Nyeri County. From each site, 3 sub sites were considered about one meter from each other and were packed differently. Six green (breaker) fruits and twenty ripe fruits were picked and from the 20 ripe fruits 10 were kept to overripe. This was repeated for the 10 different sites. The variation of the number of fruits depended on the stage of ripening because some red fruits were kept to overripe. These fruits were then put in cartons and then transported to Jomo Kenyatta university food science laboratory where the extraction and analysis took place. The unripe and

ripe fruits were washed thoroughly with distilled water and the very small spikes removed from the skin covering since the spikes are harmful to the human skin. The red cactus fruits were then peeled and the cactus pulp put in labeled separate plastic containers for analysis. Some of the ripe fruits were kept for about 4-5 days at room temperature and then prepared like the unripe and ripe fruits. Figure 3.1, 3.2 and 3.3 represents red cactus fruits at the stages of ripening.



**Figure 3.2: Red Cactus Fruits at the Breaker Stage**

**Source: Self Photo**



**Figure 3.3: Red Cactus Fruits at Completely Ripe Stage**

Source: Self Photo



**Figure 3.4: Red Cactus Fruits at Overripe Stage**

Source: Self Photo

### **3.4 Chemicals, Reagents and Apparatus**

All chemicals, reagent and the standards used in this analysis were all of analytical or HPLC grade and they were purchased from Sigma- Aldrich, Germany. Reagents included cold acetone, butylated hydroxyl toluene (BHT), 10% analytical grade nitric acid, dichloromethane, Petroleum spirit (40-60<sup>0</sup>c), acetone, tetra hyrofuran (THF), acetonitrile, and methanol. Distilled-de-ionized water was used throughout the analysis. Apparatus included filter funnel, glass columns, 50 ml volumetric flasks, and pestle and mortal, cotton wool defatted, HPLC separating funnels. Chemicals included Celite, anhydrous sodium carbonate, ascorbic acid and silica gel for column. The standards that were used were Beta-carotene, retinol and lycopene standard. All these were purchased from Merck, Germany.

### **3.5 Apparatus and Instrumentation**

All glass apparatus and vials that were used during this analysis were soaked overnight in 10% analytical grade nitric acid to ensure that any firmly held chemicals

or adsorbed metal ions were removed then washed with liquid detergent and rinsed with water that is de-ionized. To dry the apparatus they were put in an oven with a temperature set at 105 °C. Plastic apparatus was also thoroughly washed with detergent and rinsed with distilled de-ionized water, dried and stored in open racks in a lockable drawer. This procedure was employed every time the apparatus both plastic and glass were cleaned. To prevent oxidation and isomerization of the antioxidants, all the apparatus used for the extraction and storage were flushed with nitrogen gas (Kimura and Rodriguez-Amaya, 2004).

The HPLC system that was used in the analysis was a Model LC-20AS, Shimadzu Corp., Kyoto from Japan which had the following specification: The HPLC column used during the analysis was ODS C-18, Phenomenox (RP) make, and pin number of 00F-4143-E0. The size of the column was 250 x 4.60mm x 5.0 µmm and the serial number was 390336-1. The HPLC pump was of prominence (LC), LC-20A model, with a pin number of 228-45001-38 and a serial number of L20114200796LP. The HPLC detector was (deuterium lamp) was prominence UV-VIS, SPD-20AV make with a catalogue number 228-45004-38 and serial number L20144300254LP. The HPLC oven was of CTO-10AS make catalogue number of 228-45059-38 and a serial number of C21044304471SS. The settings used in HPLC are given in Table 3.1.

**Table 3.1: RP-HPLC settings**

| SETTINGS                       | Beta-Carotene | Vitamin A  | Lycopene   |
|--------------------------------|---------------|------------|------------|
| Injection volume               | 10 µL         | 10 µL      | 10 µL      |
| Flow rate                      | 1.0 ml/min    | 1.0 ml/min | 1.0 ml/min |
| Wave length( $\lambda_{max}$ ) | 440 nm        | 325 nm     | 475 nm     |
| Temperature                    | 25 °c         | 25 °c      | 25 °c      |

### **3.6 Method Validation 3.6.1 The Calibration Curves for Vitamin A, Beta-Carotene and Lycopene Standards**

#### **3.6.1.1 Vitamin A (Retinol) Standard Solution**

Vitamin A standard solution of 1 mL and 0.1 mL of peanut oil added to prevent retinol from oxidation and was saponified using the procedure for vitamin A in 5 g fruit samples. Then 5 mL of 95% mobile phase added. The 5 mL of the upper phase of the standard was used. Aliquots (working solutions) of 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 µg/mL were measured and brought to volume in separate low-actinic volumetric flasks of a volume of 50 mL. HPLC was calibrated by injecting 10 µL of the working standard in triplicate to attain the reproducibility of the detector response at different concentration. This procedure of standard preparation was done for the unripe and repeated for the ripe and over ripe cactus fruit extract analysis.

#### **3.6.1.2 Beta-Carotene Standard Solutions**

A Stock solution of beta-carotene 100 mL was prepared by weighing 0.01 g of betacarotene standard in 100 m mobile phase consisting of acetonitrile: methanol: dichloromethane (70:10:20). This solution was used to prepare working standard solutions ranging of 10, 20, 40, 60, 80 and 100 mg/mL (ppm). Then 10 µL of standard were injected in the HPLC connected with PDA (UV-visible) detector at 440 nm and the peak area against their corresponding concentration was plotted to give a standard curve. The samples were run under the same conditions as standards and peak area obtained which were compared with the standard curve. HPLC was calibrated by injecting 10 µml of the working standard in triplicate. This procedure of standard preparation was repeated for the unripe, ripe and overripe red cactus fruit extract analysis.

### 3.6.1.3 Lycopene Standard Solution

Lycopene stock standard was prepared by weighing accurately 0.01g in 100 mL volumetric flask and filled with mobile phase which consisted of THF, acetonitrile and methanol (THF: ACN: Me-OH) the ratio of 15:30:55. Other set of standard solutions of 100, 80, 60, 40 and 20 µg/mL were prepared using the stock dilution. This procedure was repeated during the analysis of the red cactus when ripe, overripe and unripe. The standard solutions of lycopene, VA and BC were used to come up with calibration curves and the linear regression analysis.

### 3.6.2 Limit of Detection

Limit of detection (LOD) for lycopene, beta-carotene and vitamin A standards were determined using standards calibration procedures where by the results and the coefficients of determination ( $r^2$ ) validated the method. The LODs were determined by comparing signals from samples that had the lowest concentration of the analyte with the blank samples. The ratio of signal to noise for the LOD was in the ratio of 3:1. The working equation of LOD is as shown in equation 3.1.

$$\text{LOD} = \text{Mean} + (3 \times \text{standard deviation}) \quad \text{Equation 3.1}$$

### 3.6.3 Reproducibility

Reproducibility test was done by analyzing a known standard for a particular antioxidant under study ten times then the relative standard deviation (RSD) was calculated by using equation 3.2 (Glen,2014).

$$\text{RSD} = \frac{\underline{S}}{X} \times 100\% \quad \text{Equation 3.2}$$

Where  $\bar{X}$  = Mean

S = Standard deviation.

The analytical instrument used for sample analysis was also used in the reproducibility test. Different volumes of standard solutions were used to plot calibration curves and the linearity of the HPLC was determined by regression analysis using the calibration curves.

### 3.6.4 Recovery Analysis

This was done by standard addition method whereby 3.8 g, 1.5 g and 0.09 g samples of Lycopene, retinol and beta-carotene respectively were spiked with 1.9 g, 0.75 g and 0.045 g of lycopene, retinol and beta-carotene respectively. This was done for the ten samples in the different sites to determine whether the method is reliable. Unspiked sample was also analyzed in triplicates using HPLC. The recovery in percentage was then calculated based on the antioxidant content initially (unspiked) and the amount added during spiking and this was done using equation 3.3. This was done for the unripe, ripe and overripe red cactus.

$$\text{Recovery (\%)} = \frac{(C_f - C_u)}{C_A} \times 100 \quad \text{Equation 3.3}$$

Where,

$C_f$  is the amount of the antioxidant in the analyte after spiking,

$C_u$  is the amount of the antioxidant in the unspiked analyte,

$C_A$  is the amount of the standard added for spiking.

### 3.7 Sample Extraction

#### 3.7.1 Lycopene Extraction

Lycopene extraction was done using the method of Fish *et al.*, (2002) with a few modifications. The lycopene extracting material was changed from 5 ml BHT : 5 ml ethanol: 10ml hexane to 13 ml 0.05 % (w/v) hexane, 13 ml butylated hydroxyl toluene (BHT) in acetone and 13 ml 95% ethanol in the ratio of 1:1:1. Vita-Mix 3600 stainless steel blender was used to grind the unripe (green) red cactus fruits pulp samples for 1 minute to form a puree. The unripe ground tissues were then kept on ice and away from light waiting for the analysis using HPLC. Aluminum foil was used to wrap the 50 mL PTFE test tubes and while on ice, exactly 1 g of the unripe puree sample was put in to each wrapped test tube. The extraction solution (39 mL) was then added and a tabletop shaker was used to shake the test tubes for 10 minute at 180 rpm while still on ice. This was done in triplicate.

To ensure efficient separation of the polar and the non-polar compounds, 6 ml of cold double distilled water was measured and then added to each of the tubes and agitation was done for 5 more minutes. They were then allowed to stand for 15 minutes at room temperature and this allowed the polar and non-polar to separate into layers. The supernatant layer which was the hexane layer containing lycopene was removed and kept in 15 ml aluminum wrapped test tubes at  $-80^{\circ}\text{C}$  for further analysis. It was evaporated to dryness in a rotary evaporator and then 1mL mobile phase which was made of tetra hydro furan (THF), acetonitrile (ACN) and methanol in the ratio 70:20:10(v/v) respectively was added and then a 0.45  $\mu\text{L}$  PTFE filter was used to filter the mixture. 10  $\mu\text{L}$  was then injected in the HPLC. The elution took 8 min with an elution flow rate of 1 mL/min. The absorbance peaks from lycopene standard and

unripe red cactus sample were compared with their retention times. This procedure was repeated for the ripe and overripe samples of the red cactus fruits.

### **3.7.2 Beta-Carotene Extraction**

Beta-carotene was extracted using the extraction method of Heinonen *et al.*, (1990) without doing saponification. A 5 grams of the unripe (green) red cactus puree sample were weighed and transferred into a mortar and 1.5 g of celite and some cold acetone added and ground with a pestle. The sample was extracted gradually with up to 50 mL of cold acetone and then transferred into a 50 mL volumetric flask by the use of a glass funnel with a cotton wool plug. The residue obtained was washed with some cold acetone several times until it turned white then it was topped up to 50 mL with cold acetone.

Petroleum ether (40 mL) was put into a separating funnel of capacity 250-500 mL and the acetone extract slowly added into the separating funnel without shaking. Distilled water was then added slowly to the neck so that all the acetone can be removed. The mixture obtained was left to stand for 2-3 minutes so that it can separate into two phases and then the lower aqueous layer was removed and then discarded. This mixture was then washed about 3 times with distilled water to remove all the acetone and leave the carotenoids in the petroleum ether layer.

This layer having the carotenoids was put in a conical flask using a funnel plugged with anhydrous  $\text{Na}_2\text{SO}_4$  to ensure that it was free from water and then petroleum ether was used to top up to 50 mL. The extract was then put on a rotary evaporator and evaporated to dryness after which 2 mL of a mobile phase consisting of ACN:

Dichloromethane: Me-OH in the ratio of 70:20:10 respectively was added to the residue and then filtered using a 0.45  $\mu\text{L}$  PTFE micro filter. 10  $\mu\text{L}$  was then injected in the HPLC. Absorbance peaks from the standard and sample were compared with their retention time. This procedure was repeated for the ripe and overripe samples of the red cactus fruits.

### **3.7.3 Vitamin A(Retinol) Extraction**

The retinal content (VA) was determined by the method of Zahar and Smith (1990). Five grams of ground unripe (green) sample was put into a 50 ml glass stoppered centrifuge tube then 10ml of absolute ethanol which contained 0.1 % (wt./vol) of ascorbic acid was added and finally 4 ml of 50 % KOH (wt./vol) was added. The tube were then stoppered and agitated carefully so that the contents can mix well for complete digestion of fat (saponification) and then placed in water at a temperature of 80  $^{\circ}\text{C}$  for about 20 minutes.

The tube was then cooled with running water from the tap and then placed in an iced cold water bath and then 15 ml of hexane which had 0.01% (wt. /vol) BHT was then added and the tube stoppered. A vortex was used to mix them vigorously for about 1 min, allowed to stand for 2 minutes and then vortexed again for another 1 minute. 5 ml of cold water (1 $^{\circ}\text{C}$ ) was added to each tube and stoppered. It was then inverted 10 times and centrifuged at 25 $^{\circ}\text{C}$ , 10000 rpm for another 10 minutes. 10 ml of the upper layer (organic) was accurately removed using a pipette and put in a rotary flask whereby the solvent was evaporated using rotary evaporator at a temperature of 40 $^{\circ}\text{C}$  under vacuum. After evaporation the residue left was immediately re-dissolved into 1 ml of absolute methanol. The sample and standard were injected at standard volume

of 10  $\mu\text{L}$ . This procedure was repeated for the ripe and overripe samples of the red cactus fruits.

### 3.8 The Elution, Identification and the Quantification of the Antioxidants

Elution, identification and the quantification of the antioxidants was carried out under reverse phase column with isocratic elution. The antioxidants were identified based on their retention time and absorption spectra when they were compared to those of their standards. The antioxidant standards and the analytes were filtered using a 0.45 micron single membrane into dark reagent vials at room temperature. The UV-Vis maximum absorbance of the analytes was determined and this was to ensure that the column would not overload during the elution. An auto sampler was used to inject the standard and the analytes in triplicates into the injection loop into the column.

The elution of lycopene took 8 min, vitamin A took 12 min and beta-carotene took 10 min at 1.0 mL/min flow rate of over a C18 column. The lycopene, beta-carotene and vitamin A were detected at a wavelength of 475 nm, 440 nm and 325 nm respectively. Identification was done by comparing the spectrum and retention time of each antioxidant with those of their respective standards. Quantification was done by using their peak areas which were converted by the use of linear calibration curves obtained from standards to a concentration  $C_x$  in mg/100 g of lycopene and beta-carotene and in  $\mu\text{g}/100$  g sample of vitamin A using equation 3.4.

$$C_x \text{ (mg/100gm)} = \frac{(P_x - B)}{S} \times D_f \times 100g \quad \text{Equation 3.4}$$

Whereby:

$C_x$  is the concentration of the sample (analyte) in mg/100g,

$P_X$  represents the Peak area of the sample (analyte),

B is the intercept,

S is the slope and  $D_f$  represents the dilution factor.

In the vitamin A quantification, a division of 1000 was done to convert to g/100g of sample. Analysis was done in triplicate for the different antioxidant at the three stages of ripening.

### **3.9 Statistical Analysis of Data**

Data analysis of beta-carotene, vitamin A and lycopene of the unripe, ripe and overripe samples was done statistically and the results expressed as mean  $\pm$  standard deviation of the antioxidants in red cactus at different stages of ripening. Anova is classified as one-way when it is used to analyse two groups so that any difference between them can be found out. It has one independent variable. The other type is the two-way that can be or without replication and it is used when testing one group later double-test it. It has two independent variables (Gurchetan, 2018). Analysis of variance (ANOVA) using the statistical package for social sciences (IBM, SPSS, Version 21) at 95 % confidence level was employed in comparison of measurements using p-value  $< 0.05$  considered significant.

## CHAPTER FOUR

### RESULTS AND DISCUSSION

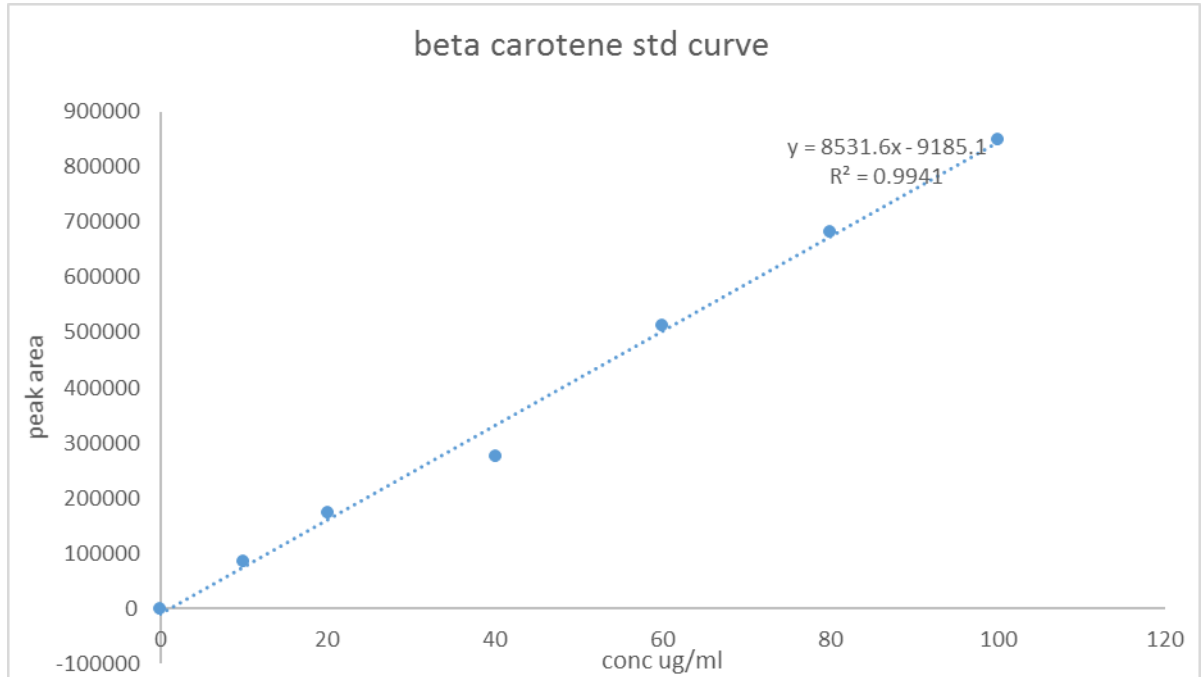
#### 4.1 Introduction

Vitamin A, lycopene and beta-carotene were analyzed in red cactus at three stages of maturity using HPLC method and the levels were reported in mg/100 gFW. The results of the analysis, identification of beta-carotene, lycopene and vitamin A are represented by different chromatograms, and their mean content at different stages of ripening are represented in different tables. The data obtained is also represented in bar graphs and in line graphs and they are discussed in this chapter.

#### 4.2 Method Validation

##### 4.2.1 Regression Analysis

A calibration line of best fit was drawn for the peak areas (maximum wavelength absorbance of each standard material) against concentrations of standards. The coefficient of determination ( $r^2$ ) and the linear regression equation were obtained from the calibration curve and they were used to determine whether the data was linear. Equation  $y = mx + C$ , was used to come up with calibration curves. The values of coefficient of determination ( $r^2$ ) and regression equation obtained from standard reading for lycopene, vitamin A and beta-carotene are 0.999, 0.999, 0.9941 and  $Y=59274x$ ,  $y =71833x$  and  $y = 8531.6x - 9185.1$  respectively. The regression equation indicates that there exists a linear relationship between the response from the instrument and the concentration of each of the standards used. The peak area plotted against the concentration of BC standard is presented in Figure 4.1. The calibration curve obtained for vitamin A and that of lycopene standards, are presented on appendix 5 and appendix 6 respectively.



**Figure 4.1: Beta-Carotene Standard Curve**

#### 4.2.2 Limit of detection

The limit of detection (LOD) refers to the lowest concentration that can be detected in a peak that has a height of 3 times that of base line noise (Marin *et al.*, 2007). The LOD values were obtained using equation 3.1 in 3.6.2 whereby the values ranged from 0.002 to 0.21  $\mu\text{g}/100\text{g}$  and were as shown in Table 4.1. Yuan *et al.*, (2010) reported LOD of VA as 0.0002  $\mu\text{g}/100\text{g}$  and this compared closely with that obtained in this analysis. Mariel *et al.*, (2015) reported high LOD of 0.1  $\mu\text{g}/\text{mL}$  for lycopene and 0.2  $\mu\text{g}/\text{mL}$  for retinol from the analysis of human plasma. According to Yuan *et al.*, (2010), the LOD of retinol was 0.01  $\mu\text{g}/\text{mL}$ . LOD of beta-carotene and lycopene was reported by Imeda *et al.*, (2018) to be 0.0081  $\mu\text{g}/\text{mL}$  and 0.034  $\mu\text{g}/\text{mL}$  in a study done on tomato and tangerine peels respectively.

**Table 4.1: LOD Values for Beta-Carotene, Lycopene and Vitamin (Retinol)**

| <b>ANTI-OXIDANT</b> | <b>Retinol</b> | <b>Lycopene</b> | <b>Beta-Carotene</b> |
|---------------------|----------------|-----------------|----------------------|
| SD                  | <b>0.002</b>   | <b>0.01</b>     | <b>0.002</b>         |
| LOD                 | <b>0.02</b>    | <b>0.21</b>     | <b>0.02</b>          |

The above values were within the range of the recommended levels. From Table 4.1 retinol had the lowest detection limit and also the highest LOD value of 0.023 and 0.023 µg/100g respectively.

#### **4.2.3 Recovery Studies**

This was done by standard addition procedure as described in the recovery analysis 3.7.4. The results obtained were expressed as mean content  $\pm$  SD of the three extractions done. Recovery done for retinol, beta-carotene and lycopene were within the recommended range of 95-102 showing that the method is very efficient. The recovery values from this analysis compared closely with those of Hussein *et al.*, (2013) who reported a recovery of between 87-97%, Mariel *et al.*, (2015) a recovery of retinol at 96% and Yuan *et al.*, (2010) a recovery of > 90%. The following Table 4.2 shows the recovery means of beta-carotene, vitamin A (retinol) and lycopene.

**Table 4.2: Recovery Means for Beta-Carotene Vitamin A and Lycopene**

| <b>Antioxidant</b> | <b>Amount of standard added in mg</b> | <b>The mean content <math>\pm</math> SD of antioxidant recovered<br/>In mg/100g</b> | <b>Mean content <math>\pm</math> SD recovered in %.</b> |
|--------------------|---------------------------------------|---|---|
| Beta-carotene      | 0.09                                  | 0.09 $\pm$ 0.14   | 98.89 $\pm$ 0.01  |
| Vitamin A          | 1.7                                   | 1.71 $\pm$ 0.19   | 100.59 $\pm$ 1.9  |
| Lycopene           | 1.9                                   | 1.89 $\pm$ 0.28   | 99.47 $\pm$ 0.36  |

#### 4.2.4 Reproducibility

The precision test was done by analyzing a homogenized sample 3 times intraday and then expressed as relative standard deviation (RSD). Low values of RSD show a high precision. The RSD values for red cactus at the different stages of ripening ranged from 1.48 to 4.6 showing that the method of extraction was reproducible. These results are shown in Table 4.3.

**Table 4.3: RSD Values for Lycopene, Beta-Carotene and Retinol n=3**

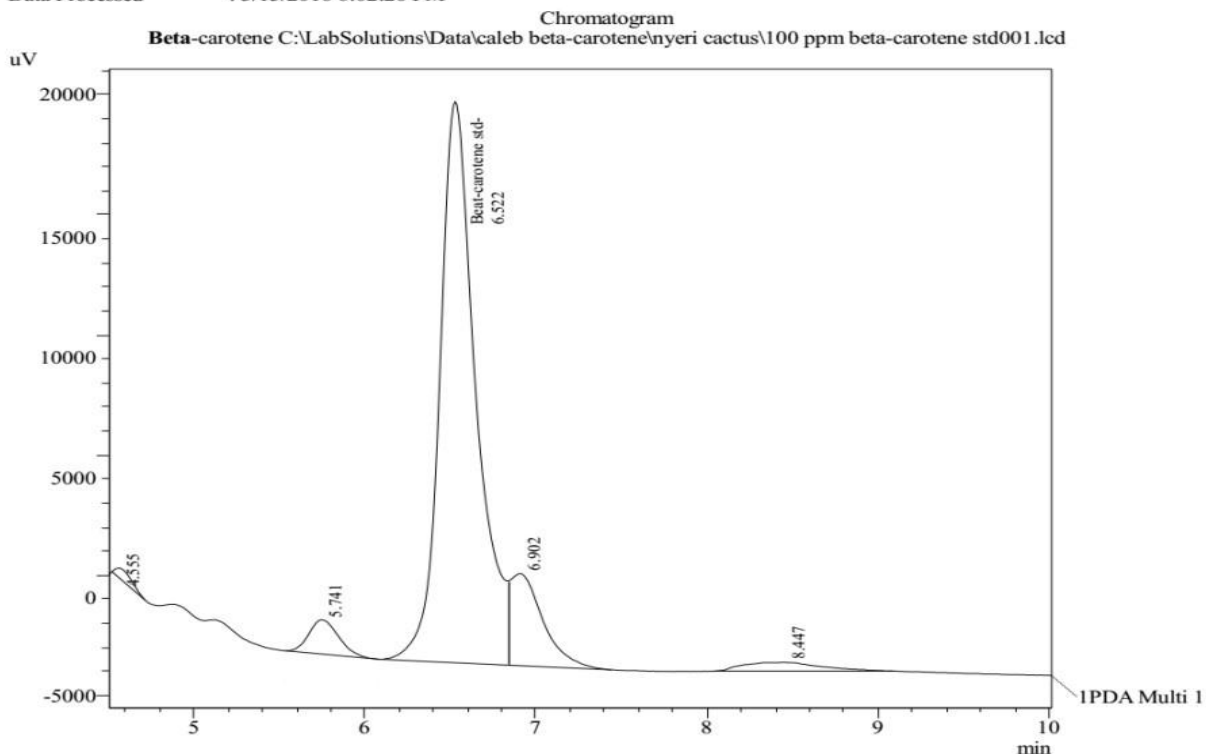
| Antioxidant |          | Mean(mg/100g) | SD     | RSD % |
|-------------|----------|---------------|--------|-------|
| Lycopene    | Unripe   | 3.257         | 0.1153 | 3.54  |
|             | Ripe     | 7.056         | 0.1126 | 1.59  |
|             | Overripe | 13.553        | 0.5034 | 3.71  |
| BC          | Unripe   | 0.0413        | 0.0019 | 4.6   |
|             | Ripe     | 0.0663        | 0.0014 | 2.11  |
|             | Overripe | 0.0856        | 0.0026 | 3.04  |
| VA          | Unripe   | 1.269         | 0.0510 | 4.02  |
|             | Ripe     | 2.142         | 0.0318 | 1.48  |
|             | Overripe | 1.474         | 0.0609 | 4.13  |

#### 4.3 Chromatogram Representation

Beta-carotene, lycopene and Vitamin A were identified in all the stages of maturity and quantified using standards and their retention times as described in the analytical procedures of vitamin A by Zahar and Smith (1990), beta-carotene by the method of (Heinonen *et al.*, 1990) and lycopene was done by the method of Fish *et al.*, (2002). The chromatogram of beta-carotene is as shown in figure 4.2 while the chromatogram of lycopene and beta-carotene are represented in appendix 7 and 8 respectively. Retention times were reproducible for similar analysis carried out for both standards and samples. The retention time of lycopene, beta-carotene and vitamin A (retinol)

was 5.45, 6.522 and 3.261 minutes respectively. These retention times and the spectra of standards were used to identify the peaks.

Injection Volume : 40 uL  
 Data Filename : 100 ppm beta-carotene std001.lcd  
 Method Filename : nyeri beta-carotene.lcm  
 Batch Filename : joyce beta carotene.lcb  
 Report Filename : Kahenyacaff.lcr Date  
 Acquired : 3/15/2016 5:52:16 PM  
 Data Processed : 3/15/2016 6:02:20 PM



1 PDA Multi 1 / 254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area   | Height | Area %  | Height % |
|-------|-----------|--------|--------|---------|----------|
| 1     | 4.555     | 2698   | 354    | 0.633   | 1.208    |
| 2     | 5.741     | 17179  | 1421   | 4.033   | 4.844    |
| 3     | 6.522     | 343815 | 23360  | 80.712  | 79.654   |
| 4     | 6.902     | 50794  | 3834   | 11.924  | 13.072   |
| 5     | 8.447     | 11491  | 358    | 2.697   | 1.221    |
| Total |           | 425976 | 29327  | 100.000 | 100.000  |

**Figure 4.2: Chromatogram for Beta-Carotene**

#### 4.4 Beta-Carotene Content in Red Cactus Fruits from Thegu Chaka Nyeri County in mg/100g at Different Stages of Ripening

The levels of beta-carotene in red cactus from Thegu area in Chaka, Nyeri County at unripe ripe and overripe stages of ripening are presented in Table 4.4.

**Table 4.4: Beta-Carotene Content in Red Cactus Fruits from 10 Sampling Sites**

| Sample sites | UNRIPE                     | RIPE                        | OVERIPE                     |
|--------------|----------------------------|-----------------------------|-----------------------------|
| 1            | 0.04 ± 0.003 <sup>c</sup>  | 0.06 ± 0.004 <sup>def</sup> | 0.09 ± 0.005 <sup>hij</sup> |
| 2            | 0.03 ± 0.006 <sup>bc</sup> | 0.06 ± 0.002 <sup>def</sup> | 0.11 ± 0.01 <sup>k</sup>    |
| 3            | 0.04 ± 0.004 <sup>c</sup>  | 0.06 ± 0.002 <sup>def</sup> | 0.11 ± 0.01 <sup>jk</sup>   |
| 4            | 0.04 ± 0.001 <sup>c</sup>  | 0.06 ± 0.002 <sup>def</sup> | 0.07 ± 0.02 <sup>efg</sup>  |
| 5            | 0.04 ± 0.006 <sup>c</sup>  | 0.07 ± 0.007 <sup>de</sup>  | 0.08 ± 0.004 <sup>gh</sup>  |
| 6            | 0.01 ± 0.006 <sup>a</sup>  | 0.05 ± 0.008 <sup>d</sup>   | 0.09 ± 0.01 <sup>hi</sup>   |
| 7            | 0.02 ± 0.13 <sup>ab</sup>  | 0.09 ± 0.004 <sup>ij</sup>  | 0.09 ± 0.005 <sup>hi</sup>  |
| 8            | 0.04 ± 0.03 <sup>c</sup>   | 0.06 ± 0.006 <sup>def</sup> | 0.09 ± 0.002 <sup>hi</sup>  |
| 9            | 0.09 ± 0.002 <sup>hi</sup> | 0.09 ± 0.008 <sup>hij</sup> | 0.10 ± 0.007 <sup>ijk</sup> |
| 10           | 0.09 ± 0.003 <sup>ij</sup> | 0.07 ± 0.001 <sup>fg</sup>  | 0.06 ± 0.002 <sup>de</sup>  |
| <b>Mean</b>  | <b>0.04 ± 0.002</b>        | <b>0.07 ± 0.001</b>         | <b>0.09 ± 0.003</b>         |

Means that are within the same column having a difference in the superscripts were significantly different ( $p < 0.001$ ). The different values were presented as mean ± SD, n=3. S.D means standard deviation)

From Table 4.4 the levels of beta-carotene ranged from 0.01 ± 0.006 to 0.09 ± 0.003 mg/100 gFW in unripe fruit with a mean of 0.04 ± 0.0019 mg/100 gFW, while in ripe

fruit the levels ranged from  $0.05 \pm 0.008$  mg/100 gFW to  $0.09 \pm 0.004$  mg/100 gFW with a mean of  $0.07 \pm 0.001$  mg/100gFW. In the overripe fruit the levels ranged from  $0.06 \pm 0.002$  to  $0.11 \pm 0.01$  mg/100gFW, with a mean of  $0.09 \pm 0.003$  mg/100g (Mean  $\pm$  SD). The levels of beta-carotene increased with each stage of ripening. As the fruit ripened there was a significant difference in the levels of beta-carotene as the starch converted more to form the beta-carotene. Beta-carotene mean lowest level was  $0.01 \pm 0.006$  mg/100g when unripe, at sampling site 6, and highest at sampling 10 with  $0.09 \pm 0.004$  mg/100g. At the ripe stage, the levels ranged from  $0.05 \pm 0.008$  mg/100g at site 6 to  $0.09 \pm 0.008$  mg/100g at sampling site 9 ( $p < 0.001$ ). For the overripe fruits the levels ranged from  $0.06 \pm 0.002$  at sampling site 10 to  $0.11 \pm 0.012$  mg/100g at site 2 when just overripe.

In all the samples from the 10 different sites the concentration of beta-carotene increased with increased stage of ripening except in site 7 and 10. In sample site 7 the content of beta-carotene increased from  $0.02 \pm 0.13$  mg/100g when unripe to  $0.09 \pm 0.004$  mg/100g when ripe but dropped to  $0.09 \pm 0.005$  mg/100g in the overripe. Analysis done on sample 10 showed a different trend whereby the beta-carotene concentration decreased from  $0.09 \pm 0.003$  mg/100g when unripe to  $0.07 \pm 0.001$  mg/100g when ripe and further to  $0.06 \pm 0.002$  mg/100g when overripe. The differences could be due to stage of maturity during picking since the color of the fruit was the basis of picking and some could have been too immature to ripen normally affecting the levels of the nutrient at different stages as a result of low levels enzymes responsible for production of the antioxidants

The content of beta-carotene in unripe red cactus were significantly different in all the sample sites apart from samples 1, 3 to 5 and 8 which had a concentration of  $0.04 \pm 0.003$ ,  $0.04 \pm 0.004$ ,  $0.04 \pm 0.001$ , and  $0.04 \pm 0.03$  mg/100g respectively. In the ripe red cactus, the content of beta-carotene was not significantly different in sample sites 1 to 4 and 8 which had a content of  $0.06 \pm 0.004$ ,  $0.06 \pm 0.002$ ,  $0.06 \pm 0.002$ ,  $0.06 \pm 0.002$  and  $0.06 \pm 0.006$  mg/100g respectively. The overripe red cactus content was significantly different in sample sites 1 to 5, 9 and 10 while in sample sites 6 to 8 the concentration was not significantly different with a content of  $0.09 \pm 0.011$ ,  $0.09 \pm 0.005$  and  $0.09 \pm 0.002$  mg/100g respectively as shown in bar graph in appendix 2.

Fatma *et al.*, (2012) did an analysis on the orange-yellow cactus pear fruit and stated a content of 0.017.54 mg/100g which was lower than the content of unripe red cactus in this study while *opuntia stricta* was reported a mean of 0.056 mg/100g (Kunyaga *et al.*, 2014) which was lower than what was reported in this study for the ripe and overripe. The daily recommended average intake of BC is in the range 2–7 mg/100g (Koushik *et al.*, 2006). The red cactus fruit at the overripe stage provides 4.5 % while at the ripe stage can provide 3.5 %. Though low levels of beta-carotene, the fruit can still be used like supplement to other fruits. About 3 fruits can provide 0.09 mg of beta-carotene at the overripe stage and 0.07 mg at the ripe stage.

The beta-carotene concentration increased by 0.03 mg/100g since the content was 0.04 mg/100g when unripe and 0.07 mg/100g when just ripe. This translated to 75 % increase from the unripe to the ripe stage of ripening. From the ripe (0.07 mg/100g) to the overripe stage (0.09 mg/100g), the content increased by 0.02 mg/100g which was 28.57 %. This means that the conversion of starch by enzymes was higher from the

unripe to the ripe stage than from the ripe to the overripe stage. The daily recommended intake of beta-carotene is 2-7 mg/100g. Murkovic (2002) stated the daily dietary recommended intake of VA to be 1-3 mg/day of retinol equivalent. The body is able to transform 2 µg of beta-carotene from the diet to 1µg of retinol hence the red cactus fruit at the overripe stage of ripening can provide about 45 µg of retinol which is equal to 45 RE. This can provide about 0.05 % of the daily recommended retinol equivalent.

A study done on seedless and seeded watermelon revealed the same trend that the level of beta-carotene increases with ripening. When freshly analyzed the amount was 0.1 mg/100g and when stored at a temperature of 5 °c, 13 °c and 21 °c the levels were 0.05, 0.08 and 0.17 mg/100g respectively (Perkins *et al.*, 2007). This trend has also been reported in a study done on *Solanum Indicum L.* (African nightshade) which showed that the levels of beta-carotene increases at different ripening stages. When green (unripe) the mean values were reported to be 0.02±0.01 mg/100g FW, when ripe the values were 0.06 ± 0.06 mg/100g and when overripe the values raised to 1.16±0.34 mg/100g FW (Pollyanna *et al.*, 2009). The level of beta-carotene when unripe in *Solanum Indicum L.* was lower than what was found in this study since red cactus had 0.04 mg/100g. In the winter, mean beta-carotene content ranged from 2.566 mg/100g in curly lettuce to 7.962 mg/100g in kale, (Pollyanna *et al.*, 2009). The concentration of carotenoids present in a fruit is determined by ripeness. In an analysis done on different tomato cultivars at different levels of ripening starting with the breaker stage through the stages to the red stage revealed an increase in the levels of beta-carotene (Shiva and Jun, 2016). Unicorn tomato cultivar increased from 6.52, 9.24, 11.27 to 12.78 mg/100 g and then dropped to 10.20 mg/100g at the red stage of

ripening (Shiva and Jun, 2016). This trend was similar to what was determined in this study. Analysis done on banana types at different stages of ripening showed that the levels of beta-carotene increased in Dwarf kalapua and Ds 11AA from 0.341, 0.344 to 0.459 and from 0.704, to 1.278 and to 1.577 mg/100 g respectively (Gerald *et al.*, 2009) which was similar to what was determined in this study.

The recommended dietary allowance (RDA) of retinol in the human body is set at 900µg/day retinol equivalents for male which translates to 5,400 µg/day beta-carotene equivalents. In female the RDA is 700 µg retinol equivalents which relates to 4,200 µg/day of beta-carotene equivalent (Trumbo *et al.*, 2001). Considering the betacarotene content of red cactus at the overripe stage, 100 g of the fruit can provide about 9.51 % of RDA in males and 12.23 % in females.

#### 4.5 Lycopene Content in Red Cactus Fruits from Thegu Chaka Nyeri County in mg/100 g at Different Stages of Ripening

The levels of lycopene obtained from the red cactus fruit at different stages of ripening are as presented in the following Table 4.5

**Table 4.5: Lycopene Content in Red Cactus Fruits from 10 Sampling Sites**

| Sample sites | UNRIPE                      | RIPE                          | OVERIPE                     |
|--------------|-----------------------------|-------------------------------|-----------------------------|
| 1            | 1.46 ± 0.15 <sup>a</sup>    | 5.54 ± 0.14 <sup>def</sup>    | 8.46 ± 0.06 <sup>jk</sup>   |
| 2            | 1.89 ± 0.03 <sup>ab</sup>   | 5.87 ± 0.07 <sup>efg</sup>    | 12.66 ± 0.36 <sup>n</sup>   |
| 3            | 2.58 ± 0.04 <sup>bc</sup>   | 6.30 ± 0.07 <sup>fghi</sup>   | 17.86 ± 1.146 <sup>o</sup>  |
| 4            | 5.52 ± 0.09 <sup>def</sup>  | 7.05 ± 0.219 <sup>fghij</sup> | 19.14 ± 1.54 <sup>0</sup>   |
| 5            | 3.26 ± 0.002 <sup>abc</sup> | 7.14 ± 0.19 <sup>fghij</sup>  | 23.92 ± 3.87 <sup>p</sup>   |
| 6            | 3.09 ± 0.22 <sup>abc</sup>  | 7.68 ± 0.13 <sup>ghijk</sup>  | 9.52 ± 0.36 <sup>kl</sup>   |
| 7            | 3.29 ± 0.15 <sup>abc</sup>  | 8.26 ± 0.04 <sup>ijk</sup>    | 10.56 ± 0.30 <sup>lm</sup>  |
| 8            | 3.56 ± 0.05 <sup>bc</sup>   | 5.93 ± 4.02 <sup>efgh</sup>   | 10.52 ± 0.25 <sup>lm</sup>  |
| 9            | 4.26 ± 0.43 <sup>cde</sup>  | 7.94 ± 0.05 <sup>hijk</sup>   | 10.82 ± 0.43 <sup>lmn</sup> |
| 10           | 3.66 ± 0.05 <sup>bcd</sup>  | 8.85 ± 0.07 <sup>jkl</sup>    | 12.08 ± 0.28 <sup>mno</sup> |
| <b>Mean</b>  | <b>3.26 ± 0.12</b>          | <b>7.06 ± 0.11</b>            | <b>13.55 ± 0.50</b>         |

Means that are within the same column having a difference in the superscripts were significantly ( $p < 0.001$ ) different. The values were presented as mean  $\pm$  SD,  $n=3$ .

From Table 4.5, the level of lycopene increases as the fruit ripens with the lowest being  $1.46 \pm 0.14$  mg/100g when unripe at sampling site 1 to  $23.92 \pm 3.866$  mg/100g FW when overripe at sampling site 5 ( $p < 0.001$ ). The levels of lycopene varied from one sampling site to another but in all the sampling sites the levels increased with the increase in stage of ripening. This analysis showed that the amount of lycopene in unripe red cactus ranged from  $1.46 \pm 0.14$  to  $5.52 \pm 0.09$  mg/100g FW, while in ripe red cactus the amount ranged from  $5.54 \pm 0.140$  to  $8.85 \pm 0.07$  mg/100g FW and in overripe red cactus the amount ranged from  $8.46 \pm 0.06$  to  $23.92 \pm 3.87$  mg/100g FW. The mean content (Mean  $\pm$  SD) of lycopene increased as the fruit ripened from  $3.26 \pm 0.12$  mg/100g FW when unripe,  $7.06 \pm 0.11$  mg/100g FW when just ripe and  $13.55 \pm 0.50$  mg/100g FW when just overripe as shown in Table 4.5.

The content of lycopene increased by 3.8 mg/100g (116.56 %) from the unripe to the ripe stage. From ripe to unripe stage, the concentration of lycopene increased by 6.49 mg/100g which is 91.93 %. This means that at the unripe stage the red cactus mainly had a lot of starch and acids with less sugars and carotenoid content at the unripe stage but during ripening the starch and acids were converted to sugars and more lycopene formed by enzymes. As the fruit was kept to ripen the lycopene content increased and this is attributed to more cellular respiration for more carotenoid since red cactus fruit is a climacteric fruit. More chloroplast was converted to chromoplast and this contributes to the increase in the levels of lycopene (Shiva and Jun, 2016).

The content of lycopene in unripe red cactus was not significantly different in sample sites 5, 6 and 7 which had a concentration of  $3.26 \pm 0.002$ ,  $3.09 \pm 0.22$  and  $3.29 \pm 0.15$  mg/100g respectively. In the ripe red cactus, the content of lycopene was significantly different in all sample sites except in sample sites 4, 5 and 6 which had a content of  $7.05 \pm 0.219$ ,  $7.14 \pm 0.19$  and  $7.68 \pm 0.13$  mg/100gFW respectively and this could be same physiological factors. The overripe red cactus lycopene content was significantly different in sample sites 1 to 6, 9 and 10 while in sample sites 7 and 8 the concentration was not significantly different with a content of  $10.56 \pm 0.30$  and  $10.52 \pm 0.25$  mg/100g( $p < 0.001$ ) respectively.

Red cactus content of lycopene increases as the fruit ripens. The content of lycopene in all the sites increased and sample 5 recorded the highest levels of 23.92 mg/100g at overripe stage of ripening while site 1 had the lowest content of 8.46 mg/100g at unripe stage of ripening. The content of lycopene in unripe red cactus were not significantly different in sample sites 5, 6 and 7 but were significantly different in sample sites 1 to 4, 8, and 9. In the ripe red cactus the content of lycopene was significantly different in all sample sites except in sample sites 4, 5 and 6. The overripe red cactus lycopene content was significantly different in sample sites 1 to 6, 9 and 10 while in sample sites 7 and 8 the concentration was not significantly different.

A study done on seeded and seedless (Black Diamond) watermelon revealed the same trend. The levels of seeded (Black Diamond) watermelon increased from 2.1 mg/100g when unripe (green) to 4.34 mg/100g when ripe and 4.82 mg/100g when overripe. Seedless (TriX313) watermelon had a content of 5.5 mg/100g when unripe 7.12

mg/100g when ripe and 7.85 mg/100g when overripe (Perkins-Veazie, 2007). These contents are a lot lower than what was found in this study. The content of lycopene in red cactus as the fruit ripen is shown in the bar graph in appendix 3.

A study done on Unicorn cultivar of tomatoes at different stages of ripening revealed the same trend as what was reported in this study though the contents of lycopene in tomatoes was higher (97.11 mg/100g) than what the study of red cactus revealed. The content of lycopene from the breaker stage, turning, pink, light red and to the red stage increased from 1.67, 9.26, 34.53, 63.48 to 97.11 mg/100g respectively (Shiva and Jun, 2016).

Red cactus at overripe stage showed a high concentration of lycopene (13.55 mg/100g) than when unripe and just ripe. The recommended daily intake of lycopene is about 10 mg/100g per day which is 100 % provided by consuming one red cactus fruit at the overripe stage of ripening. The red cactus fruit can be a very good alternative source since it has high levels than most fruits and vegetables and also above the recommended dietary intake.

#### **4.6 Retinol Content in Red Cactus Fruits from Thegu Chaka Nyeri County in $\mu\text{g}/100\text{g}$ at Different Stages of Ripening**

Unripe (breaker), ripe and overripe red cactus fruits were analyzed for retinol (VA) and the concentration obtained for each stage of ripening in  $\mu\text{g}/100\text{g}$  are shown in Table 4.6.

**Table 4.6: Retinol Content in Red Cactus Fruits from Ten Sampling Sites**

| Sample sites | UNRIPE                      | RIPE                       | OVERIPE                    |
|--------------|-----------------------------|----------------------------|----------------------------|
| 1            | 2.35 ± 0.05 <sup>j</sup>    | 1.90 ± 0.06 <sup>i</sup>   | 1.57 ± 0.27 <sup>gh</sup>  |
| 2            | 1.35 ± 0.07 <sup>efg</sup>  | 1.55 ± 0.20 <sup>gh</sup>  | 0.99 ± 0.03 <sup>bcd</sup> |
| 3            | 0.88 ± 0.007 <sup>abc</sup> | 1.16 ± 0.12 <sup>def</sup> | 0.71 ± 0.01 <sup>a</sup>   |
| 4            | 0.93 ± 0.18 <sup>abcd</sup> | 1.89 ± 0.08 <sup>i</sup>   | 1.72 ± 0.12 <sup>hi</sup>  |
| 5            | 1.10 ± 0.23 <sup>cde</sup>  | 1.48 ± 0.08 <sup>gh</sup>  | 1.42 ± 0.06 <sup>fg</sup>  |
| 6            | 0.82 ± 0.02 <sup>ab</sup>   | 5.63 ± 0.21 <sup>l</sup>   | 1.32 ± 0.01 <sup>efg</sup> |
| 7            | 0.83 ± 0.04 <sup>ab</sup>   | 1.88 ± 0.04 <sup>i</sup>   | 1.36 ± 0.02 <sup>gef</sup> |
| 8            | 1.03 ± 0.08 <sup>bcd</sup>  | 2.97 ± 0.23 <sup>k</sup>   | 3.01 ± 0.04 <sup>k</sup>   |
| 9            | 1.58 ± 0.02 <sup>gh</sup>   | 1.41 ± 0.04 <sup>fg</sup>  | 1.32 ± 0.03 <sup>efg</sup> |
| 10           | 1.83 ± 0.02 <sup>i</sup>    | 1.56 ± 0.04 <sup>gh</sup>  | 0.32 ± 0.008 <sup>ef</sup> |
| <b>Mean</b>  | <b>1.27 ± 0.05</b>          | <b>2.14 ± 0.03</b>         | <b>1.47 ± 0.06</b>         |

Mean appearing in the same column with a different superscripts were significantly (p<0.001).

The values were presented as mean ± SD, n=3. Each value is mean of triplicate. SD (Standard Deviation).

From Table 4.6 the analysis of Vitamin A (retinol) in red cactus increased in the amount as the fruit turns from unripe to ripe stage with the unripe having an overall mean (Mean ± SD) of 1.27 ± 0.05 µg/100gFW and the ripe having an overall mean of 2.14 ± 0.13 µg/100gFW but after the fruit was kept for 5 days, the mean Vitamin A (retinol) concentration decreased to 1.47 ± 0.06 µg/100gFW. The content of retinol increased by 0.87 µg/100g from the unripe to the ripe stage which is 68.34 % while from the ripe stage to the overripe stage the content decreased by 0.67 µg/100g which was 31.31 %. A comparison of the levels of vitamin A (Retinol) at the three stages of maturity is represented in bar graph in appendix 4.

The drop in the content of retinol was contributed by oxidative degradation of retinol. As the fruit ripens, the cells increase the amount of oxygen absorption leading to a

high rate of cell respiration which leads to a drop in retinol in climacteric fruits (Abushita *et al.*, 2000). The analysis of banana (*Musa spp*) hybrid cultivars reported the same trend where the retinol content decreased with ripening. Hybrid Mbouroukou n<sup>0</sup>1 had 147.99 when unripe, 78.63 when ripe and 33.49  $\mu\text{g}/100\text{gFW}$  when overripe. Hybrid Grande Naine had the lowest contents which also decreased with ripening. It contained 43.54 when unripe, 20.19 when ripe and 21.41  $\mu\text{g}/100\text{gFW}$  when overripe (Newilah *et al.*, 2009).

The Vitamin A (retinol) content of the various sites ranged from  $0.82 \pm 0.02$  to  $2.35 \pm 0.05$   $\mu\text{g}/100\text{gFW}$  when unripe stage of ripening. The concentration of the Vitamin A (retinol) ranged from  $1.16 \pm 0.12$  to  $5.63 \pm 0.21$   $\mu\text{g}/100\text{gFW}$  when ripe stage of ripening and from  $0.33 \pm 0.008$  to  $1.72 \pm 0.12$   $\mu\text{g}/100\text{gFW}$  when overripe stage of ripening. The sample site 2 to sample site 8 revealed an increase in Vitamin A (retinol) concentration from unripe red cactus to ripe red cactus and a drop in the overripe red cactus except in sample sites 1, site 9 and site 10 where the concentration dropped as the fruit matured. The trend in sample sites 1 was  $2.35 \pm 0.051$  to  $1.90 \pm 0.06$  to  $1.57 \pm 0.27$ , sample site 9 was  $1.58 \pm 0.02$  to  $1.41 \pm 0.04$  to  $1.32 \pm 0.34$  and sample site 10 the concentration dropped from  $1.83 \pm 0.02$  to  $1.56 \pm 0.04$  and finally to  $0.33 \pm 0.008$   $\mu\text{g}/100\text{gFW}$ . The content of vitamin A in unripe red cactus was significantly different in all the sample sites apart from samples 6 and 7 which had a concentration of  $0.82 \pm 0.02$  and  $0.83 \pm 0.04$   $\mu\text{g}/100\text{gFW}$  respectively. In the ripe red cactus, the content of vitamin A (retinol) was not significantly different in sample sites 2, 5 and 10 which had a content of  $1.55 \pm 0.20$ ,  $1.48 \pm 0.078$  and  $1.56 \pm 0.04$   $\mu\text{g}/100\text{gFW}$  respectively. The overripe red cactus content was significantly different in sample sites 1 and 5, 8 and 10 while in sample sites 6, 7 and 9 the concentration

was not significantly different with a content of  $1.32 \pm 0.01$ ,  $1.36 \pm 0.18$  and  $1.32 \pm 0.34 \mu\text{g}/100\text{gFW}$  respectively.

Ahmad *et al.*, (2007) analyzed and found vitamin A in carrots to be  $11210 \mu\text{g}/100\text{g}$  while in tomato it was reported as  $1610 \mu\text{g}/100\text{g}$ . Vitamin A has been analyzed in and found in different fruits such as guava  $504.10$ , pawpaw  $683.93$ , water melon  $350.12$ , tomato  $542.86$ , mango  $301.61$  and carrot  $2054.10$  in  $\mu\text{g}/100\text{g}$  (Aremu and Nweze, 2017). The red cactus content when ripe is  $2.14 \mu\text{g}/100\text{g}$  and this is lower than what is provided by the above fruits but can still be used as a supplement.

The content of vitamin A in unripe red cactus were significantly different in sample sites 1 to 5, 8, 9 and 10 but were not significantly different in sample sites 6 and 7. In the ripe red cactus the content of vitamin A was significantly different in all sample sites except in sample sites 2, 5 and 10. The overripe red cactus lycopene content was significantly different in sample sites 1 to 5, 8 and 10 while in sample sites 6, 7 and 9 the concentration was not significantly different. This is largely contributed to soil type in the different sample sites. The content of vitamin A in overripe red cactus was lower than in unripe and ripe in sample sites 1, 2 and 3. In sample sites 4 to 8 the concentration of vitamin A (retinol) in unripe red cactus was lower than overripe. In sample sites 1, 9 and 10, the vitamin A (retinol) concentration in unripe was the highest.

Gerald *et al.*, (2009) did an analysis on different varieties of banana when unripe, ripe and overripe and reported a decrease in the levels of retinol equivalent activities though the levels are higher than what is provided by the red cactus. Mbouroukou

cultivar when unripe had 147.99  $\mu\text{gRE}$ , 78.63 when ripe and 33.49  $\mu\text{g RE}$  when overripe. Grande Naine cultivar had 16.02  $\mu\text{g RE}$  when unripe which dropped to 9.74  $\mu\text{g RE}$  when ripe and finally to 8.38  $\mu\text{g RE}$  when overripe. To compare the VA activity, 1  $\mu\text{g}$  of retinol is equivalent to 1  $\mu\text{gRE}$  and the retinol activity equivalents (RAE) are used (Aremu and Nweze, 2017). The retinol equivalent provided by the red cactus fruit are a lot lower than what is provide by the banana cultivars.

Analysis done in plums reported a content of 0.88 mg/100gFW, while in green pepper, sweet pepper and hot pepper the content was 0.5, 1.03 and 0.71 mg/100gFW respectively (Adebisi *et al.*, 2014). Trumbo *et al.*, (2001) reported that in male human beings, the recommended dietary allowance (RDA) of vitamin A is which has been given in terms of retinol equivalent while in female the RDA is stated as 700  $\mu\text{g}$  day retinol equivalents. The red cactus at the ripe stage would provide 2.14  $\mu\text{g}/100\text{gm}$  which is equivalent to 0.24 % of the recommended 900  $\mu\text{g}/\text{day}$  for male adults and 0.31 % of the recommended 700  $\mu\text{g}/\text{day}$  for female which is quite low and a person would have to consume so many but the fruit can still be used as a supplement or as a source of vitamin A especially in the semi-arid regions.

## CHAPTER FIVE

### CONCLUSION AND RECOMMENDATIONS

#### 5.1 Conclusion

This study was carried out to quantify beta-carotene, vitamin A and lycopene levels in red cactus fruit at different stages of ripening. The results of this study reveal that there is significant difference ( $P < 0.001$ ) in the levels of these antioxidants as the fruit ripens except in vitamin A (retinol) where the content dropped as the fruit over ripened. The following conclusions were drawn from this study.

- (i) The unripe (breaker) red cactus had low levels of the antioxidant with lycopene being the highest at  $3.26 \pm 0.12$  mg/100gFW and retinol the lowest mean level at  $1.27 \pm 0.05$   $\mu$ g/100gFW. The mean content of beta-carotene was  $0.04 \pm 0.002$  mg/100gFW.
- (ii) At the ripe stage the levels of the antioxidant increased with lycopene being the highest mean level of  $7.06 \pm 0.11$  mg/100 g FW, beta-carotene had a mean level of  $0.07 \pm 0.001$  mg/100 g and retinol had the lowest mean level of  $2.14 \pm 0.01$   $\mu$ g/100 g FW.
- (iii) At the overripe stage, the mean level of lycopene and beta-carotene increased but those of retinol decreased. The lycopene still had the highest mean level of  $13.55 \pm 0.50$  mg/100 g FW while retinol had the lowest level of  $1.47 \pm 0.06$   $\mu$ g/100 g FW. Beta-carotene had a mean content of  $0.09 \pm 0.003$  mg/100 g FW at the overripe stage.

#### 5.2 Recommendation from this Study

Red cactus at the ripe and overripe stage can be a very good supplement of vitamin A, lycopene and beta-carotene. The results from this study recommend the consumption

of this fruit when it's just ripe to get the maximum benefits of vitamin A and when overripe to get the maximum benefits of lycopene and beta-carotene. The findings should also be made available to the already established red cactus juice and jam industries so that they can target the overripe stage for maximum nutrients. Since the plant is drought resistance it can be used provide alternative sources of these antioxidants.

### **5.3 Recommendation for Further Work**

There is need to evaluate further the antioxidants contents of other varieties like the green, yellow and the purple skinned cactus fruits at different stages of ripening from diverse geographical regions in the country to enable their utilization. The Kenyan government needs to develop a nation- wide carotenoid database and to make the RDA of several carotenoids so as to encourage intake of antioxidant food components to bring down the risks of terminal cases such as cancer and heart conditions. The data obtained to be availed to large scale industries so that the right stage of maturation can be utilized in industries.

Levels of macro and micro-nutrients should be evaluated and the presence of other antioxidants and the effect of longer storage of the fruit on the levels of the antioxidant should also be evaluated and made available.

Finally, there is need to enclose the cactus shrubs which grow wildly in the arid and semi-arid regions so that they can be well utilized to raise the health standard of the community around where they grow.

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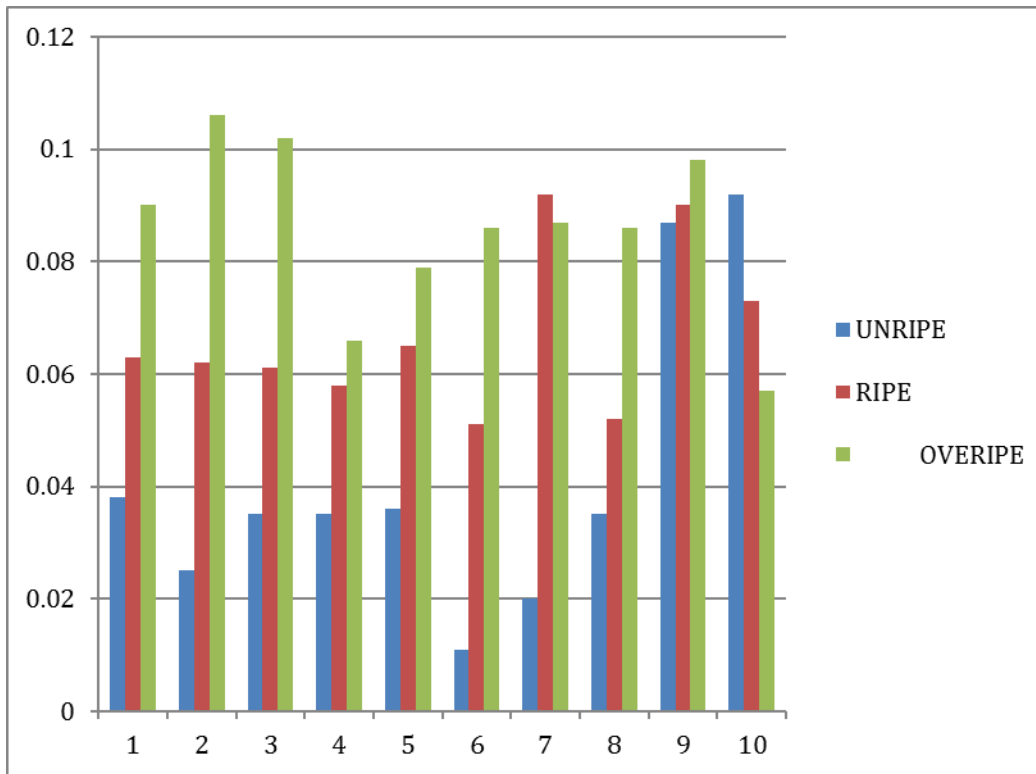
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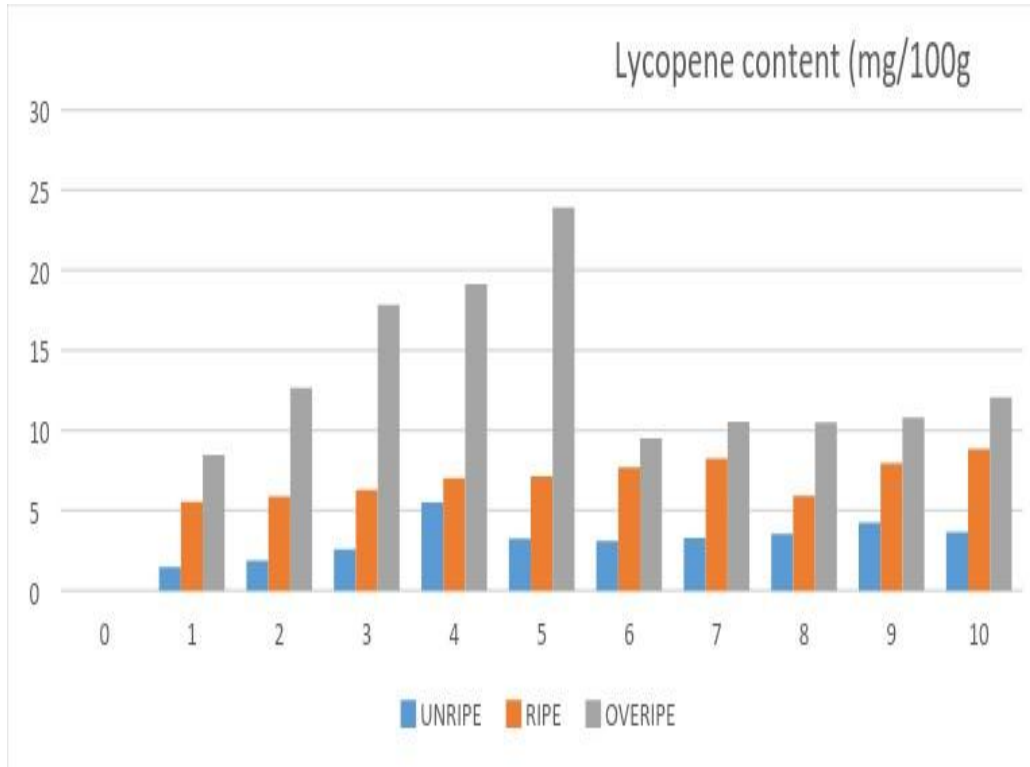
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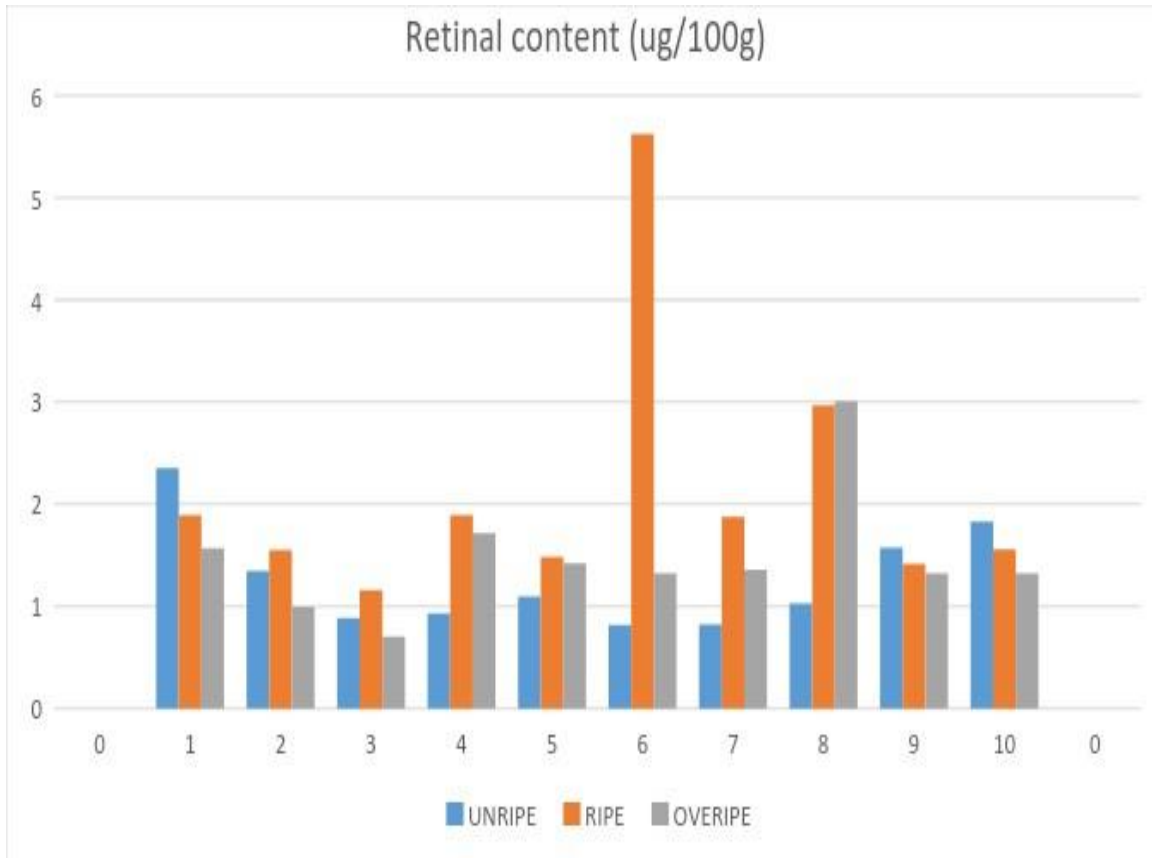
**APPENDIX**

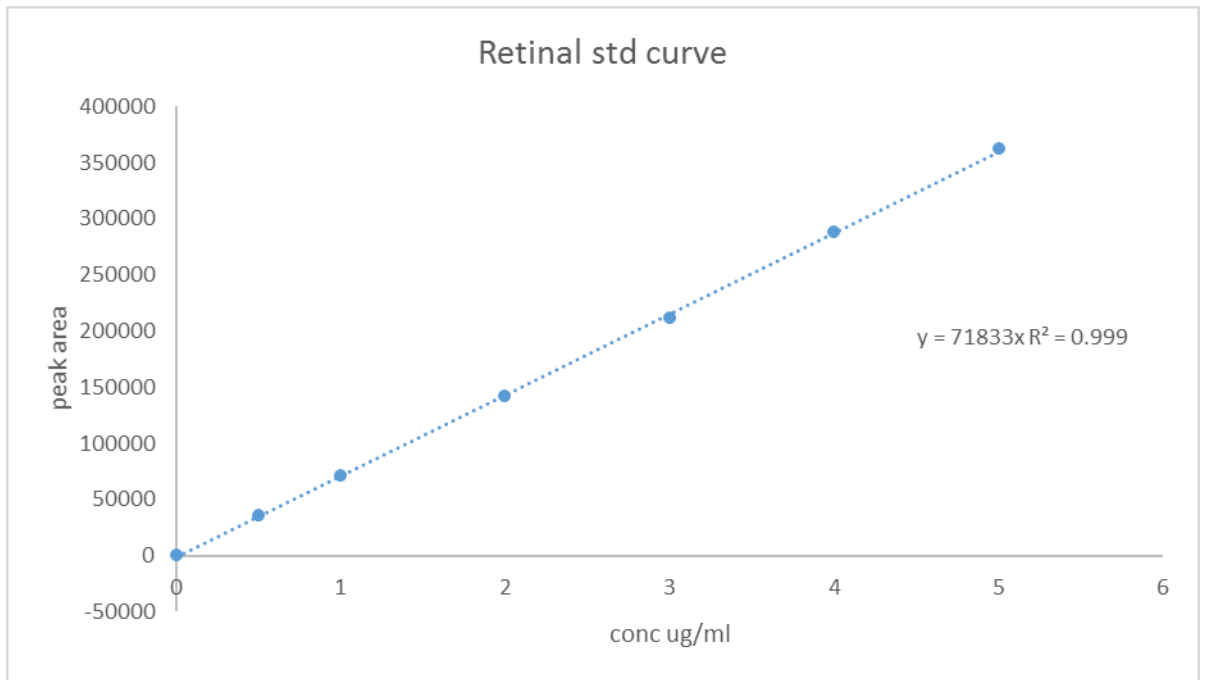
**Appendix 1: In the Field Picking the Fruits**

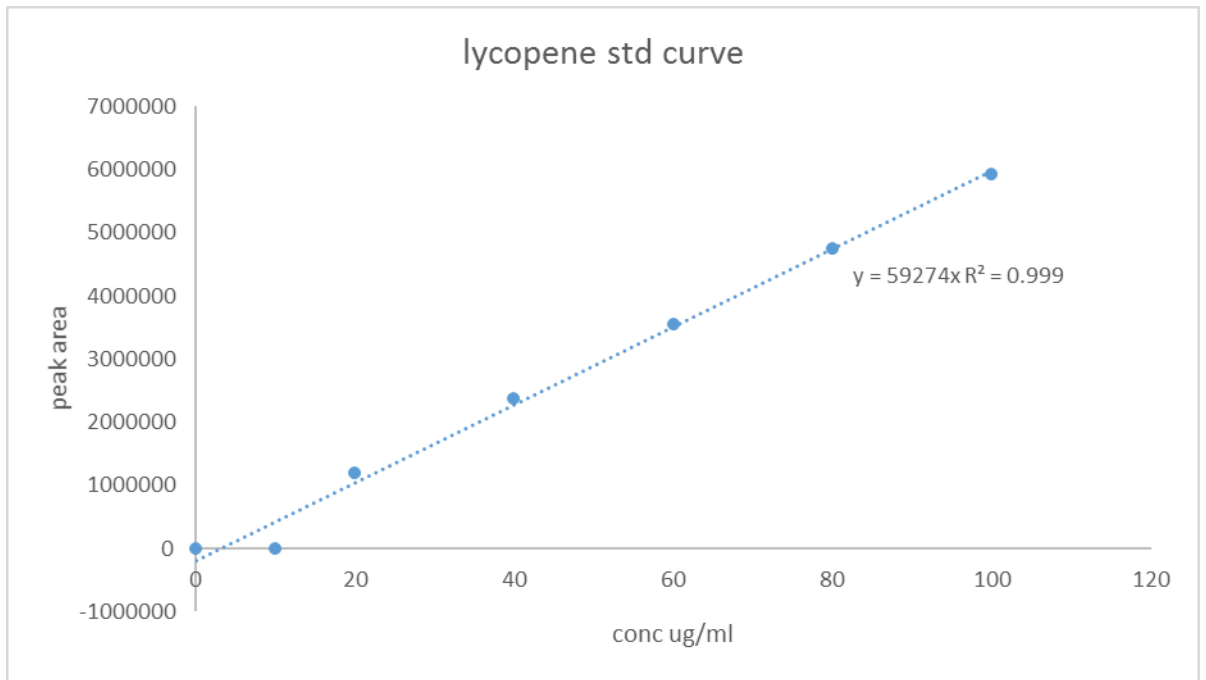


**Appendix 2: Bar Graph of the Beta-Carotene Content**

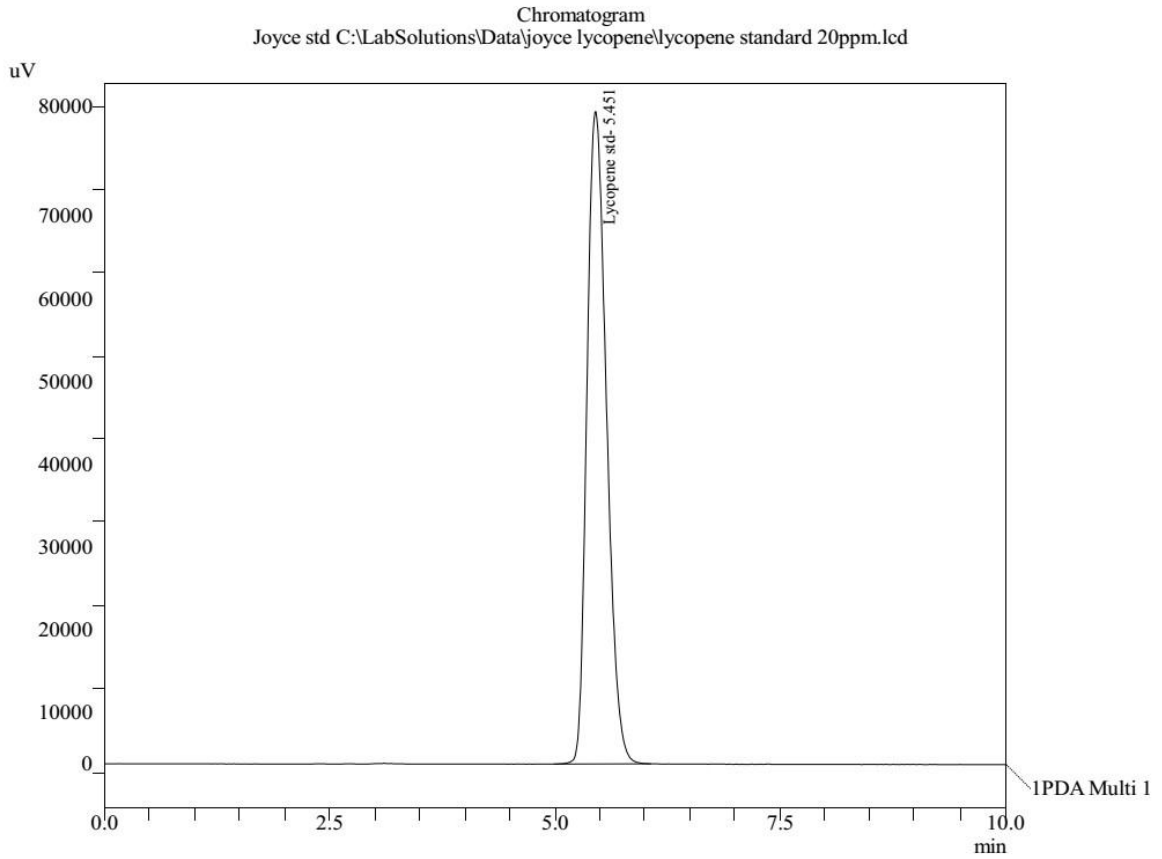
**Appendix 3: Bar Graph of the Lycopene Content**

**Appendix 4: Bar Graph of the Retinol Content**

**Appendix 5: Retinol Standard Curve**

**Appendix 6: Lycopene Standard Curve**

**Appendix 7: Chromatogram for Lycopene**



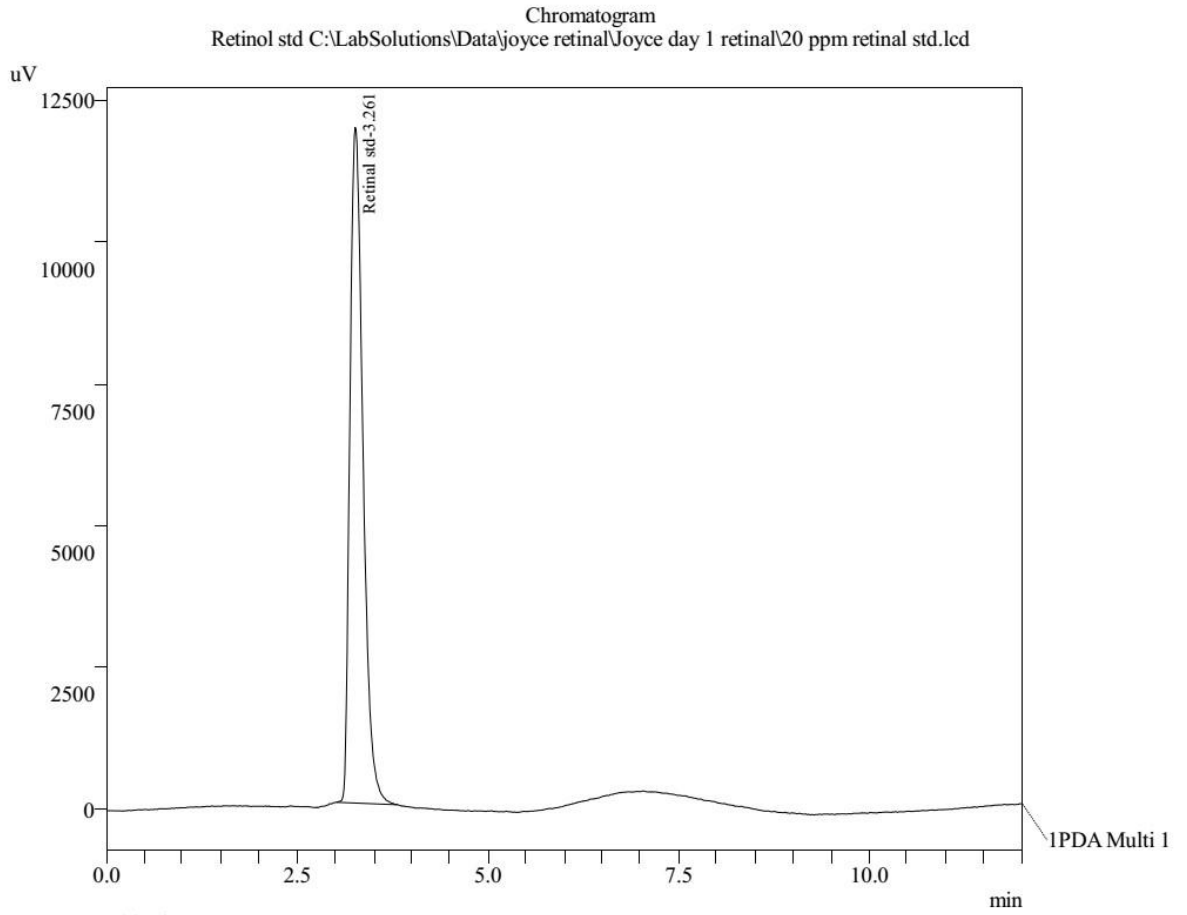
1 PDA Multi 1 / 254nm 4nm

Peak Table

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area    | Height | Area %  | Height % |
|-------|-----------|---------|--------|---------|----------|
| 1     | 5.451     | 1182441 | 78376  | 100.000 | 100.000  |
| Total |           | 1182441 | 78376  | 100.000 | 100.000  |

**Appendix 8: Chromatogram for Retinol**



Peak Table

PDA Ch1 254nm 4nm

| Peak# | Ret. Time | Area   | Height | Area %  | Height % |
|-------|-----------|--------|--------|---------|----------|
| 1     | 3.261     | 141802 | 11925  | 100.000 | 100.000  |
| Total |           | 141802 | 11925  | 100.000 | 100.000  |