RETENTION OF EXTRACTABLE BETA CAROTENE FROM *Asystasia mysorensis* AND *Solanum nigrum* VEGETABLES STORED IN EDIBLE SUNFLOWER AND PALM OILS

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I56/CE/26891/2011

A Thesis Submitted in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science (Applied Analytical Chemistry) in the School of Pure and Applied Sciences of Kenyatta University

September, 2017

DECLARATION

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

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SUPERVISORS' DECLARATION

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

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DEDICATION

This work is dedicated to my beloved wife Pauline Wairimu, my lovely children Daphne Nyakabete, Morris Nderitu, Lynn Wanjiru and Roslyn Wanjiru, my mother Lucy Wanjiru and also to the loving memory of my late dad Paul Nderitu Maina.

ACKNOWLEDGEMENTS

Special recognition goes to my supervisors, Dr Mildred Nawiri and Prof. Hudson Nyambaka for their continuous guidance and support throughout this research. I am particularly grateful to Dr. Nawiri for her availability for consultation and financial support that went a long way in facilitating data collection and analysis. I wish to thank Kenyatta University for giving me a chance to study in this prestigious institution. Special thanks to the entire chemistry department, the lecturers and the laboratory staff particularly Dennis Osoro and Kevin Were for facilitating my work during preparation of samples. I would like to acknowledge the assistance of Peter Langat of Vetcare Kenya limited during HPLC analysis.

I am grateful to my peers Ziporah Mungai, Maxwell Obumba and John Hena whom we worked with closely the whole period and for their continuous encouragement. Special thanks to my mum Lucy Wanjiru, my brothers Martin Maina, Peter Waititu and Joseph Kimotho, my sisters Martha Maina, Esther Karuge, Agatha Muthoni and Ann Karare for their prayers, encouragement, financial and moral support.

Finally, I wish to thank the Almighty God for the care and protection during the study period. All glory and honour to His Name.

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ABBREVIATIONS AND ACRONYMS

AIVs	African indigenous vegetables
ANOVA	Analysis of variance
AV	Acid value
BHT	Butylatedhydroxytoluene
CVDs	Cardiovascular diseases
DCM	Dichloromethane
DGLVs	Dark green leafy vegetables
DM	Dry matter
FAO	Food agricultural organization
GDP	Gross domestic product
HIV/AIDS	Human immune virus- Acquired immune deficiency syndrome
HPLC	High performance liquid chromatography
KOH	Potassium hydroxide
KNH	Kenyatta national hospital
LOD	Limit of detection
NCDs	Non-communicable diseases
OCC	Open column chromatography
PE	Petroleum ether
PET	Polyethylene terephthalate
PV	Peroxide value
RAE	Retinol activity equivalent
RDA	Recommended daily allowance
RE	Retinol equivalent
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
RP-HPLC	Reverse phased high performance liquid chromatography
SNK	Student Newman Keul test
THF	Tetrahydrofuran
TLC	Thin layer chromatography
UV-VIS	Ultraviolet-visible
VA	Vitamin A
VAD	Vitamin A deficiency
VADDs	Vitamin A deficiency disorders
WHO	World health organization

ABSTRACT

Hidden hunger, caused by lack of micronutrients in the diet afflicts billions of people especially in developing nations. Vitamin A deficiency (VAD) is among the top ten risk factors contributing to the global burden of disease, with WHO estimating 1.4 % of all deaths worldwide attributed to it. The deficiency can be addressed by intake of foods rich in preformed vitamin A such as eggs, meat or dairy products, however these are expensive and out of reach for the poor population. A suitable alternative would be vegetables which are rich in beta carotene (a provitamin A carotenoid) but they are seasonal, ignored or underutilized. In Kenya, indigenous vegetables *Solanum nigrum* and *Asystasia mysorensis*, though rich in beta carotene and locally available are grossly underutilized yet they can offer a solution to address VAD. The concern on the perishability of these vegetables calls for a cheaper way of preservation compared to conventional methods including refrigeration, canning and sun-drying. Despite numerous reports showing that oils increase bioavailability and bio-accessibility of beta carotene, no information is available on preservation of extractable beta carotene in edible oils. This study investigated the retention of beta carotene extracted from *S. nigrum* and *A. mysorensis* and preserved separately in sunflower and palm oils for a period of 180 days. The peroxide and acid values of the oils were determined by iodometric titration while RP-HPLC was employed for quantitative determination of beta carotene. One way ANOVA was used for data analysis while SNK was used for separation of means. The mean levels of beta carotene extracted from *A. mysorensis* and preserved in sunflower and palm oils reduced from 24.83 ± 0.002 to 6.67 ± 0.002 mg/100g DM (73.14 % decrease) and 24.82 ± 0.001 to 8.45 ± 0.001 (69.95 % decrease) respectively for the duration of 180 days. Levels of beta carotene from *S. nigrum* preserved in sunflower and palm oils reduced from 41.48 ± 0.003 to 7.65 ± 0.003 (81.56 % decrease) and 41.46 ± 0.003 to 14.28 ± 0.001 (65.56 % decrease) respectively for the same duration. This translates to 0.513 ± 0.0002, 0.650 ± 0.0001, 0.588 ± 0.0002 and 1.098 ± 0.0001 mg/100g DM of retinol activity equivalent (RAE) respectively at the end of storage duration. These values show considerable degradation of beta carotene in sunflower and palm oils though the final amounts retained provided more than the recommended daily allowance (RDA) of retinol in 100 mg consumed. Peroxide values in sunflower and palm oils increased from 3.93 ± 0.03 to 6.84 ± 0.04 and 2.00 ± 0.01 to 2.4 ± 0.01 mEq/kg oil respectively while acid values rose from 0.34 ± 0.01 to 0.64±0.04 and 0.11 ± 0.01 to 0.49 ± 0.04 mg KOH/g respectively. These values indicate that oxidation of the oil matrices occurs with time but not to critical levels (10 mEq/ kg oil and 0.6 mg KOH/g respectively) for the 28 days studied. The findings indicate that edible oils can preserve beta carotene if peroxide and acid values are kept at allowable levels.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Globally, diseases continue to cause untold suffering and untimely deaths to human beings. Non-communicable diseases (NCDs) such as cancers, cardiovascular diseases, diabetes and mental health pose a substantial economic burden worldwide (Bloom *et al*., 2012). Macro-economic simulation in 2010 suggested a cumulative output loss of US$ 47 trillion over the next two decades due to NCDs. This loss represented 75 % of the global gross domestic product (GDP) of US$ 63 trillion that year (Bloom *et al.*, 2012). Another major health concern to developing nations is vitamin A deficiency (VAD). Worldwide, VAD affects approximately 21.1 % of pre-school age children and 5.6 % of pregnant women (RiCE *et al.*, 2004). Statistics show that between 20-24 % of child mortality from measles, diarrhea and malaria can be attributed to this easily preventable condition. In Kenya, 1.8 million pre-school aged children representing 40.6 % and 992000 pregnant women accounting for 9.1 % are vitamin A deficient (West, 2002).

The effects of public health consequences attributed to VAD, the vitamin A deficiency disorders (VADDs) include xerophthalmia which leads to blindness, stunted growth, weakened innate and acquired host defenses, exacerbated infections and increased risk of death (West Jr and Darnton-Hill, 2008). Among women of reproductive age, VAD may increase morbidity and mortality during pregnancy and if severe, it may disadvantage the new born leading to increased mortality in the first months of life. Rich sources of preformed vitamin A include eggs, meat and dairy products but these are economically out of reach for most of the population especially in developing countries (Codjia, 2001). For many communities in developing countries, the major source of VA in the diet is pro-vitamin A carotenoids (Ahamad *et al*., 2007) found in cheap and locally available dark green leafy vegetables (DGLV's).

Carotenoids are tetraterpenoids synthesized in plants and other photosynthetic organisms as well as in non-photosynthetic bacteria, yeast and moulds (Stahl and Sies, 2005). Most of the carotenoids are composed of a central carbon chain of alternating single and double bonds and carry different cyclic or acyclic end groups. Classification based on chemical composition, are the carotenes with beta carotene, alpha carotene and lycopene as prominent members of this group (Rodriguez-Amaya and Kimura, 2004). Carotenoids can also be divided into pro-vitamin A and non-provitamin A compounds (O'Byrne *et al.*, 2004). Provitamin A carotenoids includes beta carotene, alpha carotene, delta carotene and beta cryptoxanthin (Bohn, 2008).

Beta carotene, found in large quantities in dark green leafy vegetables is a fat-soluble member of the carotenoids which is considered vitamin A precursor because it can be converted to active vitamin A. Structurally, vitamin A (retinol) is essentially one half of the molecule of beta carotene with an added water molecule at the end of the lateral polyene chain making it a potent pro-vitamin A to which 100 % activity is assigned

(Yeum and Russell, 2002; Rodriguez-Amaya and Kimura, 2004; Stahl and Sies, 2005). Other than being a major source of vitamin A, beta carotene has other benefits such as a remedy to cancer prevalence due to its antioxidant properties (Stahl and Sies, 2003; Young and Lowe, 2001). Observational studies have consistently found that people who consume large amounts of vegetables have lower rates of some cancers (Pomerleau *et al*., 2005).

Vegetables can be categorized as either exotic or indigenous. Indigenous vegetables are those that have evolved within and spread throughout an area unassisted by humans otherwise exotic. Kenya is home to more than 210 species of indigenous vegetables that are underutilized such as Black nightshade (*Solanum nigrum*), cowpeas (*vigna unguiculata*), spider plant (*cleome gynandra*), *Asystasia mysorensis* among others (Mbugua *et al*., 2006). African indigenous vegetables (AIVs) have been found to be superior to the exotic ones in both human nutrition and medicinal value. These vegetables constitute valuable natural resources that needs to be preserved and their consumption encouraged (Mbugua *et al*., 2006). However, many people in Kenya are still unaware of the importance of AIVs and are yet to include them in their diets. Moreover, local varieties have been replaced by improved or exotic varieties and species (Ngugi *et al*., 2007).

Africa indigenous vegetables are rich in beta carotene, however, their content is likely to be affected by many factors such as cultivar or variety, climate of production, stage of maturity, geographic conditions, harvesting and postharvest handling as well as part of the plant consumed (Rodriguez-Amaya and Kimura, 2004). The availability of AIVs is season dependent, being plenty during the rainy season and scarce during the dry season. This challenge is alleviated by a number of ways such as improved home gardening or preservation methods such as refrigeration, caning and drying that prevent massive loss of the perishable vegetables. Efforts have been put in place to preserve the valuable carotenoids in vegetables such as beta carotene which undergo degradation easily. The mechanisms of any carotenoid degradation involves among others, the reaction with atmospheric air (autooxidation), heat (thermal degradation), light (photodegradation) as well as interaction with singlet oxygen, acid, metals and free radicals (Moura *et al.*, 2015).

A study done on dark green leafy vegetables showed that open sun dried vegetables improves the bioavailability of beta carotene, retinol and hemoglobin levels among preschool children as dehydration retained beta carotene levels at over 60 % (Nawiri *et al.,* 2013). Further findings indicate that the amounts of provitamin A carotenes in traditionally treated vegetables were extensively reduced by open sun-drying than solar drying (Mulokozi *et al.,* 2000). Other preservation methods like encapsulation and phospholipids manufacture that aims at preserving the extractable carotenoids have been explored though expensive. Owing to the many health benefits of beta carotene, it is absolutely necessary to come up with cheaper and viable preservation methods.

Many factors influence carotenoid bioavailability. One such factor is vegetable oils as a source of specific fatty acid (Reddy, 2011). Edible vegetable oils are triglycerides, natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acids with different levels of unsaturation (Dauqan *et al.*, 2011). Some common edible oils include: sunflower oil, palm oil, corn oil, and olive oil among others. Vegetable oils are rich in polyunsaturated fatty acids which are prone to oxidation giving peroxides and other compounds that give oils objectionable odour (Kamau and Nanua, 2008). Oxidation of edible oils is influenced by factors such as light, heat, composition of fatty acids, contact with oxygen and minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and diacylglycerols, thermally oxidized compounds, and antioxidants (Choe and Min, 2006). Peroxide values, acid values and thiobarbituric acid values are useful parameters in assessing the extent to which spoilage or deterioration has advanced in vegetable oils.

Since edible oil matrices stabilize beta carotene and consequently slow down degradation process (Choe and Min, 2006), storage of extractable beta carotene from locally available vegetables will not only provide a cheaper preservation method which avails enough beta carotene to address VAD in target population but also utilize fully AIVs. Furthermore, the findings regarding peroxide and acid values will be useful to edible oils manufacturers in ensuring oils of high quality in order to promote and maintain healthy dietary practices.

1.2 Problem statement and justification

Kenya and other developing nations spend a lot of resources managing diseases caused by lack of proper nutrition. Hidden hunger, caused by lack of micronutrients in the diets, afflicts billions of people leading to high cost expenditure on drugs to correct the health situation. Nutritional approach is cheaper and a more sustainable solution in the long term. An estimated 250 million preschool children and a substantial proportion of pregnant women in developing countries suffer from VAD which can be addressed cheaply by consumption of foods rich in beta carotene such as the underutilized indigenous vegetables. However, vegetable availability all year around is a great problem especially during the dry seasons.

Many methods to preserve vegetables have been explored. These include refrigeration, solar drying, canning, storage under oxygen absorbers among others. This research seeks to explore preservation of beta carotene extracted from the vegetables as opposed to preserving the whole vegetable as is the case for the methods above. Edible oils on one hand increase bioavailability of beta carotene while on the other hand beta carotene slows down oil oxidation by light filtering, singlet oxygen quenching, sensitizer inactivation and free radical scavenging (Reddy, 2011). This study projected that the extractable beta carotene from *A. mysorensis* and *S. nigrum* stored in edible sunflower and palm oils would be retained in sufficient levels enough to provide RDA to target groups such as pre-school children and lactating mothers especially in developing countries.

1.3 Hypothesis

There is no significant reduction of beta carotene extracted from *A. mysorensis* and *S. nigrum* and preserved in edible oils for up to 180 days.

1.4 Objectives

1.4.1 General objectives

To monitor levels of extractable beta carotene from *A. mysorensis* and *S. nigrum* stored in edible vegetable oils for a period of 180 days.

1.4.2 Specific objectives

i. To monitor the peroxide values of sunflower and palm oils stored under room temperature for 28 days.

ii. To monitor the acid values of sunflower and palm oils stored under room temperature for 28 days.

iii. To monitor levels of extractable beta carotene from *A. mysorensis* and *S. nigrum* vegetables preserved in sunflower and palm oils for a period of 180 days**.**

1.5 Significance of the study

The findings of this research will provide information on an alternative way of preserving beta carotene. Beta carotene is not only a pro-vitamin A carotenoid that splits enzymatically to provide vitamin A but also an anti-oxidant that is able to reduce oxidative stress that would otherwise be the genesis of degenerative diseases. Vegetable oils are used by virtually all homesteads the world over. This means that beta carotene, once preserved in these oils can reach the target population hence curb VAD. The oxidation status of oils greatly interacts with the stability of beta carotene once the carotenoid is stored in them thus delaying its oxidation and eventual degradation. The findings are expected to promote edible oils as matrices for storing the carotenoid and consequently addressing the challenge of massive waste of perishable vegetables available during rainy seasons. Of utmost long term vision generated from the findings is the contribution of such retained values of beta carotene to fighting nutritional related illness among them VAD, diabetes, CVDs, cancers among others.

1.6 Scope and limitations

The quantity of beta carotene in vegetables is affected by such factors such as cultivar or variety, stage of maturity, geographic site of production and postharvest handling that were not considered in this study. Degradation of carotenoids may involve reaction with atmospheric oxygen, light, heat, acid, metals and free radicals. Other than exposure to acid, free radicals and metals that were not monitored in the procedures, efforts were made to minimize the effects of light, air and heat although uncontrollable. Products of beta carotene degradation were not investigated. Though peroxide values, acid values and thiobarbituric acid values are used in assessing the extent to which spoilage or deterioration has advanced in vegetable oils, thiobarbituric acid values were not investigated in this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Malnutrition and vitamin A deficiency in Kenya

Hidden hunger, a term used to refer to micronutrient malnutrition is an important public health problem that afflicts billions of people especially the poor (Moura *et al*., 2015). A diet with chronically insufficient vitamin A leads to a major nutritional concern known as vitamin A deficiency (VAD) (Sommer and Vyas, 2012). An estimated 250 million preschool children and a significant proportion of pregnant women suffer from VAD in developing countries, Kenya included (Moura *et al*., 2015). Table 2.1 presents basic demographics and health-related indicators in Kenya in 2010.

Table 2-1: Demographics and health-related indicators for Kenya

Population	37 million
GDP per capita	US $ 760
Population living in rural areas	78%
Infant mortality	55/100 live births
Life expectancy	54 years
Total national budget dedicated to health	8.4%
Government expenditure annually on health per capita	US $ 8.30

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

Vitamin A deficiency is a major public health problem in Africa, especially in the Sahelian countries (Codjia, 2001; WHO, 2009). It occurs mainly in young children and women of child bearing age. It is caused by a dietary pattern providing too little bioavailable vitamin A to support physiological needs under the prevailing circumstances. Vitamin A deficiency is determined clinically through signs of xerophthalmia in an individual or by measurement of serum retinol where values less than 20µg of retinol per gram indicate VAD (Sommer and Davidson, 2002). Human and animals do not synthesize Vitamin A (VA) hence it must be provided from the diet in sufficient amounts to meet all physiological needs (West Jr and Darnton-Hill, 2008). Rich sources of preformed VA include liver, eggs and milk products but these are economically out of reach for most of the population especially in developing countries (Codjia, 2001). For many communities in developing countries, the major source of VA in the diet is pro-vitamin A carotenoids (Ahamad *et al.*, 2007). Table 2.2 shows the main animal sources of VA including liver, eggs, milk and milk products

Table 2-2: Vitamin A content of the main animal vitamin A sources

Food source	Vitamin A µg RE / 100g edible part
Fresh whole milk (goat, cow, sheep, camel)	25-95
Eggs	90.5
Dairy butter (cow)	130
Cheese	297
Cow liver	810
Goat liver	7490
Chicken liver	8235

(Source: Wu Leung *et al.*, 1968)

Even though these sources are rich in highly bioavailable VA, their consumption among the African population is still low. Plants rich in provitamin A represent more than 80 % of the total food intake of VA because of their low cost, high availability and diversity. Fruits, roots, tubers and leafy vegetables are the main providers of provitamin A carotenoids (Codjia, 2001).

2.2Carotenoids

Carotenoids are tetraterpenoids synthesized in plants and other photosynthetic organisms. They are also synthesized by some species of bacteria, yeast and fungi which are not photosynthetic (Stahl and Sies, 2005). The yellow, orange and red colour of many fruits and flowers is caused by carotenoid containing chromoplasts. Carotenoids are also found in animal species and are important colorants in birds, insects, fish and crustaceans (Britton *et al*., 2004; Stahl and Sies, 2005; Ahamad *et al*., 2007). Food carotenoids are usually C_{40} tetraterpenoids built from eight C_5 isoprenoid units joined so that the sequence is reversed at the centre. This results to a symmetrical molecule which can be cyclized at one or both ends and has lateral methyl groups separated by six carbon atoms at the centre and five carbon atoms elsewhere (Rodriguez-Amaya, 2001). Cyclization and other modifications such as halogenations, dehydrogenation, double bond migration, chain shortening or extension, rearrangement, isomerisation, introduction of oxygen functions or combination of these processes result in a myriad of structures (Rodriguez-Amaya and Kimura, 2004). More than 700 carotenoids with different structures have been isolated from natural sources (Djuikwo *et al*., 2011).

Carotenoids are categorized according to their chemical composition as either carotenes or xanthophylls (Stahl and Sies, 2005). Hydrocarbon carotenoids, generally known as carotenes include β-carotene, α-carotene and lycopene. Xanthophylls which are essentially oxidation products of the carotenes include lutein, zeaxanthin, β-cryptoxanthin among others (Rodriguez-Amaya and Kimura, 2004; Stahl and Sies, 2005). The principal carotenoids in foods include β-carotene **(1),** α-carotene **(2),** β-cryptoxanthin**(3),** lycopene **(4),** and lutein **(5)** shown in figure 2.1 (Rodriguez-Amaya and Kimura, 2004; Stahl and Sies, 2005).

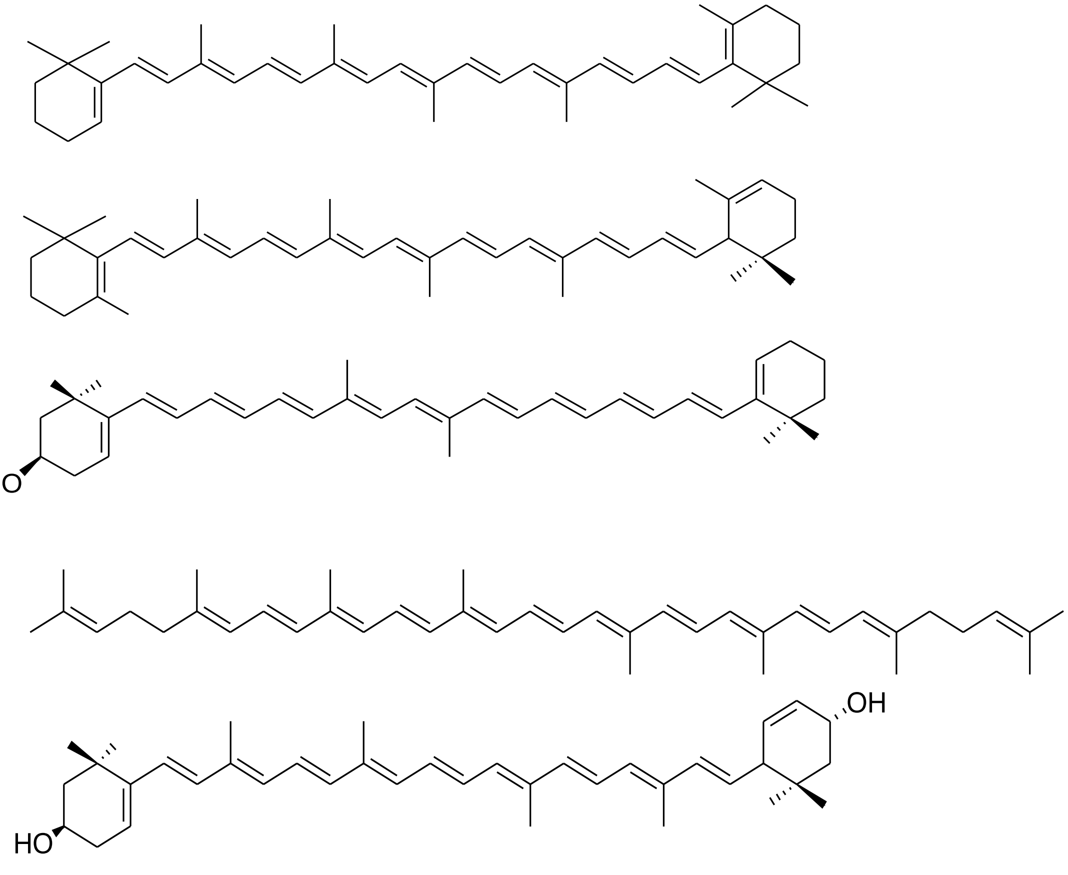

Figure 2-1: Structures of some carotenoids

Carotenoids can also be categorized into provitamin A and non-provitamin A compounds. The major provitamin A carotenoid is β-carotene with 100 % vitamin A activity. Others include α-carotene and β-cryptoxanthin. Non-provitamin A carotenoids include lutein, zeaxanthin and lycopene (Yeum and Russell, 2002).

2.2.1 Physiochemical properties of carotenoids

Carotenoids are lipophilic compounds, they have virtually no solubility in water but are soluble in organic solvents. Carotenes are soluble in petroleum ether, hexane and toluene while xanthophylls dissolve better in methanol and ethanol (Rodriguez-Amaya, 2001). The highly conjugated double bond system constitutes the light absorbing chromophore that provides the visible absorption spectrum that serves as the basis for their identification and quantification. Loss or change of colour at any time gives indication of degradation or structural modification (Rodriguez-Amaya, 2001). The highly unsaturated carotenoid is prone to isomerization and oxidation. Factors such as heat, light, air, acid and adsorption on an active surface promote isomerization of all trans carotenoids to the cis forms. Oxidative degeneration, the principal cause of extensive loses of carotenoids depends on the availability of oxygen and is stimulated by light, enzymes, metals and co-oxidation with lipid hydroperoxides (Rodriguez-Amaya, 2001).

2.2.2 Carotenoids and health

Carotenoids are unique constituents of a healthy diet and suitable photoprotectants not only to plants but also to humans. Epidemiological studies demonstrate that an increased consumption of a diet rich in carotenoids is associated with reduced risk for different kind of cancers (Tapiero *et al*., 2004; Rao and Rao, 2007). Carotenoids are known to scavenge for free radicals involved in the pathobiochemistry of degenerative diseases. Other health benefits of carotenoids include protection against age related macular degeneration, source of vitamin A and intercellular signaling which is a prerequisite for coordinating biochemical functions in multicellular organisms (Stahl and Sies, 2005). Carotenoids are rarely used as a specific therapy per se but commonly recommended as part of a healthy diet or as purified supplements.

2.3 Beta carotene

2.3.1 Sources

Beta carotene, the principal carotenoid in carrots is a hydrocarbon with a formula $C_{40}H_{56}$ whose semi systemic name is β,β-carotene. The bicyclic beta carotene is the most widespread of all carotenoids in foods. Studies show that dark green leafy vegetables (DGLV's) such as spider plant, kale, amaranths, cowpeas, African nightshade among others are rich in beta carotene (Koech *et al*., 2005). Table 2.3 shows some selected indigenous vegetables and their levels of beta carotene.

Table 2-3: Mean beta carotene from selected indigenous vegetables

Vegetable	Mean BC content in µg per 100g edible portion
Spider plant	10452
African nightshade	9047
Cowpeas	5880
A. mysorensis	5783
Amaranths	5387
Cats whiskers	13170

(Source: AGEA *et al.*, 2014).

2.3.2 Chemistry

2.3.2.1 Provitamin A activity

Vitamin A is a fat soluble vitamin that is essential for various physiological processes in the body with the greatest function being in vision. Naturally occurring vitamin A compounds are considered a subset of a much larger family of retinoids that share a common monocyclic, double bonded chemical structure with various functional terminal groups (West, 2002). Preformed VA is found exclusively in animal foods with high levels reported in fish livers and fish oils (West and Mehra, 2010; Kraemer *et al.*, 2012). The main forms include retinol, retinal and retinoic acid. Lipid soluble yellow and orange carotenoids provide the precursor form of VA to animal diets. β-carotene, α-carotene and β-cryptoxanthin are provitamin A carotenoids. The all trans isomer of beta carotene is the most potent provitamin A carotenoids due to its high provitamin A activity. Table 2.4 shows relative vitamin A activity of different carotenoids.

Table 2-4: Relative vitamin A activity of provitamin A carotenoids

Carotenoid	% Activity
All trans-β-carotene	100
All trans- cryptoxanthin	57
13 cis-β-carotene	53
All trans-α-carotene	53
β-carotene 5,8-epoxide	50
Gamma carotene	42-50
15-cis-cryptoxanthin	42
9-cis-β-carotene	38
9-cis-cryptoxanthin	27
β-zeacarotene	20-40
β-carotene 5,6-epoxide	21
13-cis-α-carotene	16
9-cis-carotene	13

(Source: Lietz *et al.*, 2010)

Vitamin A is formed from all trans beta carotene through an enzymatic reaction where beta-carotene 15, 15’monooxygenase cleaves the molecule centrally to form retinol. A secondary mechanism involves beta carotene 9’,10’-dioxygenasewhich cleaves beta carotene, α-carotene and β-cryptoxanthin eccentrically to form two apocarotenals. The longer of the two is then oxidized to one molecule of retinal (Chichili *et al.*, 2005; Lietz *et al.*, 2010). Carotenoid conversion to VA in the body has been estimated to be 6 µg BC: 1 µg VA (FAO and WHO, 2005).

2.3.2.2 Antioxidant activity of beta carotene

Reactive oxygen and nitrogen species (ROS and RNS) are generated in the course of anaerobic metabolism and pathological processes. They cause damage to important molecules like DNA, lipids and proteins contributing to pathobiochemistry of degenerative diseases (Stahl and Sies, 2005). Beta carotene is one of the most efficient singlet molecular oxygen quencher. This ability is linked to the highly conjugated double bond system (Rodriguez-Amaya and Kimura, 2004, Stahl and Sies, 2005). Singlet oxygen quenching by beta carotene occurs through both physical and chemical quenching. Physical quenching involves the transfer of excitation energy from singlet oxygen to the carotenoid resulting in ground state oxygen and an excited triplet state carotenoid. The energy is dissipated between the excited beta carotene and the surrounding solvent to yield ground state carotenoid and thermal energy (Packer *et al*., 2005). In the process of physical quenching, the beta carotene remains intact and can undergo further cycles of singlet oxygen quenching. Beta carotene efficiently scavenges peroxy radicals and contributes to the defense against lipid peroxidation. Carotenoids interact with free radicals in three main ways. These include electron transfer, hydrogen abstraction and addition of a radical species (Krinsky *et al*., 2004; Rao and Rao, 2007).

2.4 Vegetables

2.4.1 African indigenous vegetables and nutrition

African indigenous vegetables (AIVs) is a term used to refer to those plants whose leaves or aerial parts have been integrated in African communities culture for use as food over a long span of time (Koech *et al.*, 2005). Some of these AIVs include African nightshade (*S. nigrum*), *A. mysorensis*, Amaranths, Crotalaria, spider plant, jute mallow, African eggplant among others (Karanja *et al*., 2011). Studies show that AIVs are superior to exotic ones in human nutrition and medicinal value. They are rich in micronutrients such as vitamin A, iron, zinc and contain non-nutrients called phytochemicals which help protect human beings against NCD’s (Abukutsa-Onyango *et al*., 2010). Compared to the conventionally well known and widely cultivated *Brassica oleraceavar p capitata* L (cabbage), AIVs have been reported to be richer sources of macro-nutrients, vitamin C and beta carotene hence can help improve household nutrition especially during periods of social unrest, droughts, famine and other natural calamities (AGEA *et al*., 2014).

2.4.2 Utilization of African indigenous vegetables

Socio-economic changes that have taken place in Africa have influenced people's eating habits in both rural and urban set-ups. Most people prefer exotic foods to traditional foods, including plant foods whose consumption is widely regarded as a primitive culture manifesting poor lifestyles. However, recent studies on traditional plant foods have shown that some are highly nutritious; containing high levels of both vitamins and minerals (Orech *et al*., 2005). They also have potential as a remedy to counter food insecurity since most are well adapted to the local environment, enabling them to resist pests, drought and diseases (Orech *et al*., 2005)**.**

Globally, Food and Agriculture Organization, FAO reports that at least one billion people are thought to use wild foods in their diets (Burlingame, 2000). As accompaniment to carbohydrate-based staples, AIVs are highly valued in the African diet and have advantages over other crops such as shorter cycles, fast growing, require little space, maximize scarce water supplies and soil nutrients and are less risk-prone (Weinberger and Lumpkin, 2007). Studies show that AIVs are rarely affected by common pests and diseases that affect other vegetables (Abukutsa-Onyago, 2002). Studies done in Uganda reported that wild vegetables are mainly consumed during dry seasons and early in the rainy seasons when cultivated food resources are least available. It can thus be concluded that AIVs are mainly used as substitute for cultivated species during the lean periods of the year.

African indigenous vegetables are becoming important as commercial crops and as items in diet for Kenyans of all economic status (Onyango and Jasper, 2007). Kenya is home to more than 200 different indigenous plant species which are used as leafy vegetables. Of these, only a few have been fully domesticated while more are semi-domesticated and majority are collected from the wild. The most commonly consumed AIV’s include *Amaranthus spp* (pig weed), *Vigna spp* (cowpea leaves), *Solanum spp* (nightshade), *Cleomegynandra* (cats whiskers), *Cucurbita spp* (pumpkin leaves) and *Corchous spp* (jute/bush okra) (Onyango and Jasper, 2007). Among the many AIVs in Kenya, this study was limited to *Asystasia mysorensis* and *Solanum nigrum.*

2.4.2.1 *Asystasia mysorensis*

Asystasia mysorensis (appendix I) is a small herb that grows to a height of 30-75 cm. It is widely distributed in Kenya and other parts of Africa. Among the different communities of Kenya, it is known by different names for example *muhika-na-ihu* (Kikuyu), *kisuvu* (Luhya), *atipa* (Luo), *karimi-ka-nthia* (Mbeere), *orongwo* (Pokot), *esidiba* (Teso) among others. It is common in Nyanza, Western and Nairobi where the leaves are cooked and eaten as vegetables. It is mainly available during the rainy season and soon after (Maundu *et al*., 1999).

2.4.2.2 *Solanum nigrum*

African nightshades play an important role in satisfying the nutritional needs of many rural households. They are reported to be particularly rich in proteins, vitamin A, iron and calcium (Mwai*et al.*, 2007). In Kenya, nightshades occur in many areas where they are known by different local names such as *managu* (Kikuyu), *ndulu* (Kamba), *osuga* (Luo), *lisuta* (Luhya), *mnavu* (Giriama), *ksoyo* (Pokot), *kisochot* (Elgeyo) among others. According to Mwai *et al*. (2007), nightshades are known to grow well under high moisture conditions, rainfall of about 1500 mm and temperature between 20-30 ^{o}C. They do well in fertile soils that are rich in nitrogen, phosphorous and high organic matter. *Solanum nigrum* (appendix II) is an erect herbaceous plant that grows to 1 meter or more with elliptic leaves and small white flowers borne on a branched inflorescence. Its fruits are green, turning orange, red, yellow or shiny purplish depending on the species and the leaves are used as vegetables normally eaten with ugali (Maundu *et al*., 1999).

2.4.3 Preservation and storage

In Kenya, AIVs are grown for their nutritional, medicinal and commercial value (Koech *et al.*, 2005). Their availability and consumption, however is seasonal with supply being high during the rainy season and limited during the dry season. The AIVs are highly perishable due to their high moisture content hence preservation methods should be explored to enhance availability during the dry months of the year (Kendall *et al*., 2004; Mulokozi and Svanberg, 2003). Many methods have been applied to preserve vegetables, the common ones being freezing, fermentation, canning and dehydration (Kendall *et al*., 2004). Dehydration is the most applicable in developing countries like Kenya due to the cost implication as it is cheap and therefore affordable (Mulokozi *et al*., 2000; Nyambaka and Ryley, 2001; Kendall *et al*., 2004).

Drying is one of the oldest form of food preservation. It works through removal of enough moisture from food to prevent decay and spoilage through microbial attack from bacteria and fungi (Kendall *et al*., 2004). Successful drying involves removal of moisture at a temperature that does not seriously affect the flavor, texture and colour of the food. According to Ndawula *et al*. (2004), open sun drying done on cowpeas leaves caused 58 % loss while visqueen-covered solar drying caused 34.5 % loss of beta carotene. Blanching is done before dehydration as it helps slow down or stop the enzyme activity that can cause undesirable changes during dehydration period. Blanching cowpeas leaves improved beta carotene retention by 15 % (Ndawula *et al*., 2004). It also relaxes tissues such that foods dry faster, protects the vitamins and colour and reduces time needed to refresh vegetables before cooking (Kendall *et al*., 2004).

Different dehydration methods have been explored such as open sun drying, solar drying and oven drying. Study done by Mulokozi *et al.* (2000) on eight different vegetables reported between 57-90 % retention in beta carotene in blanched solar dried compared to between 42-64 % of blanched open sun dried. The relatively higher retention is attributed to favorable conditions in solar driers such as shorter drying time and shielding from direct ultra violet radiation (Mulokozi and Svanberg, 2003). The study reported that the amount of all trans beta carotene in traditionally treated vegetables was highly reduced by open sun drying with significantly higher retentions reported in solar dried vegetables. Sun drying is the oldest and easiest dehydration method but has many shortcomings such as contamination by dirt or rodents, infestation by insects, easy spoilage from exposure to weather elements and uncontrolled drying conditions (Nyambaka and Ryley, 2001).

Dehydration is widely used to preserve dark green leafy vegetables but storage in normal atmosphere conditions causes beta carotene loss through oxidation. A study done on dehydrated dark green leafy vegetable samples of amaranthus (*amarathus hybridus*), nightshade (*solanum nigrum*), and cowpea leaves (*vigna unguiculata*) stored with oxygen absorber for a period of up to six months showed beta carotene loss ranging between 16.41 and 32.28 % while a loss of 61.4 to 81.3 % was noted for samples stored under normal conditions (Nyambaka *et al*., 2012). Dehydrated cowpea vegetables stored in Kraft paper was found to have higher loss of beta carotene than samples stored in polyethylene paper (Muchoki *et al.*, 2007). This can be attributed to the fact that Kraft paper is permeable to air. Amounts of pro-vitamin A carotenoids in traditionally treated vegetables were highly reduced by open sun-drying while solar drying resulted in significantly more retention of the carotenoids (Mulokozi and Svanberg, 2003).

A study done on extractable beta carotene samples of amaranthus (*amarathus spp*) preserved in virgin coconut oil and unadulterated honey for a period of up to six months reported beta carotene loss of 90.77 % and 82.81 % respectively (Mungai *et al*.,2016). While the loss was significant, the findings indicated that the retained beta carotene was enough to meet RDA needs for target groups. Powdered beta carotene encapsulated in dry phosholipid particles and preserved in refrigerated vacuum conditions reported a 10 % loss as opposed to a 30 % loss in normal conditions (Moraes *et al*., 2013). It is therefore imperative that methods with better retention, hygienically safe and those that make beta carotene bioavailable and bioaccessible be explored to enable more beta carotene to be availed to the target group.

Fortification of edible oils with micronutrients such as vitamin A is considered a cost effective and simple to implement strategy to combat VAD but the stability of VA remains a limiting factor (Laillou *et al*., 2012). Many published studies as well as manufacturers reports indicate that VA in edible oils has good storage stability when protected from light and oxygen and that only minor losses are incurred during normal cooking procedures (Gao *et al*., 2001; Dary and Mora, 2002; Viana *et al*., 2007). The oxidation status of edible oil as assessed by peroxide values greatly affects the stability of VA in the oil. Vitamin A oxidizes faster and losses its activity in the presence of oxidized oils (Laillou *et al*., 2012)**.**

During oil production, formation of peroxides generates oil rancidity and undesirable changes in oil quality which causes deterioration of added micronutrients such as VA (Viana *et al*., 2007). In most cases, fortification standards do not take into account the potential effect of different quality of edible oils on stability of VA added. The aim of this study was to investigate the stability of beta carotene extracted from two underutilized indigenous vegetables, *Asystasia mysorensis* and *Solanum nigrum* and used to fortify two edible (sunflower and palm) oils with different chemical characteristics.

2.5 Edible oils

Many factors influence carotenoid bioavailability. One such factor is vegetable oils as a source of specific fatty acid (Reddy, 2011). Oleic and linoleic acids in mixed micelles of olive oil and sunflower oil in a diet, significantly elevate the plasma and tissue levels of beta carotene (Lakshminarayana *et al.*, 2005). Edible vegetable oils are natural products of plant origin consisting of ester mixtures derived from glycerol with chains of fatty acids with different levels of unsaturation (Dauqan *et al.,* 2011). Some edible oils are extracted from seeds by pressing or by extraction with hexane. Some oils, such as virgin olive oil are used without further treatment other than filtering but most are refined to some extent before use (Gunstone, 2011). Vegetable oils play important functional and sensory roles in food products and act as carriers of fat-soluble vitamins A, D, E and K. Some common edible oils include: corn oil, sunflower oil, palm oil, olive oil among others.

Oxidative stability of edible oils is the resistance to oxidation during storage (Gullen and Cabo, 2002). Oxidative stability of edible oils is affected by temperature, light, oxygen concentration, processing and fatty acid composition (Choe and Min, 2006). Edible oils consists of mainly triacylglycerols but also contains minor components such as carotenoids, free fatty acids, mono and diacylglycerols, metals, phospholipids, peroxides, chlorophylls, phenolic compounds and tocopherols. Some of the components accelerate oil oxidation and others act as antioxidants (Choe and Min, 2006). Edible oils, especially unrefined oils, contain beta carotene which can slow down oil oxidation by light filtering, singlet oxygen quenching, sensitizer inactivation and free radical scavenging (Choe and Min, 2006).

2.6 Methods of analysis

Since analysis of carotenoids is complicated and prone to errors, the analyst must be highly skilled and well conversant with analytical procedures, laboratory conditions and properties of the carotenoid being analysed for accurate results to be obtained (Rodriguez-Amaya and Kimura, 2004). The technique available for the extraction of carotenoids is solvent extraction with water miscible organic solvents such as acetone, methanol, ethanol or mixtures of the same. This is so because the food matrix contain large amounts of water (Rodriguez-Amaya and Kimura, 2004). Acetone has been frequently used but with the advent of high performance liquid chromatography (HPLC), tetrahydrofuran (THF) has also become a popular extracting solvent. Exhaustive extraction is confirmed by sequential extraction until residue is colorless. Stability of carotenoids must be taken into account to avoid isomerisation and degradation.

Partitioning is an integral part of open-column chromatography (OCC) so that chromatography can be started at low mobile phase polarity and then increased during extraction process. In HPLC, the extract is evaporated to dryness and then dissolved in mobile phase or a solvent compatible with the mobile phase. Solvents with low melting points such as petroleum ether (PE) (b.pt 40-60 ^{o}C), dichloromethane (DCM) (b.pt 42^{o}C) are preferred for extraction and partitioning. Saponification effectively removes chlorophyll and other unwanted lipids which may interfere with chromatographic separation and shorten the column's life in HPLC.

Several methods for separation and detection can be employed. Thin layer chromatography (TLC) is a good method as it is simple, accurate and reproducible. Unlike other chromatographic processes, TLC is capable of separating large number of samples in a single run (Preedy, 2012)**.** TLC however is not adequate for quantitative analysis because of the danger of degradation and isomerisation on the highly exposed plate (Rodriguez-Amaya and Kimura, 2004).

Chromatography in descending, gravity-flow columns commonly referred as open column chromatography (OCC) is a powerful classical method of separating carotenoids for quantitative analysis. Though the method is simple and inexpensive, reproducibility and efficiency of the separation largely depends on the analyst's skill, patience and experience in packing the columns, adjusting the volumes and proportions of the eluting solvent and keenness for detecting the separation (Rodriguez-Amaya and Kimura, 2004).

HPLC is a highly improved form of column chromatography where there is possibility of reproducible separations with a reusable column under controlled conditions and without undue exposure to air or light. In addition, smaller particle sizes for the column packing material gives a greater surface area for interactions between the stationary phase and analyte molecules. This allows better separation of components (Lough and Wainer, 1995). The detectors employed in HPLC are highly automated and extremely sensitive. Reverse phased high performance liquid chromatography (RP-HPLC) is the most commonly used form of HPLC. It uses the same column size but the silica is modified to make it non-polar hence attaching long hydrocarbon chains to its surface.

RP-HPLC can have isocratic elution or gradient elution. Isocratic elution is where the composition of mobile phase remains the same throughout the analysis while gradient elution is where polarity of mobile phase is changing thus varying the retention time (t_r) (Rouessac and Rouessac, 2007). HPLC has a number of advantages such as compatibility with most solvents and polarity range of carotenoids, weak hydrophobic interactions with the analytes, high speed resolution, sensitivity and accuracy (Ficarro *et al*., 2005)**.** Separation of analytes occurs in columns packed with silica gel in form of spherical particles of between 2 µm-5 µm diameters. Most carotenoid analysis has been carried out using 5 µm C_{18} spherical particles packed in a 250 × 4.6 mm column (Rodriguez-Amaya and Kimura, 2004).

Identification of carotenoids is done by detectors. An effective detector should be sensitive, have a wide linear range and capable of filtering background noise (Rouessac andRouessac, 2007). HPLC gives data images in form of chromatograms. The chromatogram displays a peak indicating presence of a chemical in the sample. Each peak is associated with a certain retention time (r_t) indicating how long it takes for a compound to elute out of a HPLC column. Using calibration curves of standards, the amount of the analyte can be quantified by integrating peak areas in the HPLC chromatograms (Perry *et al*., 2009)**.**

Traditional iodometric titration with thiosulfate is an accurate assay used to determine lipid oxidation. It is stoichiometric, linear and useful for high peroxide concentrations. Unclear endpoints limit sensitivity and therefore handling issues must be well controlled to ensure reproducible results (Steltzer, 2012). Results ultimately depend on the analysts' perception of colour change when endpoint is reached. Potassium iodide used during peroxide value determination should always be freshly made to enhance reaction performance. The biggest problem with iodometric titration is oxygen. This can be overcome by bubbling argon in every step of the analysis. Spurging argon in and over the reaction solution followed by vigorous mechanical stirring increases reproducibility of the method (Steltzer, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Research Design

The research involved monitoring levels of extractable beta carotene from *A. mysorensis* and *S. nigrum* leaves stored in sunflower and palm oils for a period of six months. Analysis of beta carotene was done at intervals of 14 days for the first month and 30 days for the subsequent period of five months. Peroxide and acid values of the sunflower and palm oils used for preservation of beta carotene were monitored for a period of one month.

3.2 Sampling and pretreatment

Five different vendors selling *A. mysorensis* and *S. nigrum* respectively were randomly selected at Githurai market in Nairobi County. From each, 5 fresh bundles of each vegetable were bought to make approximately 600 g in total. The vegetables were transported to Kenyatta University Department of Chemistry Laboratory packed in crates. In the laboratory, the bundles of each vegetable were thoroughly mixed up to make a homogeneous sample. The vegetables were washed thoroughly using distilled water to remove soil particles and other matter attached to them. From each clean vegetable, 500g of laboratory sample was weighed accurately awaiting extraction. Sunflower and palm oils (Appendix III) were purchased from a supermarket in Nairobi County and taken to Kenyatta University department of chemistry laboratory for use in the study. Oils bought had two weeks from the date of manufacture.

3.3 Cleaning of apparatus

All apparatus were soaked in 10 % analytical grade nitric acid overnight, washed using clean water and liquid detergent and rinsed several times with running tap water. The glassware's were dried in an oven at 105 ^{o}C for 12 hours. Rinsing was done using distilled water every time the apparatus were in use.

3.4 Chemicals and reagents

Glacial acetic acid, chloroform, potassium iodide, soluble starch, sodium thiosulfate, phenolphthalein, and potassium hydroxide were purchased from Kobian laboratories Nairobi Kenya. Acetonitrile, methanol, deionised water and dichloromethane were of HPLC grade, while all the other chemicals used including the solvents were of analytical grade.

3.5 HPLC and specifications

Separation was performed using HPLC (Shimadzu Prominence LC-20A) with a LC-20AD pump and UV-VIS SPD-20VA deuterium lamp detector. Samples were injected on to a Gemini-NX 18110A column (5 µm; 250 × 4.6 mm) at 30 ^{o}C. Volumes of standard and samples injected were 1 µL and 20 minutes elution time was allowed. A mobile phase consisting of acetonitrile: methanol: dichloromethane (70:10:20) was isocratically eluted at a flow rate of 0.8 ml\min.

3.6 Recovery studies

The accuracy of the extraction method was investigated by spiking five samples of beta carotene from each vegetable with 1 ppm of beta carotene standard. Determination of the analyte concentration was done from peak areas of the resulting chromatograms. The percentage recovery was calculated according to Equation 1;

$$\%R = \left(\frac{a-b}{c} \right) .100 \text{ ....(1)}$$

Where:
R- recovery
a- concentration of beta carotene in sample after spiking.
b- concentration of beta carotene in sample before spiking.
c- concentration of beta carotene standard used for spiking.

Repeatability of the procedure used was calculated for five replicates using 10 g of each vegetable. After extraction of beta carotene, the peak areas obtained from resulting chromatograms were used to work out the concentration using the calibration curve.

3.7 Laboratory procedures

3.7.1 Peroxide value determination

To measure peroxide value, the AOCS Cd 8-53 method was followed (Steltzer, 2012). Five (5) g of vegetable oil was dissolved in 30 ml of glacial acetic acid: chloroform solution (3:2). Exactly 0.5 ml of saturated potassium iodide solution was added and the flask stoppered with occasional shaking of the mixture. After exactly 1 minute, 30 ml of distilled water was added and the headspace flushed with nitrogen. Three drops of 1 % starch solution was added to produce a bluish purple colour. The solution was then titrated with 0.01 Nsodium thiosulfate until the blue colour disappears. Peroxide value was calculated according to Equation 2;

$$\mathrm{PV} = \frac{1000 \times \mathrm{Vs} \times \mathrm{Ns}}{\mathrm{g}} \ldots\ldots\ldots\ldots\ldots\ldots. (2)$$

Where:

PV is peroxide value in mEq/kg of vegetable oil

Vs is the volume of $Na_2S_2O_3$ used in the vegetable oil sample until yellow colour fades (ml)

Ns is the normality of $Na_2S_2O_3$ (mEq/ml used for titration)

g is the mass of vegetable oil sample used in grams

3.7.2 Acid value determination

To measure acid value, the AOCS Cd 3d-63 method (Steltzer, 2012) was used. Exactly 2.5g of oil was dissolved in 25 ml of petroleum ether and the contents of the flask heated on a steam bath for 2 minutes before 2 drops of phenolphthalein indicator was added. The solution was titrated with 0.1 N potassium hydroxide until a pink colour appeared. Acid value was calculated according to Equation 3;

$$\mathrm{AV} = \frac{\mathrm{Vs} \times \mathrm{Ns} \times 56.1}{\mathrm{g}} \ldots\ldots\ldots\ldots\ldots.(3)$$

Where:

AV is acid value in mg KOH/g in vegetable oil sample.

Vs is the volume of potassium hydroxide (KOH) used in the vegetable oil sample until pink colour appears (ml).

Ns is the normality of KOH

56.1 is the molecular weight of KOH.

g is the mass of vegetable oil sample in grams.

3.7.3 Preparation of beta carotene standard and calibration curve

Stock solution for beta carotene was prepared by dissolving 1 mg of pure beta carotene standard in exactly 2 ml of methanol to make 0.5 mg\ml (500 parts per million) of solution. Four serial standards of 250, 125, 62.5 and 31.25 ppm were prepared through dilution for purposes of calibration of HPLC. All the five were injected into HPLC in triplicate to confirm the reproducibility of the detector response at each concentration level. The peak area was plotted against the concentration to obtain the calibration graph. The five concentrations were subjected to regression analysis to calculate calibration equation and correlation coefficients

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3.7.4 Extraction of beta carotene

3.7.4.1 Extraction

Extraction of beta carotene from the vegetables was performed as described by Rodriguez-Amaya and Kimura (2004) with slight modifications. The clean vegetables were blanched in hot water at 90 ^{o}C for 3 minutes. The vegetable leaves were then detached from the stems and placed in a blender. Celite powder was added to the vegetables and blended to make a smooth paste. About 20 g of the homogeneous paste was placed in a conical flask and 50 ml acetone refrigerated for 2 hours added. The mixture was swirled for 10 minutes in a sonicator and the extract filtered using a Buchner funnel. This procedure of extraction was repeated until there was no more colour change to the residue.

3.7.4.2 Partitioning

Exactly 25 ml of petroleum ether (PE) was placed in a separating funnel and 10 g of butylatedhydroxytoluene (BHT) added. Small portions of the acetone extract were added at a time until all the extract was transferred to PE. Distilled water was added slowly along the walls of the funnel and the two phases allowed to separate. The lower aqueous-acetone phase was discarded since it majorly contained chlorophyll. Washing was repeated 5 times with distilled water to remove any residual acetone.

3.7.4.3 Saponification and concentration

Saponification was done by washing the beta carotene solution in PE with potassium hydroxide in ethanol (0.1 % potassium hydroxide in ethanol) the mixture was allowed to stand for 15 minutes to allow further separation of the carotenoid from chlorophyll. Washing was done 5 more times using distilled water to remove impurities and allow beta carotene to be collected. The carotenoid solution collected was dried using anhydrous sodium sulphate and then placed in a round bottomed flask connected to a rotary evaporator for concentration at 30 ^{o}C. Beta carotene was obtained in solid form which was weighed accurately awaiting storage.

3.8 Preservation of beta carotene in oils

Exactly 425 mg of the beta carotene extract obtained from *A. mysorensis* was stored in sunflower and palm oil by placing it in 15 cm^3 of the oils. A homogenized mixture was made using a magnetic stirrer in a stream of nitrogen gas. Using a syringe the mixture was placed into eight 1.5 cm^3 amber air-tight vial and kept at room temperature. The procedure was done in an enviroment with subdued light to prevent degradation. The levels of betacarotene was determined on thefirst day (day 0), after two weeks in the first month (day 14) and then monthly (day 28, 60, 90, 120, 150 and 180), up to six months employing RP-HPLC. Beta-carotene obtained from *S. nigrum* was preserved in sunflower and and palm oilsby placing 448 mg in 15 cm^3 of each oil and same treatment repeated as for beta carotene from *A. mysorenis.*

For purposes of control, dry nitrogen was blown into 8 empty 1.5 cm^3 dark bottles to drive out air. 425 and 448 mg of extracted beta-carotene from *A. mysorensis* and *S. nigrum* was dissolved in 15 cm^3 petroleum ether, stirred and placed in each bottle and nitrogen blown again into the bottle and closed tightly. The level of beta carotene was determined using HPLC for a period of six months by injecting into HPLC to obtain chromatograms.

3.9 Data analysis

Data obtained was subjected to one way analysis of variance (ANOVA) where significant difference between the concentration means of beta carotene was found, separation of means using multiple range tests was done using Student Newman Keul Test (SNK). The level of confidence (α) for all the values analyzed was performed at 95 %. The peroxide and acid values are reported as means and the student t-test used to compare them.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction

This chapter gives tabulations, graphs and discussions of results obtained in method validation, peroxide and acid values of preservative matrices and concentration levels of beta carotene preserved in sunflower and palm oils for six months.

4.2 Method validation

A calibration curve (Figure 4.1) was derived from plotting peak areas from chromatograms of beta carotene standard against concentrations with optimized instrument conditions. The beta carotene calibration curve shown in the figure shows the linearity of the values obtained.

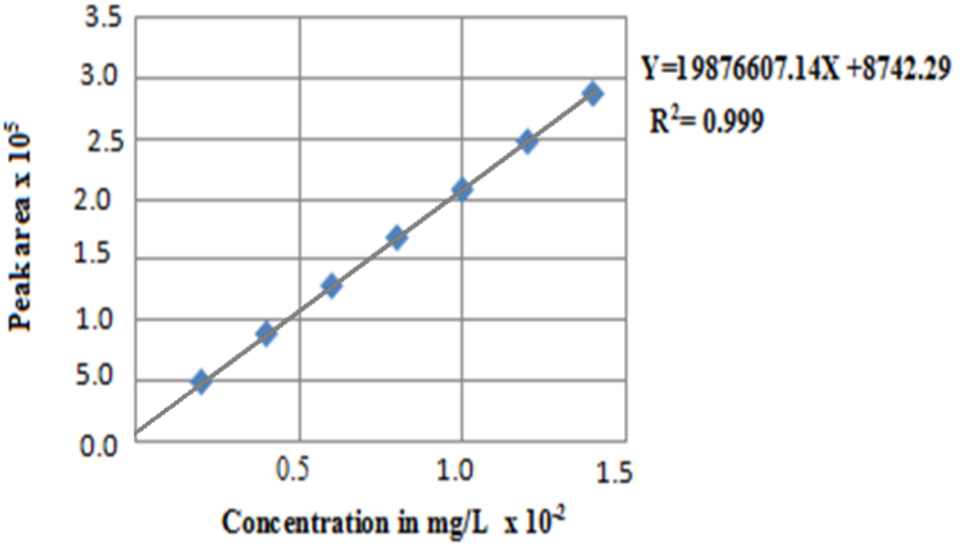

Figure 4-1: HPLC calibration curve

The chromatogram obtained from the standard is presented in figure 4.2

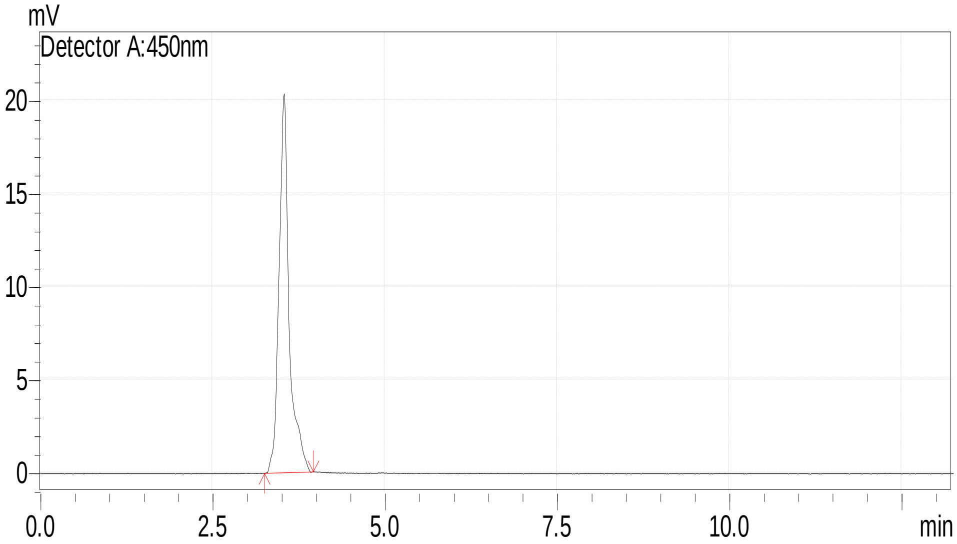

Figure 4-2: Chromatogram of beta carotene standard

The R^2 value, 0.999 indicate that the established calibration curve is linear over the range of concentration (Cortés *et al*., 2004)**.** The closeness of the value of R^2 to 1 indicates that 99.9 % of the peak areas were directly proportional to the concentration. The limit of detection (LOD), defined as the lowest concentration obtained by the instrumental signal and determined as the blank signal plus three times the standard deviation of blank using the regression equation was 1.084 mg/L. The value obtained indicates that the method of analysis can only detect samples containing higher values**.**

The accuracy of the method for beta carotene analysis was evaluated by recovery studies. The concentration of un-spiked sample, spiked sample and the percentage recovery (% R) of beta carotene is shown in table 4.1

Table 4-1: Accuracy by % recovery test for HPLC

Standard	Beta Carotene (mg)
Added standard	0.422 ± 0.01
Un-spiked sample	0.048 ± 0.01
Spiked sample	0.465 ± 0.06
% R ± SD (n = 3)	98.82 ± 0.03

(Source: Barba *et al*.,2006)

The mean % recovery obtained was 98.82 ± 0.03 which confirms that the HPLC procedure used gave accurate results.

4.3 Levels of peroxide and acid values of oils

4.3.1 Peroxide values

Peroxide values of sunflower and palm oils were determined to assess their oxidative rancidity. A control experiment was done where storage conditions were controlled to keep away oxygen, light and direct heat. The values obtained are given in table 4.2

Table 4-2: Peroxide values in sunflower and palm oils.

	Peroxide values (mEq/ kg oil), Mean ± SE (n=3)			
Days	Sunflower oil	Palm oil	Sunflower in nitrogen	Palm oil in nitrogen
0	3.93 ± 0.03^{A}	2.00 ± 0.01^{A}	3.92 ± 0.03^{A}	2.00 ± 0.01^{A}
7	4.80 ± 0.01^{B}	2.10 ± 0.00^{B}	3.94 ± 0.02^{A}	2.00 ± 0.01^{A}
14	5.40 ± 0.06^{C}	2.17 ± 0.03^{C}	3.95 ± 0.02^{A}	2.04 ± 0.01^{A}
21	6.03 ± 0.03^{D}	2.27 ± 0.03^{D}	3.96 ± 0.01^{A}	2.08 ± 0.03^{A}
28	6.84 ± 0.04^{E}	2.40 ± 0.00^{E}	3.98 ± 0.01^{A}	2.09 ± 0.02^{A}
p-value	<0.001	<0.001	> 0.05	> 0.05

Mean values followed by the same letter within the same column do not differ significantly. (One-way ANOVA, SNK-test, α =0.05)

Peroxide values increased significantly ($p < 0.001$) from 3.93 ± 0.03 to 6.84 ± 0.04 and 2.00 ± 0.01 to 2.40 ± 0.01 mEq/kg oil in sunflower and palm oils respectively for the 28 days of storage. This represents 74.05 % and 20 % increase in peroxide value for sunflower and palm oils respectively. This increase can be attributed to factors such as contact with oxygen, light and level of unsaturation. During the 28 days, oil containers were opened daily for five seconds simulating the daily activity in the kitchen hence contact with oxygen. Oxygen concentration in the oils depends on the oxygen partial pressure in the headspace of the oil container. A high partial pressure dissolves more oxygen in the oil hence higher peroxide value. A study done on soybean oil found that one gram of the oil dissolves 55 µg oxygen at room temperature, an amount that is sufficient to oxidize the oil to a peroxide value of approximately 10 mEq/kg in the dark (Choe and Min, 2006).

The peroxide values obtained confirms that lipid oxidation had set in as early as the initial day of the study (day 0) where values of 3.93 ± 0.03 and 2.00 ± 0.01 milli-equivalent of peroxide oxygen per kilogram of oil were reported. This can be attributed to autoxidation and photosensitized oxidation during processing and storage period the oils took on the shelves before purchase (Choe and Min, 2006). The results obtained in this study show higher increase in peroxide values for sunflower than palm oil. This can be attributed on one hand to packaging of the two oils. Sunflower oil was packaged in transparent while palm oil was in opaque plastic containers. The reported values in this study agree with Velasco and Dobarganes (2002) who reported that transparent plastic bottles increase oil oxidation due to singlet oxygen ($^1O_2$) oxidation which occurs in the presence of light. Oils with more unsaturation are oxidized more than less unsaturated oils (Tan *et al*., 2002; Parker *et al*., 2005). Iodine value is used to show the level of unsaturation in oils. The higher the iodine value the higher the unsaturation. Sunflower oil with iodine value greater than 130 showed a significantly higher oxidation than palm oil whose iodine value is less than 20 when stored for 28 days. The increase in peroxide values recorded in this research was at varying degrees with sunflower and palm oils being 74.05 % and 20% respectively. The results differed from those obtained by Kamau and Nanua (2008) whose peroxide values increased from 0.75-6.66 and 0.75 - 2.92 mEq/kg for semi-refined sunflower oil packed in containers with a headspace and full respectively for one month. This increase translates to (Steltzer, 2012) 788 % and 289.3 % respectively.

Peroxide values of oils kept in an inert environment (nitrogen) and away from light, air and heat did not increase significantly ($p > 0.05$). This is probably because of limited atmospheric oxygen, light and high temperatures that cause increased oxidation reactions. The peroxide values obtained were well within the FAO recommended limits of up to 10 milli-equivalents of active oxygen/kg oil (Alimentarius, 1999).

4.3.2 Acid values for sunflower and palm oils

The two matrices used for beta carotene preservation were assayed for acid values to establish the level of hydrolytic rancidity. The oils tested were stored at room temperature while a control experiment was done limiting atmospheric oxygen. The results obtained are tabulated in table 4.3

Table 4-3: Acid values in the oils

	Acid values (mg in KOH/ g), (Mean ± SE), (n=3)			
Days	Sunflower oil	Palm oil	Sunflower (nitrogen)	Palm (nitrogen)
0	$0.34±0.01^{A}$	$0.11±0.01^{A}$	$0.33±0.01^{A}$	$0.11±0.01^{A}$
7	$0.41±0.04^{B}$	$0.19±0.04^{A}$	$0.34±0.01^{A}$	$0.13±0.01^{A}$
14	$0.49±0.04^{C}$	$0.30±0.04^{B}$	$0.35±0.02^{A}$	$0.13±0.02^{A}$
21	$0.56±0.01^{D}$	$0.37±0.04^{B}$	$0.35±0.01^{A}$	$0.14±0.01^{A}$
28	$0.64±0.04^{E}$	$0.49±0.04^{C}$	$0.37±0.01^{A}$	$0.16±0.01^{A}$
p-value	<0.001	<0.001	> 0.05	> 0.05

Mean values followed by the same capital letter(s) within the same column do not differ significantly from one another. (One-way ANOVA, SNK-test, α =0.05)

The acid values ranged from 0.34 ± 0.01 to 0.64 ± 0.04 and 0.11 ± 0.01 to 0.49 ± 0.04 mg in KOH/g, indicating an increase of 88.23 % and 345.45 % in sunflower and palm oils respectively over the 28 days of storage. There was a significant difference in acid values for the one month of storage ($p < 0.001$). Hydrolytic rancidity is mainly encouraged by presence of moisture and lipolytic enzymes in oils (Kamau and Nanua, 2008). The storage conditions did not seem to cause a very high rate of acid development hence values remained within the FAO recommended limits of 0.6 mg KOH/g of oil (Alimentarius, 1999). The findings are close to those given by Kamau and Nanua. (2008) who obtained values ranging from 0.56-0.62 mg in KOH/g for ram press extracted semi-refined sun flower oil.

4.4 Retention of beta carotene stored in oil matrices.

Retention of beta carotene extracted from *A. mysorensis* and stored in sunflower and palm oils was monitored for 180 days. Table 4.4 gives the mean levels of extracted beta carotene stored in the edible oils.

Table 4-4: Mean levels of beta carotene from *A. mysorensis*

	Concentration mg/100g DM	Mean ± SD, n=3	
Days	Nitrogen	Sunflower oil	Palm oil
0	24.85 ±0.002	24.83 ± 0.002^{a}	24.82 ± 0.001^{a}
14	24.82 ± 0.007	23.58 ± 0.008^{b}	23.49 ± 0.001^{b}
28	24.78 ± 0.003	18.61 ± 0.002^{c}	20.58 ± 0.002^{c}
60	24.61 ± 0.001	15.38 ± 0.001^{d}	18.89 ± 0.001^{d}
90	24.50 ± 0.003	12.89 ± 0.001^{e}	15.24 ± 0.002^{e}
120	24.38 ± 0.002	10.16 ± 0.002^{f}	12.38 ± 0.002^{f}
150	24.25 ± 0.004	7.42 ± 0.002^{g}	9.74 ± 0.002^{g}
180	24.05 ± 0.008	6.67 ± 0.002^{h}	8.45 ± 0.001^{h}
P-value	> 0.05	< 0.001	<0.001

*Mean values followed by different letters are significantly different (α=0.05), One-way ANOVA, SNK-test

Storage over the days showed a significant decrease ($p < 0.001$) in the levels of beta carotene as days progressed. In sunflower oil, beta carotene decreased from 24.83 ± 0.02 to 6.67 ± 0.002mg/100g DM (73.14 %) while in palm oil the reduction was from 24.82 ± 0.001 to 8.45 ± 0.001mg/100g DM (65.95 %). Further, the trend in the reductions was lowest in the first 14 days, 5.03 % and 5.36 % in sunflower and palm oils respectively. This can be attributed to the low peroxide and acid values of the matrices during this period. It is evident that the increased peroxide and acid values of the oils with storage impacted highly on the degradation of preserved beta carotene. This can be deduced from the correlation studies of peroxide and acid values of sunflower and palm oils done on beta carotene whose values obtained were r = -0.854, -0.908 and -0.839,-0.850 respectively.

The percentage retention from this study differs from findings reported by Moura *et al.* (2015) where beta carotene content in oven-dried cassava reduced from 72 % to 40 % and 32 % after two and four weeks of storage at room temperature respectively, perhaps because the whole plant was preserved and beta carotene was only extracted at the end of storage duration. Moraes *et al*. (2013) reported 90 % and 70 % retention of beta carotene encapsulated in phospholids by the 100th day in vacuum and at normal room temperatures respectively

The chemistry of carotenoids supports the reduction of beta carotene over days. Beta carotene has a conjugated system that degrades when exposed to atmospheric oxygen, heat, acids, free radicals and other components in the matrices (Rodriguez-Amaya, 2001; Moura *et al*., 2015). Factors such as oxygen, acids from within and heat cannot be therefore ruled out as having direct effect to the reduction of the carotenoid as observed despite the procedures taking precaution on minimal reaction with oxygen. Acid factor effects from without can be ruled out since in the laboratory procedures no acid was used. It was within the study limitation to control products with molecular weight lower than beta carotene such as epoxides, apocarotenals and apocarotenones that would be formed and have effect on the results (Moura *et al.*, 2015). According to Choe and Min, (2006), both sunflower and palm oils contain palmitic, stearic, oleic, linoleic and traces of free fatty acids which react with beta carotene causing degradation. Further, autoxidation of oils result to lipid alkyl radicals which could have initiated free radical chain reaction with beta carotene hence degradation. Minor components present in the oils such as metals, phospholipids and phenolic compounds acts as prooxidants which accelerate beta carotene degradation (Choe and Min, 2006).

Retention of beta carotene preserved under different conditions has been studied (Nyambaka *et al.*; 2012, Moraes *et al*., 2013; Mungai *et al*., 2016). The degradation has been found to range between 16.41 %-32.28 % when dehydrated dark green leafy vegetables were stored under oxygen absorbers for a period of six months. Mungai *et al*. (2016) reported 9.23 % and 17.19 % retention of beta carotene extracted from *Amaranthus spp* and stored for a period of 180 days in virgin coconut oil and honey respectively. Beta carotene encapsulated in phospholipids was retained at 90 % and 70 % in both vacuum and room temperatures respectively for a period of 100 days (Moraes *et al*., 2013). The results above agree with the findings in this research though at different extents.

The retention of beta carotene preserved in an inert medium (nitrogen) devoid of factors that cause beta carotene degradation indicated that there is no major degradation of beta carotene. The levels of beta carotene in mg/100g dry matter ranged from 24.85 to 24.05. This translates to 96.78 % retention hence no significant difference ($p > 0.05$). The slight variation reported may have been caused by presence of residual air in the head space of the vial as well as inherent random errors. Nyambaka *et al*. (2012) reported 93.8 % retention of beta carotene preserved in nitrogen for a period of six months.

Levels of beta carotene extracted from indigenous *A. mysorensis* preserved in sunflower were compared to the levels of beta carotene preserved in Palm oil. Figure 4.3 shows the trend observed.

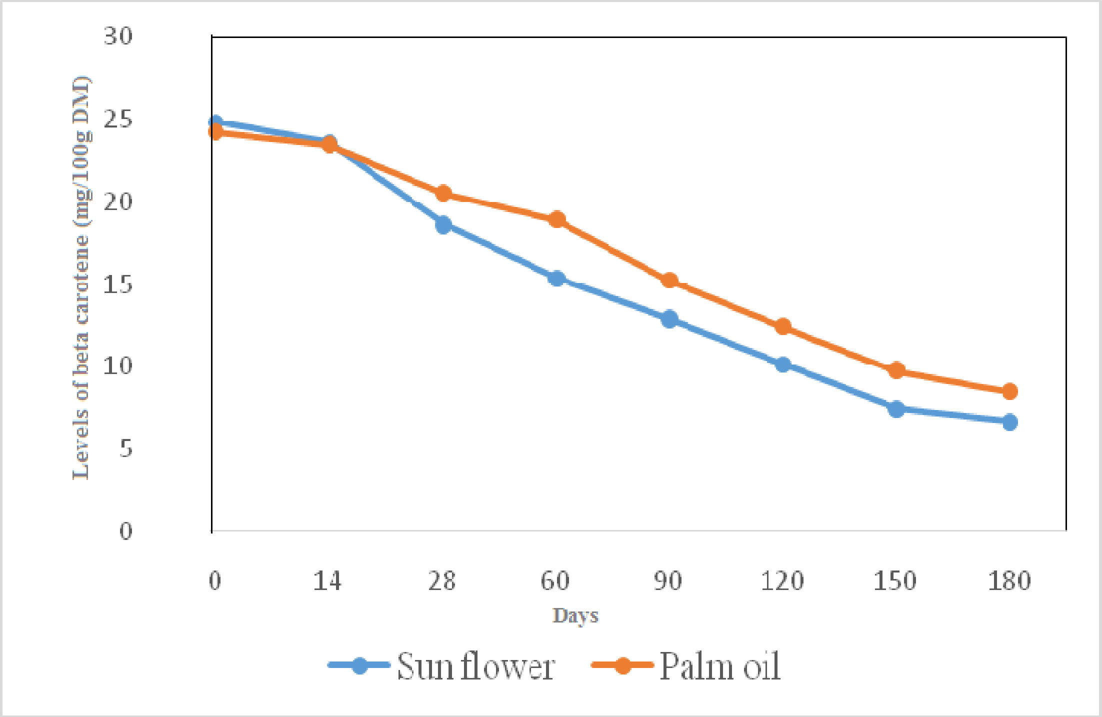

Figure 4-2: Retention trend of beta carotene extracted from *A. mysorensis*

The general trend shows a gradual decrease in beta carotene with storage time. Similar findings reported that deterioration of vitamin A stored in vegetable oils of different characteristics increased when peroxide and acid values were greater than 2 mEq/kg and 0.6 mg KOH/g (Laillou *et al*., 2012a). Using a paired sample t-test, the trend showed a decline in beta carotene mean levels for both matrices, however, there was a significantly higher level of beta carotene preserved in palm oil (mean 16.62 ± 0.0028) than that preserved in sunflower oil (mean 14.94 ± 0.0017), t = 3.49, P = 0.010. This could be attributed to the relatively higher peroxide and acid values reported in sunflower oil compared to palm oil. Oils that are more unsaturated are oxidized faster than less conjugated ones (Parker *et al.*, 2003). Palm oil which is less unsaturated has higher oxidative stability than sunflower oil (Gullen and Cabo, 2002). This leads to higher peroxide and acid values thus increased beta carotene degradation.

4.5 Retention of beta carotene extracted from *S. nigrum* stored in oil matrices

Beta carotene was extracted from *S. nigrum* and stored in sunflower and palm oils. Retention of beta carotene in the matrices was monitored for 180 days. Table 4.5 gives the mean levels of analyte in the matrices.

Table 4-5: Mean levels of beta carotene extracted from *S. nigrum*

Concentration mg/100g DM Mean ± SD, n=3			
Days	nitrogen	Sunflower oil	Palm oil
0	41.49±0.02	41.48± 0.003^{g}	41.46± 0.003^{g}
14	41.44±0.07	35.55 ±0.003^{f}	40.42± 0.003^{g}
28	41.38±0.03	27.62 ±0.002^{e}	35.20 ± 0.002^{f}
60	41.21±0.01	21.82 ±0.003^{d}	30.38 ± 0.001^{e}
90	41.10±0.03	16.81 ± 0.002^{c}	26.09 ±0.002^{d}
120	40.98±0.02	13.07 ± 0.003^{b}	19.65 ± 0.001^{c}
150	40.65±0.20	8.91 ± 0.003^{a}	17.51± 0.002^{b}
180	40.14 ±0.08	7.65 ± 0.003^{a}	14.28 ± 0.001^{a}
P-value	> 0.05	< 0.001	<0.001

*Mean values followed by different small letter are significantly different (α=0.05, One-way ANOVA, SNK-test)

The mean concentration of beta carotene obtained on day 0 was 41.49 ± 0.02 mg/100g DM indicating clearly that *S. nigrum* vegetables are rich sources of beta carotene. The levels however reduced significantly day by day during the storage period. After 180 days, the levels of beta carotene in *S. nigrum* stored in sunflower oil and in palm oil were significantly lower than the levels in the previous days ($P < 0.001$). Mean levels of beta carotene stored in sunflower and palm oils ranged from 41.48 ± 0.003-7.65 ± 0.003 and 41.46 ± 0.003-14.28 ± 0.001 mg/100g dry matter respectively. This translates to 18.44 % and 34.44 % of beta carotene being preserved in the matrices after 180 days of storage respectively. It was noted that the reductions between days was 14.30 %, 22.31 %, 21.00 %, 22.96 %, 21.18 %, 22.25 %, 31.83 % and 14.14 % in sunflower oil and 2.51 %, 12.91 %, 13.69 %, 14.12 %, 24.68 %, 10.89 % and 18.45 % in palm oil at 14, 28, 60, 90, 120, 150 and 180 days respectively. Factors influencing the reduction of beta carotene are similar to those affecting beta carotene extracted from *A. mysorensis* highlighted above. Increased peroxide and acid values of the oils with storage affected the beta carotene retained. This is evident from the correlation studies of peroxide and acid values done on sunflower and palm oils on beta carotene whose coefficients obtained were r = -0.837, -0.856 and -0.966, -0.873 respectively.

Levels of beta carotene extracted from indigenous *S. nigrum* preserved in sunflower were compared to the levels of beta carotene preserved in palm oil. Figure 4.4 shows the trend observed.

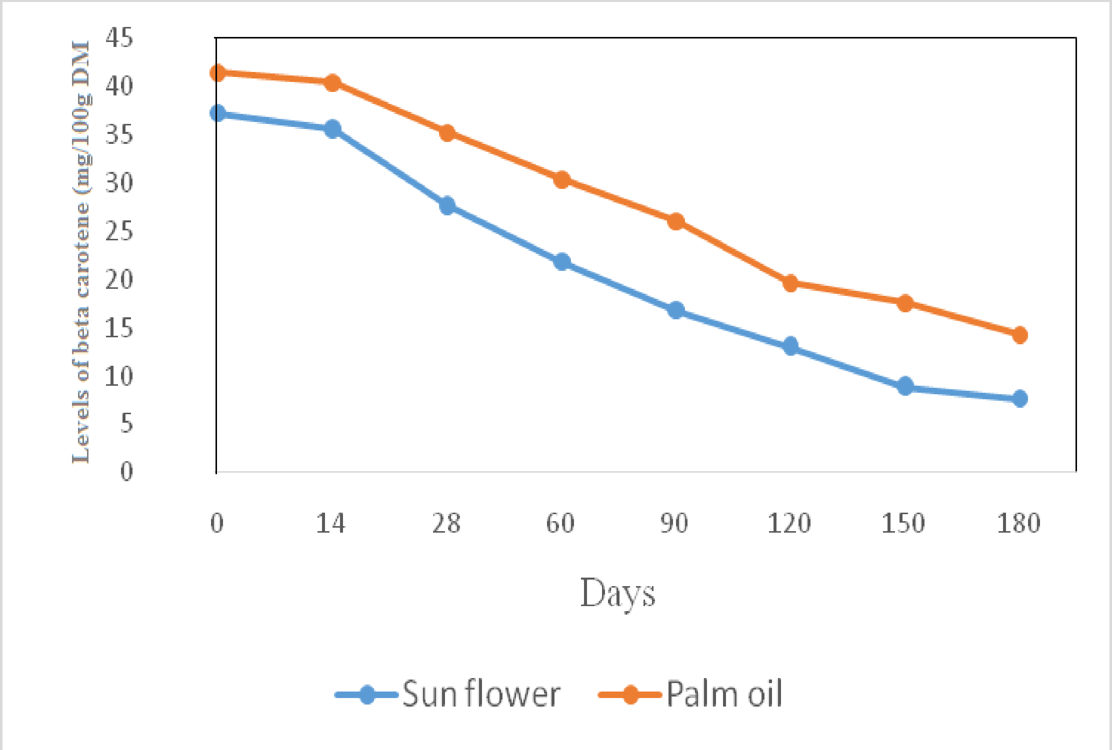

Figure 4-3: Retention trend of mean levels of beta carotene extracted from *S. nigrum*

The general trend of beta carotene retention with storage indicates a decline; however, this reduction is not uniform. Within the first two weeks of storage, 5.93 mg (14.30 %) and 1.04 mg (2.5%) degraded in sunflower and palm oil respectively. Highest loss of beta carotene was reported between day 120 to 150 in sunflower oil (31.83 %) and day 90 to 120 in palm oils (24.68 %). The degradation is attributed to increased peroxide and acid values in addition to other factors such as oxygen, light and heat.

Using a paired sample t-test, the trend showed a gradual decline in beta carotene mean levels for both matrices, however, there was a significantly higher levels of beta carotene preserved in palm oil (mean 28.12 ± 0.0009) than those preserved in sunflower oil (mean 21.08 ± 0.0021), $t = 11.05$, $P = 0.0001$. This can be attributed to high levels of peroxide and acid values in sunflower oil than in palm oil. The results indicate an increase in beta carotene degradation with increase in peroxide levels in the oils. The results show that once oxidation starts, it increases exponentially with time. Oxidation induces increase in peroxide and free radicals hence accelerated beta carotene decay (Laillou *et al.*, 2012).

Through symmetric cleavage with the enzyme β-carotene -15,15’monooxygenase, a molecule of beta carotene splits forming vitamin A (retinol). Table 4.6 presents the calculated retinol activity equivalent for the retained beta carotene.

Table 4-6: Retinol activity equivalent (RAE) of beta carotene retained in the matrices

	Calculated retinol activity equivalent (RAE)			
	A. mysorensis		*S. nigrum*	
Days	Sunflower oil	Palm oil	Sunflower oil	Palm oil
0	1.910±0.0002^{h}	1.863±0.0001^{h}	2.860±0.0002^{h}	3.189±0.0002^{h}
14	1.814±0.0006^{g}	1.806±0.0001^{g}	2.735±0.0002^{g}	3.109±0.0002^{g}
28	1.432±0.0002^{f}	1.583±0.0002^{f}	2.125±0.0002^{f}	2.708±0.0002^{f}
60	1.183±0.0001^{e}	1.453±0.0001^{e}	1.678±0.0002^{e}	2.337±0.0001^{e}
90	0.992±0.0001^{d}	1.172±0.0002^{d}	1.293±0.0002^{d}	2.007±0.0002^{d}
120	0.782±0.0002^{c}	0.952±0.0002^{c}	1.005±0.0002^{c}	1.512±0.0001^{c}
150	0.571±0.0002^{b}	0.749±0.0002^{b}	0.685±0.0002^{b}	1.347±0.0002^{b}
180	0.513±0.0002^{a}	0.650±0.0001^{a}	0.588±0.0002^{a}	1.098±0.0001^{a}
P-value	< 0.001	< 0.001	< 0.001	< 0.001

*Mean values followed by different small letter are significantly different (α=0.05, One-way ANOVA, SNK-test)

The structure of beta carotene molecule makes it the most potent provitamin A carotenoid (Yeum and Russell, 2002). Retinol activity equivalent (RAE) of beta carotene is 12 µg (1 RAE=12 µg of all-trans β-carotene in a food matrix). The recommended daily allowance of beta carotene range for infants and adults (0-50 yrs) are from a minimum of 180 µg and 390 µg per day while the maximum is 3600 µg to 18,000 µg respectively (Sizer *et al.*, 2012). Although there were great losses of beta carotene in the oils, enough beta carotene, 0.513 ± 0.002, 0.650 ± 0.001, 0.588 ± 0.002 and 1.098 ± 0.001 mg/ 100g DM was preserved to provide recommended daily allowance (RDA) hence curb vitamin A deficiency.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The research established that peroxide values increased significantly with storage for the period of one month studied. The values are slightly higher in sunflower oil than in palm oil. The increase was however noted to be within allowable levels for the time in consideration. There is need to keep the values as low as it is practically possible through exclusion of oxygen and light, reduction of temperatures and removal of metals and oxidized compounds during processing and storage of edible oils.

Acid values increased significantly for both matrices with storage time. The values are slightly higher in sunflower oil than in palm oil. The values recorded were within allowable levels (0.6 mg KOH/g). Therefore, treatment and storage did not seem to affect the rate of acid development. Effort should be made to maintain the acid values as low as possible for preservation of beta carotene in the oils to be more successful.

The study established that the AIVs studied are rich in beta carotene (24.83 and 41.48 mg/100 DM reported on day 0), a precursor of vitamin A. After storage in sunflower and palm oils for a duration of 180 days, beta carotene degraded significantly in both matrices. However, retinol activity equivalent (RAE) calculated, though little was sufficient to provide recommended daily allowance (RDA) for the target populations.

Therefore, addition of beta carotene in edible oils of good quality with low peroxide and acid values for preservative purposes can provide vitamin A to help fight VAD in Kenya and other countries.

5.2Recommendations

5.2.1 Recommendations from the study

Fortification of vegetable oils with beta carotene should be encouraged since the findings of this study show that the retained beta carotene can provide the RDA to the target population. In order to optimize retention of beta carotene in the matrices, the quality of the oils should be ensured by keeping peroxide and acid values below allowed levels of less than 10 mEq/kg and 0.6 mg KOH/g respectively. Fortification of oils with beta carotene should be explored since it can ensure maximum utilization of the highly perishable indigenous vegetables such as *A. mysorensis* and *S. nigrum* which would otherwise go to waste due to overproduction during the rainy seasons and scarcity during droughts.

5.2.2 Recommendations for further work

Levels of other provitamin A carotenoids present in matrices should be investigated since they also provide vitamin A. In addition to peroxide and acid values, thiobarbituric acid value of the vegetable oils is also useful in assessing the extent to which spoilage or deterioration has advanced hence it ought to be determined.

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APPENDICES

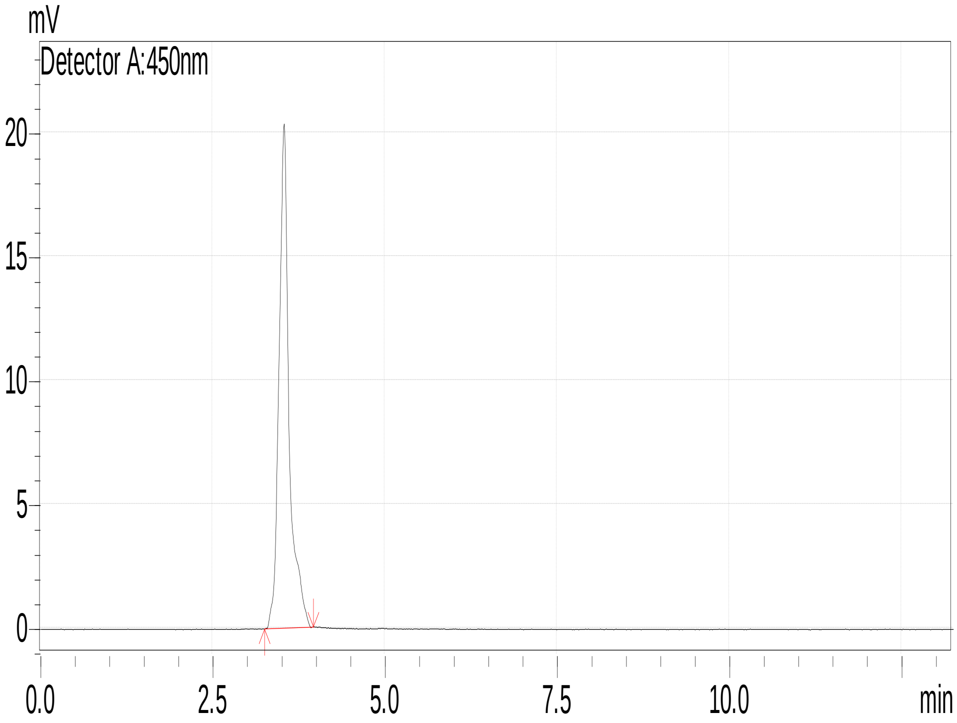
Appendix I: ***Asystasia mysorensis***

Appendix II: ***Solanum nigrum***

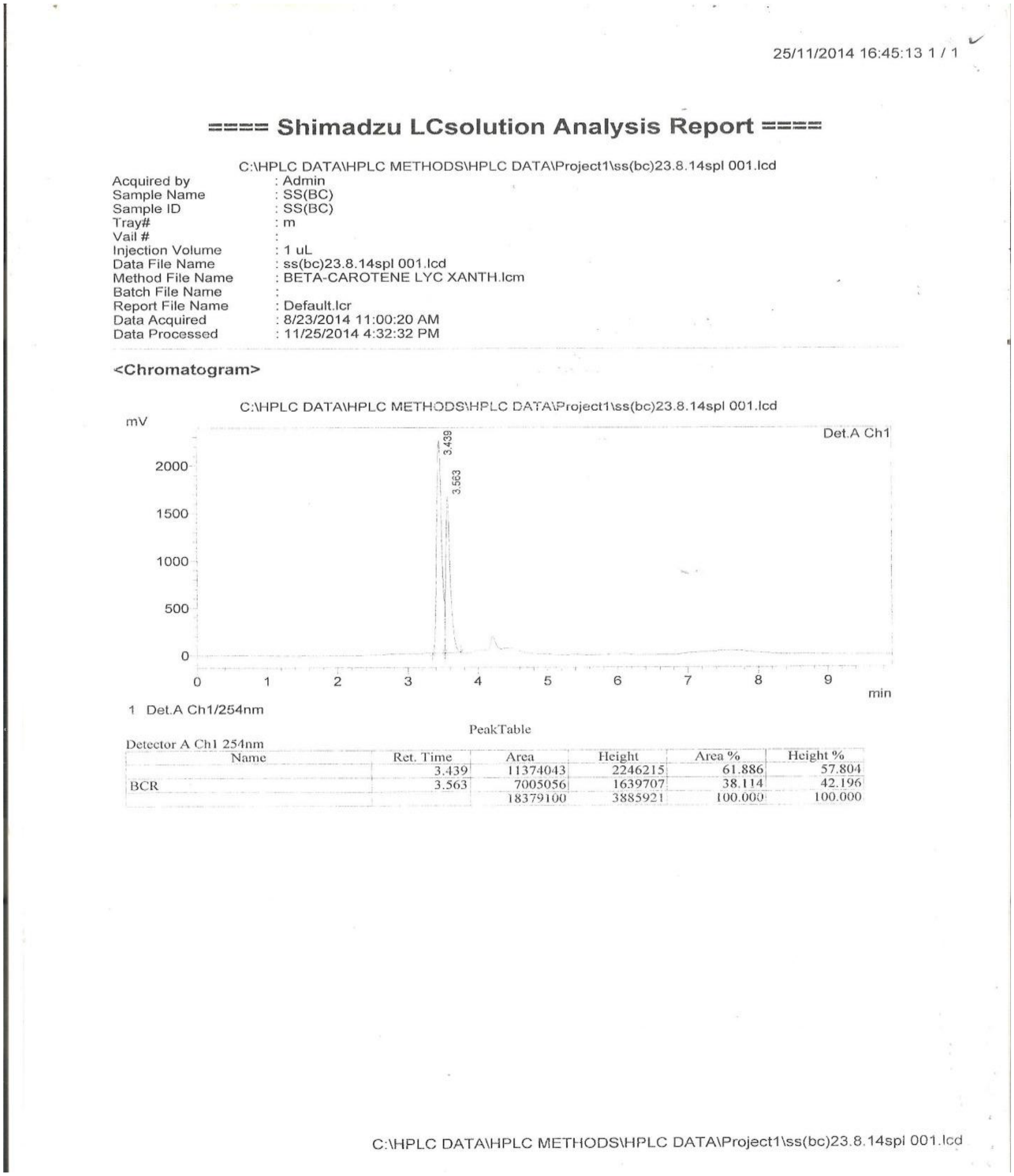
[redacted]

[redacted]

Appendix III: Oils used



Appendix IV: Beta carotene chromatogram from standard



25/11/2014 16:45:13 1 / 1

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Tray# : m
Vail # :
Injection Volume : 1 uL
Data File Name : ss(bc)23.8.14spl 001.lcd
Method File Name : BETA-CAROTENE LYC XANTH.lcm
Batch File Name :
Report File Name : Default.lcr
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1 Det.A Ch1/254nm

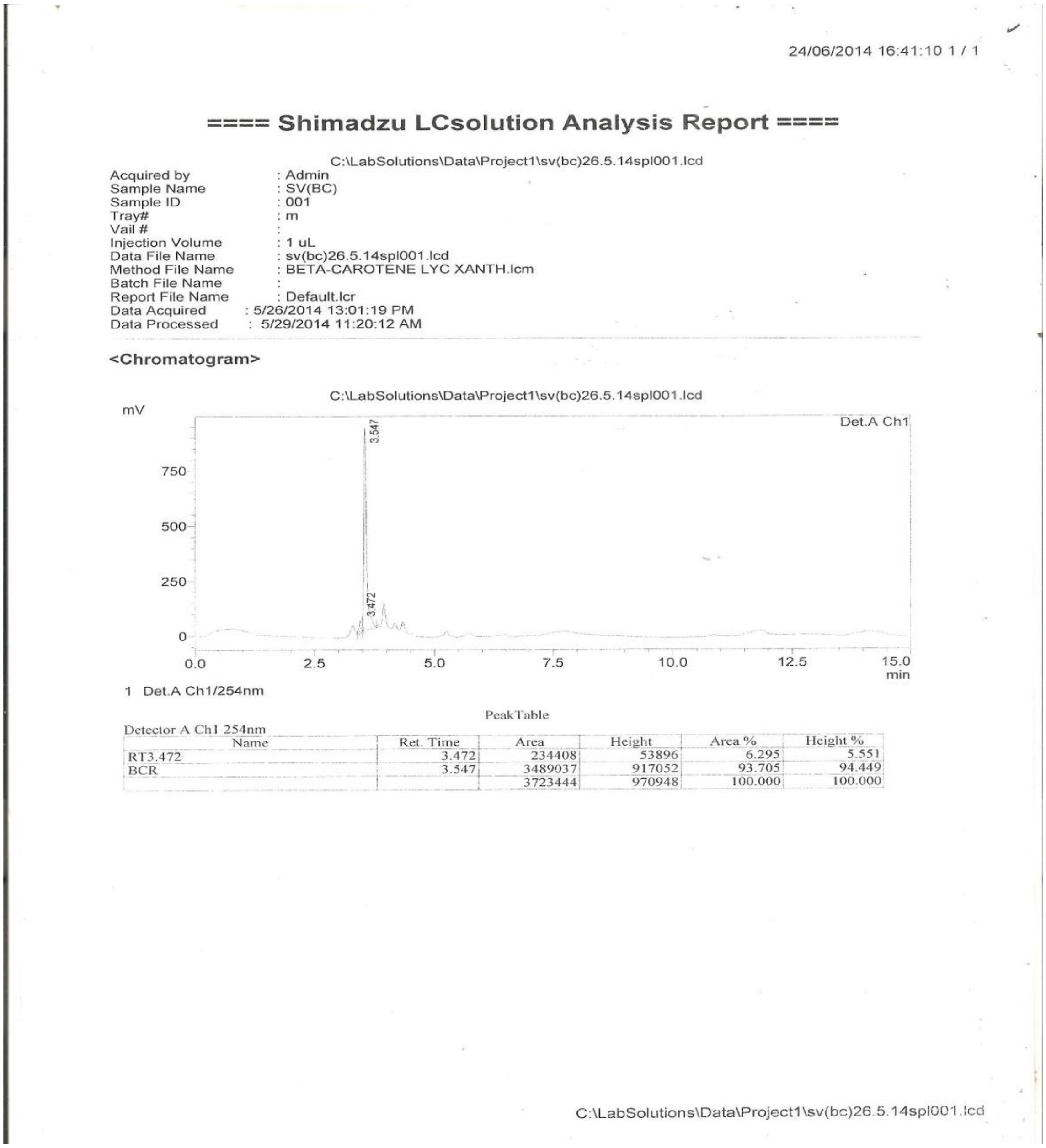
PeakTable

Detector A Ch1 254nm

Name	Ret. Time	Area	Height	Area %	Height %
	3.439	11374043	2246215	61.886	57.804
BCR	3.563	7005056	1639707	38.114	42.196
		18379100	3885921	100.000	100.000

C:\HPLC DATA\HPLC METHODS\HPLC DATA\Project1\ss(bc)23.8.14spl 001.lcd

Appendix V: Chromatogram of beta carotene from *S. nigrum* preserved in sunflower oil



24/06/2014 16:41:10 1 / 1

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Vail # :
Injection Volume : 1 uL
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Method File Name : BETA-CAROTENE LYC XANTH.lcm
Batch File Name :
Report File Name : Default.lcr
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1 Det.A Ch1/254nm

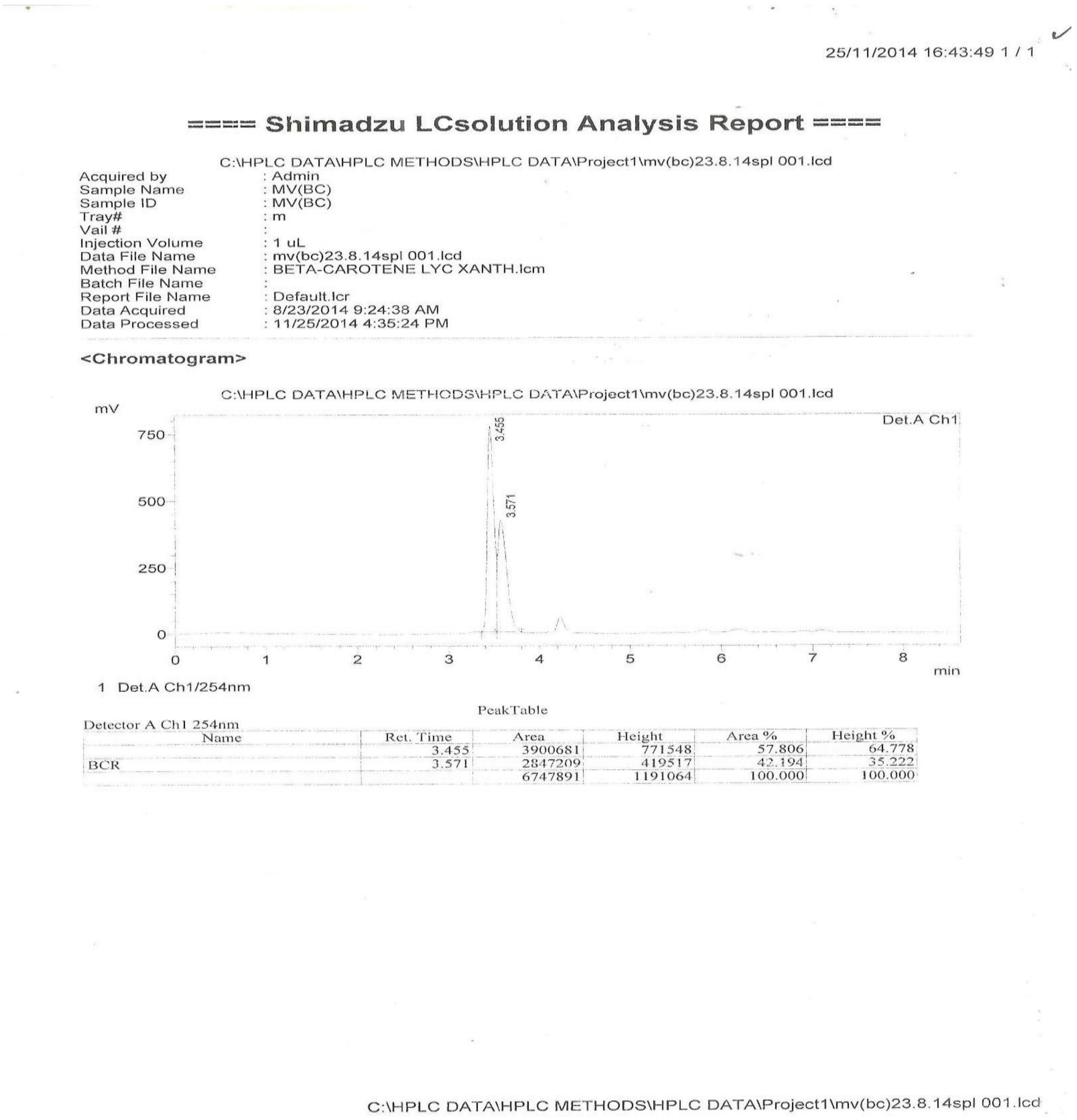
PeakTable

Detector A Ch1 254nm

Name	Ret. Time	Area	Height	Area %	Height %
RT3.472	3.472	234408	53896	6.295	5.551
BCR	3.547	3489037	917052	93.705	94.449
		3723444	970948	100.000	100.000

C:\LabSolutions\Data\Project1\sv(bc)26.5.14spl001.lcd

Appendix VI: Chromatogram of beta carotene from *S. nigrum* preserved in palm oil



25/11/2014 16:43:49 1 / 1

==== Shimadzu LCsolution Analysis Report ====

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Sample ID : MV(BC)
Tray# : m
Vail # :
Injection Volume : 1 uL
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Method File Name : BETA-CAROTENE LYC XANTH.lcm
Batch File Name :
Report File Name : Default.lcr
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<Chromatogram>

C:\HPLC DATA\HPLC METHODS\HPLC DATA\Project1\mv(bc)23.8.14spl 001.lcd

1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Name	Ret. Time	Area	Height	Area %	Height %
	3.455	3900681	771548	57.806	64.778
BCR	3.571	2847209	419517	42.194	35.222
		6747891	1191064	100.000	100.000

C:\HPLC DATA\HPLC METHODS\HPLC DATA\Project1\mv(bc)23.8.14spl 001.lcd

Appendix VII: Chromatogram of beta carotene from *A. mysorensis* preserved in palm oil