QUANTIFICATION OF β-CAROTENE, LYCOPENE AND β-CRYPTOXANTHIN IN DIFFERENT VARIETIES AND STAGES OF RIPENING OF MANGOES GROWN IN MWALA, MACHAKOS COUNTY

BY

MULI DAVID MUSYOKA (BSc)

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Muli, David Musyoka
Quantification of
ß-carotene, lycopene and

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DECLARATION

I hereby declare that this thesis is my original work and has not been presented in any other institution for the award of a degree or any other award.

David Musyoka Muli

Chemistry Department

Kenyatta University

Signature .... Date 23/05/13

We confirm that the work reported in this thesis was carried out by the candidate under our supervision.

Dr. Sauda Swaleh

Chemistry Department

Kenyatta University

Signature .... Date 23/05/2013

Dr. Rotich Kibet Henry

Kenya Bureau of Standards

Signature .... Date 29/05/2013

Prof. Ahmed Hassanali

Chemistry Department

Kenyatta University

Signature .... Date 23rd May 2013

DEDICATION

This research work is lovingly dedicated to my wife Beatrice and my children Eliezer and Pharez for their encouragement, moral and spiritual support as I travelled through the research journey.

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ABBREVIATIONS AND ACRONYMS

ACS	American Cancer Society
ACN	Acetonitrile
ADA	American Dietetic Association
AJCN	American Journal of Clinical Medicine
ANOVA	Analysis of Variance
AORTIC	African Organization for Research and Training Cancer
BHT	Butylated hydroxyl toluene
CAS	Chemical Abstracts Service
CFNI	Caribbean Food and Nutrition Institute
CPP	Committee on Commodity Problems
DAFS	Days After Fruit Set
DMRC	Days at Market Ripening Conditions
DNA	Deoxyribonucleic Acid
DRI	Dietary Reference Intake
MF	Molecular Formula
HPLC	High Performance Liquid Chromatography
HPV	Human Papillomavirus
IQF	Individually Quick Frozen
KEBS	Kenya Bureau of Standards
LSD	Least Significant Difference

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MDG	Millennium Development Goals
MetOH	Methanol
FW	Formula Weight
MSDS	Material Safety Data Sheet
NAS	National Academy of Sciences
NWE	New World Encyclopedia
PSDA	Private Sector Development in Agriculture Kenya
RAE	Retinol Activity Equivalents
RDA	Recommended Dietary Allowance
RP-HPLC	Reverse Phase High Performance Chromatography
RNI	Recommended Nutrient Intake
TEA	Triethylamine
USDA	USA Department of Agriculture
UV-VIS	Ultra Violet-Visible Spectrum
WHO	World Health Organization

ABSTRACT

Mangoes are rich in antioxidants, mainly β-carotene, β-cryptoxanthin, violaxanthin and lycopene. β-Carotene, β-cryptoxanthin and lycopene have been shown to play an important role in the prevention of several forms of cancer, rheumatoid arthritis, diabetes, heart diseases and macular degeneration. However, there is scarce data on nutraceutical levels in the various local and exotic mango varieties at different stages of ripening. The study was carried out to determine the levels of β-carotene, β-cryptoxanthin and lycopene in six varieties of mangoes in Mwala, Machakos County. The compounds were analyzed on a reverse phase isocratic HPLC system with a UV detector. There was a significant difference ($p<0.05$) in the levels of β-carotene, β-cryptoxanthin and lycopene among the six varieties at the mature unripe and ripe stages. β-Carotene was the predominant carotenoid in ripe mangoes with levels ranging from 3.74±0.44 mg/100g in Tommy Atkins mango to 10.47±2.04 mg/100g in Apple mango. However, in mature unripe mangoes it was significantly lower, ranging from 0.21±0.04 mg/100g in Tommy Atkins mangoes to 2.22±0.39 mg/100g in Keit mangoes. The level of β-cryptoxanthin in ripe mangoes was highest in Apple cultivar (107.23±16.34 μg/100g) and lowest in Ndoto cultivar (36.67±11.63 μg/100g). In mature unripe mangoes, the levels of β-cryptoxanthin mangoes were also lower ranging from 28.66±3.63 μg/100g in Apple cultivar to 7.21±1.42 μg/100g in Ngowe cultivar. Similarly, lycopene contents were higher in ripe mangoes (ranging from 16.96±4.07 μg/100g in Keit to 53.98±9.23 μg/100g in Tommy Atkins cultivar) than in unripe mangoes (4.34±0.62 μg/100g in Keit cultivar to 8.01±1.41 μg/100g in the Kent cultivar). The study reveals that mangoes have significant levels of β-carotene, β-cryptoxanthin and lycopene and their widespread consumption may contribute toward alleviating incidences of degenerative diseases such as cancer, cardiovascular diseases, and inflammatory diseases of the skin, as well as help boost immune system of persons living with HIV/AIDS. Further work is needed to assess other nutraceutical compounds such as violaxanthin and zeaxanthin in mangoes. The study lays down some groundwork for more comprehensive analyses of mango varieties growing in different agro-ecological zones in Kenya and Eastern Africa. In addition, the methodology and results could provide a basis for quality control of growing number of commercial products of mango fruits by bodies such as the Kenya Bureau of Standard.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Mango (*Mangifera indica L.*) is a popular tropical fruit due to its unique taste, affordability and nutritional qualities (Krenek, 2009; Morton, 1987). Mangoes are predominantly grown in tropical and sub-tropical climates in places such as Asia, Africa and the Americas (NWE, 2008). The fruit is an excellent source of Vitamin-A and nutraceutical compounds like β-carotene, lycopene, and β-cryptoxanthin (Mateljan, 2010). A 100 g serving of fresh fruit provides 765 mg or 25% of recommended daily levels of vitamin A required for maintaining healthy mucus membranes and skin (Balch, 2006). Consumption of natural fruits rich in nutraceuticals is known to protect the body from various forms of degenerative diseases (LaChance, 2008). Mango falls in the category of under-utilized fruits whose nutrient composition data is insufficient coupled with little consumer knowledge about its nutritional value (FAO, 2008). In some West African countries loss of mangoes exceed an annual total of 100,000 metric tonnes (ADA, 2008). In Kenya, most of the fruit goes to waste in the peak of the production season (PSDA, 2010).

The nutraceutical content and antioxidant capacity of tropical fruits such as mangoes is taking greater importance in evaluations of fruit quality in marketing for human health benefits (Manthey and Perkin-Veazie, 2009). Although the antioxidants of mangoes have been the subject of earlier studies, complete information is still lacking about the influence of cultivar type, production practice, and harvest locations and dates on antioxidant capacities in mango pulp (Barreto *et al.*, 2008).

Despite the important relationship between fruits and health, most countries lack nation-wide data on the consumption of carotenoids as health indicators (Padovani and Amaya-Farfan, 2006). To date, no recommended dietary intake levels have been established for β-cryptoxanthin despite a lot of research linking the carotenoid to great health benefit (Mateljan, 2010). Most of the papers presenting quantitative data on food carotenoids are limited to pro-vitamin A carotenoids and total antioxidant capacity (Rodriguez-Amaya, 2001; Manthey and Perkins-Veazie, 2009; Wall, 2006). Further, reports indicate that there is little research focusing on quantitative changes associated with variety and ripening and especially nutraceutical compounds that are linked to anti-cancer and anti-inflammatory activities in the body (FAO-UN, 2006). This has largely been the case for Sub-Saharan Africa where it is estimated that 667,000 new cases and 518,000 cancer related deaths were registered in 2008 (ACS, 2011).

Most reviews indicate that there is little development in the provision of cancer prevention and control facilities (AORTIC, 2010; Bhattacharya, 2005). Cancer treatment continues to be out of reach for many Africans and estimates show that by 2020, cancer will kill about 20 million people with 70 per cent of the deaths happening in developing countries (WHO/ICO, 2010; Richard and Denis, 2007). According to a recent study, medications do not have a significant impact on reducing heart attacks and may even increase the risk of heart attacks in some individuals (Pugliese, 2008). Nutritional studies indicate that about one-third of all cancer deaths could be prevented by simply exploiting a variety of readily available fruits including mangoes (Bowden, 2008). Mangoes are rich in pre-biotic dietary fiber, vitamins, minerals, and poly-phenolic flavonoid antioxidant compounds and an excellent source of vitamin-A and flavonoids like β-carotene, α-carotene and β-cryptoxanthin (Shah *et al.*, 2010).

According to several trial studies, nutraceutical anti-oxidant compounds such as those present in mangoes have been found to protect against colon, breast, leukemia and prostate cancers (CCP, 2003; Hamid *et al.,* 2010).

1.2 Nutraceuticals as antioxidants and their role in disease prevention

Antioxidants are molecules that can interact with free radicals -Highly reactive species with unpaired pair of electrons (Hamid *et al.,* 2010). They are capable of inhibiting or terminating the free radical chain reaction, increasing immune competence, and inhibiting the occurrence of mutagenesis and premalignant lesion (Kivilompolo, 2009; Padovani and Amaya-farfan, 2006). β-Carotene, β-cryptoxanthin and lycopene are among the most extensively studied nutraceutical compounds known to have antioxidant properties and are essential in combating diseases such as cancer, arthritis and cardiovascular diseases (Bowden, 2008; Naguib, 2003; Karen and Lutz, 2001; Shah *et al.,* 2010). In addition, β-carotene, β-cryptoxanthin have the added advantage of being able to be converted to Vitamin A hence essential for maintaining healthy mucus membranes skin, and enhance immune functions (Hamid *et al.,* 2010). Consumption of natural fruits rich in nutraceuticals is known to protect the body from various forms of degenerative diseases (Israel, 1994).

Considerable laboratory evidence from chemical, cell culture, and animal studies indicates that antioxidants may slow or possibly prevent the development of cancer (Borek, 2004).The increased interest in natural antioxidants has led to the antioxidant evaluation in many species of fruits, vegetables, herbs, spices and cereals (Karen and Lutz, 2001). There is convincing scientific evidence in support of the association between diet and chronic diseases via several mechanisms that include metabolism, immune modulation and hormonal induction (Rao and Rao, 2007).

Based on such evidence, dietary guidelines have been formulated around the world for the prevention of chronic diseases such as cancer, cardiovascular disease, diabetes and osteoporosis. A research study carried out at the University of Manchester supports the view that dietary antioxidants such as β-carotene, β-cryptoxanthin and zeaxanthin as well as lycopene may be related to reduction in the occurrences of arthritis (Pattison, 2005).

In view of the great number of carotenoids which have been detected in foods, research should be concentrated on nutraceuticals which dominate in terms of quantity, and those with either provitamin A structure or a minimum number nine or more conjugated double bonds in the chromophore responsible for the antioxidative potential (radical-scavenging capacity and quencher of singlet oxygen). The phenomenon is explained by resonance stabilization of carotenoid radicals being formed (Muller, 1997). This makes β-carotene, β-cryptoxanthin and lycopene nutraceuticals of great antioxidant significance (Rao and Rao, 2007; Hamid *et al.*, 2010).

1.3 Statement of the problem

There are limited data bases on levels of some important antioxidants in most local and exotic mango varieties, their levels at different ripening stages and recommended intake levels. In Kenya, we lack nation-wide data to provide critical information on consumption of nutraceuticals as health indicators. There is need to quantify β-carotene, β-cryptoxanthin and lycopene content in local and exotic mango cultivars and establish quantitative changes associated with cultivar differences and ripening to assess their nutritional benefits and potential in modulating cellular processes to counter risk of degenerative diseases including various forms of cancer, cardiovascular diseases, age related macular degeneration and inflammatory disorders such as rheumatoid arthritis.

1.4 Justification

More extensive and accurate data on nutraceutical composition of foods are required for promotion of better human health. In particular, there is need to provide the general population with the necessary information on nutrient composition of different mango varieties and the variations associated with ripening.

This will enhance cultivation of varieties which will give maximum health benefits and promote their incorporation into diet. quantify nutraceuticals which dominate in terms of quantity, and those with either provitamin A structure or a minimum number nine or more conjugated double bonds in the chromophore responsible for the antioxidative potential to evaluate their role in the fight against certain kinds of cancers, arthritis, diabetes and heart diseases.

1.5 Hypothesis

The levels of β-carotene, β-cryptoxanthin and lycopene vary significantly depending on the variety and the ripening stage of the mango.

1.6 Objectives

1.6.1 General objective

To determine the levels of β-carotene, β-cryptoxanthin and lycopene in six varieties of mangoes collected in Mwala, Machakos County at the mature unripe stage and after full ripening.

1.6.2 Specific objectives

The specific objectives of the study were:

i) To quantify the levels of β-carotene, β-cryptoxanthin and lycopene in six varieties of mangoes in Mwala, Machakos County.

ii) To determine the levels of β-carotene, β-cryptoxanthin and lycopene in each of the six mango varieties at the mature unripe stage and after full ripening.

1.7 Significance of the study and anticipated out-puts

The study provides useful nutraceutical composition data which may be a guide as to the best source(s) of β-carotene, β-cryptoxanthin, and lycopene; the stage of maturity associated with the greatest nutraceutical benefits and their contribution to health. Nutraceutical profile data from this study is expected to encourage more consumption of mango by the general population if the data is incorporated in our nation's nutraceutical data base. Such data may also provide a basis for choice of best cultivars for mango processing and export markets as well as quality control of processed commercial fruit products by the Kenya Bureau of Standards for local consumption and export. Finally, the profiled data will help in sensitizing the general population and create awareness on the need to adopt better feeding and dietary habits that promote good health.

1.8 Potential beneficiaries of the research results and the impacts

The results of this study will be published in an appropriate international journal. These could also be publicized to the local populations through local newspapers and broadcasting. This will provide the general population with information regarding nutraceutical levels of β-carotene, β-cryptoxanthin and lycopene from different varieties of mangoes and their contribution to health to encourage their incorporation into diet to counter incidences of diseases including cancer, cardiovascular disease, rheumatoid arthritis and other inflammatory diseases based on experimental findings of this study. Further, the study will help in sensitizing industries and manufacturers on the best stage of maturity from which to derive maximum benefit from mangoes.

The study is expected to create a positive awareness on dietary practices among the poor rural population given that some communities have a practice where green unripe mangoes are boiled for use as food. Mango farmers, mango processing industries and the export market will also obtain valuable information on the choice of best cultivars based on mango nutraceutical compositional data. Medical practitioners, nutritionists and researchers will be encouraged to explore the role of nutraceuticals in heath and especially in control of terminal illnesses such as cancer and age related macular degeneration. Based on the study findings, standards bodies such as the Kenya Bureau of Standards can initiate quality control and standardization of Kenyan fruit juices and processed products to protect consumers from undue exploitation by dishonest manufactures and juice vendors.

1.9 Scope and limitations of the study

The nutraceutical content of mango was not assessed in the peel and some varieties of mango also present in the study area such as Van Dyke, Batawi, Maya, Sensation, Boribo, and Dodo were not investigated due to the constraints of time and destruction of the mango fruits by pests and diseases in many farms targeted for this study. Nutraceutical compositional variation associated with geographical location, soil type and farming practices was not investigated due to limitations of time and laboratory space. Mangoes have other nutraceutical compounds including violaxanthin and zeaxanthin, also known to have substantial health benefits which were not assessed in this study due to lack of pure standards and the need to carry out the analytical procedure within the shortest time possible due to the labile nature (easily oxidized and isomerized) of the compounds analysed.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin of the mango and their characteristics

Mango (*Mangifera indica* L.) is a tropical fruit grown worldwide with excellent nutritional value and widely attributed health promoting properties (Manthey and Perkins-Veazie, 2009). Mangoes are members of the family Anacardiaceae with a large intraspecies diversity (Morton, 1987). They originated from Southeast Asia where it has been grown for over 4,000 years (Kehlenbeck *et al.*, 2010). Over the years mango groves have spread to many parts of the tropical and sub-tropical world places such as Asia, Africa, and the Americas where the climate allows mangoes to grow best (Morton, 1987; Kimberly, 2009). Mango trees are evergreens that will grow to 60 feet tall and the tree will fruit 4 to 6 years after planting (Griesbach, 2003). Mango trees require hot dry periods to set and produce a good crop (Morton, 1987). There are over 1,000 different mango varieties throughout the world (Kehlenbeck *et al.*, 2010).

Mango fruit is a drupe in which an outer fleshy part surrounds a shell (the pit or stone) of hardened endocarp with a seed inside (NWE, 2008). Mature, mango fruit hang from the tree on long stems and varies in size from six to 25 cm long, seven to 12 cm wide, and with a weight of up to 2.5 kg (Morton, 1987). The ripe fruit is variably colored -yellow, orange, and red (most red) on the side facing the sun and yellow in the shade (ECHO, 2009). Green usually indicates that the fruit is not yet ripe but this depends on the cultivar or variety (Griesbach, 2003). Some mangos have a turpentine odor and flavor while others have a rich and pleasant fragrance (NWE, 2008). The flesh ranges from pale yellow to deep orange and is extremely juicy, with a mostly sweet flavor (Barret, 2010).

Mango consists of between 33-85% edible pulp, with 9-40% inedible kernel and 7-24% inedible peel (Ajila *et al.*, 2007).

2.2 Description of mango cultivars and their characteristics

There are over one thousand different cultivars of mangos growing world-wide (Kimberly, 2009). In Kenya, the crop has been cultivated in the Coast province since the 14th century before spreading to central, eastern and other parts of the country (Msabeni *et al.*, 2010). Two types of mangoes are grown in Kenya; local and exotic or improved varieties, the latter being cultivars grafted on local mangoes and grown for the export market (FAO, 2008). The local varieties are usually left to grow naturally without much crop husbandry and tend to have high fiber content (Msabeni *et al.*, 2010). Exotic varieties are grown mainly in Meru, Makueni, Machakos, Kitui, Mwingi and parts of central province in Kenya where they have become an essential source of local and foreign income from employment in addition to combating degenerative disorders (Kehlenbeck *et al.*, 2010). Commercial mango production is presently well developed in Kenya based on locally adapted and newly imported cultivars (Chegeh, 2010). Local varieties include Ngowe, Dodo, Boribo, Ndoto and Batawi (Griesbach, 2003). Exotic varieties are Apple, Kent, Keitt, Tommy Atkins, Haden, Van dyke and others (Kehlenbeck *et al.*, 2010).

2.2.1 Apple

The cultivar originated from the Coastline around the Malindi area. Fruits are medium to large and almost round in shape with an average weight of 397g. Has a yellow orange to red colour when ripe (Griesbach, 2003). It is a non-polyembryonic cultivar and trees are propagated by seed. Fruits are varied in shape, colour and quality (Kehlenbeck *et al.*, 2010). Photograph 1 shows mature unripe Apple mango fruits.

Plate 1: Mature fruits of Apple mango variety (Source: http://www.redfox.executive.com)

2.2.2 Keitt

The cultivar has its origin from Homestead, Florida (Griesbach, 2003). It yields large fruits with a greenish yellow colour, a pink or red blush and a lavender bloom (Mukherjee, 1997). The fruit weighs 456g on average. It has a small mono-embryonic seed (Crane, 2005). The flesh is deep yellow with little fibre and a rich sweet flavour with a pleasant aroma (Griesbach, 2003). Photograph 2 shows mature unripe fruits of Keitt mango cultivar.

Plate 2: Mature unripe fruits of Keitt mango variety (Source: http://www.21food.com)

2.2.3 Kent

It is a seedling of the cultivar Brooks with its origin in Miami, Florida and is quite similar to Keitt (Griesbach, 2003). Kent is reportedly a cross between Brooks and Haden (Olano *et al.*, 2005).

The fruit has a regular oval shape, with plump cheeks, greenish-yellow color with red shoulder and weighs approximately 545g. The seed is mono-embryonic (Griesbach, 2003). The skin is thick and tough with numerous small yellow lenticels. The fruit is very sweet with rich flavour and fiber-free flesh (Kehlenbeck *et al.*, 2010). Photograph 3 shows a mango tree of Kent variety with mature fruits.

Plate 3: Mature unripe fruits of Kent mango variety (http://toptropicals.com)

2.2.4 Tommy Atkins

The cultivar originated from Florida. The fruit has a regular oval shape with medium to large sized fruits weighing approximately (Griesbach, 2003). The ripe fruit is yellowish-orange with deep red to purple blush and an average weight of 522g. It is thickly skinned, juicy but firm with yellow to deep yellow flesh with medium fiber.

The cultivar is highly resistant to diseases and is developed and grown for commercial export. The seed is mono-embryonic (Griesbach, 2003). Photograph 4 shows ripe mango fruits of Tommy Atkins variety.

Plate 4: Mature fruits of Tommy Atkins variety (http://toptropicals.com)

2.2.5 Ngowe

Has its origin from Zanzibar from where it spread, first to Lamu then to the medium altitude locations along the coastline. The fruit is large, oblong and slender with a prominent hook-like beak at the apex and weighs approximately 523g. The seeds are poly-embryonic. Has a pale green colour when unripe but turns to yellow and finally orange when fully ripe and forms a yellow fibre free flesh (Griesbach, 2003). Photograph 5 shows ripe mango fruits of Ngowe.

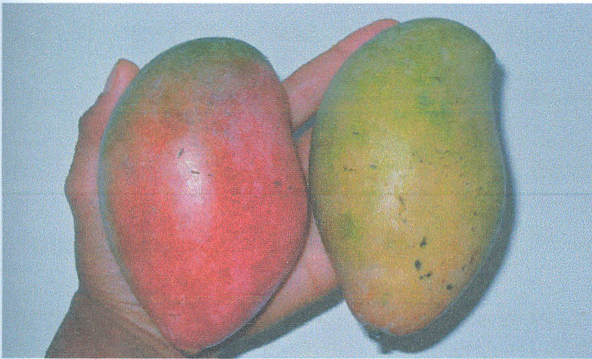

Plate 5: Ripe fruits of Ngowe mango variety (http://encyclopedia.mitrasites.com)

2.2.6 Ndoto

The cultivar is widely cultivated in many parts of the lower Eastern especially Machakos, Mwala, Kitui and the larger Makueni District.

This indigenous cultivar has not been fully characterized and though popular in the districts mentioned, there is lack of sufficient information to help in its identification. The cultivar has been mentioned in a previous research study to determine β-carotene in mangoes (Muoki, 2007). The cultivar is quickly been replaced by exotic varieties through grafts such as Apple on Ndoto parent stocks in Machakos county. The mango weighed 181.65±13.67g in this study. Photograph 6 show mature mango fruits of Ndoto variety taken during field work.

Plate 6: Mature fruits of Ndoto mango variety (Field photograph)

2.3 Mango production, availability and marketing in Kenya

Mango output in Kenya has increased steadily over the past decade compared to other major fruits such as bananas and oranges to the current level of 10 tonnes per hectare (FAO-UN, 2006). Mango production is primarily rain fed but new orchards under irrigation have been established for production of exotic varieties where water is available. Mangoes are available almost all year round due to variation in climatic conditions (Griesbach, 2003). Mango supply peaks between October and February with Eastern and Coast provinces being the leading mango production regions in Kenya (Kehlenbeck, 2010). Local varieties such as Ngowe are predominantly produced in the whole of Coast province while apple is the main exotic variety cultivated in Lamu, Malindi and Kilifi districts (Moturi *et al.*, 2010). Only one crop is produced per year in Eastern province while two harvesting seasons occur in Coast province.

The mangoes are mostly consumed locally while a small proportion comprising high quality fruits of exotic varieties are preserved for the export market (FAO, 2001). A small proportion of Kenyan mangoes are processed due to lack of processing facilities (Msabeni *et al.*, 2010). Large quantities of ripe and unripe mango are processed into various other forms, such as puree, juices, nectars, concentrates, pickles and chutneys, canned slices, and dried fruit, products which have worldwide popularity.

2.4 Mango growing in Mwala

Mwala Division of Machakos County, Kenya, lies within the semi-arid areas and is characterized by a topography comprising of undulating hills and seasonal streams (UNEP, 2010). The region relies mainly on rainwater for agricultural activities including mango growing. Farmers have diversified to include exotic mango varieties in addition to local varieties, which has greatly contributed to food security, better nutrition and family income in this region (The Anchor, 2011). Some of the local mango varieties grown in Mwala include Ndoto, Ngowe, Boribo and Batawi among others. Exotic varieties include (Apple, Tommy Atkins, Haden, Sensation and Van Dyke (KARI, 2012).

2.5 Nutraceutical composition and variation in mango (*Mangifera indica L.*)

Nutraceutical content in mangoes appears to vary across mango varieties (FAO-UN, 2006). Carotenoid composition is affected by factors such as cultivar or variety, part of plant sample, stage of maturity, climate or geographic site of production, harvesting and post-harvest handling and processing (Kim *et al.*, 2007; Rodriguez-Amaya, 2001) . Variation in harvest period as well as the analytical methods may impact on carotenoid levels (Tokusogulu and Hall, 2008). However stage of maturity is the main factor that affects carotenoid composition in mangoes and other fruits (Rodriguez-Amaya *et al.,* 2007).

Maturation and ripening in fruits is accompanied by enhanced carotenogenesis leading to a markedly increase in both number and quantity during ripening (Rodriguez-Amaya and Kimura, 2004). There is a considerable variation in mango carotenoid content between varieties of mangoes (Perkins-Veazie, 2007 and Rodriguez-Amaya, *et al.*, 2007). Literatures discrepancies are likely due to a number of factors including the stage of fruit ripening and cultivar (Laura, *et al.*, 2010, Perkins-Veazie, 2007).

Among mango cultivars imported into US, Ataulfo had the highest amount of β-carotene (26 mg/Kg) compared to Kent, Haden, Tommy Atkins and Keitt with β-Carotene content raging between 5mg/Kg and 16mg/Kg of puree (Perkin-Veazie, 2007). A FAO nutrient composition survey of different fruits indicated β-carotene and β-cryptoxanthin levels of 4520 and 40 μg/100g pulp of Badami mango, 670 and 20 μg/100g pulp in Keitt and 580 and 30 μg/100g pulp of Tommy Atkins respectively with significant variation at different stages of ripening (FAO, 2008). Mercadante *eta al.*, reported increases in β-carotene from approximately 2 to 7 μg/g during ripening of Keitt mango cultivar (Mercadante *eta al.*, 1997). During ripening of Tommy Atkins cultivar, increases in β-carotene were approximately 6-22 μg/g (Mercadante and Rodriguez-Amaya, 1998).

Significantly higher average levels of β-carotene concentration have been reported in Ataulfo cultivar compared to other mango cultivars tested (Manthey and Perkin-Veazie, 2009). A comparison of β-carotene levels by Perkins-Veazie had a similar trend; Ataulfo 27.9 μg/g, Kent 16.6 μg/g, Keitt 8.3 μg/g, Haden 6.7 μg/g and Tommy Atkins 4.6 μg/g (Perkins-Veazie, 2007). Studies show that composition of carotenoids in fruits and vegetables depend on several factors such as cultivar type, stage of maturity and climate or geographic part of production (Kudachikar *et al.*, 2001).

Other factors include farming practices, part of the plant consumed, processing and food storage (Rodriguez-Amaya, 2001). In most carotenoid-containing fruits, ripening is accompanied by enhanced carotenoid biosynthesis as chlorophylls decompose and the chloroplasts are transformed into chromoplasts (Rodriguez-Amaya *et al.*, 2007). Maturity of the plant food when harvested is the major factor that affects carotenoid content when harvested and offered for consumption (Maria, 2008). Carotenoid biosynthesis in the flesh of ripening Indian Alphonso mango was observed to be maximal at tropical ambient temperatures (28-32°C) (Thomas and Janave, 1975). Some studies indicate that climatic effects could surpass cultivar differences and that greater exposure to sunlight and elevated temperatures heighten carotenoid biosynthesis in fruits (Rodriguez-Amaya, 2001).

2.6 Mango maturity and postharvest handling

Mango maturity depends mainly on the cultivar and environmental conditions (Kader, 2008). It takes 90 to 160 days after flowering for Kenya mangoes to reach maturity (Griesbach, 2003). Maturity of mangoes occurs at different times even for mangoes of the same cultivar on the same tree (Kader and Mitcham, 2008). The precise stage at which the fruits are ripe for picking is not easy to determine (Kudachikar, 2001). However the fruit develops its best flavor when allowed to ripen on the tree (Saranwong *et al.*, 2005). The fruit may be picked when colour on the fruit's surface begins to change (Sirinnapa *et al.*, 2004). A destructive maturity test may also be carried out by cutting the fruit into two halves and examining the colour of the flesh around the seed (Slaughter, 2009). When the colour changes from greenish white to yellow or orange, it indicates the fruit is ripening (Saranwong *et al.*, 2005). The swelling of the shoulders above the stalk attachment is an indicator of maturity and commencement of ripening (Slaughter, 2009). Table 1 shows the maturity seasons of various mango varieties found in Kenya.

Table 1: Maturity seasons of various mango cultivars found in Kenya.

SEASON	PERIOD	CULTIVAR
Early season cultivars	November to mid-January	Apple, Ngowe, Haden and Ndoto
Mid-Season Cultivars	Mid-January to Late February	Boribo, Van-Dyke and Tommy Atkins
Late-season Cultivars	March to June	Kent, Keitt and Sensation

Source: Griesbach, 2003

The fruit is highly perishable and requires proper postharvest handling and care. It is picked from the tree by cutting the fruit stalk about 2 cm from the fruit and placing the mangoes in shallow single layered plastic trays, packed in cartons and transferred into a cold storage compartment at 7-10°C (Griesbach, 2003).

2.7 Nutritional and health benefits of mangoes

The mango is known to be an excellent source of many vitamins such as ascorbic acid, thiamine, riboflavin, and niacin, and essential dietary minerals (Kimberly, 2009). The antioxidant vitamins A, C and E comprise 25%, 76% and 9% of the dietary reference intake (DRI) in a 165 grams serving of the pulp, Vitamin B6 (pyridoxine, 11% DRI), Vitamin K (9% DRI), other B vitamins and essential nutrients such as potassium, copper and 17 amino acids are at good levels (Fowomola, 2010). Mango peel and pulp contain other phytonutrients, such as the pigment antioxidants -carotenoids and polyphenols, as well as omega 3 and 6 polysaturated fatty acids (Shah *et al.*, 2010). Lupeol, a triterpene in mangoes is an effective inhibitor in laboratory models of prostate and skin cancers (Fowomola, 2010). Vimang, an extract of mango branch bark isolated by Cuban scientists contains numerous polyphenols with antioxidant properties in vitro and on blood parameters of elderly humans (IQF, 2010).

Mango phenolic compounds have been demonstrated to have anti-proliferative and pro-apoptotic activities against human leukemia as well as human lung, breast, colon and prostate cancer cells (Masibo and He, 2008). Mango is particularly useful food when it comes to the fight against diabetes (GDC, 2006). In addition mangoes also contain high levels of oils (Fowomola, 2010). Recently much attention has been given to phytochemicals and the distinctive roles they play in anti-inflammatory and anti-cancer properties related to the consumption of fruits and vegetables (Kimberly, 2009).

2.8 Nutraceuticals and role in human health

Nutraceuticals are non-nutritive plant chemicals or phytochemicals that have been shown to have protective or disease preventive properties (CFNI, 2005). There are more than a thousand known nutraceutical compounds (Whitney and Rolfes, 1999). It is well known that plants produce these nutraceutical compounds for their own protection but recent research demonstrates that they can also protect humans against diseases (Shah *et al.*, 2010). Some of the well-known nutraceutical compounds are β-carotene, β-cryptoxanthin, lycopene and violaxanthin in mangoes and other fruits (Balch, 2006). Foods containing nutraceutical compounds are already part of daily diet and some foods such as whole grains, vegetables, beans, fruits and herbs contain many nutraceutical components (CFNI, 2005).

Nutraceutical components function in the body as antioxidants (Krinsky *et al.*, 2004). Antioxidants are substances that protect cells from the damage caused by very reactive molecular species with odd number of electrons known as free radicals (Shah *et al.*, 2010; Krinsky *et al.*, 2004). Free radicals mainly arise during oxidation reactions and readily attach to other molecules with which they come into contact (Whitney and Rolfes, 1999).

The action of free radicals increases the risk of diseases such as Alzheimer, cancer and heart problems and could accelerate aging (Manukau, 2009). The action of antioxidants is often described as "mopping" of free radicals (NCI, 2004). That is, they neutralize the electrical charge and prevent the free radical from taking electrons from other molecules by receiving or donating an electron because they are stable in both forms (Krinsky *et al*, 2004). They neutralize free radicals as the natural by-product of normal cell processes (NCI, 2004).

2.9 Carotenoid nutraceutical compounds

2.9.1 General characteristics

Carotenoids are yellow, orange and red pigments synthesized by plants (Irwandi *et al.*, 2011). They are valuable antioxidants (Krinsky *et al.*, 2004). Food carotenoids are usually C_{40} tetraterpenoids built from eight C_5 isoprenoid units (Rodriguez-Amaya, 2001). They have a distinctive extensive conjugated double-bond system as the light absorbing chromophore (Krinsky *et al*, 2004). This physical property imparts the characteristic red/yellow color of the pigments responsible for the yellow, orange, or red colour on many carotenoid foods (Rodriguez-Amaya, 2001). Generally, the deep color of the fruit or vegetable is an indication of a higher concentration of carotenoids (Krinsky *et al.*, 2004).

More than 600 carotenoids have been found in plants (Balch, 2006). Carotenoids also give colour to salmon, goldfish, flamingoes and autumn leaves (Rodriguez-Amaya, 2001). The human diet contains approximately 50 carotenoids, half of which are absorbed in the blood stream. Carotenoids are further transported by blood in lipid rich cholesterol particles known as low-density lipoproteins (Borek, 2004).

Carotenoids are nearly insoluble in water and are best absorbed when associated with oils with β-carotene and lycopene constituting about 30% each of plasma carotenoids (Krinsky *et al.*, 2004).

2.9.2 The structure of carotenoids

Carotenoids have a basic structure made up of isoprene units joined end-to-end to give a conjugated chain which is common to all carotenoids (Rodriguez-Amaya and Kimura, 2004). The two centre isoprene units are joined differently to the others ("head-to-head" rather than "head-to-tail") so that the chain has a centre of symmetry (Rodriguez-Amaya, 2001).

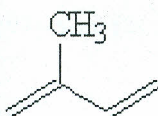

Figure 1 (a): An isoprene unit

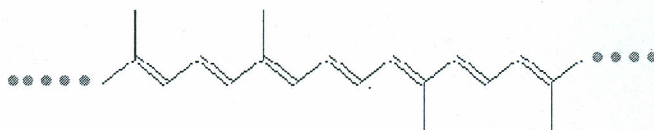

Figure 1(b): The central part of a carotenoid

Hydrocarbon carotenoids are collectively called carotenes while those containing oxygen are termed xanthophylls (Genox Inc., 2010). In nature, they exist primarily in the more stable all-trans isomeric form, but cis isomers do occur in plants as well as in some animal foods (Rodriguez-Amaya, 2001). As many as 50 carotenoids may be absorbed and metabolized by the human body and only 14 have been identified in the human serum (Genox Inc., 2010).

The ability of carotenoids to quench singlet oxygen is related to the conjugated double-bond system, and maximum protection is given by those having nine or more double bonds (Rodriguez-Amaya, 2001).

2.9.3 Importance of carotenoids to human health

Carotenoids have been shown to have numerous health promoting effects which are related to their structure and reactivity.

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Pro-vitamin A carotenoids are important sources of vitamin A or retinol (LaChance, 2008). Most carotenoids are associated with reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and macular degeneration (Rodriguez-Amaya and Kimura 2004). In addition carotenes also offer protection from heart disease since their antioxidant behavior protects the lining of the arteries and the fats in the blood from free radicals' oxidative damage (Jacob, 2009). Age-related macular degeneration of the eye, which leads to vision loss, may be counteracted by carotenes' antioxidant power (Nikki, 2010). There is no known carotenoid deficiency but rather, deficiency symptoms are instead linked to vitamin A deficiency (Rodriguez-Amaya, 1999).

2.10 β-Carotene

2.10.1 Characteristics and structure of β-carotene

The carotenoid is the most widespread and potent pro-vitamin A carotenoids (Bowden, 2008). The structure of vitamin A represents one half of the β-carotene. Cyclization occurs at both ends. The long conjugated chain is responsible for the orange colour of β-carotene (Krinsky *et al*, 2004).

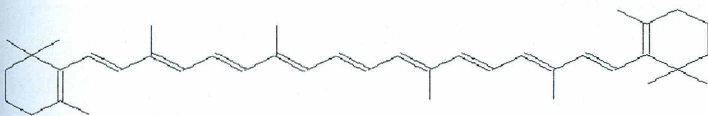

Figure 2: The structure of β-carotene

2.10.2 Health benefits of β-carotene

β-Carotene is the most efficient pro-vitamin A carotenoid. It can boost the activity of natural killer immune cells; stimulate DNA repair enzymes, gives protection against UV-light (Rodriguez-Amaya *et al.,* 2006).

It acts as an antioxidant reducing the risk of heart diseases, strokes, eye and skin disorders (Krinsky *et al.*, 2004). It stimulates the immune system, lowers serum cholesterol, and is converted into vitamin A as needed (Bowden, 2008). Epidemiology studies have reported an inverse correlation between β-carotene and cancer, cardiovascular diseases and cataract (Karen and Lutz, 2001). Average daily intake of β-carotene is 2.4-3.0 mg for career adults. It is abundant in human serum and a variety of tissues (LaChance, 2008).

2.11 β–Cryptoxanthin

2.11.1 Characteristics and structure of β-cryptoxanthin

β-Cryptoxanthin is a widely distributed xanthophyll, although at generally low levels (Rodriguez-Amaya, 2001). It is a fat soluble phytochemical substance present in many vegetables and yellow/orange fruits, mainly papaya, peaches, oranges, tangerines, pumpkin, mangoes, carrots, bell peppers, corn and water melon (Mateljan, 2010). It is also found in some yellow coloured animal products such as egg yolk and butter (Rodriguez-Amaya, 2001).

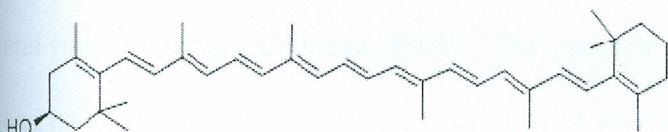

Figure 3: The structure of β-cryptoxanthin

2.11.2 Health benefits of β-cryptoxanthin

β-Cryptoxanthin is a strong antioxidant that counters the action of free radicals which can damage the body cells and DNA (Krinsky *et al.*, 2004). It is an important source of vitamin A (Bowden, 2008). Research suggests that β-cryptoxanthin may promote the health of the respiratory tract and serum concentrations of this carotenoid have been found to be associated with improved lung function as measured by functional tests (Mateljan, 2010).

It provides health benefits to the people with various chronic diseases such as lung cancer, colon cancer, laryngeal cancer (cancer of the larynx), cervical cancer, prostate cancer, skin cancer, heart disease, photosensitivity, rheumatoid arthritis or polyarthritis (Rodriguez-Amaya *et al.*, 2006). Studies show that it can reduce the risk of lung and colon cancer by more than 30% and the risk of rheumatoid arthritis by 41% (Wilkinson, 2008). One daily additional serving of a food high in β-cryptoxanthin helped reduce arthritis risk (Mateljan, 2010).

2.12 Lycopene

2.12.1 Characteristics and structure of lycopene

Lycopene is one of the most common acyclic carotene and a principal pigment of many red-fleshed fruits and fruit vegetables (Rodriguez-Amaya, 2001). It has 11 conjugated double bonds in the all-*trans* configuration which accounts for the red colour of lycopene and its powerful antioxidant activity (Bramley, 2000). It is a natural phytochemical pigment responsible for the deep red colour of tomatoes, apricots, papayas, mangoes, guava, water melon and pink grape (Mateljan, 2010). Its structure consists of a long chain of conjugated double bonds with two open end rings (Rodriguez-Amaya, 2001). The structure of lycopene is the longest of all carotenoids and its antioxidant activity is much stronger (Kin-Weng, 2010).

Figure 4: The structure of lycopene

2.12.2 Health benefits of lycopene

Lycopene is a powerful antioxidant which reduces damage to DNA and proteins and gives better protection against UV-light compared to β-carotene (Rao and Rao, 2007).

The carotenoid concentrates in the skin, testis, adrenal and prostrate where it protects against cancer (Rai, 2011; Roy *et al.*, 2005). It reduces cholesterol levels and suppresses Insulin-like Growth Factor 1 (IGF-1) stimulation of tumor growth. Lycopene is generally known for its protective action against prostate cancer (Kong, 2010).

In-vitro studies have shown the anti-cancer properties of lycopene against many cancer cells, including cancer cells of prostate, stomach, lung, colon and skin (Bramley, 2000). Lycopene also shows anti-mutagenic action against chemically induced DNA damage (Krinsky *et al.*, 2004). Lycopene possesses antibacterial and antifungal properties (Sue, 2010). Lycopene helps to lower the risk of diabetes and may improve immune responses (Ilfeld, 2005). Treatment of cells with Lycopene protects cells against DNA damage and lipid peroxidation (Krinsky *et al.*, 2004). Lycopene inhibits platelet aggregation and reduces the production of foam cells which play an important role in the development of arteriosclerosis (Duttaroy, 2005). Intake of lycopene and lycopene rich foods can reduce the risk of heart diseases and atherosclerosis (Omoni, 2008). Reduced blood levels of lycopene are linked to colorectal cancers (Mateljan, 2010). A daily intake requirement for protection is about 6.5 mg (Lycored, 2010). Lycopene has also been widely used as a food colorant for many years (Bramley, 2000).

Though there are no official recommended nutrient intake (RNI) level set by health professionals and government regulatory agencies, based on reported studies, a daily intake level of 5–7 mg lycopene in normal healthy human beings may be sufficient to maintain circulating levels of lycopene at levels sufficient to combat oxidative stress and prevent chronic diseases (Rao and Rao, 2007).

2.13 Stability of carotenoids

Carotenoids are naturally protected in plant tissues and cutting, shredding, chopping and pulping of fruits and vegetables increase exposure to oxygen and bring together carotenoids and enzymes that catalyze their oxidation (Rodriguez-Amaya, 2001). Carotenoids have different stabilities and susceptibilities to degradation. Storage and processing are the main sources of carotenoids degradation in fruits and vegetables (Mercadante and Rodriguez-Amaya, 1998).

Retention of carotenoids is greatly increased by reducing the processing time, lowering the temperature and time between peeling, cutting or pureeing and analysis (Rodriguez-Amaya, 1999). Isomerization of trans-carotenoids to cis-carotenoids occurs quite often during heat treatment (Rodriguez-Amaya and Kimura, 2004).

2.14 Physicochemical properties of carotenoids

2.14.1 Solubility

Most carotenoids are lipophilic (Krinsky *et al.*, 2004). They are insoluble in water but soluble in organic solvents, such as acetone, alcohol, ethyl ether, chloroform and ethyl acetate. Carotenoids are readily soluble in petroleum ether, hexane and toluene; xanthophylls dissolve better in methanol and ethanol (Rodriguez-Amaya, 2001).

2.14.2 Light absorption and identification

The conjugated double-bond system constitutes the light absorbing chromophore that gives carotenoids their attractive colour and provides the visible absorption spectra used for their identification and quantification (Krinsky *et al.*, 2004). The greater the number of conjugated double bonds, the higher the λ_{max} values (Rodriguez-Amaya and Kimura, 2004). The wavelength of maximum absorption (λ_{max}) and the shape of the spectrum are characteristic of the chromophore (Krinsky *et al.*, 2004).

Most carotenoids absorb maximally at three wavelengths, resulting in three-peak spectra (Rodriguez-Amaya, 2001). Thus, lycopene being the most unsaturated acyclic carotenoid with 11 conjugated double bonds is red and absorbs at the longest wavelengths of λ_{max} at 444, 470 and 502 nm. β-Cryptoxanthin absorbs at lower values of 425, 449 and 476 nm in petroleum ether while β-Carotene absorbs at 425, 450 and 477 nm in petroleum ether and hexane (Rodriguez-Amaya, 2001). However, the absorption spectra of carotenoids are highly solvent dependent (Krinsky *et al.*, 2004).

2.14.3 Isomerization and oxidation of carotenoids

Light, heat, acids and adsorption on an active surface (e.g. alumina) promote isomerization and oxidation of carotenoids (Krinsky *et al.*, 2004). Highly unsaturated carotenoids are prone to isomerization from their usual trans-configuration to the cis form, resulting in some loss of colour and provitamin activity (Rodriguez-Amaya, 2001). Different carotenoids have different susceptibilities to isomerization (FAO, 2008). Conditions necessary for isomerization and oxidation of carotenoids exist not only during preparation, processing and storage of food, but also during analysis and thus preventive measures must be taken to guarantee the reliability of analytic results (Rodriguez-Amaya, 2001).

2.15 Carotenoid analysis

2.15.1 Steps and procedures in carotenoid analysis

Carotenoid analysis is a difficult and error prone procedure and accuracy of analytical results is highly dependent on the analyst's experience, understanding of the carotenoid properties, analytical procedures and laboratory conditions (Rodriguez-Amaya and Kimura, 2004).

However, the analytical procedure is carried out efficiently in several steps that take into consideration the unique reactions of carotenoids and include sampling, isolation or extraction from plant or animal material, identification, chromatographic separation and quantification of the carotenoids (Rodriguez-Amaya, 2001).

2.15.2 Sampling and sample preparation

Sampling refers to the identification of the population from which the sample is to be obtained, selection and withdrawal of valid gross samples and reduction of the gross sample to suitable laboratory analytical samples (Rodriguez-Amaya and Kimura 2004). Sampling is carried out to secure a portion of the material that satisfactorily represents the whole while the purpose of sample preparation is to homogenize the large samples in the laboratory and subsequently reduce it in size and amount for analysis (Pomeranz and Meloan, 1994).

2.15.3 Extraction of carotenoids

This refers to the procedure carried out in order to release all the carotenoids from the food matrix and bring them into solution without altering them (Rodriguez-Amaya, 2001). Different methods of extraction may be carried out depending on the type and nature of food material being handled. Solvents with low boiling points should be used to enable efficient recovery of carotenoids and avoid prolonged heating (Rodriguez-Amaya and Kimura, 2004).

Water miscible or more polar solvents such acetone and tetrahydrofuran are preferred for better interactions with the food matrices especially biological samples containing large amounts of water (Kimura and Rodrigues-Amaya, 2002). A pestle and mortar or mechanical blender is used for homogenization of the sample with the solvent (Kimberly, 2009). Calcium carbonate or magnesium carbonate and butyrated hydroxytoluene are normally added to neutralize cytosolic acids and prevent degradation of carotenoids (Khachik, 1991).

Filtration and re-extraction is carried out, at least three times until residue is colourless (Muller, 1997). Water is subsequently removed from the extract by partitioning to a suitable solvent such as hexane or petroleum ether, the lower phase discarded and extract washed several times with water to remove residue extractant (Rodriguez-Amaya, 2001).

Saponification is usually carried out to remove chlorophylls and unnecessary lipids which may interfere with chromatographic separation (Pomeranz and Meloan, 1994). This may be achieved by adding an equal volume of 10% methanolic potassium hydroxide to the extract in hexane or petroleum ether. The mixture is left overnight in the dark at room temperature after which the mixture is washed three times with water to remove the alkali (Rodriguez-Amaya, 2001).

2.15.4 Chromatographic separation and identification of carotenoids

Open Column Chromatography (OCC) and High Performance Liquid Chromatography (HPLC) have been successfully used for separation of carotenoids (Rodriguez-Amaya and Kimura, 2004). In OCC, a column has to be packed for each analysis while HPLC allows separations with a reusable column, under controlled conditions, without undue exposure to air or light. Reverse-Phase HPLC on C_{18} columns is preferred due to its weak hydrophobic interactions with the analytes. In addition they are compatibility with most carotenoid solvents and the polarity range of carotenoids, and have wide commercial availability (Scott, 2003). Many different C_{18} reversed-phase materials are available from different manufacturers and vary in the degree of carbon loading, end capping, and the nature of the bonded phase (i.e., monomeric or polymeric). Most carotenoid separations have been carried out with 5-µm C18 spherical particles packed in a 250 × 4.6 mm column (Rodriguez-Amaya, 2001). Carotenoids are identified on the basis of retention time and absorption spectra compared to those of carotenoid standards (Laura *et al.*, 2010).

The ultraviolet and visible spectrum is the first diagnostic tool for the identification of carotenoids and the wavelength of maximum absorption (λ_{max}) and the shape of the spectrum are characteristic of a particular chromophore (Brittons, 1995).

2.15.5 Quantification of carotenoids

Carotenoids are quantified spectrophotometrically by either Open Column Chromatography (OCC) or High Performance Liquid Chromatography (HPLC). This is because in solution they obey the Beer-Lambert law, that their absorbance is directly proportional to concentration (Rodriguez-Amaya, 2001). Carotenoids are quantified by integrating peak areas in the HPLC chromatograms (Perry *et al.*, 2009). However, quantification depends on availability of accurate absorption coefficients (Rodriguez-Amaya, 2001). In HPLC the availability of the photodiode array detector allows the acquisition of the spectra on-line, making the use of this criterion easier. The spectra is taken, stored, and subsequently compared with those of standards. Spectra taken at points across the peak provide a means of verifying peak purity (i.e., absence of interfering substances) while in OCC, enough isolated carotenoids are collected to submit to chemical tests (Rodriguez-Amaya, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 The study area

Sampling was carried out in two mango farms in the sub-county of Mwala located within Machakos County. A large number of local and exotic mango varieties are grown in this area for local consumption, commercial processing and the horticultural export market. Two farms were selected on the basis of availability of particular varieties identified for this study labelled 'Farm 1' and 'Farm 2'. All the six varieties used in this study were present in 'Farm 1' but Keitt, Kent and Ndoto were not present in 'Farm 2'. The two farms were separated by a distance of about 33 km but within the same geographical region of Mwala.

3.2 Pre-sampling procedures

Identification and monitoring of the mango maturing process was carried out with the help of a taxonomist from KARI and a mango growing specialist from Mwala. To enhance uniformity and validity of the analytical procedure, tags were placed on the mango tree blooms at fruit set to prepare for mango collection exactly 140 days (DAFS) when the fruits had attained market maturity. Fruit shape ('fullness' of cheeks or shoulders) and flesh colour were also used to enhance identification of the mangoes which had attained the 'mature unripe' stage over the same period (Kader *et al.,* 2008; Slaughter, 2009). At this stage the mangoes were referred to as 'mature unripe' to distinguish them from the 'ripe stage' after keeping the 'mature unripe' mangoes at a storage facility for 14 days at market ripening conditions (DMRC).

3.3 Sample collection

Sampling was carried out according to the maturity seasons of the mango varieties used in this study. A sample size of 30 mangoes for each variety at each of the two stages of maturation was chosen by purposive sampling, with due consideration to analytical protocols, time and laboratory space. The first set of sixty (30x2) 'mature unripe' mangoes consisting of Apple, Ngowe, Ndoto and Tommy Atkins were picked for each variety in January 2011. The second set of sixty 'mature unripe' mangoes consisting of Keitt and Kent were picked for each variety in March 2011. The samples were transported to the KEBS laboratory in an air conditioned sample kit. Both sets were collect early in the morning and transported to the laboratory immediately to conserve their moisture natural attributes. For each cultivar, the fruits were divided into two sets of thirty mangoes each. One set of the mature unripe mangoes from each variety were kept in a special laboratory storage and ripening facility at 21 °C and at 90-95% relative humidity to allow them to ripen fully over a period of 11 days for analysis at the ripe stage (Slaughter, 2009). Sample pretreatment and preparation was carried out on the second set for each variety and immediately analysed for β-carotene, β-cryptoxanthin and lycopene content. Laboratory analytical samples were prepared and analysed in triplicate.

3.4 Apparatus and instruments

Amber coloured volumetric flasks, beakers, amber coloured vials, conical flask, micro-liter syringe, pestle and mortar, round bottomed. Other apparatus included: separatory funnel with Teflon stopcock, glass funnels, Whatman no. 42 filter paper, aluminum foil, labels, freezer, rotary evaporator, orbital shaker, reverse phase HPLC system with variable UV-VIS detector.

3.5 Reagents

HPLC grade solvents: methanol and hexane (Panreac), tetrahydrofuran (Fisher Scientific), acetonitrile(Lab-Scan) and triethylamine (Merck, SA), reagent grade acetone (Sigma-Aldrich, USA), butylated hydroxyl toluene (BHT) -Loba Chemie, calcium carbonate (Merck, Germany), HPLC grade water (Panreac), distilled water, source of nitrogen/argon, anhydrous sodium sulphate. The standards: all-trans-β-carotene, all-trans-β-cryptoxanthin, and all-trans-lycopene were purchased from Sigma-Aldrich, USA.

3.6 Cleaning of apparatus

Apparatus were soaked in 2M nitric acid for 48 hours and then cleaned with a detergent then rinsed with distilled water and finally in acetone. The vials were first soaked in dichloromethane before the cleaning. Extraction and storage vessels were flushed with nitrogen before use to protect carotenoids from oxidation and isomerization brought about by oxygen in air (Rodriguez-Amaya and Kimura, 2004).

3.7 Experimental and analytical procedures

3.7.1 Sample preparation and pretreatment

The mango fruits were peeled, cut into four quarters and opposite quarters lyophilized (freeze dried at -25 ºC) then ground up using a pestle and mortar after removal of the seed prior to extraction and analysis (Jaroslaw *et al.*, 2000; Manthey and PerkinsVeazie, 2009).

3.7.2 Extraction of the carotenoids

Extraction procedure was carried out according to previously published methods with some modifications (Yahia, 2006; Rodriguez-Amaya and Kimura, 2004). Saponification was avoided to reduce analytical time, loss of xanthophyll carotenoids through washing and minimize carotenoid degradation and isomerization (Rodriguez-Amaya, 2001).

A 5.0g of ground sample was placed in a 100 mL low actinic extraction vessel and a 5 mL aliquot of distilled water added to rehydrate the sample. The sample was then mixed with 20 mL methanol containing 0.1% BHT (antioxidant) and 0.5g calcium carbonate (acid neutralizing agent). The mixture was sonicated for 2 minutes then extracted with 50 mL acetone-hexane mixture (2:3 v/v) stabilized with 0.1% BHT and homogenized on an orbital shaker for 10 minutes on ice. The liquid phase was separated from the solid phase by filtration using Whatman no 42 filter paper. The solid phase was separated cleanly from filter paper and both placed in the extraction vessel and extracted two more times using 10 mL of the acetone-hexane mixture until no appreciable colour was observed in the filter paper and the solid phase. All filtrates were saved and pooled. The pooled filtrates were placed in a 125 mL separatory funnel and 40 mL of 10% sodium chloride added for phase separation. The bottom layer was discarded and upper layer dried by filtering over 5g of anhydrous sodium sulphate. The extracts were evaporated to near dryness in a 250 mL round bottomed flask with a rotary evaporator attached to a vacuum pump at 35ºC. The residue was re-dissolved in 10 mL of hexane and stored under nitrogen gas at -20 °C until HPLC analysis. Samples were prepared and analysed in triplicate.

All operations were carried out within the shortest time possible at 21 ºC and in dim (subdued) light; equipment and glassware covered by black cloth or aluminium foil and analytical samples placed in low actinic (Amber coloured) glassware to protect carotenoid extracts from light oxidation. Each extraction solvent was stabilized with 0.1% BHT (as an antioxidant). Extracts, samples and standard residues were stored at very low temperatures (-21ºC) in hexane. Acetone rinsing of glassware was carried out before and after the usual laboratory washing to avoid contamination with carotenoids of previous analyses.

3.7.3 Preparation of β-carotene standard stock and working solutions (100ppm)

3.7.3.1 Description of β-carotene standard

The analytical description of β-carotene standard is given in Table 2

Table 2: Description of β-carotene standard

Source	CAS	MF	FW	Purity (%)	MSDS
Sigma-Aldrich, USA	7235-40-7	$C_{40}H_{56}$	536.9	· 93	C-9750

3.7.3.2 Preparation of all-trans- β-carotene standard stock solution (100ppm)

About 5 mL of Ethanol (98%) were injected into the product vial and shaken for 2 minutes or until fully dissolved then transferred into a 10 mL amber coloured volumetric flask. The flask was filled to the mark with ethanol washings from the product vial, capped properly and store under -20°C until required for use.

3.7.3.3 Preparation of all-trans-β-carotene standard working solution (10ppm)

Exactly 1 mL of standard stock solution was placed into a 10 mL amber coloured volumetric flask, dried with nitrogen and reconstituted in 10 mL methanol: acetonitrile (90:10, v/v) solvent mixture, shaken and made to the mark. The standard working solution was well capped and stored under -20°C until HPLC analysis. The solution was used within 2 weeks.

3.7.3.4 UV-VIS spectrophotometric determination of all-trans- β-carotene standard stock solution

The spectrophotometer was warmed up as per the manufacturer's instructions and zeroed with ethanol in a cuvette. A cuvette containing the carotenoid standard working solution was placed into the sample cell holder and scanned for wavelength of maximum absorbance, λ_{max}. The absorbance measured immediately at the λ_{max}.

Three runs were taken and the mean absorbance calculated. The concentration of the standard working solution for all trans-β-carotene was calculated using the formula below:

$$C_X\,(\mu g\,/\,mL) = \frac{A_X\ X\ 10000\ X\ Purity\ of\ std}{Molar\ Extinction\ Coefficient,\ \varepsilon\ X\ 100}$$

Where; $\mathbf{C_x}$ is the concentration of the β-carotene standard stock solution in μg/mL, $\mathbf{A_X}$ is the mean absorbance of the standard stock solution and $\mathbf{\varepsilon}$ is the Molar extinction coefficient of β-carotene in ethanol ($\varepsilon_{\beta\text{-Carotene}}$ in ethanol at λ_{max} of 451nm = 2560).

3.7.4 Preparation of all-trans-lycopene standard stock and working solution (100ppm)

3.7.4.1 Description of all-trans-lycopene standard

The analytical description of lycopene standard is given in table 3

Table 3: Description of lycopene standard

Source	CAS	MF	FW	Purity (%)	MSDS
Sigma-Aldrich, USA	502-65-8	$C_{40}H_{56}$	536.87	96	L9879-1MG

3.7.4.2 Preparation of all-trans-lycopene standard stock solution (100ppm)

About 5 mL of hexane (98%) was injected into the product vial and shaken until fully dissolved (no particles seen) then transferred into a 10 mL amber coloured volumetric flask. The flask was filled to the mark with hexane washings from the product vial, capped properly and stored at about -20 ^{o}C until required for use.

3.7.4.3 Preparation of all-trans-lycopene standard working solution (10ppm)

Exactly 1 mL of standard working solution was placed into a 10 mL amber coloured volumetric flask and evaporated to dryness using nitrogen gas.

Approximately 5 mL methanol: acetonitrile (90:10, v/v) mobile phase mixture was added into the flask and contents sonicated over ice to dissolve. The flask was then filled to the mark using with the mobile phase, capped well and stored under -20 °C until HPLC analysis. The solution was used within 2 weeks.

3.7.4.4 UV-VIS spectrophotometric determination of all-trans-lycopene stock solution

The procedure was carried out as described in the uv-vis spectrophotometric determination of the β-carotene standard working solution. $\varepsilon_{Lycopene}$ in hexane at λ_{max} of 472nm = 3450.

3.7.5 Preparation of all-trans-β-cryptoxanthin standard stock and working solution (100ppm)

3.7.5.1 Description of β-cryptoxanthin standard

The analytical description of β-cryptoxanthin standard is given in table 3

Table 4: Description of standard β-cryptoxanthin

Source	CAS	MF	FW	Purity (%)	MSDS
Sigma-Aldrich, USA	472-70-8	$C_{40}H_{56}0$	552.88	98	C 6368-1MG

3.7.5.2 Preparation of all-trans-β-cryptoxanthin standard Stock solution (100ppm)

The procedure was carried out as described in the preparation of the β-carotene standard stock solution.

3.7.5.3 Preparation of all-trans-β-cryptoxanthin standard working solution (10ppm)

The procedure was carried out as described in the preparation of the β-carotene standard working solution.

3.7.5.4 UV-VIS Spectrophotometric determination of all-trans-β-cryptoxanthin standard stock solution

The procedure was carried out as described in the uv-vis spectrophotometric determination of the β-carotene standard stock solution ($\varepsilon_{\beta\text{-cryptoxanthin}}$ in ethanol at λ_{max} of 452nm = 3456)

Each standard working solution was filtered using a 0.45µm pores syringe filter into 20 mL amber coloured vials or 10 mL beaker and covered with aluminium paper prior to injection into the HPLC system.

3.8 HPLC Analysis

3.8.1 Equipment description

A description of the equipment used is provided in tables 5-8

Table 5: HPLC column specifications

MODEL/ MAKE	P/No	Size	Serial number
Phenomenox (RP)	00F-4143-E0	150 x 4.60mm x 5.0µm	390336-1

Table 6: HPLC pump description

MODEL/ MAKE	Pin number	Serial Number
Prominence (LC), LC-20AT	228-45001-38	L201142 00796LP

Table 7: HPLC detector (Duterium lamp)

MODEL/ MAKE	Catalogue number	Serial number
Prominence UV-VIS, SPD-2OAV	228-45004-38	L201443 00254LP

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Table 8: HPLC Oven

MODEL/ MAKE	Catalogue number	Serial number
CTO-10AS	228-45059-38	C21044304471 SS

3.8.2 HPLC settings

HPLC conditions were set as indicated in the table 9 below.

Table 9: RP-HPLC settings

Settings	β-Carotene	β-Cryptoxanthin	Lycopene
Injection Volume	5 μL	5 μL	5 μL
Flow rate	2.0 mL/min	1.20 mL/min	1.0 mL/min
Wavelength (λ_{max})	451 nm	452 nm	471 nm
Temperature	25°C	25 °C	25 °C

3.8.3 Injection of standards and samples

The mobile phase (MetOH: ACN, 90:10 v/v containing 0.05% TEA) was degassed on a sonicator before use. Prior to HPLC analysis, stored standards and samples were brought to room temperature and filtered through a polyethylene membrane filter of 0.45 μm pores syringe filter into 20 mL amber coloured vials. A 5.0 μL aliquot of the standard was injected into the HPLC using a glass syringe followed by samples and at least three injections were made, each time taking the average. Triethanolamine was necessary to minimize the effects of acidity generated by the free silanol groups present on the silica support.

3.8.4 Identification and quantification

Identification and quantification was carried out under reverse phase isocratic HPLC conditions (RP-HPLC).

The carotenoids were identified on the basis of retention time and absorption spectra compared to those of carotenoid standards (β-carotene, β-cryptoxanthin and lycopene). Carotenoids were quantified using single point calibration and the results expressed in mg/100g of carotenoid sample, C_x using the formula below:

$$C_x \text{ (mg/100g)} = \frac{A_x \ X \ C_{st} \ X \ V_{Ext} \ X \ V_x \ X \ 100}{A_{st} \ X \ W_x \ X \ V_{st} \ X \ 1000}$$

Where; A_{st} is the peak area of the standard solution, A_x is the peak area of the carotenoid X, C_{st} is the concentration of the standard solution, V_{Ext} is the total volume of the carotenoid extract, V_{st} is the standard injection volume in μL, V_x is the sample injection volume in μL, W_x is the sample weight in grams, 1000 is the conversion factor from μg to mg, 100 is the multiplication factor for carotenoid concentration per 100g of sample.

Each sample was analyzed in triplicate for the different carotenoids at the two stages of ripening.

3.9 Data analyses

The mean β-carotene, β-cryptoxanthin and lycopene content for each of the six varieties of mangoes was subjected to analysis of variance (ANOVA) and Tukey's multiple comparison tests at a confidence level of 95% ($P<0.05$) using MS excel. The mean β-carotene, β-cryptoxanthin and lycopene content for each variety between the two stages of ripening process were compared using the student T-test. Excel bar charts were obtained for the means of the three carotenoids for each variety at the two stages of ripening. Each data point on bar graphs represented a mean with standard error bars and the bars labelled to indicate whether the means were significantly different or not.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

In this chapter, the results of the study are provided and discussed. Identification of β-Carotene, β-cryptoxanthin and lycopene is represented by sample chromatograms and their mean content in the different varieties of mangoes and stages of ripening summarized in a table and bar graphs with standard error bars.

4.1 Chromatographic separation

The analytical method provided satisfactory separation of constituents in the mango samples. β-Carotene, β-cryptoxanthin and lycopene were positively identified in all the samples and quantified using the pure standards as described in the analytical procedure. Retention times were reproducible for similar analyses carried out for both standards and samples. Three representative chromatograms from analysis of β-carotene β-cryptoxanthin and lycopene ripe in sample and standard are shown in figures 5-7. The three chromatograms represent ripe Apple mango, ripe Kent mango and ripe Tommy Atkins mango respectively. The peak representing the compound of interest is shown by an arrow.

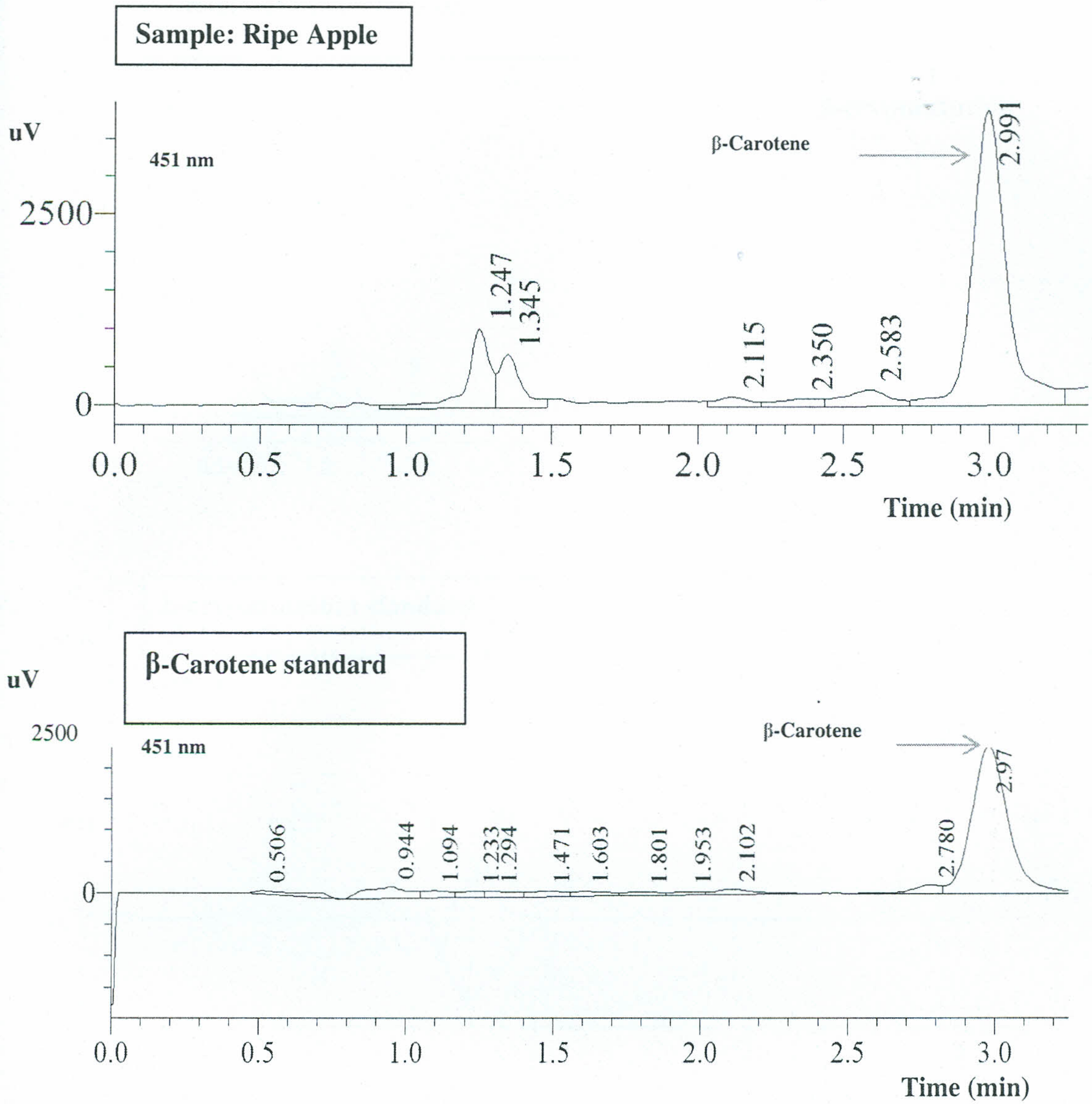
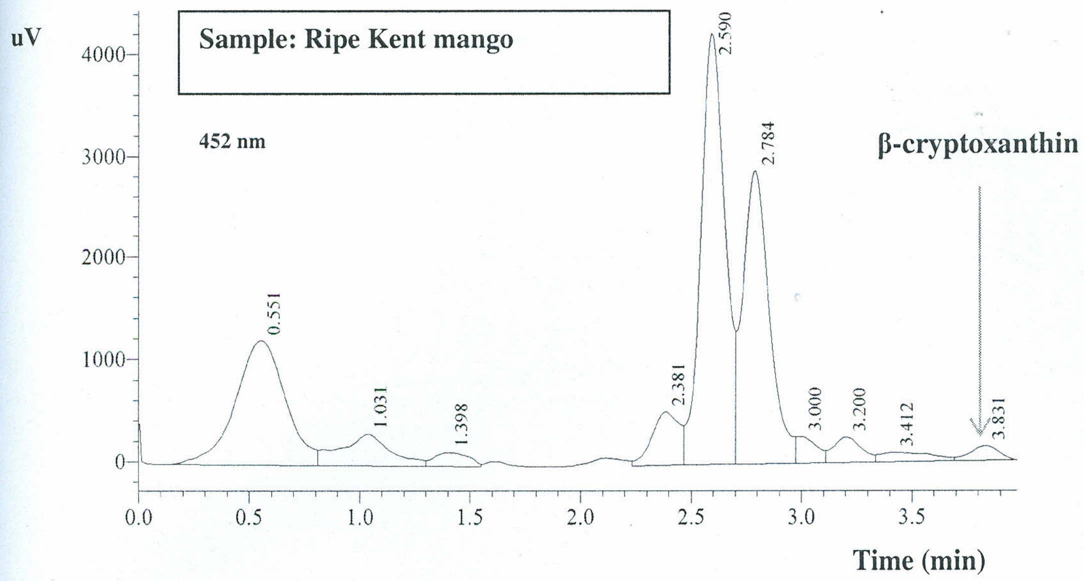


Figure 5: Chromatogram obtained from analysis of β-carotene in ripe Apple mango and standard.



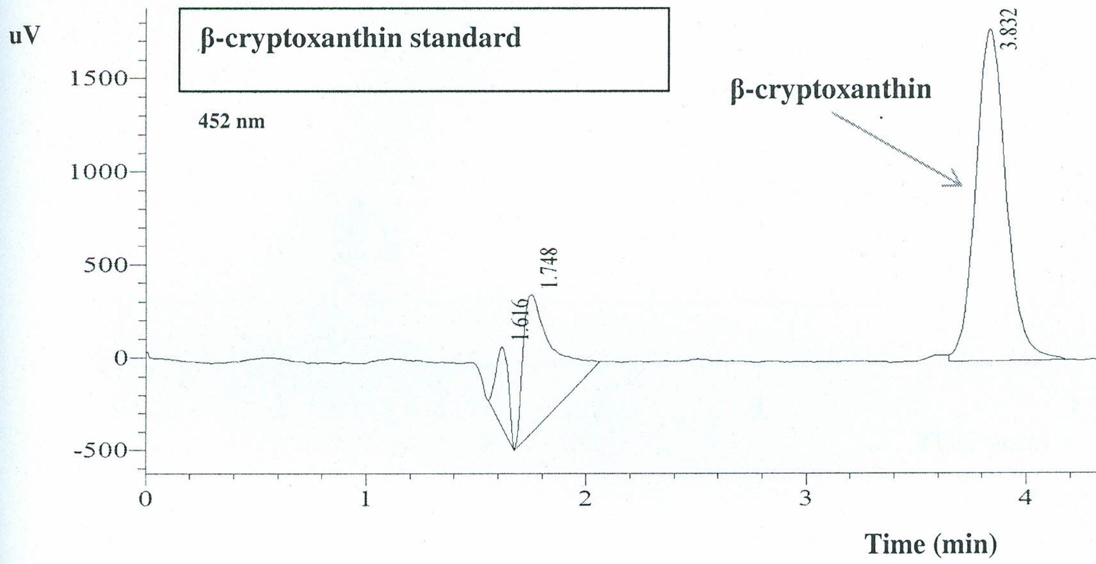
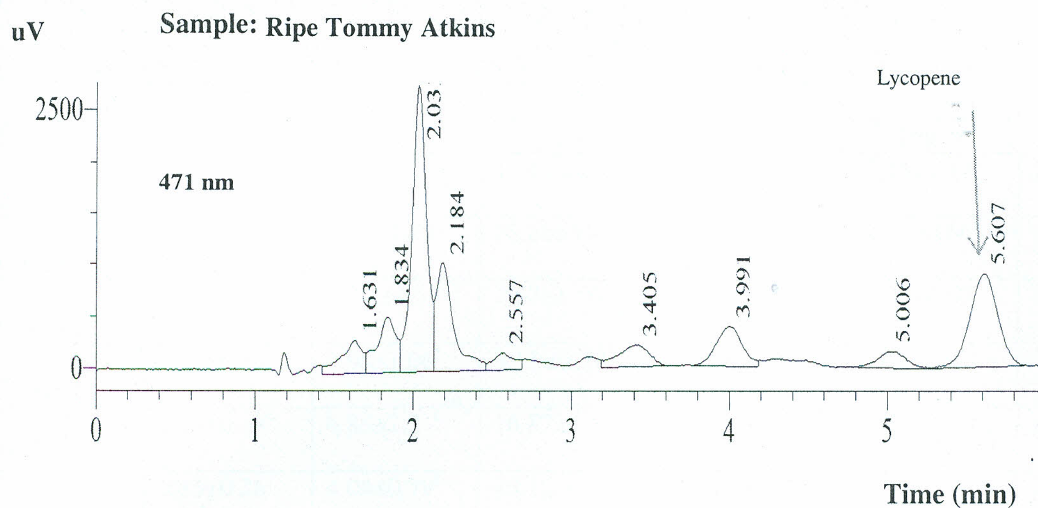


Figure 6: Chromatogram obtained from the analysis of β-cryptoxanthin in ripe Kent mango and standard.



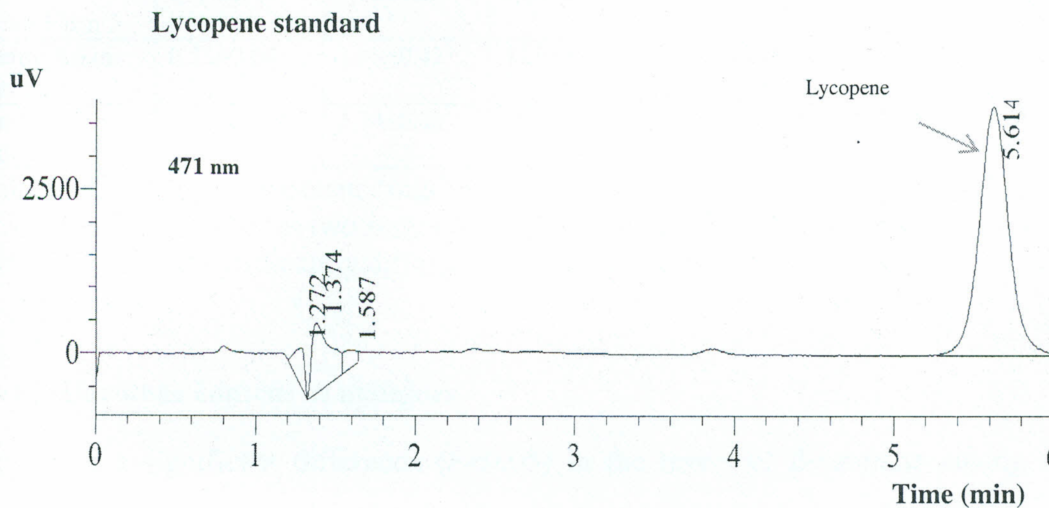


Figure 7: Chromatogram obtained from the analysis of lycopene in ripe Tommy Atkins mango and standard.

4.2 Carotenoid content in mangoes

Carotenoid content of the six mango varieties are shown in Table 10 and Figures 8-10.

Variety	β-carotene		β-cryptoxanthin		Lycopene	
	Unripe	Ripe	Unripe	Ripe	Unripe	Ripe
Apple Farm 1	0.40±0.08^{d}	10.47±2.04^{a}	11.61 ±1.93^{d,e}	54.06±21.27^{f}	7.16±1.19^{a}	25.95±4.98^{e}
Apple Farm 2	1.92±0.23^{a}	7.34±1.80^{b}	28.66±3.63^{a}	107.23±16.34^{a}	5.31±0.66^{b}	18.98±3.28^{f}
Keitt Farm 1	2.22±0.39^{a}	4.74 ±1.59^{d}	7.67±1.46^{f}	57.30±5.36^{f}	4.34±0.62^{d}	16.96±4.07^{f}
Kent Farm 1	1.19±0.40^{b}	4.05±1.06^{e}	9.15 ±2.28^{e}	78.81±8.65^{c}	8.01±1.41^{a}	32.40±6.05^{d}
Ndoto Farm 1	0.61±0.18^{c}	6.83±2.21^{c}	16.87±4.16^{c}	36.67±11.63^{h}	6.37±0.99^{b}	46.08±7.40^{b}
Ngowe Farm 1	0.65±0.26^{c}	4.04±0.79^{e}	19.12 ±4.78^{b}	66.00±16.49^{e}	7.36±1.18^{a}	36.68±6.60^{c,d}
Ngowe Farm 2	1.07±0.45^{b}	4.85±1.23^{d}	7.21 ±1.42^{f}	54.71±14.07^{f}	5.90±1.71^{b}	39.10±5.29^{c}
Tommy Atkins Farm 1	0.21±0.04^{e}	3.80±0.42^{f}	12.76 ±3.23^{d}	44.66±13.61^{g}	7.46±1.00^{a}	53.98±9.23^{a}
Tommy Atkins Farm 2	0.39±0.16^{d}	3.74±0.44^{f}	10.54 ±2.54^{e}	80.15±9.18^{b}	7.08±1.60^{a}	45.87±4.41^{b}

Table 10: Content of β-carotene (mg/100g), β-cryptoxanthin and lycopene (μg/100g) in six varieties of mangoes at two stages of maturity (Mean±SD). Means in a column followed by a different letter are significantly different (Tukey's test $P<0.05$).

4.2.1 β-Carotene content in mangoes

There was a significant difference ($P<0.05$) in the levels of β-carotene among the different cultivars as well as between the two stages of maturity. Effect of maturation on β-cryptoxanthin content was most significant ($P<0.05$) in Apple cultivar. β-carotene content of the six mango varieties ranged from 0.21±0.04 to 2.22±0.39 at the mature unripe stage and 3.74±0.44 to 10.47±2.04 mg/100g in the ripe fruits. Keitt had the highest β-carotene content (2.22±0.4 mg/100g) and Tommy Atkins the lowest (0.21±0.04 mg/100g) at the mature unripe stage. The Apple variety had the highest β-carotene content (10.47±2.04 mg/100g) while Tommy Atkins had the lowest (3.74±0.44 mg/100g) in the ripe fruit. Unripe mature green mangoes of Tommy Atkins cultivar had the lowest amount of β-carotene in this study (0.213±0.039 mg/100g).

Figure 8 shows a chart summary of the results obtained for β-carotene.

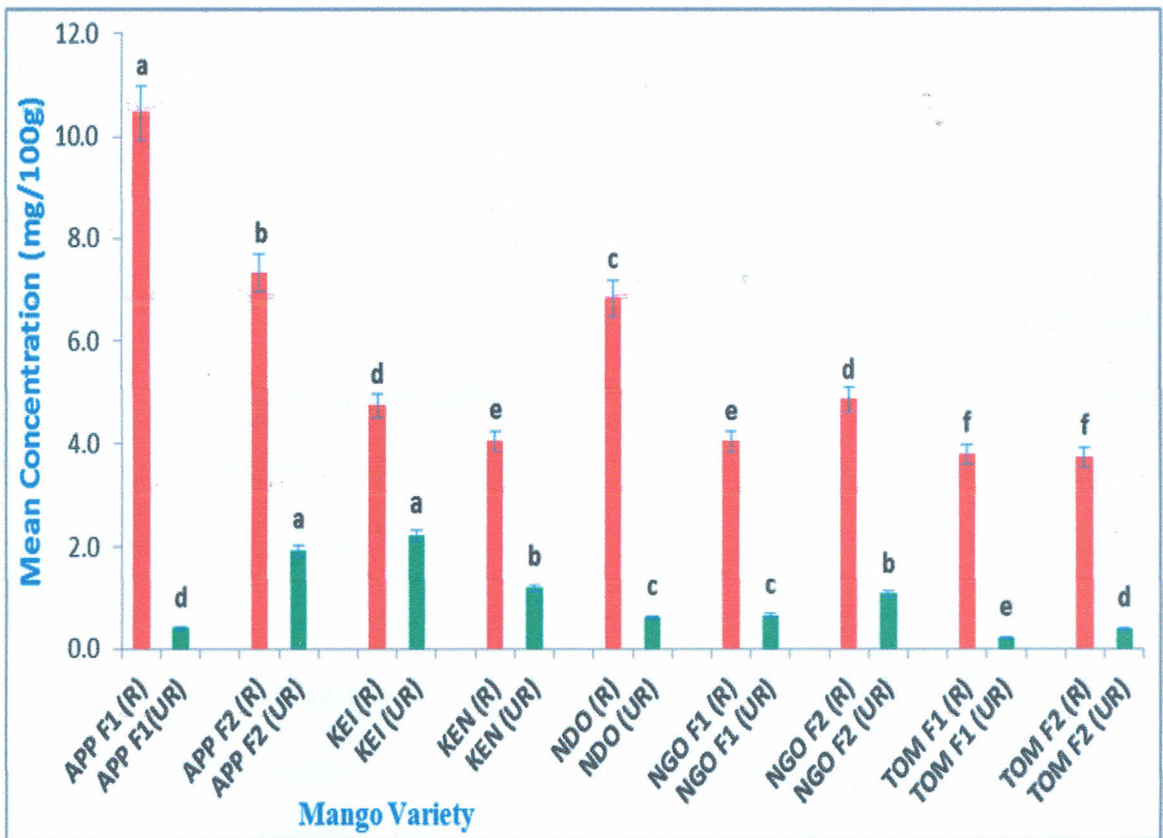


Figure 8: β-carotene content in unripe and ripe mango fruits of different cultivars. Means in bars of the same colour followed by a different letter are significantly different, *P*□0.05. (Refer to the key in appendix i).

One study reported lowest amount of 0.17±0.03 mg/100g in mature green mangoes of Keitt cultivar and 0.2±0.08 mg/100g in Tommy Atkins (Mercadante and Rodriguez-Amaya, 1998). β-carotene levels were comparable to those reported other studies (John *et al.*, 1970; Rodriguez-Amaya, 1987; Perry *et al.*; Mercadante and Rodriguez-Amaya; Muoki *et al.*, 2007) and were mostly in the range of normal fluctuations. Most carotenoid studies on mangoes have reported significantly high levels of β-carotene in mangoes when compared with β-cryptoxanthin and lycopene (Muoki 2007, Mercadante and Rodriguez –Amaya, 1998 and Perry *et al.* 2009).

However this study reported relatively higher levels of this antioxidant compared to other related studies. The ripe mangoes of apple cultivar in this study had highest β-carotene levels of 10.47±2.04 mg/100g. The highest content of β-carotene was reported in ripe Badami mango from Brazil by Rodriguez-Amaya (1987) having 4.52 mg/100g. Perkin-Veazie (2007) reported highest levels of 2.6 mg/100g in Ripe Ataulfo mango from Brazil.

The differences in the study results may be attributed to the differences in cultivars, soils, farming practices, differences in geographical and climatic conditions, degrees of ripeness and different analytical methods (Rodriguez-Amaya, 2001). In tropical regions where is greater exposure of fruits to sunlight and elevated temperature there is enhanced carotenoid biosynthesis that may explain high levels of β-carotene in this study compared to other studies carried out in places like Brazil and other areas with a moderate tropical climate (Mercadante and Rodriguez-Amaya, 1998 and Perkin-Veazie, 2007). A recent study gave much lower levels of the carotenoid which may be attributed to the differences in analytical procedures such as exclusion of saponification or methodology of research or uneven distribution of carotenoids in different part of the fruit sampled (Muoki, 2007).

Though official recommendations for the egestion of nutraceuticals are lacking, the Institute of Medicine (IOM) suggests a daily prudent ingestion of 3–6 mg of β-carotene from food sources in order to maintain plasma levels within the range that is associated with a low risk of chronic diseases. Food guides in the USA and the National Cancer Institute advice that adhering to the proposed from a varied diet would be equivalent to consuming from 5.2-6mg/day of provitamin A carotenoids, from a varied diet containing fruits and vegetables (Padovani and Amaya-Farfan, 2006).

β-Carotene is a major source of retinoids due to its high provitamin A activity (Rodriguez-Amaya, 2001). The recommended daily carotenoid intake still refers exclusively to ß-carotene ranging from 2-6mg (NCI, 2004). One study in Indonesia calculated the relative vitamin A equivalency of β-carotene to be 26 μg of β-carotene from leafy vegetables and carrots corresponding to 12 μg of β-carotene from fruit, and equaled 1 μg of pre-formed vitamin A in vitamin A-rich foods. As a consequence of the reported lower bioavailability of provitamin A carotenoids than previously thought, the conversion factors were increased from 6:1 to 12:1 for β-carotene [12 β-carotene = 1 μg retinol = 1 RAE (IOM, 2001; Rodriguez-Amaya *et al.,* 2006). About 100-500g/day of fruits such as mangoes and vegetables incorporated in the human diet can ensure the intake of a sufficient quantity of antioxidants (Muller, 1997).

4.2.2 β-Cryptoxanthin content in mangoes

The study reported substantial levels of β-cryptoxanthin content in mangoes across various cultivars investigated in this study. β-Cryptoxanthin content of all the mango cultivars was in the range 7.21±1.42 to 28.66±3.63 μg/100g in mature unripe mangoes and 36.67±11.63 to 107.23±16.34 μg/100g in the ripe mango fruits. In the mature unripe mango fruit, Apple cultivar had the highest β-cryptoxanthin content (28.66±3.63 μg/100g) and Ngowe the lowest (7.21±1.42 μg/100). The Apple cultivar had the highest β-cryptoxanthin content (107.23±16.34 μg/100g) while Ndoto had the lowest (36.67±11.63 μg/100g) in the ripe fruit.

It was also observed that for all the cultivars, β-cryptoxanthin content increased significantly ($P<0.05$) as the fruits progressed from mature unripe stage to the ripe stage. Effect of the stage of ripening on β-cryptoxanthin content was much more significant ($P<0.05$) in Apple and Kent cultivars. Figure 9 shows a summary of the results obtained for β-cryptoxanthin.

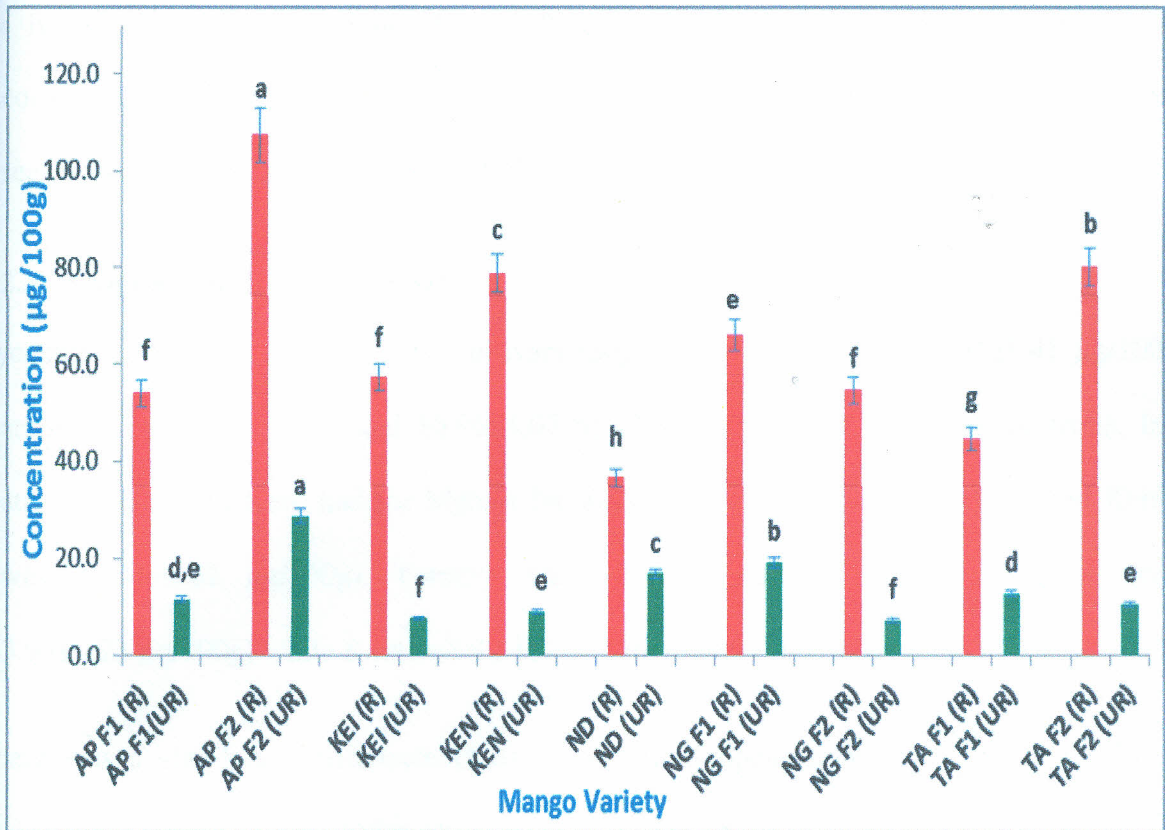


Figure 9: β-cryptoxanthin content in unripe (■) and ripe (■) mango fruits of different cultivars. Means in bars of the same colour followed by a different letter are significantly different, *P*□0.05. (Refer to the key in appendix i).

Some studies reported the levels of β-cryptoxanthin in mangoes as undetected (Perry *et al*., 2009) or trace levels in the unripe mango fruits (Rodriguez-Amaya). In a study carried by Rodriguez-Amaya (1987) Badami mango from Brazil had the highest amounts (40 µg/100g) and Keitt from the same region had the lowest amount (20 µg/100g). A number of research studies reported trace levels of the antioxidant in the mature unripe stage of different cultivars. The differences may be attributed to the same factors described for β-carotene. β-Cryptoxanthin is also converted to retinol (vitamin A) in vivo. RAE for β-cryptoxanthin is given as 24 µg of provitamin A carotenoid = 1 µg retinol = 1 RAE (IOM, 2001).

As is the case with β-carotene about 300g/day of fruits such as mangoes and vegetables incorporated in the human diet can ensure protection of the body from oxidative stress related diseases by this nutraceutical (Muller, 1997).

4.2.3 Lycopene content in mangoes

Lycopene content of all the mango cultivars ranged from 4.34±0.62 to 8.01±1.41 μg/100g in mature unripe mango fruits and 16.96±4.07 to 53.98±9.23 μg/100g in the ripe fruits. In the mature unripe fruit, Kent had the highest lycopene content (8.01±1.41 μg/100g) and Keitt the lowest (4.34±0.62 μg/100g). Tommy Atkins variety had the highest lycopene content (53.98±9.23 μg/100g) while Keitt had the lowest (16.96±4.07 μg/100g) in the ripe fruit.

There was a significant difference ($P<0.05$) in the lycopene contents between the different varieties in this study. It was also observed that for all the varieties, lycopene content increased significantly ($P<0.05$) as the fruits progressed from mature unripe stage to the ripe stage. Figure 10 shows a chart summary of the results obtained for lycopene.

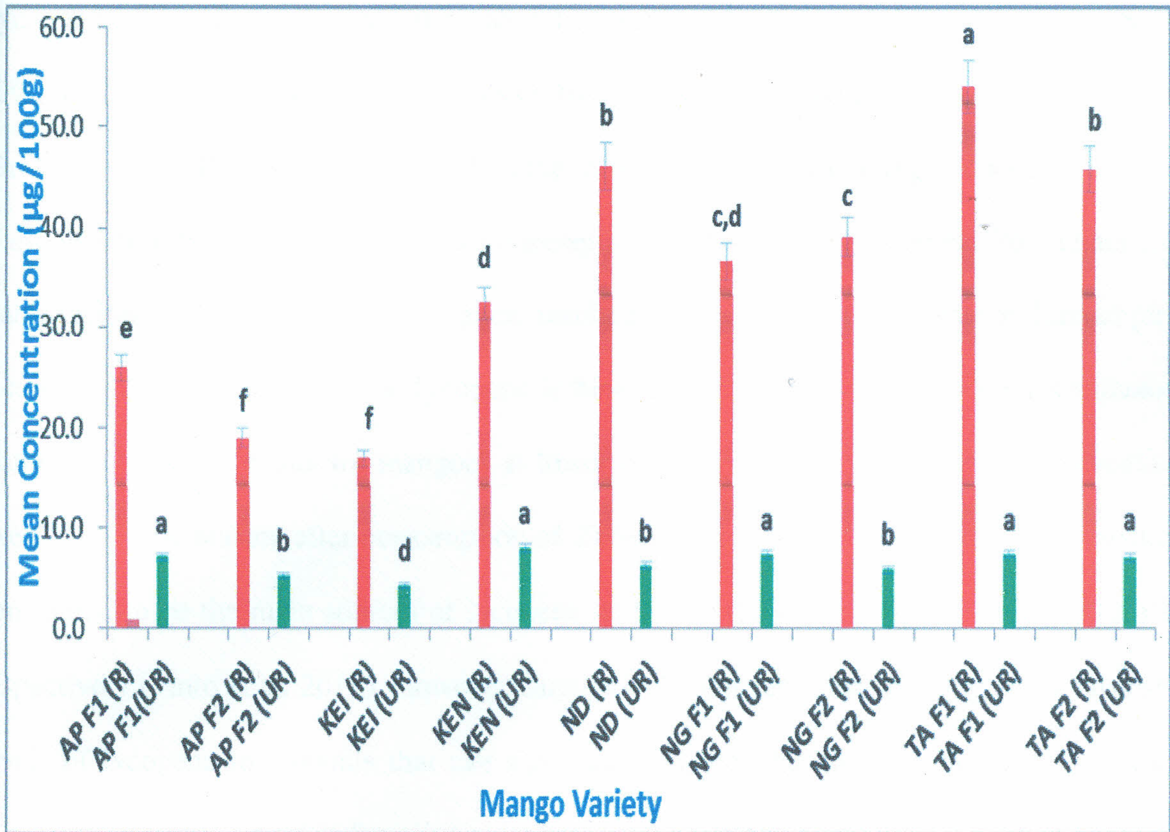


Figure 10: Lycopene content in unripe (■) and ripe (■) mango fruits of different cultivars. Means in bars of the same colour followed by a different letter are significantly different, *P*□0.05. (Refer to the key in appendix i).

Lycopene content was the lowest among the three carotenoids in all mangoes cultivars investigated in this study but were quite substantial given that lycopene is majorly present in tomatoes, guavas and water melon (Perkins-Veazie, 2007 and Charoensiri *et al.*, 2009).

Lycopene content in mangoes though not reported in other studies was present in significant amounts (53.98±9.23 µg/100g –Tommy Atkins F1) in mangoes. Some of the studies reviewed indicated presence of lycopene in mangoes though the levels were not stated (Gayle *et al.*, 2010 and Mateljan, 2010).

Lycopene is the most studied and most abundant antioxidant in blood plasma (accounts for about 50% of all carotenoids in serum) and about 10-30% of dietary lycopene is absorbed by humans (Bramley, 2000; Kivilompolo, 2009). It is the most effective singlet oxygen quencher of (Hamid *et al.,* 2010). It has been observed that consumption of 20mg/day of lycopene from either frozen water melon juice or canned tomato juice, resulted in a 100 to 200% increase in human plasma lycopene, thus showing that that lycopene is bioavailable from non-heat processed tomato and water. This may hold true for mangoes at lower per cent increments. Recent reports indicate a rise in lycopene plasma after consumption of 21-9mg of lycopene. Papaya and fresh tomato are considered to be the main sources of lycopene with 2000-5300 μg/100g and 900-4200 μg/100g respectively (Pinto et al, 2010). However cumulatively, consumption of mango may raise serum levels of lycopene to amounts that can significantly lower the levels of degenerative diseases especially prostate and other forms of cancer (Mateljan, 2010).

Several studies have demonstrated that the risk of cancer is reduced significantly with increased intake of lycopene and increased levels of lycopene (Rao and Rao, 2007).

Reports about the daily intake levels of lycopene have varied significantly due to the methods of estimation used. In general they range from 3.7 to 16.2 mg in the United States of America, 25.2 mg in Canada, 1.3 mg in Germany, 1.1 mg in United Kingdom and 0.7 mg in Finland (Rao, 2002). Based on reported studies, a daily intake level of 5–7 mg in normal healthy human beings may be sufficient to maintain circulating levels of lycopene at levels sufficient to combat oxidative stress and prevent chronic diseases (Rao and Shen, 2002).

Under the condition of disease such as cancer and cardiovascular diseases, higher levels of lycopene ranging from 35 to 75 mg per day may be required (Heath et al., 2006; Rao and Rao, 2007).

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CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The objectives of this study were to quantify β-carotene, β-cryptoxanthin and lycopene levels in different varieties of mango (*Mangifera indica L.*) from two farms in Mwala, Machakos County and determine the influence of cultivar and maturation stage on the levels of the carotenoids. The study reveals that there are significant variations in the levels of these antioxidants in different cultivars. Moreover, ripening from mature unripe to the ripe stage is accompanied by a significant increase in β-carotene, β-cryptoxanthin and lycopene content in Apple, Keitt, Kent, Ndoto, Ngowe and Tommy Atkins mango cultivars. The results show that β-carotene, β-cryptoxanthin and lycopene are present in ripe mangoes in significant amounts. β-Carotene was the predominant carotenoid in mangoes with β-cryptoxanthin and lycopene occurring in relatively lower amounts. In other studies undertaken in South America, lycopene level in mangoes was not reported. This study provides evidence that the nutraceutical is present in this fruit in significant amounts.

Apple and Tommy Atkins mangoes have relatively higher levels of β-carotene, β-cryptoxanthin and lycopene compared to other cultivars investigated in this study.

Levels of β-carotene, β-cryptoxanthin and lycopene in local (indigenous) mangoes are comparable to those of exotic (improved) varieties. Epigenetic factors may also be important in inducing differences in the content of anti-oxidants in different varieties of mangoes.

5.2 Recommendations

5.2.1 Recommendations from the study

Mangoes can be useful for public health interventions to alleviate various forms of cancer, cardiovascular diseases, macular degeneration and rheumatoid arthritis as well as help boost immune system of persons living with HIV/AIDS given its richness in β-carotene and significant amounts of β-cryptoxanthin and lycopene. To ensure the intake of a sufficient quantity of this nutraceutical compounds, the human diet should contain a high proportion of mangoes in addition to other fruits and vegetables.

Considering the large variation in nutraceutical levels associated with ripening, maximum benefit is derived when mangoes are allowed to attain full maturity (140 days after fruit set) before plucking and full ripening at market conditions in order to derive maximum health benefits.

Small scale farmers, fruit processing industries, and horticultural export industries can exploit Apple, and Kent and Tommy Atkins mangoes for their relatively high levels of β-carotene and substantial levels of β-cryptoxanthin and lycopene.

The study recommends local industrial and small scale processing of Kenyan mangoes to juices, pickles, jams, mango paste, puree, pulp, solar dried flakes and powder to promote their utilization and minimize their losses especially during the peak harvesting season.

5.2.2 Recommendations for further work

There is need to evaluate the carotenoid contents of other mango cultivars from diverse geographical regions in the country and investigate other nutraceutical compounds, such as violaxanthin in Kenyan mangoes of different cultivars to enable promotion, cultivation and utilization of cultivars with highest carotenoid content and greatest health benefits.

There is need for assessment and standardization of nutraceuticals in mango juices and processed fruit products by KEBS to authenticate claims on their levels and protect consumers from undue exploitation. It is also hoped that the Kenyan government will develop a nation-wide carotenoid database and a policy on consumption of nutraceuticals as a health indicator and regulation of their use in fruit juices and processed fruit products. Nutraceutical composition data need to be published in national food composition tables to encourage intake of antioxidant food components and research linking diet and disease, health and nutrition agricultural policies, food labeling and consumer education.

Finally there is need to evaluate existing methods with regard to carotenoid analysis so as to harmonize reported levels of β-carotene, β-cryptoxanthin and lycopene in mangoes which appear to have wide variations.

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APPENDICES

APPENDIX I: Key for figures 8, 9 and 10

P F1 (R)	-Ripe Apple mangoes in Farm 1
P F1 (UR)	-Unripe Apple mangoes in Farm 1
P F2 (R)	-Ripe Apple mangoes in Farm 2
P F2 (UR)	-Unripe Apple mangoes in Farm 2
EI (R)	-Ripe Keit mangoes in (Farm 1)
EI (UR)	-Unripe Keit mangoes in (Farm 1)
EN (R)	-Ripe Kent mangoes in (Farm 1)
EN (UR)	-Unripe Kent mangoes in (Farm 1)
D (R)	-Ripe Kent mangoes in Farm 1
D (UR)	-Unripe Kent mangoes in Farm 1
G F1 (R)	-Ripe Ngowe mangoes in Farm 1
G F1 (UR)	-Unripe Ngowe mangoes in Farm 1
G F2 (R)	-Ripe Ngowe mangoes in Farm 2
G F2 (UR)	-Unripe Ngowe mangoes in Farm 2
A (R)	-Ripe Tommy Atkins mangoes in (Farm 1)
A (UR)	-Unripe Tommy Atkins mangoes in (Farm 1)
A (R)	-Ripe Tommy Atkins mangoes in (Farm 2)
A (UR)	-Unripe Tommy Atkins mangoes in (Farm 2)

APPENDIX II: ANOVA results for analysis of β-carotene in ripe mangoes

Test for equal variance (β-Carotene in ripe mangoes)						
F	DFn	DFd	P			
2.642669648	8	81	0.012584			
FAIL - equal variance CANNOT be assumed (p <= 0.05).						
Analysis of variance						
ANOVA	SS	DF				
Between	410.9028	8				
Within	165.9421	81				
F	25.07134					
P	5.79E-19	****				
Multiple comparisons: Tukey test						
Group 1	Group 2	Delta means	SE	Q	P	Significant?
APPLE F1	TOMMY ATKINS F2	6.7305	0.452622	14.87002	6.16E-05	Yes
APPLE F1	TOMMY ATKINS F1	6.6747	0.452622	14.74674	6.16E-05	Yes
APPLE F1	NGOWE F1	6.4326	0.452622	14.21185	6.16E-05	Yes
APPLE F1	KENT	6.4211	0.452622	14.18645	6.16E-05	Yes
APPLE F1	KEIT	5.7316	0.452622	12.6631	6.16E-05	Yes
APPLE F1	NGOWE F2	5.6161	0.452622	12.40792	6.16E-05	Yes
APPLE F1	NDOTO	3.6452	0.452622	8.053517	6.62E-05	Yes
APPLE F1	APPLE F2	3.126	0.452622	6.906423	0.000219	Yes
APPLE F2	TOMMY ATKINS F2	3.6045	0.452622	7.963596	6.78E-05	Yes
APPLE F2	TOMMY ATKINS F1	3.5487	0.452622	7.840315	7.09E-05	Yes
APPLE F2	NGOWE F1	3.3066	0.452622	7.305432	0.00011	Yes
APPLE F2	KENT	3.2951	0.452622	7.280024	0.000114	Yes
APPLE F2	KEIT	2.6056	0.452622	5.756678	0.003359	Yes
APPLE F2	NGOWE F2	2.4901	0.452622	5.501499	0.00611	Yes
APPLE F2	NDOTO	0.5192	0.452622	1.147094	0.996282	No
NDOTO	TOMMY ATKINS F2	3.0853	0.452622	6.816503	0.000265	Yes
NDOTO	TOMMY ATKINS F1	3.0295	0.452622	6.693221	0.000348	Yes
NDOTO	NGOWE F1	2.7874	0.452622	6.158338	0.001276	Yes
NDOTO	KENT	2.7759	0.452622	6.13293	0.001357	Yes
NDOTO	KEIT	2.0864	0.452622	4.609585	0.041077	Yes
NDOTO	NGOWE F2	1.9709	0.452622	4.354405	0.066575	No
NGOWE F2	TOMMY ATKINS F2	1.1144	0.452622	2.462098	0.719647	No

APPENDIX III: ANOVA results for analysis of β-carotene in unripe mangoes

Test for equal variance (β-Carotene in mature unripe mangoes)						
F	DFn	DFd	P			
3.013809824	8	81	0.005177			
FAIL - equal variance CANNOT be assumed (p <= 0.05).						
ANOVA	SS	DF				
Between	39.79853	8				
Within	6.271752	81				
F	64.25002					
P	7.03E-32	****				
Multiple comparisons: Tukey test						
Group 1	Group 2	Delta means	SE	q	p	Significant?
KEIT	TOMMY ATKINS F1	2.0033	0.087994	22.76639	6.16E-05	Yes
KEIT	TOMMY ATKINS F2	1.8279	0.087994	20.77306	6.16E-05	Yes
KEIT	APPLE F1	1.8165	0.087994	20.64351	6.16E-05	Yes
KEIT	NDOTO	1.6039	0.087994	18.22743	6.16E-05	Yes
KEIT	NGOWE F1	1.5689	0.087994	17.82967	6.16E-05	Yes
KEIT	NGOWE F2	1.1426	0.087994	12.98501	6.16E-05	Yes
KEIT	KENT	1.0239	0.087994	11.63605	6.16E-05	Yes
KEIT	APPLE F2	0.2984	0.087994	3.391149	0.299359	No
APPLE F2	TOMMY ATKINS F1	1.7049	0.087994	19.37524	6.16E-05	Yes
APPLE F2	TOMMY ATKINS F2	1.5295	0.087994	17.38191	6.16E-05	Yes
APPLE F2	APPLE F1	1.5181	0.087994	17.25236	6.16E-05	Yes
APPLE F2	NDOTO	1.3055	0.087994	14.83628	6.16E-05	Yes
APPLE F2	NGOWE F1	1.2705	0.087994	14.43852	6.16E-05	Yes
APPLE F2	NGOWE F2	0.8442	0.087994	9.593861	6.16E-05	Yes
APPLE F2	KENT	0.7255	0.087994	8.244902	6.41E-05	Yes
KENT	TOMMY ATKINS F1	0.9794	0.087994	11.13033	6.16E-05	Yes
KENT	TOMMY ATKINS F2	0.804	0.087994	9.137011	6.17E-05	Yes
KENT	APPLE F1	0.7926	0.087994	9.007456	6.18E-05	Yes
KENT	NDOTO	0.58	0.087994	6.591376	0.000442	Yes
KENT	NGOWE F1	0.545	0.087994	6.19362	0.001172	Yes
KENT	NGOWE F2	0.1187	0.087994	1.348959	0.988881	No
NGOWE F2	TOMMY ATKINS F1	0.8607	0.087994	9.781375	6.16E-05	Yes
NGOWE F2	TOMMY ATKINS F2	0.6853	0.087994	7.788052	7.26E-05	Yes
NGOWE F2	APPLE F1	0.6739	0.087994	7.658497	7.81E-05	Yes
NGOWE F2	NDOTO	0.4613	0.087994	5.242417	0.010976	Yes
NGOWE F2	NGOWE F1	0.4263	0.087994	4.844661	0.025609	Yes
NGOWE F1	TOMMY ATKINS F1	0.4344	0.087994	4.936713	0.021131	Yes
NGOWE F1	TOMMY ATKINS F2	0.259	0.087994	2.94339	0.49308	No

APPENDIX IV: ANOVA results for analysis of β-cryptoxanthin in ripe mangoes

Test for equal variance (β-cryptoxanthin in ripe mangoes)

F	DFn	DFd	P
1.739937123	8	81	0.101628

PASS - equal variance may be assumed ($p > 0.05$).

ANOVA	SS	DF
Between	37028.25	8
Within	15274.31	81
F	24.5452	
P	1.05E-18	****

Multiple comparisons: Tukey test

Group 1	Group 2	Delta means	SE	Q	p	Significant?
APPLE F2	NDOTO	70.564	4.342485	16.24968	6.16E-05	Yes
APPLE F2	TOMMY ATKINS F1	62.568	4.342485	14.40834	6.16E-05	Yes
APPLE F2	APPLE F1	53.174	4.342485	12.24506	6.16E-05	Yes
APPLE F2	NGOWE F2	52.526	4.342485	12.09584	6.16E-05	Yes
APPLE F2	KEIT	49.932	4.342485	11.49849	6.16E-05	Yes
APPLE F2	NGOWE F1	41.233	4.342485	9.495254	6.16E-05	Yes
APPLE F2	KENT	28.422	4.342485	6.5451	0.000493	Yes
APPLE F2	TOMMY ATKINS F2	27.08	4.342485	6.236061	0.001053	Yes
TOMMY ATKINS F2	NDOTO	43.484	4.342485	10.01362	6.16E-05	Yes
TOMMY ATKINS F2	TOMMY ATKINS F1	35.488	4.342485	8.172279	6.47E-05	Yes
TOMMY ATKINS F2	APPLE F1	26.094	4.342485	6.009002	0.001838	Yes
TOMMY ATKINS F2	NGOWE F2	25.446	4.342485	5.859778	0.002628	Yes
TOMMY ATKINS F2	KEIT	22.852	4.342485	5.262425	0.010501	Yes
TOMMY ATKINS F2	NGOWE F1	14.153	4.342485	3.259194	0.351813	No
KENT	NDOTO	42.142	4.342485	9.704581	6.16E-05	Yes
KENT	TOMMY ATKINS F1	34.146	4.342485	7.863239	7.02E-05	Yes
KENT	APPLE F1	24.752	4.342485	5.699962	0.003841	Yes
KENT	NGOWE F2	24.104	4.342485	5.550739	0.005444	Yes
KENT	KEIT	21.51	4.342485	4.953385	0.020401	Yes
NGOWE F1	NDOTO	29.331	4.342485	6.754427	0.000303	Yes
NGOWE F1	TOMMY ATKINS F1	21.335	4.342485	4.913085	0.022207	Yes
NGOWE F1	APPLE F1	11.941	4.342485	2.749808	0.585718	No
KEIT	NDOTO	20.632	4.342485	4.751197	0.030995	Yes
KEIT	TOMMY ATKINS F1	12.636	4.342485	2.909855	0.508984	No
NGOWE F2	NDOTO	18.038	4.342485	4.153843	0.095062	No

APPENDIX V: ANOVA results for analysis of β-cryptoxanthin in unripe mangoes

Test for equal variance (β-cryptoxanthin in mature unripe mangoes)

F	DFn	DFd	P
2.891229707	8	80	0.007003

FAIL - equal variance CANNOT be assumed (p <= 0.05).

Analysis of variance

ANOVA	SS	DF
Between	3765.576	8
Within	732.3193	80
F	51.41986	
P	2.16E-28	****

Multiple comparisons: Tukey test

Group 1	Group 2	Delta means	SE	q	p	Significant?
APPLE F2	NGOWE F2	21.449	0.956765	22.41825	6.19E-05	Yes
APPLE F2	KEIT	20.983	0.956765	21.9312	6.19E-05	Yes
APPLE F2	KENT	19.507	0.956765	20.3885	6.19E-05	Yes
APPLE F2	TOMMY ATKINS F2	18.119	0.956765	18.93778	6.19E-05	Yes
APPLE F2	APPLE F1	17.044	0.956765	17.8142	6.19E-05	Yes
APPLE F2	TOMMY ATKINS F1	15.895	0.956765	16.61328	6.19E-05	Yes
APPLE F2	NDOTO	11.78167	0.982983	11.98563	6.19E-05	Yes
APPLE F2	NGOWE F1	9.537	0.956765	9.967966	6.19E-05	Yes
NGOWE F1	NGOWE F2	11.912	0.956765	12.45029	6.19E-05	Yes
NGOWE F1	KEIT	11.446	0.956765	11.96323	6.19E-05	Yes
NGOWE F1	KENT	9.97	0.956765	10.42053	6.19E-05	Yes
NGOWE F1	TOMMY ATKINS F2	8.582	0.956765	8.96981	6.21E-05	Yes
NGOWE F1	APPLE F1	7.507	0.956765	7.846232	7.12E-05	Yes
NGOWE F1	TOMMY ATKINS F1	6.358	0.956765	6.64531	0.000394	Yes
NGOWE F1	NDOTO	2.244667	0.982983	2.283527	0.794044	No
NDOTO	NGOWE F2	9.667333	0.982983	9.834695	6.19E-05	Yes
NDOTO	KEIT	9.201333	0.982983	9.360628	6.19E-05	Yes
NDOTO	KENT	7.725333	0.982983	7.859075	7.08E-05	Yes
NDOTO	TOMMY ATKINS F2	6.337333	0.982983	6.447046	0.000631	Yes
NDOTO	APPLE F1	5.262333	0.982983	5.353435	0.008622	Yes
NDOTO	TOMMY ATKINS F1	4.113333	0.982983	4.184544	0.090315	No
TOMMY ATKINS F1	NGOWE F2	5.554	0.956765	5.804979	0.00302	Yes
TOMMY ATKINS F1	KEIT	5.088	0.956765	5.317921	0.009334	Yes
TOMMY ATKINS F1	KENT	3.612	0.956765	3.775222	0.175726	No
APPLE F1	NGOWE F2	4.405	0.956765	4.604057	0.041656	Yes
APPLE F1	KEIT	3.939	0.956765	4.116999	0.101454	No

APPENDIX VI: ANOVA results for analysis of Lycopene in ripe mangoes

Test for equal variance (Lycopene in ripe mangoes)						
F	**DFn**	**DFd**	**P**			
1.500684462	**8**	**81**	**0.169929**			
PASS - equal variance may be assumed (p > 0.05).						
ANOVA	**SS**	**DF**				
Between	**12913.03**	**8**				
Within	**2876.073**	**81**				
F	**45.45935**					
P	**7.94E-27**	********				
Multiple comparisons: Tukey test						
Group 1	**Group 2**	**Delta means**	**SE**	**q**	**P**	**Significant?**
TOMMY ATKINS F1	KEIT	37.019	1.884332	19.64569	6.16E-05	Yes
TOMMY ATKINS F1	APPLE F2	34.998	1.884332	18.57316	6.16E-05	Yes
TOMMY ATKINS F1	APPLE F1	28.024	1.884332	14.87211	6.16E-05	Yes
TOMMY ATKINS F1	KENT	21.573	1.884332	11.44862	6.16E-05	Yes
TOMMY ATKINS F1	NGOWE F1	17.293	1.884332	9.177257	6.17E-05	Yes
TOMMY ATKINS F1	NGOWE F2	14.873	1.884332	7.892982	6.94E-05	Yes
TOMMY ATKINS F1	TOMMY ATKINS F2	8.111	1.884332	4.304443	0.072908	No
NDOTO	KEIT	29.122	1.884332	15.45481	6.16E-05	Yes
NDOTO	APPLE F2	27.101	1.884332	14.38228	6.16E-05	Yes
NDOTO	APPLE F1	20.127	1.884332	10.68124	6.16E-05	Yes
NDOTO	KENT	13.676	1.884332	7.257744	0.000118	Yes
NDOTO	NGOWE F1	9.396	1.884332	4.986382	0.019022	Yes
NDOTO	NGOWE F2	6.976	1.884332	3.702107	0.195609	No
TOMMY ATKINS F2	KEIT	28.908	1.884332	15.34124	6.16E-05	Yes
TOMMY ATKINS F2	APPLE F2	26.887	1.884332	14.26872	6.16E-05	Yes
TOMMY ATKINS F2	APPLE F1	19.913	1.884332	10.56767	6.16E-05	Yes
TOMMY ATKINS F2	KENT	13.462	1.884332	7.144176	0.00014	Yes
TOMMY ATKINS F2	NGOWE F1	9.182	1.884332	4.872814	0.024159	Yes
NGOWE F2	KEIT	22.146	1.884332	11.7527	6.16E-05	Yes
NGOWE F2	APPLE F2	20.125	1.884332	10.68018	6.16E-05	Yes
NGOWE F2	APPLE F1	13.151	1.884332	6.97913	0.000189	Yes
NGOWE F2	KENT	6.7	1.884332	3.555636	0.240885	No
NGOWE F1	KEIT	19.726	1.884332	10.46843	6.16E-05	Yes
NGOWE F1	APPLE F2	17.705	1.884332	9.395902	6.16E-05	Yes
NGOWE F1	APPLE F1	10.731	1.884332	5.694856	0.003887	Yes
KENT	KEIT	15.446	1.884332	8.197068	6.45E-05	Yes
KENT	APPLE F2	13.425	1.884332	7.12454	0.000145	Yes
KENT	APPLE F1	6.451	1.884332	3.423494	0.287237	No

APPENDIX VII: ANOVA results for analysis of Lycopene in unripe mangoes

Test for equal variance (Lycopene in mature unripe mangoes)						
F	DFn	DFd	P			
1.063957533	8	79	0.396808			
PASS - equal variance may be assumed (p > 0.05).						
Analysis of variance						
ANOVA	SS	DF				
Between	110.3034	8				
Within	111.9302	79				
F	9.731483					
P	2.69E-09	****				
Multiple comparisons: Tukey test						
Group 1	Group 2	Delta means	SE	q	p	Significant?
KENT	KEIT	3.662	0.376409	9.728779	6.22E-05	Yes
KENT	APPLE F2	2.693	0.376409	7.154451	0.000142	Yes
KENT	NGOWE F2	2.10725	0.399242	5.278126	0.010262	Yes
KENT	NDOTO	1.638	0.376409	4.351649	0.067249	No
TOMMY ATKINS F1	KEIT	3.118	0.376409	8.283542	6.45E-05	Yes
TOMMY ATKINS F1	APPLE F2	2.149	0.376409	5.709215	0.003818	Yes
TOMMY ATKINS F1	NGOWE F2	1.56325	0.399242	3.915545	0.141587	No
NGOWE F1	KEIT	3.019	0.376409	8.020531	6.76E-05	Yes
NGOWE F1	APPLE F2	2.05	0.376409	5.446203	0.007026	Yes
APPLE F1	KEIT	2.817	0.376409	7.48388	9.18E-05	Yes
APPLE F1	APPLE F2	1.848	0.376409	4.909553	0.022567	Yes
TOMMY ATKINS F2	KEIT	2.734	0.376409	7.263376	0.00012	Yes
TOMMY ATKINS F2	APPLE F2	1.765	0.376409	4.689048	0.035368	Yes
NDOTO	KEIT	2.024	0.376409	5.37713	0.008217	Yes
NDOTO	APPLE F2	1.055	0.376409	2.802802	0.560314	No
NGOWE F2	KEIT	1.55475	0.399242	3.894254	0.146442	No

APPENDIX VIII: RAW DATA FOR β-CAROTENE CONTENT IN MANGOES (mg/100g)

1. β-CAROTENE CONTENT IN MANGOES (mg/100g)

a) Ripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	7.496	8.9	11.56	10.201	10.67	14.681	8.787	11.896	11.304	9.204	10.470	2.041
APPLE F2	5.816	5.102	10.102	6.708	7.985	10.717	7.371	6.837	6.371	6.43	7.344	1.801
KEIT F1	4.638	3.554	3.931	4.826	6.179	2.288	4.29	7.913	5.926	3.838	4.738	1.586
KENT F1	4.018	6.183	3.396	3.078	4.297	5.207	3.526	2.787	4.675	3.321	4.049	1.060
NDOTO F1	9.498	5.1	8.389	7.028	9.631	7.259	7.333	7.115	3.481	3.413	6.825	2.207
NGOWE F1	5.688	4.035	3.725	3.327	4.806	3.742	4.405	3.812	2.83	4.003	4.037	0.792
NGOWE F2	3.343	4.262	5.095	4.177	4.108	3.208	5.167	6.275	6.474	6.429	4.854	1.231
TOMMY ATKINS F1	3.858	3.744	4.195	3.798	3.37	4.109	3.445	3.297	3.5	4.636	3.795	0.423
TOMMY ATKINS F2	3.912	4.088	3.514	3.538	3.302	4.324	3.248	3.355	3.628	4.485	3.739	0.439

b) Mature unripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	0.343	0.445	0.358	0.333	0.276	0.397	0.474	0.392	0.431	0.551	0.400	0.079
APPLE F2	1.727	2.227	2.197	2.203	1.626	1.748	2.045	1.683	1.797	1.928	1.918	0.234
KEIT F1	1.662	1.557	2.555	2.902	2.161	2.359	2.2	2.11	2.333	2.326	2.217	0.393
KENT F1	1.093	0.864	1.068	0.966	2.203	0.968	0.964	1.261	1.058	1.481	1.193	0.396
NDOTO F1	0.837	0.284	0.577	0.888	0.72	0.484	0.504	0.54	0.703	0.589	0.613	0.179
NGOWE F1	0.42	0.584	0.564	0.666	0.737	0.676	0.37	0.904	1.192	0.363	0.648	0.257
NGOWE F2	0.644	0.763	0.615	0.656	1.797	1.5	1.027	1.715	0.922	1.1	1.074	0.448
TOMMY ATKINS F1	0.216	0.156	0.195	0.169	0.266	0.243	0.204	0.187	0.225	0.271	0.213	0.039
TOMMY ATKINS F2	0.377	0.428	0.506	0.392	0.599	0.463	0.361	0.413	0	0.347	0.389	0.156

APPENDIX IX: RAW DATA FOR β-CRYPTOXANTHIN CONTENT IN MANGOES (μg/100g)

2. β-CRYPTOXANTHIN (μg/100g)

a) Ripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	38.04	34.46	50.95	33.81	62.64	106.21	55.34	59.25	41.14	58.74	54.06	21.27
APPLE F2	95.91	105.25	77.09	93.49	126.8	94.66	120.65	120.23	119.12	119.12	107.23	16.34
KEIT F1	56.18	58.52	63.11	50.9	58.55	55.86	48.2	54.51	61.63	65.54	57.30	5.36
KENT F1	90.14	87.77	77.16	81.88	76.29	64.01	68.21	81.36	87.75	73.53	78.81	8.65
NDOTO F1	58.9	35.47	48.15	37.33	46.1	33.79	19.91	25.25	33.46	28.32	36.67	11.63
NGOWE F1	56.32	80.650	65.72	69.33	64	95.39	43.97	82.88	46.88	54.85	66.00	16.49
NGOWE F2	36.92	58.36	79.64	59.83	67.49	29.67	51.92	52.8	55.94	54.49	54.71	14.07
TOMMY ATKINS F1	66.64	52.35	57.9	37.22	31.56	26.67	57.99	37.29	31.95	47.07	44.66	13.61
TOMMY ATKINS F2	77.75	93.35	77	79.59	65.54	90.1	76.03	77.08	72.12	92.96	80.15	9.18

b) Mature unripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	10.73	12.35	11.45	12.19	7.79	10.89	13.69	9.79	12.9	14.33	11.61	1.93
APPLE F2	26.57	25.14	33.45	28.71	27.07	28.24	30.1	36.1	25.85	25.32	28.66	3.63
KEIT F1	9.26	4.86	6.99	8.75	6.24	9.43	7.46	8.08	6.89	8.76	7.67	1.46
KENT F1	8.33	6.2	7.97	10.11	11.98	12.52	6.54	9.8	11.09	6.94	9.15	2.28
NDOTO F1	17.06	12.35	13.32	22.98	15.1	23.5	12.43	17.8	17.32	ND	16.87	4.16
NGOWE F1	23.36	26.16	13.24	21.85	22.11	13.4	13.7	22.65	15.6	19.11	19.12	4.78
NGOWE F2	6.8	8.6	6.47	8.32	5.59	9.85	6.62	5.64	6.14	8.03	7.21	1.42
TOMMY ATKINS F1	14.61	10.19	15.54	15.87	18.74	10.01	10.95	11.27	8.68	11.74	12.76	3.23
TOMMY ATKINS F2	10.27	9.52	11.31	6.98	12.49	10.34	7.74	9.21	11.56	15.94	10.54	2.54

APPENDIX X: RAW DATA FOR LYCOPENE CONTENT IN MANGOES ($\mu g/100g$)

3. LYCOPENE CONTENT IN MANGOES ($\mu g/100g$)

a) Ripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	25.43	24.54	29.56	26.61	29.02	19.38	18.8	35.94	24	26.24	25.95	4.98
APPLE F2	16.72	18.58	15.4	19.2	18.15	23.5	17.9	20.02	25.29	15.02	18.98	3.28
KEIT F1	16.47	17.49	15.01	16.33	19.7	11.33	13.86	13.21	24.86	21.31	16.96	4.07
KENT F1	34.92	28.5	41.22	28.5	21.04	36.88	36.15	31.91	27.37	37.54	32.40	6.05
NDOTO F1	58.59	52.13	45.68	47.21	51.27	36.23	35.19	39.12	48.79	46.58	46.08	7.40
NGOWE F1	27.3	37.11	35	32.98	44.2	34.93	40.52	35.06	30.13	49.6	36.68	6.60
NGOWE F2	40.83	36.02	38	41.12	31.7	34.33	44.84	46.1	45.14	32.95	39.10	5.29
TOMMY ATKINS F1	65.42	69.82	48.95	62.47	50.84	49.88	57.65	45.23	44.76	44.74	53.98	9.23
TOMMY ATKINS F2	44.71	50.64	47.26	44.63	42.47	53.29	40.44	40.54	44.29	50.38	45.87	4.41

b) Mature unripe mangoes

VARIETY	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8	Sample 9	Sample 10	MEAN CONC	STDEV
APPLE F1	7.13	6.92	6.37	6.11	4.91	7.48	8.69	7.54	8.95	7.51	7.16	1.19
APPLE F2	4.09	5.07	5.97	5.46	6.45	5.46	5.17	4.61	5.6	5.25	5.31	0.66
KEIT F1	4.33	3.46	3.75	5.18	5.03	4.93	4.2	4.46	3.51	4.59	4.34	0.62
KENT F1	9.13	9.73	8.57	7.94	8.54	8.25	8.25	5.3	5.71	8.64	8.01	1.41
NDOTO F1	5.22	6.13	5.31	7.27	5.44	6.02	7.91	7.76	5.97	6.65	6.37	0.99
NGOWE F1	9.11	6.8	8.32	6.4	6.98	8.97	5.52	7.05	6.49	7.99	7.36	1.18
NGOWE F2	4.08	4.52	8.45	5.8	5.06	8.6	5.63	5.05	ND	ND	5.90	1.71
TOMMY ATKINS F1	9.08	5.97	6.34	7.28	9.01	7.4	7.84	7.5	6.97	7.23	7.46	1.00
TOMMY ATKINS F2	7.9	5.57	6.8	5.04	8.13	6.73	9.7	5.14	6.75	9.02	7.08	1.60