CAROTENOID CONTENT AND VITAMIN A VALUE OF SWEETPOTATO (*Ipomoea batatas* (L.) Lam.) CULTIVARS AS INFLUENCED BY ROOT AGE, FARMING SITE AND COOKING

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A Thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in Plant Physiology and Biochemistry at the Department of Botany of the University of Nairobi.

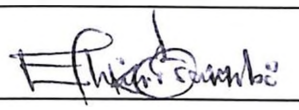
Faculty of Science

1999

Declaration

This Thesis is my original work and has not been presented in any other Institution for an award of a degree.

Date: APRIL 27th 1999

Sign 

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This Thesis has been submitted for an award of a Master of Science degree with my approval as the supervisor.

Date 27/4/99

Sign

Dr. Misra A.K.

Dedication

A sip for those who thirst for the prosperity of knowledge.

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Acknowledgement

This work was supported by the University of Nairobi scholarship and International Center for Research in Women (ICRW) project. Much support was also granted by International Potato Center (CIP). I also pay tribute to International Livestock Research Institute for allowing me to use its HPLC facility.

My supervisors, Dr. Misra and Dr. Carey deserve utmost acknowledgement. This Thesis is a product of their tutelage, sacrifice and patience. Dr. Vital Hagenimana of CIP can never be forgotten for his insight and critique that shaped and set the standard of this work.

Much appreciation goes to ILRI's Biochemist, Dr. Jon Wilkes for his technical support in HPLC analyses. I also appreciate the input of Rose Nasaka (formerly of K.A.R.I.- Tigoni) who expedited my laboratory work.

Much thanks to Dr. Peter Molnar of the Department of Medical Chemistry in the University Medical School of Pecs for donating β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide. I also thank Dr. W. Shuep and Dr. J. Schierle of Hoffmann La Roche Ltd. for their donation of β-Cryptoxanthin and ζ-Carotene.

ABSTRACT

This study involved establishment of the main carotenoids in the storage roots of a number of sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivars and determination of the effect of root age, trial location and cooking on their carotenoid content. Carotenoid content was evaluated by isocratic non-aqueous reverse phase high performance liquid chromatography (NARP HPLC) and spectrophotometry. Carotenoids in four selected cultivars were also analysed with HPLC Photodiode array analyser (HPLC PDA). Vitamin A value was quantified from the concentration of provitamin A carotenoids and expressed as retinol equivalent (RE/100g) root fresh weight basis (fwb).

It was revealed that 10 carotenoids were present in the sweetpotato cultivars studied. Of these, 5 were found to occur in significant quantities. The NARP HPLC elution pattern of these 5 main carotenoids was consistent among the cultivars studied. Three of these carotenoids that eluted at 5.36, 8.61 and 14.60 minutes were positively identified using pure carotenoids to be β-carotene-5,6,5',6'-diepoxide, β-carotene-5,6-monoepoxide and all *trans*-β-carotene, respectively. Only β-carotene and β-catotene-5,6-monoepoxide were quantified.

Significant variation in carotenoid content among cultivars was evident. Total carotenoid, β-carotene content and provitamin A varied from 0-8,923 μg/100g, 0-7,984 μg/100g and 1,258.33 RE/100g (fwb), respectively. The percentage of β-carotene to total carotenoids ranged between 0-90% (fwb). Orange fleshed sweetpotatoes contained higher total carotenoid and β-carotene content and provitamin A value than white to cream fleshed lines.

The effect of root age on carotenoid content was studied in 5 cultivars: KEMB 10 (CIP 440169), Kakamega 4 (SPK 004), Zapallo (CIP 420027), KSP 20 (TIS 2534) and Japanese (CIP 420009). Root age was found to exert a significant effect on carotenoid content. The time taken to attain maximum carotenoid content was noted to vary with cultivars. Cultivars, Japanese and Kakamega 4 took 24 weeks while KEMB 10, KSP 20 and Zapallo required up to 24 weeks to achieve peak carotenoid titre.

The effect of farming site on total carotenoid and beta-carotene content was investigated in KSP 20, LM 88.002 (CIP 188001.2), Japanese and KEMB 10, procured from Kabete, Kiboko and Kisii. Significant variation in total carotenoid content was observed across farming sites. However, trial location did not exert a significant effect on β-carotene content.

Three boiling regimes were used to assess the effect of cooking. This involved boiling for 30 minutes, 45 minutes and 60 minutes. Boiling caused significant reduction in total carotenoids and beta-carotene, and had more severe effect on the latter than the former. HPLC studies showed that boiling resulted in an increase in the proportion of an unidentified carotenoid eluting at 3.05 minutes.

INTRODUCTION

Sweetpotato is an important food crop in many parts of the World. It is grown in 111 countries of which 101 are classified as 'developing countries'. Sweetpotato is grown mostly for its edible storage roots though its foliage is often also utilised both for human (vegetable) and animal feed. Sweetpotato combines a number of advantages that give it a vital role in combating food shortages and malnutrition (Woolfe, 1992). Sweetpotato is a source of a number of vitamins, notably carotenoids that act as precursors of vitamin A (Picha, 1985; Simonne *et al.*, 1993).

Carotenoids constitute one of the most widespread classes of pigments found in nature. More than 600 carotenoids have been established to occur naturally (Scita, 1992). Carotenoids are composed of the carotenes which are strictly hydrocarbons, and xanthophylls which are oxidated carotenes. Carotenoids are exclusively synthesized by higher plants, algae and some photosynthetic bacteria (Goodwin, 1980). Carotenoids found in animals are derived from their diet (Lakshman and Okoh, 1993). Carotenoids are distributed in various plants' organs and tissues such as, leaves, stems, fruits, seeds, and roots (Khachik *et al.*, 1992; Goodwin, 1980). Carotenoids are accessory pigments in radiant energy capture and also protect photosynthetic systems from photochemical oxidation (Lawlor, 1990).

Of the more than 600 carotenoids that occur in nature, about 40 are precursors of Vitamin A (Scita, 1992). Such provitamin A carotenoids are cleaved in the mucosa of animals' small intestines into Vitamin A. The main provitamin A carotenoid is β-carotene (Lakshman and Okoh, 1993).

Vitamin A plays a fundamental role in growth and development, regulation and stability of biological membranes, maintenance of mucus secreting cells of epithelia, biosynthesis of glycoproteins, and prevention of keratinization (Wong, 1989). As precursors of the visual pigment rhodopsin, carotenoids are also intimately associated with the mechanisms by which we perceive colours (Olson, 1989). Recent research findings have indicated that there is possible association between consumption of foods rich in carotenoids and the development and prevention of cancer (Gester, 1993; Mascio *et al.*, 1991). The role of carotenoids as anti-ageing nutrients in humans, their role in immunity, fertility and early prophylaxis of cardiovascular diseases in livestock has also generated much interest in these compounds (Pfander, 1992).

Deficiency of Vitamin A in the diet causes debilitating health problems such as xerophthalmia, corneal lesions, keratomalace, and in many instances deaths (Olson, 1989; Dowdey, 1974). It has been estimated that there are more than 500,000 cases each year of new, active corneal lesions and 6 to 7 million cases of non-corneal xerophthalmia in children World-wide (*FAO*, 1988).

Carotenoids from vegetable are the main dietary source of vitamin A in developing world since the retinol containing dairy and other animal products are often too expensive (Temalilwa, *et al.*, 1986, Simpson, 1983). Provitamin A carotenoids present in fruits and vegetables are estimated to provide 30 to 100% of the vitamin A requirements in human populations (Fennema, 1996).

Carotenoids, especially β-carotene, are responsible for the yellow-orange flesh colour of sweetpotato roots (Ameny and Wilson, 1997; Takahata *et al.*, 1993). A number of sweetpotato cultivars are under cultivation and trial in Kenya. Unfortunately, most commonly cultivated

sweetpotato clones in Kenya are white-fleshed with low provitamin A value (Ngare *et al.*, 1995). Efforts are underway to test and disseminate orange-fleshed ones to improve vitamin A nutrition. This study was developed to identify provitamin A rich sweetpotato cultivars grown in Kenya.

Sweetpotato farming in Kenya is carried out mainly in the Western and Eastern regions with some pockets of intensive cultivation occurring in central and coast provinces. It is noted that these areas experience different sets of environmental conditions. Notably, these sites receive variable amounts of rainfall, have different soil-types, altitude and temperature regimes. It is not established whether variations in environmental conditions have any effect on carotenoid content. It is thus important to investigate whether there is significant variation in carotenoid content across farming sites.

There are inconclusive reports that carotenoid content changes during growth and development of sweetpotato roots (Data *et al.*, 1987; Abubakar, 1981). Studying variation in carotenoid content, during root development, is relevant in recommending the appropriate harvest age. This study was undertaken to determine the effects of root age in a number of cultivars.

Carotenoids are susceptible to degradation upon exposure to heat, light, metal ions, acids and even alkali, due to their highly conjugated structure (Goodwin, 1980; Wong, 1989; Dowdey, 1974). Foods are usually exposed to these degradation agents during processing and cooking. Because sweetpotato roots are normally cooked before consumption, it is of interest to ascertain whether there is significant change in provitamin A value of sweetpotato roots during cooking as aimed in this study.

Objectives

1. Establish the types of carotenoids found in a range of sweetpotato cultivars grown in Kenya.

2. To identify provitamin A rich sweetpotato cultivars grown in Kenya.

3. To determine the effect(s) of root age, boiling and farming sites on carotenoid content of selected cultivars.

CHAPTER 1

LITERATURE REVIEW

1.1 Carotenoids - An Overview

Carotenoids comprise a large family of long chain tetraterpenoids composed of 8 C5 isoprenoid units. Carotenoids have a basic C40 structure with nine or more alternating double bonds at different positions. This backbone can be further modified by the addition of C5 units to give C45 and C50 carotenoids (homocarotenoids), or by oxidation that results in carotenoids containing less than 40 carbon atoms (apocarotenoids) (Pfander, 1992). Carotenoids occur in cyclic or aliphatic forms and are divided into two principal groups, the carotenes, which are strictly hydrocarbons, and, xanthophylls, which are oxidated carotene derivatives with hydroxyl, methoxyl, epoxide or keto groups.

1.1.1 General Carotenogenesis.

Carotenoids, like other terpenoids, are biosynthesized from acetyl-CoA precursors (Goodwin, 1993). During carotenogenesis, three molecules of acetyl CoA are condensed stepwise to form mevalonic acid (MVA, 3,5-dihydroxy-3-methylpentanoic acid) (Figure 1.1). Mevalonic acid is the first specific terpenoid precursor (Goodwin, 1993). It is now established that in the main pathway of mevalonic acid biosynthesis, acetyl-CoA and acetoacetyl-CoA are condensed by the enzyme 3-hydroxy-3-methylglutaryl coenzyme A synthetase to form the C_6 compound (*3S*)-3-hydroxy-3-methylglutaryl-CoA(HMG-CoA).

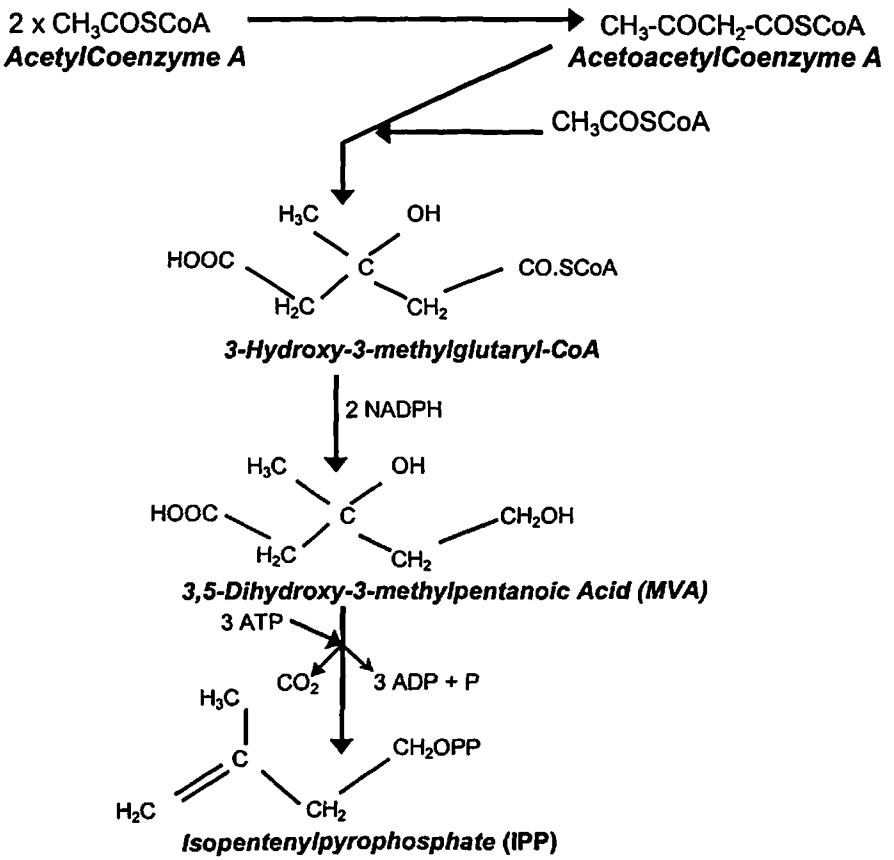

Figure 1.1: Formation of Isopentenylpyrophosphate (*From Goodwin, 1980*).

HMG-CoA then undergoes an 1`irreversible two-step reduction to give (*3R*) mevalonic acid, the reduction being catalysed by HMG-CoA reductase and requiring two molecules of NADPH (Goodwin, 1993). Studies on biosynthesis of primary plant isoprenoids including sterols and carotenoids (Goodwin, 1993) have shown that HMG-CoA reductase catalyzes a key regulatory step in the biosynthesis of all plant isoprenoids. The incorporation of MVA into carotenoids has been accomplished in many carotenogenic systems (Goodwin, 1993). Mevalonic acid undergoes decarboyxylative phosphorylation to form isopentenyl pyrophosphate (IPP). Isopentenyl pyrophosphate is the fundamental C_5 biosynthetic unit from which the carotenoids, and indeed all terpenoids, are constructed.

Isomerization of IPP to dimethylallyl pyrophosphate (DMAPP) must occur before chain elongation can begin (Goodwin, 1993). The DMAPP formed is the substrate of the prenyl transferase enzyme and condenses with a molecule of IPP to give the C_{10} compound geranyl pyrophosphate (GPP). This enzyme then adds two further IPP units to produce successively, C15 farnesyl pyrophosphate (FPP) and the C20 geranylgeranyl pyrophosphate (GGPP) (Fig. 1.2) (Goodwin, 1993). In carotenogenic systems, two GGPP molecules condense head to head to form phytoene. Phytoene is the first C40 hydrocarbon produced by biosynthetic condensation of GGPP.

Phytoene which is a colourless carotenoid is converted into coloured carotenoids through a series of reduction reactions (Bartley *et al.*, 1991). Phytoene is reduced stepwise in stereospecific reactions via phytofluene, ζ-carotene and neurosporene to lycopene (Figure 1.3) (Goodwin 1993, Luckner, 1972).

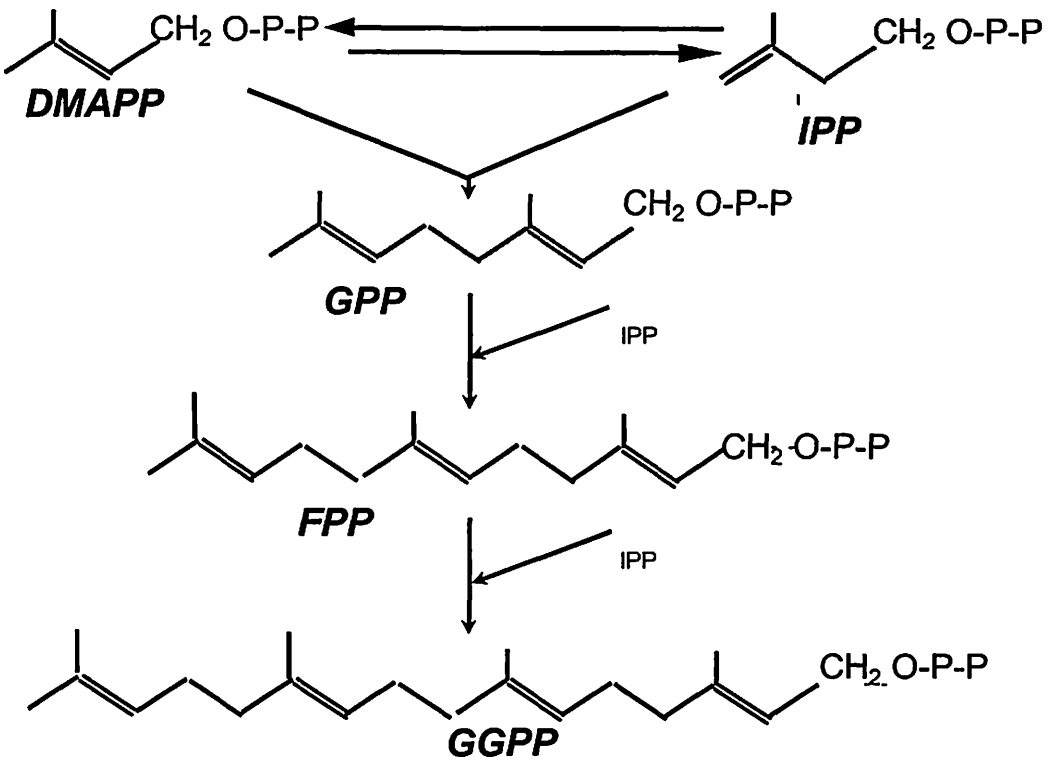

Figure 1.2: Formation of Geranylgeranylpyrophosphate (GGPP) from Isopentenylpyrophosphate (IPP) (*From Goodwin, 1993*)

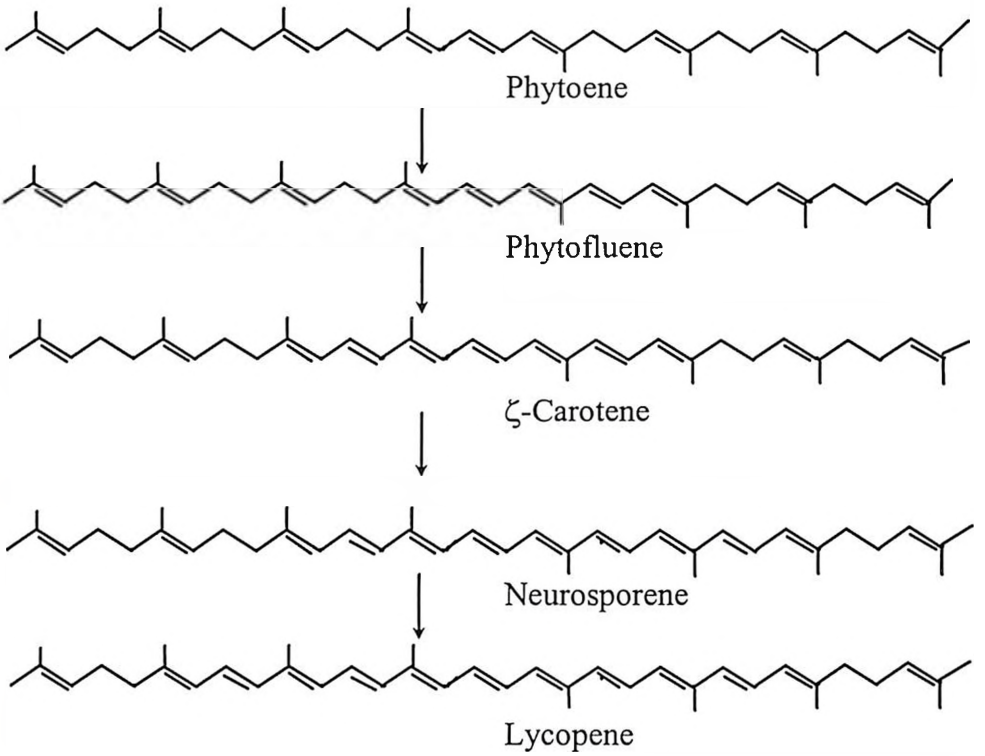

Figure 1.3: Conversion of Phytoene to Lycopene (*From Goodwin, 1993*).

During the conversion of phytofluene to ζ carotene, the central *cis*- double bond is converted to *trans*- double bond, as a result of which, the all-*trans* configuration of the carotenes is formed (Luckner, 1972). The colour of carotenoids is due to the great number of *trans*-double bonds found in these compounds (Fig 1.3).

Lycopene is an aliphatic red carotenoid abundant in tomatoes and berries (Goodwin, 1993). Inhibitors like nicotine, which causes the accumulation of lycopene in organisms normally synthesizing cyclic carotenoids, have been used in experiments that shows that lycopene is the precursor of cyclic carotenoids (Goodwin, 1993; Britton, 1988). Cyclization of such an aliphatic precursor, is initiated by H+ attack in the formation of ß- and ε-rings (Goodwin, 1993) (Fig. 1.4). Cyclization of lycopene results in the formation of cyclic carotenes like α-, β- and γ-carotenes. The mechanism for the formation of gamma ring is almost certainly the same, but it has not been proved experimentally (Goodwin, 1993).

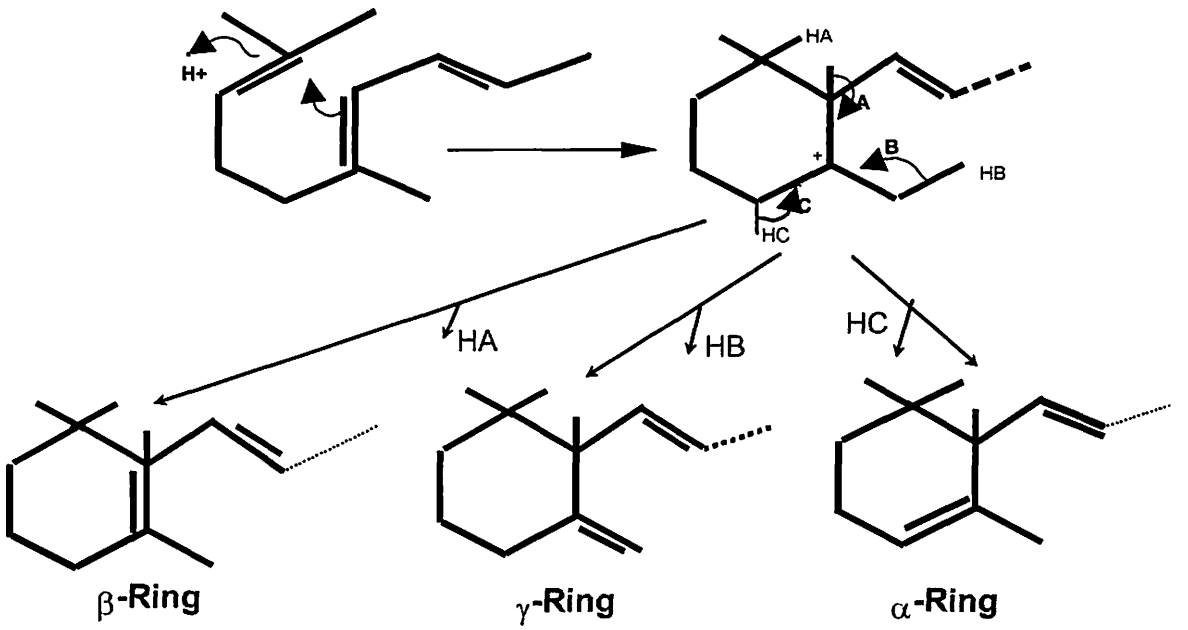

Figure 1.4: Mechanism of Formation of β-, α- and γ-Rings from a Common Precursor

(From Goodwin, 1993)

Nuclear magnetic resonance (NMR) studies with radioactive water have clearly defined the stereochemistry of the hydrogen ions removal during the cyclization leading to the formation of zeazanthin (3,3'-dihydroxy-β carotene) and lutein (3,3'-dihydroxy-α carotene) (Britton, 1988; Goodwin, 1980). Xanthophylls are formed by subsequent oxidation.

The xanthophylls arise initially by hydroxylation of carotenes to form monohydroxyl xanthophylls, like cryptoxanthin (3-Hydroxy-ß-carotene). Monohydroxyl xanthophylls are precursors of dihydroxyl xanthophylls such as lutein (3,3'-dihydroxy-α carotene) and zeaxanthin (3,3'Dihydroxy-ß-carotene). Carotenoid epoxides like antheraxanthin (5,6-Epoxy-5,6-dihydro-ß,ß-carotene-3,3'-diol), violaxanthin [(*3s,5r,6s,3s'5'r,6's*)-5,6,5',6'-diepoxy-5,6,5',6'-tetrahydro- ß,ß-carotene-3,3'-diol], and ketones such as astaxanthin [(*3s,3's*)-3,3'-dihydroxy-ß-carotene-4,4'-dione] and capxanthin, are formed in subsequent oxidation. Neoxanthin is a rare natural example of an allene. The apocarotenoids are a small group of xanthophylls in which C5 isopentenyl fragments have been lost from one or both ends of the chain.

1.1.2 Chemical and Physical Properties of Carotenoids.

The extensive conjugated double bond system constitutes the light-absorbing chromophore that gives carotenoids their attractive colours and provides the visible absorption spectrum that serves as the basis for their identification and quantification. The most unsaturated acyclic carotenoid lycopene, with 11 conjugated double bonds, is red and absorbs at the longest wavelength (λ max at 446, 472, 505 nm) (Krinsky *et al.*, 1990). At least seven conjugated double bonds are needed for a carotenoid to have perceptible colour. Thus ζ-carotene with a much lower λ max at 378, 400, 425 nm is light while the two carotenoids which precede it in the

desaturation biosynthetic pathway, phytoene and phytofluene, are colourless and absorb maximally at 276, 286, and 297 nm, and 331, 347 and 367 nm, respectively (Krinsky *et al.*, 1990). Hydroxyl substituents result in virtually no change in colour and absorption spectrum. Thus lutein resembles α-carotene; β-cryptoxanthin and zeaxanthinm are similar to β-carotene in absorption properties (Krinsky *et al.*, 1990).

The absorption spectra bear out the observation that xanthophylls are the dominant pigment in yellow tissues whereas carotenes tend to give orange colour. The colour intensity and hues of plant foods depend on which carotenoids are present and their concentration, as well as their localization in plastids (Krinsky *et al.*, 1990). In animals, complexing of carotenoids with proteins can extend the colour to green, purple, blue or black (Pfander, 1992). Carotenoids are liposoluble and dissolve in fat solvents such as acetone, alcohol, ethyl ether and hexane. Xanthophylls are more polar but still insoluble in water; they dissolve more readily in ethanol and methanol.

There is increasing evidence that carotenoids can function directly as antioxidants. The primary mechanism of action appears to be their ability to quench singlet oxygen and interact with radical species, formed enzymatically and chemically (Gester, 1993). Quenching characteristics seem to be maximal with those having nine or more double bonds.

1.1.3 Provitamin A Activity of Carotenoids and Vitamin A Biosynthesis

Plant tissues provide Vitamin A activity as provitamin A carotenoids. Provitamin A carotenoids are metabolised in the intestinal mucosa to yield vitamin A (Lakshman and Okoh, 1993). Catabolism of provitamin A carotenoids by either asymmetric fission or, terminal cleavage, yield Vitamin A aldehyde (retinal) that is eventually reduced to retinol (Lakshman and Okoh, 1993) (Figure 1.5). Retinol (Vitamin A) is a diterpene alcohol consisting of a 2,6,6-trimethyl-1-cyclohexenyl ring with a side chain of two isoprene units. β-carotene cleavage enzyme is widely held to be responsible for catalyzing the conversion of all *trans*-β-carotene to two molecules of retinol (Lakshman and Okoh, 1993).

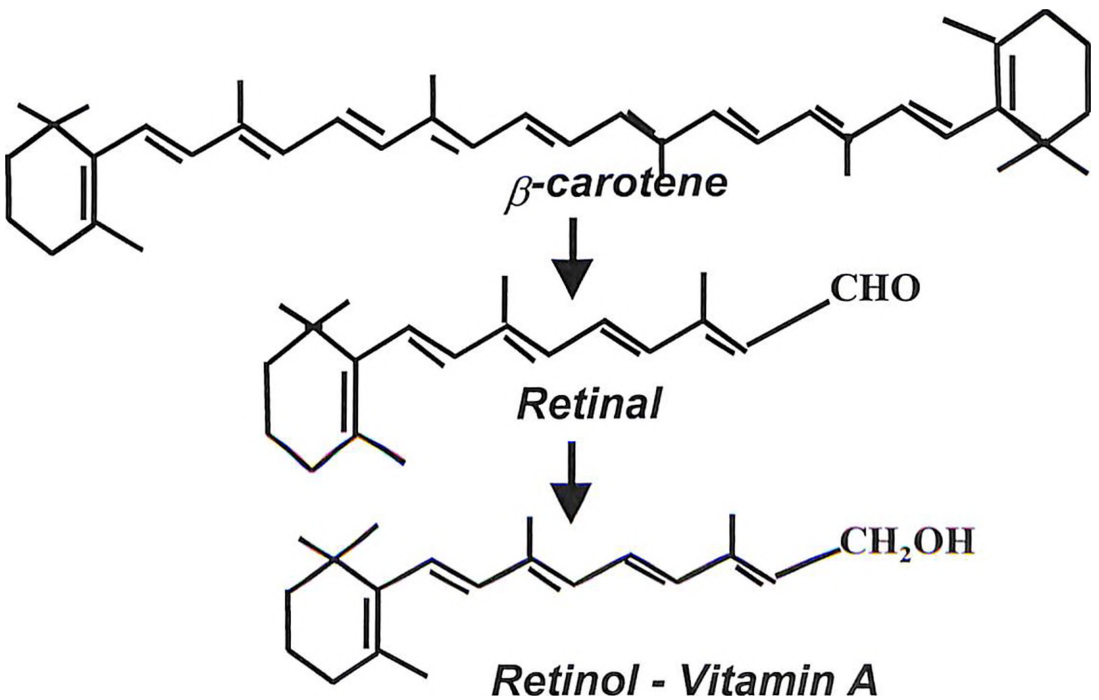

Figure 1.5: Formation of Vitamin A from β-Carotene (From Krinsky *et al.*, 1990)

Provitamin A activity of carotenoids is determined by their structure and isomeric forms. In order to serve as a precursor of vitamin A, a carotenoid must have at least one unsubstituted ß-ionone (2,6,6-trimethyl-1-cyclohexenyl) ring with a polyene side-chain of at least 11 carbon atoms. β-carotene which has two unsubstituted β-ionone rings at both ends of the molecule, has twice the vitamin A potential of α-carotene and γ-carotene, that has only one ß-ionone ring.

Canthaxanthin with keto substitution on both rings, has no provitamin A activity. Other carotenoids, such as lycopene (which has no ß-ionone ring) and the hydroxycarotenoids lutein and zeaxanthin (which are substituted on both ß-ionone rings), may have other important physiological functions, but have no provitamin A activity (Simpson, 1990). Carotenoids can be found in two isomeric forms, i.e., *cis*- and *trans*- isomers. The isomeric form of a carotenoid is also important in determining Vitamin A potential. *Trans*- forms are superior to and more stable than *cis*- forms. Normally the amount of *cis*-isomers is low but typically increases upon exposure to high temperatures such as those incurred during processing (Chandler and Schwartz, 1988).

Absolute vitamin A activity is not well understood. Generally, it seems that efficiency of conversion of carotenoids to vitamin A depends on vitamin A status. High intake of pre-formed vitamin A results in poor efficiency of conversion to vitamin A. This may be due to metabolic control of the cleavage enzymes. Excessive vitamin E intake seems to impair carotenoid cleavage (or interfere with absorption) (Krinsky *et al.*, 1990). Vitamin E deficiency decreases vitamin A formation, perhaps because adequate vitamin E is needed to protect carotenoids and vitamin A from oxidation. Conversion of β-carotene and other carotenoids to vitamin A is also decreased at high carotenoid intakes, perhaps as a result of either impaired intestinal absorption

or metabolic control of cleavage enzyme(s). Protein deficiency impairs cleavage, suggesting that protein malnutrition in humans exacerbates vitamin A deficiency (Krinsky *et al.*, 1990).

1.1.4 Occurrence and Importance of Carotenoids

Carotenoids are widely distributed in the living World (Scita, 1992; Goodwin, 1980). The presence of carotenoids in photosynthetic tissues, for example, green leaves, is often masked by chlorophyll. Carotenoids are known to provide a range of light yellow to dark red colourings (Pfander, 1992). A wide variety of foods and feeds such as yellow vegetables, tomatoes, apricots, oranges, egg yolk, chicken, butter, shrimp, lobster, salmon, trout, yellow corn, and sweetpotato roots owe their colour principally to carotenoids. Certain food colour extracts from a natural source such as palm oil owes their colour to carotenoids (Ong and Tee, 1992). Carotenoids are also responsible for the beautiful colours of many fruits and flowers (Eschscholtzia, Narcissus), as well as the colours of many birds (flamingo, cock of the rock, ibis, canary), insects (ladybird), and marine animals (crustaceans, salmon) (Scita, 1992). When complexed with proteins, carotenoids produce green and blue coloration (Pfander, 1992). A well-known example is the blue carotenoprotein crutacyanin of the lobster carapace which is an astaxanthin-protein complex. On desaturation of the protein (for example by heating), astaxanthin is released and its vivid red colour ensues.

The occurrence pattern of carotenoids may vary from relatively simple to extremely complex mixtures. The simplest carotenoids may be found in foods of animal origin, owing to the limited ability of animals to absorb, modify and deposit carotenoids. The other extreme is the formidable array of carotenoids encountered in citrus products, dehydrated alfalfa meal, and

paprika (Goodwin, 1980). The concentrations of carotenoids vary enormously from one source to another. The highest concentration of carotenes has been found in the red fringe of the corona of the narcissus pheasant's eye (*Narcissus majalis).* Here, β-carotene can constitute up to 16% of the dry weight (Scita, 1992). Furthermore, the daily rate of β-carotene formation reaches 70 μg/mg dry weight, which is more than 10,000 times the rate observed in carrot roots (Scita, 1992). The total carotenoid production in nature has been estimated at 100 million tons a year (Luckner, 1972). Most of this production is as 4 major carotenoids, viz., fucoxanthin, lutein, violaxanthin and neoxanthin. Fucoxanthin which is the characteristic pigment of many marine algae, is the most abundant natural carotenoid. Lutein, violazanthin, and neoxanthin are the principal carotenoids in green leaves (Pfander, 1992).

The best known function of carotenoids is the long established role of β-carotene and other carotenoids with unsubstituted β-ring as provitamin A. Vitamin A is a crucial nutrient whose deficiency causes serious health problems. Deficiency of Vitamin A in diet can cause a myriad of health problems such as xerophthalmia, corneal lesions, keratomalace, increased susceptibility to infections, increased severity of infections and increased mortality and morbidity (Underwood, 1994; Olson, 1989; Dowdey, 1974). In western countries the supply of vitamin A is not critical but in countries of the third world it is still a severe problem (Simpson, 1993; Temalilwa et al., 1986).

In addition to acting as provitamin A precursors, certain vital roles have been attributed to carotenoids. Research findings since the 1970s have indicated that food crops containing carotenoids, anthocyanins, and other flavonoids are believed to function as chemopreventers by providing protection against some forms of cancer and a reduction in cardiovascular disease

(Stavric, 1994). Interestingly, non-provitamin A carotenoids such as lycopene also confer chemoprevention (Stavric, 1994). The important characteristic of all chemopreventers is their antioxidant capacity.

In the plant, carotenoids in the leaves function both in photosynthesis and in preventing the chlorophyll molecule from being photooxidized in the presence of light and oxygen (Lawlor, 1990). The antioxidant properties of carotenoids that protect plants from photo-oxidation, apparently, may also protect humans from carcinogens and heart disease (Simon, 1997). In flowers, carotenoids act as attractants of pollination and dispersal agents. However, their presence in subterranean plant parts may largely represent selection by man rather than an essential biological role (Kays, 1992).

Commercial synthetic carotenoids are used as pigments for food (egg yolk, chickens, or farm-raised salmon) and for coloration of food products (margarine, cheese). Various methods have been developed for carotenoid application. A microcrystalline dispersion is used for colouring margarine. In fruit juices, a powder in which β-carotene in the form of a microdispersion in a hydrophilic protective colloid, is used. Market prices for stabilized dispersible powders containing 5-10% active substances ranged from $600 for β-carotene, $900 for β-apo-8'-carotenal, $1300 for canthaxanthin, to $2500 for astaxanthin per kilogram (Pfander, 1992). It is estimated that a production capacity for more than 500 tons of β-carotene per year is currently planned or under construction world-wide (Pfander, 1992). Total sales of synthetic carotenoids are about $300 million and may pass the $500 million mark in about 5 years (Pfander, 1992).

1.2 Analysis of Carotenoids from Plant Sources

Precise determination of carotenoid content and vitamin A value of food products is crucial in assessing the status of vitamin A and alleviating its deficiency in human populations. Thus particular emphasis has been placed on understanding the types and concentration of carotenoids in foods (Ong and Tee, 1992). It is also apparent that previously reported values of vitamin A activity in food composition tables may be unreliable because the methodologies used were not sufficiently discriminative and therefore included carotenoids that do not possess vitamin A activity (Bhaskarachary *et al.*, 1995; Ong and Tee, 1992).

In cognizance of the need for more accurate analysis of carotenoids, the International Vitamin A Consultative Group (IVACG) has called on national laboratories to develop techniques for determining accurately the provitamin A content of native fruits and vegetables in order to support vitamin A deficiency prevention and control programs (Simpson, 1990). The group also has emphasised that because carotenoids may be protective against some forms of cancer, the quantification of these compounds in foods assumes even broader importance.

Analysis of carotenoids and retinoids is complicated and beset with various problems. The main problem arises from their inherent instability. Carotenoids are especially sensitive to light, heat, oxygen, acid and in some instances, to alkali. Any failure to strictly observe a number of general precautions in handling carotenoids in the laboratory results not only in a low overall quantitative recovery but also in the possible loss of certain particularly labile carotenoids, their conversion to other carotenoids whose natural occurrence is therefore made ambiguous, and in the appearance of *cis-trans* isomers as artefacts (Craft, 1992; Khachik *et al.*, 1992). This

problem is compounded by the large number of naturally occurring carotenoids. The occurrence of *cis* and *trans* isomers of carotenoids further complicates the analysis.

Any good analytical procedure should be able to remove interfering compounds effectively, to separate the carotenoids, and to quantify them accurately. A wide variety of separation, detection and quantification procedures have been used in studies of carotenoids. These include counter-current distribution, paper chromatography, gas-liquid chromatography, gel-permeation chromatography, thin layer chromatography, spectroscopy, open-column chromatography and high performance liquid chromatography (HPLC). HPLC has proved to be the method of choice for carotenoid identification and quantification (Craft, 1992).

1.2.1 Carotenoid Extraction

Extraction procedures for quantitative removal of carotenoids from plant tissues utilize organic solvents that must penetrate a hydrophilic matrix of cell membranes. Normally this requires the use of appropriate organic solvents such as acetone, ether, methanol, or tetrahydrofuran. In tissues that contain significant amounts of water, it is desirable to use an organic solvent that is miscible with water in an attempt to facilitate the denaturing of carotenoid-protein complexes and to prevent formation of emulsions (Khachik *et al.*, 1992). In cases in which dried fruits and vegetables are to be extracted, samples should be re-hydrated for several hours prior to extraction (Khachik *et al.*, 1992). Hexane-acetone mixtures are commonly employed for this purpose, but special solvents and treatments are sometimes needed to achieve satisfactory separation (Fennema, 1996). To prevent oxidation and promotion of epoxides during extraction,

the extracting solvents should be stabilized with an antioxidant such as butylated hydroxytoluene (BHT), α-tocopherol or sodium ascorbate (Matsuno, 1992; Scita, 1992).

For a general homogenization procedure, foods are mixed with extracting solvent in the presence of sodium or magnesium carbonate using a blender. For smaller samples, a mortar and pestle can be used. The function of the carbonates is to neutralize trace levels of organic acids that are commonly present in some foods and can cause destruction and/or structural transformation of carotenoids.

Extraction is normally carried out under chilled conditions by immersing the homogenization vessel in an ice bath to prevent the degradation and isomerization of carotenoids (Khachik *et al.*, 1992). The resulting carotenoid extract is then filtered and extraction of the residue is repeated until the filtrate is devoid of yellow colour. The combined filtrate is concentrated, and the carotenoids are partitioned into an appropriate organic solvent and water. The organic layer is removed, dried and concentrated into the appropriate volume of selected solvent for further carotenoid analysis.

The saponification of carotenoid extracts can provide valuable information as to the nature of the carotenoids present in a specific food (Khachik *et al.*, 1992). Most carotenoids are stable towards saponification procedure that usually involve alkaline treatment used to remove the unwanted chlorophyll and lipids from food extracts. For most fruits and vegetables, a mild saponification that involves treatment of extracts with 10% (w/v) methanolic potassium hydroxide for 2 to 3 hours at room temperature, is employed (Khachik *et al.*, 1992). However, high-fat foods that contain a large concentration of lipids and sterols, more severe treatments

may be required. Where carotenoids sensitive to saponification are present (for example, astaxanthin and fucoxanthin) this step should be omitted. A comparison between the chromatographic profiles of the saponified and un-saponified food extracts should be a common practice in any carotenoid analysis of foods (Khachik *et al.*, 1992).

1.2.2 Measurement of Total Carotenoid Content

Total carotenoid content is commonly determined spectrophotometrically. The absorbance of total carotenoid extracts is measured by a spectrophotometer at a particular wavelength and quantity estimated from a absorbance-concentration standard curve (Bhaskarachary *et al.*, 1995). A β-carotene standard curve is commonly used for this purpose. There is a tendency to underestimate total carotenoid content by evaluating absorbance of total carotenoid extract at fixed wavelength of β-carotene λmax. Part of this underestimation may be due to the higher absorbance of β-carotene than other carotenoids at β-carotene λmax (Simon and Xenia, 1987). Since total carotenoid estimation is based upon a standard curve of pure β-carotene, all other carotenoids are less effectively estimated.

1.2.3 Non Aqueous Reversed Phase (NARP) HPLC Carotenoid Analysis

HPLC has also been shown to be an efficient technique for the analysis of sensitive compounds like carotenoids in complex food extracts (Rudatt and Will III, 1985). Since the late 1970s, HPLC has become a widely used procedure for the separation of carotenoids. HPLC effects rapid separation, it is non-destructive and, more importantly, it achieves better resolution (Craft,

1992). The ability of HPLC to separate rapidly and to quantify various carotenoids have been demonstrated (Rudatt and Will III, 1985).

A number of HPLC systems have been used in carotenoid analysis. Carotenoids have been analysed by both normal phase and reversed phase HPLC. Normal phase HPLC encompasses adsorptive phases (such as silica and alumina) and polar bonded phases (i.e., alkylamine, alkylnitrile, and alkylglycol) in conjunction with nonpolar mobile phases (Craft, 1992). Polar sites on the carotenoid molecules compete with solvent modifiers for adsorptive sites on the stationary phase. During this form of chromatography, the least polar carotenoids (hydrocarbons or carotenes) elute first while oxygenated carotenoids (xanthophylls) are retained longer (Rudatt and Will III, 1985). Reversed phase HPLC incorporates nonpolar (such as octyl - C8, octyldecyl - C18) and polymer bonded phases (for example, polystyrene, polymethacrylate) in conjunction with polar mobile phases. Carotenoids partition between the nonpolar stationary phase and the polar mobile phase. During reversed phase chromatography, xanthophylls partition more effectively into the mobile phase and, therefore elute first while the carotenes partition preferentially into the stationary phase and elute later (Rudatt and Will III, 1985). Both normal phase and reversed phase HPLC can be run with the same solvent throughout (isocratic elution), or with solvent composition changing during the run (gradient elution).

The hydrophobic interactions among non-polar stationary phase, solute, and mobile phase in reverse-phase in NARP HPLC, are much weaker than the ionic or polar forces in normal-phase chromatography. This reduces loss of carotenoid or artefact formation. Reverse phase column addresses the widest range of applications of any HPLC column type. Consequently, when an unknown mixture is to be analyzed, a reverse phase column is often tried first. The most

common column packing used for carotenoid separation is octyldecylsilane (ODS or C18) (Craft, 1992). Reasons for the popularity of C18 packing include compatibility with most solvents, usefulness for the entire polarity range of carotenoids, and wide commercial availability (Craft, 1992). Although the vast majority of reversed phases HPLC use C18 columns, not all C18 packing are the same. Factors such as particle size and shape, pore diameter, surface coverage (carbon load), end capping, and monomeric versus polymeric synthesis can influence the resultant separation (Craft, 1992).

HPLC solvents should be degassed in order to minimize baseline noise by reducing solvent out-gassing at the detector flow cell. This can be accomplished using ultrasonic agitation, vacuum filtration, or helium sparging. Injection volume should be kept to a minimum to prolong the column life and reduce band broadening. The injection solvent should be compatible with the HPLC mobile phase.

More often than not, duplication of reported HPLC conditions does not guarantee similar results. This is due to complex sample-column interactions that are very sensitive to a number of factors. These include sample impurities, solvent composition, ambient temperature, age of the column, and HPLC machination (condition of pumps, flow cell, wavelength monitor, etc.). HPLC conditions should be standardized using authentic analytes before any analysis.

1.2.4 HPLC and Photodiode Array Analyzer

Proper HPLC peak identification is of paramount importance in connection with profiling and quantification of carotenoids in biological materials. In daily HPLC practice, this is often done

by simply comparing retention times of unknown peaks with those of authentic reference substances. However, only a limited number of synthetic carotenoids are commonly available. Alternatively, fractions can be collected from the HPLC column and compounds characterized off-line by spectrophotometric techniques. This approach may be seriously hampered by the incomplete resolutions between peaks in complex carotenoids patterns encountered in biological extracts.

The most sophisticated scanning absorption detector presently available is the photodiode array detector (PDA). This instrument permits the on-line recording of complete absorption spectrum of a chromatographic peak in less than 1 second. It owes this unique performance to a reverse-optics configuration, the presence of an array of photo-diodes each dedicated at a particular band of wavelengths, and its connection to a computer for data handling (Leenheer and Nelis, 1992). The identities and quantities of the eluting carotenoids are inferred from the spectral and chromatographic data generated by the computer.

1.3 Sweetpotato - An Overview.

Sweetpotato is a creeping dicotyledoneous plant belonging to the family of convolvulaceae. Sweetpotato is the only species of major economic importance amongst the approximately 1000 species of the 50 genera in this family (Woolfe, 1992; Edmond and Ammerman, 1971).

1.3.1 General Morphology

The sweetpotato plant can be divided into three basic parts each of which has its own function (Woolfe, 1992). These consist of an above ground photosynthetic canopy, petioles and vines. The canopy absorbs light energy and converts it to carbon compounds. The petioles and vines transport the photosynthates from the canopy and nutrients from the root system from one site to another within the plant. Below ground, sweetpotato plant consists of the root system that absorbs water, nutrients and act as an anchor for the plant. It also stores excess energy that is not needed for maintenance or structural development as carbohydrate in large fleshy storage roots.

1.3.2 Sweetpotato Cultivation

Sweetpotato is a perennial plant, but it is normally grown as an annual. It is usually propagated from vine cuttings in the tropics, but in temperate regions it may also be grown from rooted sprouts pulled from bedded roots (Woolfe, 1992). Cuttings 30 - 45 cm long are taken from apical growth of mature plants. The bottom leaves are removed and the lower half to 2/3 of the cutting inserted into the soil at an angle. Sprouts are obtained by planting small or medium sized roots close together in nursery beds. When resulting sprouts reach 22 - 30 cm in length, they are removed by pulling them from the storage roots and planted in the field (Woolfe, 1992). Cuttings and sprouts are planted on mounds or ridges, or on the flat.

The optimum growth conditions occur at temperatures at or below 24 °C. The crop is damaged by frost and this restricts its cultivation in temperate regions to areas with a minimum frost-free period of 4 - 6 months (Woolfe, 1992). Sweetpotato grows best where light intensity is

relatively high, but at the same time both flowering and root formation are promoted by short day lengths such as those found in the tropics. Optimum rainfall is 750 - 1000 mm per annum, with approximately 50 cm falling during the growing season (Woolfe, 1992). Sweetpotato prefers sandy-loam soils of a high organic matter content and with a permeable subsoil, doing poorly on clay soil. Good drainage is essential as the plants do not withstand water logging. Soil with a high bulk density or poor aeration retards storage root formation and result in poor yields of sweetpotato. A soil pH of 5.6 to 6.6 is preferred, the plant being sensitive to alkaline or saline conditions (Woolfe, 1992).

The crop is, however, adaptable to a wide range of conditions owing to its broad genetic base (Woolfe, 1992). It can be cultivated on heavy clay and peat soils as well as sandy loam; on flatlands as well as slopes of up to 40°. Sweetpotato is drought resistant. Vines can remain green and healthy during severe droughts although root growth is negligible (Woolfe, 1992).

After planting the growth and development of the sweetpotato occurs in three distinct phases (Onwueme and Charles, 1994):

a) Initial phase when fibrous roots grow extensively and there is only moderate growth of the vines. This phase takes up to 9.5 weeks.
b) \Middle phase when the vines make extensive growth and the tubers are initiated. A tremendous increase in leaf area occurs during the middle phase that lasts from 9.5 - 16 weeks.
c) Final phase of tuber bulking lasting for the rest of the growth period (16 weeks to maturity)

The respective duration of these three phases in sweetpotato may vary with cultivar and environmental conditions. Under more tropical conditions storage root initiation can begin as early as 4 weeks after planting with most of it occurring 4-7 weeks after planting (Onwueme and Charles, 1994). The rest of the season is devoted to storage root enlargement. In cooler highlands these processes take longer duration. The principal tissues of the storage root are the periderm or skin, the ring of secondary vascular bundles under the periderm, the tracheids, sieve tubes, laticifers (latex ducts) and the parenchyma (Woolfe, 1992). The skin and flesh of storage roots of some cultivars contain carotenoid and anthocyanin pigments that determine their colour.

Sweetpotato genotypes are predominantly prostrate vining plants (although they can be upright and sometimes also twining) and in contrast to most agricultural plants they establish a relatively shallow and largely two-dimensional canopy. The sweetpotato's long thin stems trail along the soil surface, sending roots into the soil at the nodes. The leaves are arranged spirally on the stem and have petioles varying from 5 - 30 cm in length.

Sweetpotato are ready for harvesting 3 - 8 months after planting, often much sooner than other root and tuber crops. In many traditional settings, sweetpotato is harvested piecemeal and there is no fixed harvest time. In most parts of the tropics harvesting is done by hand. In countries where sweetpotato is grown on large farms, mechanical harvesters are used.

Since its domestication, cultivation of sweetpotato has spread to 111 countries of which 101 are classified as a 'developing nations' (Woolfe, 1992). Developing countries produce and consume nearly all of the world's sweetpotato. Approximately 90% of World's sweetpotato production comes from Asia, with Africa contributing only about 5% of this (Woolfe, 1992).

1.3.3 Carotenoid Biosynthesis in Sweetpotato Roots.

Kehr *et al.*, (*1955*) pioneered the research on carotenoid biosynthesis in sweetpotato roots. These workers studied the site of the carotenoid biosynthesis by utilizing reciprocal grafting techniques. They concluded that carotenoids are apparently synthesized *in situ* in sweetpotato roots. Later, Miller and Gaafar (1958) concurred that the synthesis of carotene actually occurs in the root, and that carotenoids are not translocated from the leaves. Edmond and Ammerman (1971) confirmed that it is the precursors that are biosynthesized in the vines, while the finished products, carotenoids are assembled in the fleshy roots.

The mechanisms for carotenoid biosynthesis is thought to be genetic in character and are either present or absent in the sweetpotato roots (Miller and Gaafar, 1958). The capacity of roots to synthesize carotenoids is dependent on inherent root genetic factors (Woolfe, 1992). Variability in carotenoid content in different cultivars is therefore thought to be genetic.

1.3.4 Carotenoids and Provitamin A Value of Sweetpotato

Carotenoids are largely responsible for the cream, yellow to orange flesh colour of sweetpotato roots (Almeida *et al.*, 1992; Takahata *et al.*, 1993). The depth of the flesh colour is mainly a function of the concentration of β-carotene (Simonne *et al.*, 1993; Woolfe, 1992; Kays, 1992). The correlation of flesh colour and β-carotene content can be used to predict provitamin A value of various sweetpotato lines on the basis of colour (Picha, 1985). The main carotenoids that have been identified in raw and/or processed sweetpotato roots include, all-trans-β-carotene,

15,15'-cis-β-carotene, 13-cis-β-carotene, 9-cis-β-carotene, neo-β-carotene, β-carotene epoxide, β-carotene furanoxide, tetrahydro-β-carotene, β-carotene, α-carotene, γ-carotene, ζ-carotene, hydroxy-ζ-carotene, β-ζ-carotene, neurosporene, phytoene, phytofluene, luteochrome, and several unspecified xanthophylls (Khachik and Beecher, 1992, Chandler and Schwartz, 1988; Almeida and Penteado, 1988; Martin, 1983; Picha 1985, Purcell, 1962). β-zeacarotene and neurosporene have been tentatively identified in light coloured sweetpotato (Martin, 1983).

It is noted that, of the over 600 carotenoids that have been identified in the plant kingdom, β-carotene and its close derivatives predominate in sweetpotato storage roots. β-carotene make up 86-90% of the carotene present in most sweetpotato cultivars especially the yellow-orange fleshed ones (Ezell *et al.*, 1952; Purcell, 1962: Purcell and Walter, 1968; Woolfe, 1992). Some intensely pigmented cultivars have been selected for high levels of β-carotene (Bhaskarachary *et al.*, 1995, MacNair *et al.*, 1956). In light fleshed lines, other carotenoids such as luteochrome and lutein (Almeida *et al.*, 1992) may predominate. Some cultivars with white flesh contain no β-carotene (Takahata *et al.*, 1993); others contain only small quantities (Bradbury and Halloway, 1988). Those with creamy or light yellow roots generally contain trace amounts (Garcia *et al.*, 1970). Most varieties grown in Kenya are white fleshed, with low β-carotene content (Ngare et al., 1995).

1.4 Root Age and Carotenoid Content.

Carotenoid content changes during root growth and development have been reported (Data *et al.*, 1987; Ezell *et al.*, 1952). To investigate the effect of root age, Ezell *et al,.* (1952) determined carotenoid content of the fleshy roots of a number of cultivars harvested at definite age intervals. They reported variety-dependent difference in the rate of carotene formation. The varieties also varied in the number of days required to obtain maximum carotene. The percentages of β-carotene and terpenes have also been noted to be significantly affected by age at harvest (Data *et al.*, 1987). Simpson (1990) reported higher yields of carotenoids are gained from mature than younger roots.

Edmond *et al.(*1950) obtained data on the relationship of time of harvest and flesh colour, in their studies of yield and grade of sweetpotato. Flesh colour intensity was observed to increase with three succeeding harvests. Flesh colour being reliable index of carotenoid content, this observation was an indication of changing carotenoid level. Edmond *et al.* (1950) pointed out that the rate of photosynthesis and the rate of carotenoid precursor formation are involved. However, there was no consistent trend in the variation of carotenoid content with root age in these studies. In another study, Kimbrough *et al.*(1946), found out that the time of planting and size of the tuber had no effect on its carotene content, while the regular harvest gave higher carotene content than the early or late harvest. Abubakar (1981) reported that carotenoids were higher at first harvest (125 days after planting) for two out of three cultivars, a third increasing in carotenoid concentration with prolonged harvest. However, Abdel-Kader (1991), maintained

that age at harvest had no effect on carotenoid level. The time of harvest of roots from the same planting showed no consistent effect on carotenoid content.

1.5 Influence of Farming Site on Carotenoid Content.

Environmental and biological stresses often limit sweetpotato production (Kays *et al.*, 1992). Different farming sites present a range of different environmental and biological conditions. There are conflicting reports on the influence of farming site on carotenoid content (Woolfe, 1992). While some researchers have noted significant influence of farming site, reports of non-significant variation in carotenoid content between farming sites abound (Speirs *et al.*,1954; Hammett, 1974, Woolfe, 1992). Speirs *et al.*, (1954) observed statistically significant differences among 4 cultivars. Their mean carotenoid content at harvest was 19 µg/100g at the first location and 31 µg/100g (fwb) at the second. The chief difference in the two locations was a mean monthly temperature difference of 5 ^{0}C. In his study Hammett (1974), noted a variation of as much as 62% to 93% in three American cultivars. Woolfe (1992) observed that farming sites do not exert consistent effect on carotenoid content in sweetpotato.

1.6 Effects of Cooking on Vitamin A Value of Sweetpotato.

Cooking methods differ across regional and cultural boundaries, but boiling, steaming, baking, roasting and frying, are typical. Cooking improves the texture and flavour of roots as well as increases the digestibility of certain nutrients, especially starch. Cooking may also serve to

reduce the levels of toxic terpenoid phytoallexins such as ipomeamarone (Cody and Haard, 1976) and anti-nutritional trypsin inhibitors (Woolfe, 1992).

Cooking losses of carotenoids arise from thermal isomerization (Chandler and Schwartz; 1988) and oxidation (Krinsky *et al*, 1990). During cooking, the all-*trans* form of β-carotene is isomerized to less active or inactive forms e.g. 13-cis-β-carotene (Lee and Ammerman, 1974; Schwartz and Catignani, 1989) lowering the provitamin A value of the product. However, isomerisation may not result in complete loss of vitamin A value. In an *in vivo* ferret model, 9-cis-β-carotene has been shown to have a good bioavailability and is a precursor of 9-cis-retinoic acid (Kays, 1992).

The effect of different cooking methods on carotenoid content has been studied, albeit with inconsistent findings. Baking was found to cause a greater loss than boiling in one such study (MacNair, 1956). In a subsequent study, however, no differences were found between baking, boiling, steaming, microwaving, and canning (Lanier and Sistrunk, 1979). Cascony *et al.*, (1988) reported that a Brazilian cultivar containing 9 mg/100g (fwb) total carotenoid (80% of which was β-carotene), lost less than 10% total carotenoids and β-carotene when placed in water already boiling and cooked for 20 minutes. In another study, 26% loss of β-carotene was computed after boiling (Almeida and Penteado, 1988).

Cultivars have been noted to respond differently to cooking. Reddy and Sistrunk (1980), reported that the cultivars they studied did not react uniformly to different cooking methods resulting in significant interactions in colour intensity.

CHAPTER 2

MATERIALS AND METHODS

Seventeen sweetpotato cultivars with a range of flesh colour from white to orange were selected for analysis (Table 2.1). Sweetpotato flesh colour were determined using a CIP colour description.

2.1. Sweetpotato Planting, Harvesting and Sampling

Vine cuttings measuring 40 cm long were taken from apical growth of mature plants from established CIP seedlot. The bottom leaves were removed and the lower half of the cutting inserted into the soil at an angle. The vines were planted at a spacing of 33cm within rows and 1m between rows. The plants were tended according to standard low input. The plants were grown under natural growth conditions prevalent at the location of cultivation. No subsidiary irrigation or fertilization was used.. Roots were manually harvested according to the aspect of study intended.

2.1.1 General Survey

Seventeen cultivars were selected for general survey. General survey plants were planted at Kabete (University of Nairobi's College of Agriculture and Veterinary Sciences) experimental plots on 18/111996. Harvesting was done on 21/6/1996. Sampling of each cultivar for carotenoid analyses was done in a 2 stage random sampling. Three plants were randomly selected and 3 roots randomly picked from each plant for carotenoid extraction and analyses.

Table 2.1: Sweetpotato Cultivars Used for General Survey of Carotenoids, and the Study of the Effect of Root age, Farming Site and Effect of Cooking.

	Cultivars	*CIP No.*	*General survey*	*Root age*	*Farming Site*	*Cooking*
1	**KSP 11**	_	X			
2	**TIS 2435 (KSP 20)**	_	X	X	X	X
3	**Ex-Diani**	_	X			
4	**Kakamega 4**	_	X	X		X
5	**LM 88.002**	188001.2	X		X	
6	**Maria Angola**	420008	X			
7	**Japanese**	420009	X		X	
8	**Zapallo**	420027	X	X		X
9	**Capadito**	420053	X			
10	**W-220**	440015	X			
11	**TIB 11**	440057	X			
12	**TIS 70357**	440078	X			
13	**Naveto**	440131	X			
14	**Phillippine**	440160	X			
15	**KEMB 10**	440169	X	X	X	X
16	**Kokei No.14?**	440240	X			
17	**NG 7370**	440377	X			

Kokei No. 14?: Identity of this cultivar was not conclusively determined.

2.1.2 Root Age Studies

Root age studies involved monthly sampling of roots planted at Kabete on 23/5/96. Root age sampling were on 19/8/96, 22/9/96, 25/10/96 and 20/11/96. At each stage of root age 3 plants were randomly selected and a systematic sampling technique involving harvesting of the largest tuber from each randomly selected plant was used. Different plants were sampled every month.

2.1.3 Effect of Farming Site.

Sweetpotato roots for the study of the effect of trial location on carotenoid content were planted at 3 farming sites of Kabete, Kiboko and Kisii on 17/1/96, 13/1/96 and 5/2/96, respectively. These plants were harvested on 10/6/96, 1/6/96 and 5/7/96, respectively. This study considered four cultivars, KEMB 10, TIS 2534, Japanese and LM88.002. Sampling was done as outlined in general the survey above.

2.1.4 Effect of Cooking

Samples used for cooking were collected from the 6 months old Kabete's root age studies samples. They were sampled on 20/11/96. Random sampling as outlined in general survey above was used. Boiling was carried out under uniform conditions. Samples were divided and subjected to three boiling regimes. This involved boiling of un-peeled roots in water for 30, 45 and 60 minutes. After boiling, the roots were pealed and carotenoids extracted.

Sampled roots were expediently transported from the fields to Plant Physiology Laboratory, Department of Botany in Chiromo Campus - University of Nairobi, for processing and analysis.

Sampled roots were peeled and about 2 cm thick medial transverse slices taken. The sampled medial slices were finely grated length-wise by a cheese grater. The finely grated samples were thoroughly mixed and packed into Nasco Whirlpacks® plastic packets under nitrogen. The packed samples were stored at -20°C for a maximum of 2 weeks.

2.2 *Carotenoid Extraction*

The extraction procedure was adopted from Khachik *et al.* (1992) with slight modification on the basis of available equipment. Two grams of the grated root sample was mixed with 4g anhydrous sodium sulphate, 0.2g magnesium carbonate and 10 ml of acetone: petroleum spirit (40-60°C) (PEP) (5:1). Thermal degradation and isomerization were minimized by extracting in a mortar placed in an ice bath. The extract was then decanted and filtered through glass wool. The residue was re-extracted and filtered until the filtrate was devoid of yellow colour.

The combined filtrate was then partitioned between distilled water and PEP to remove acetone and other aqueous extract components. Aqueous - organic phase partitioning was done in 100 ml separatory funnel partially covered with aluminium foil. It involved constant washing with water until a clear organic PEP layer was attained. Complete removal of acetone is important as its presence causes aldol condensation and clouding of extracts upon saponification. The organic PEP phase was recovered and dried through a column of anhydrous sodium sulphate. The anhydrous sodium sulphate column was thoroughly washed with PEP and eluent made up

to 50 ml. An aliquot of this was taken for spectrophotometric and HPLC carotenoid analysis. Exposure of carotenoid extracts to air and/or direct light was avoided by covering vials and tubes with aluminium foil and using amber coloured Nalgene® sample bottles.

2.3 Saponification.

The saponification procedure used was one described by Takahata *et al.* (1993). It involved fluxing 10% methanolic sodium hydroxide with an equal volume of carotenoid extract. The saponification mixture was left in a flask at room temperature overnight. The saponified extract was then washed thoroughly with distilled water in a separation funnel and dried through a column of anhydrous sodium sulphate. The dried total carotenoid extracts were reconstituted in appropriate solvents for spectrophotometric and HPLC analyses.

2.4 Total carotenoid Content Determination

Total carotenoid content was determined spectrophotometrically as described by Imungi and Potter (1983). Carotenoid concentration was evaluated from spectrophotometric absorbance of dried total carotenoid extracts using a correlative absorbance-concentration function derived from a β-carotene standard curve. A Unicam® 500 spectrophotometer with a variable wavelength monitor was used. The wavelength for carotenoid absorbance reading was set at 450 nm. The optical density readings of the extracts were taken using 1 cm X 3.5 ml LKB® quartz microcuvettes. Petroleum spirit 40 - 60 °C was used as the spectrophotometric solvent.

2.5 β-carotene Content Determination

Determination of β-carotene content was done according to the procedure described by Imungi and Potter (1983). β-carotene content was evaluated from the absorbance of β-carotene fractions separating from total carotenoid extracts. The separation was done by a silica gel column. Total carotenoid extracts were first concentrated in a Rotavapor® before separating through a silica gel column. Concentration was done by evaporating 25 ml of the total carotenoid extract to 2 ml at temperatures below 25 °C.

A 15 cm long silica gel chromatographic column was prepared from a slurry of 20g silica gel in 15 ml PEP: absolute ethanol (8:1). An anhydrous sodium sulphate was added onto the column to form a 5 mm thick drying layer. One ml of the 2 ml total carotenoid concentrated extract was eluted through the column with 100% PEP. β-carotene eluted as an intensely orange band which was collected and the eluent made up to 10 ml. Chromatography was done using pure β-carotene as a standard. β-carotene concentration was evaluated spectrophotometrically from the absorbance of the eluent.

2.6 β-carotene Standard Curve.

β-carotene standard curve was developed using standard solutions of pure β-carotene from Sigma Co. The concentrations used were of between 0.005 to 0.05µg/ml. Absorbance was read in petroleum spirit 40-60 °C (PEP) at 450 nm and plotted against corresponding β-carotene

concentration. A correlation equation of absorbance-β-carotene concentration was computed and used to quantify total carotenoid and β-carotene content in extracts.

2.7 *HPLC CAROTENOID ANALYSIS*

2.7.1 HPLC Samples Preparation

Sweetpotato carotenoid extracts for HPLC analysis were further processed to remove impurities and artefacts. One ml of total carotenoid extract from 2g of grated sweetpotato sample was freeze-dried and reconstituted in HPLC mobile phase, typically 90%MeOH:10%Tetrahydrofuran (THF). The reconstituted samples were ultra-filtered through 0.5 μm Satorious® microfilters before injection into the HPLC system.

2.7.2 HPLC System Standardization.

β-carotene standard was used to optimise the HPLC system. A range of MeOH:THF mobile phase compositions were run to determine an optimum MeOH:THF ratio for better retention and run time. MeOH:THF ratio used were 100% MeOH, 98% MeOH: 2% THF, 94% MeOH:6 % THF and 90% MeOH: 10% THF. The latter ratio was established as the most appropriate.

2.7.3 HPLC System, Conditions and Procedure

HPLC studies were done in Laboratory 1 at International Livestock Research Institute (ILRI), Nairobi. A Pharmacia® HPLC with Pharmacia LKB VWM 2141® wavelength monitor and Pharmacia LKB Gradient Pump 2249® was used. The HPLC system was fitted with a manually operated Rheodyne® 7010 sample injector. The HPLC column used was a silica-based octadecylsilyl Supelcosil® LC-18, 5 μm, 25 cm X 4.6 mm ID (pore size-100Å, surface area-170m^2/g, pore volume-0.6 ml/g, pH range-2-7, carbon-11%, frit pore size-2 μm) (catalogue number 5-8298, Supelco Co.).

Isocratic non aqueous reverse phase (NARP) HPLC was used. The mobile phase consisted of methanol 90%MeOH:10%Tetrahydrofuran. The HPLC solvents were ultra filtered using Ultipor® Nylon - 66 (catalogue number 38-111, Rainin Instrument Co.) 0.2 μm by 47 mm diameter membrane filters. Degassing of the mobile phase by helium sparging for 15 min was done before daily runs. Flow rate of the mobile phase was set at 1.5 ml/minute. A back-pressure of 7.5 ± 0.5 MPa was maintained during the HPLC runs. Chromatography was done at room temperature.

HPLC analysis procedure was adopted from Ruddat and Will III (1985). Sweetpotato samples were introduced into the Rheodyne® 7010 sample injector by a 250μl microsyringe. The injection volume was 200μl. Carotenoid detection was made at 450 nm.

HPLC was run by HPLCmanager® ©1992, Pharmacia LKB Biotechnology AB Program version 3.02. Chromatographic data was generated by a PE Nelson® 3000 series chromatography data system with 2600 revision 5.1. software ©1988 PE Nelson system, Inc.

Carotenoid concentration was quantified from HPLC chromatographic peak area. β-carotene and β-carotene-5,6-monoepoxide standard curves were constructed to quantify these carotenoids.

2.7.4 HPLC Carotenoids Identification and Quantification

Carotenoid standards were used to identify different HPLC carotenoid chromatographic peaks. β-carotene (Lot 3H9522 catalogue number C-126 Sigma Co.), α-carotene (Lot 15H9574 catalogue number C-0251 Sigma Co.), ζ-carotene, lycopene (Lot 45H9514 catalogue number. L-9879 Sigma Co), β-cryptoxanthin, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide standards were used in this study. β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide standards were donation from Dr. Peter Molnar of University Medical School of Pecs, Hungary. ζ-carotene and β-cryptoxanthin were donated by Dr. W. Schuep and Dr. J. Schierle of Hoffmann La Roche Ltd.

Identification of the various HPLC carotenoid peaks was based on consistent retention times and co-chromatography. The former method entailed comparison of the HPLC mean retention times of main sweetpotato carotenoids and those of pure carotenoid standards. Similarity and identity of sweetpotato carotenoids and pure standards were statistically evaluated by t-test.

Co-chromatography involved HPLC runs of mixtures of pure carotenoid standards and sweetpotato extracts. Equal volumes (25µl) and concentrations (0.025µg/l) of carotenoid standards and Japanese extract were injected into the HPLC and run under standard conditions. The HPLC co-chromatographic traces were compared to traces generated by carotenoid standards and Japanese extract. Elements considered during comparisons were co-elution and the number and retention times of eluting carotenoid species.

HPLC quantification of carotenoids was based on chromatographic peak areas. Absolute quantities were derived from peak areas-concentration correlation computed from HPLC standard curves of the respective carotenoids. Only provitamin carotenoids, β-carotene and β-carotene-5,6-monoepoxide, were quantified.

2.7.5 HPLC and Photodiode Array Analyser (PDA)

Four representative cultivars, Japanese, Kakamega 4, KEMB 10 and TIS 2534, were analysed by HPLC and Photodiode Array Analyzer (PDA) at the University of Wisconsin in the Laboratory of Philipp Simon. These cultivars were selected for their variation in flesh colour.

The HPLC system consisted of a Whatman Partisil 5 ODS-3 column fitted with a HC Pellosil Guard Column. An Acetonitrile:Methylene Chloride:Methanol (82.4:11.8:5.9%) mobile phase pumped at a flow rate of 2ml/min, was used. Injection volume was 25µl. Detection was done at λ range of 192.4 - 797.6nm with a Waters 996 Photodiode Array Detector. Waters Millennium Version 2.1 software handled the data.

2.8 Recovery Experiments

Normally, a certain amount of carotenoids are lost during analytical procedure. Recovery experiments were carried out to quantify these loses. This entailed subjecting carotenoid standards of known concentrations through the rigor of the analytical procedure. In this study, β-carotene standard was used. Recovery experiments involved addition of amounts of β-carotene to white fleshed LM 88.002. β-carotene was then determined at the end of each analytical procedure. Recovery was expressed as a percentage. Final carotenoid content values in this study were readjusted with the overall recovery to compensate for experimental loses.

2.9 *Vitamin A Value Calculation* (From Simpson, 1990)

1 RE = 1 μg Retinol

= 6 μg β-Carotene

= 12 μg Other Provitamin Carotenoids

$$\text{Vitamin A Value (Retinol Equivalents)} = \frac{\mu g\ \beta\text{-Carotene}}{6} + \frac{\mu g\ \beta\text{-Carotene-5,6-monoepoxide})}{12}$$

2.10 Statistical Analyses

Mstat computer statistical package (MSTAT-C, 1990) was used in analyses of the effect of root age, farming site and cooking on carotenoid content. One way and two-way ANOVA, students t-test, correlation and regression analyses were used in this investigation.

CHAPTER 3

RESULTS

3.1 Experimental Results

3.1.1 Saponification.

No significant difference was observed between carotenoid content data of saponified and unsaponified carotenoid extracts. HPLC chromatographic profiles of saponified and unsaponified extracts were found to be similar (Figure 3.1). No significant shifts in retention time nor novel peaks were noted between the saponified and unsaponified carotenoid extracts. Saponofication was skipped in subsequent analysis.

3.1.2 Recovery Experiments.

There was incomplete recovery of carotenoids after extraction, aqueous-organic phase partitioning, rotavapor sample concentrating, column elution of extracts and saponification. Mean recovery from these stages were 86.84, 81.02, 91.59, 85.41 and 96.23% respectively (Table 3.1). Spectrophotometric total carotenoid and β-carotene content determination was done with 64.44% and 55.04% recovery, respectively. HPLC losses occurred during saponification and freeze-drying. Recovery of 96.23% and 95.60% respectively, was realised (Table 3.1). HPLC analysis was done with a recovery of 86.23%. These recovery figures were used to adjust carotenoid content values.

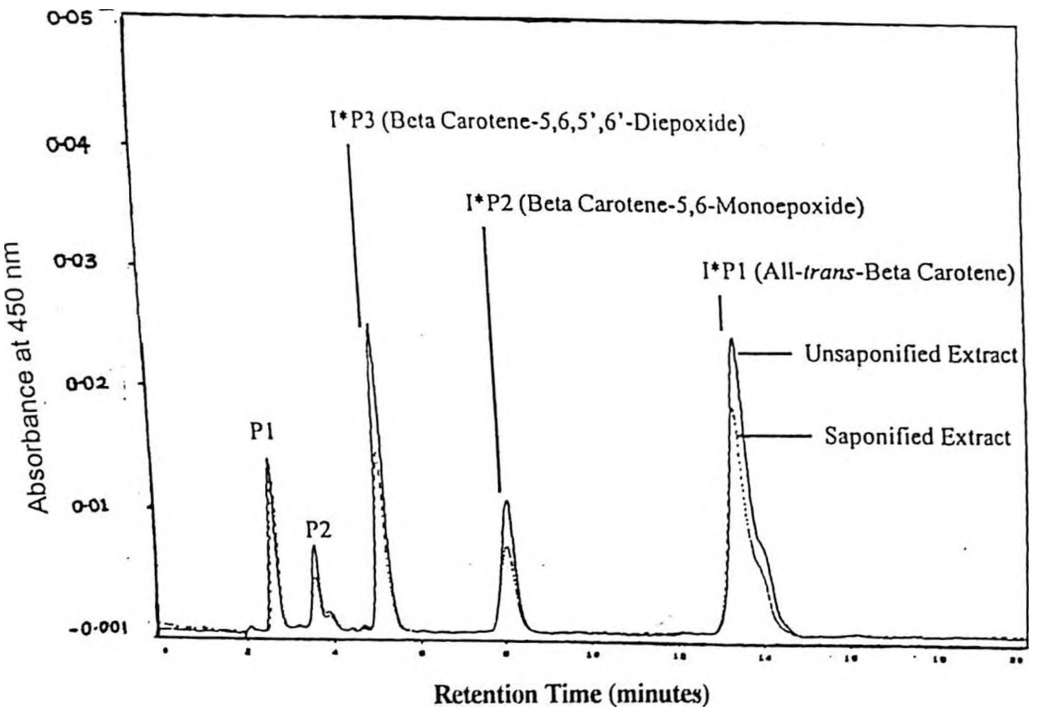

Figure 3.1: Carotenoid HPLC Profile of Saponified and Unsaponified Sweetpotato Extract, Cultivar Kokei No.14? (Mobile Phase-90%MeOH:10%THF, Flow Rate-1.5ml/min, Detection λ-450 nm).

Table 3.1: Recovery (Expressed as a %) of β-carotene Standard from Extraction, Partitioning, Rotavapor Drying, Column Elution, Saponification and Freeze-Drying

Replicates	Extraction	Partitioning	Rotavapor	Column Elution	Saponification	Freeze drying
1	89.18	83.13	90.35	89.84	95.34	95.89
2	83.65	80.08	91.67	88.05	97.95	96.00
3	88.38	82.19	95.30	86.99	98.59	98.17
4	84.0	83.55	93.23	81.20	93.92	94.99
5	84.5	78.91	92.83	80.99	97.11	95.31
Mean Recovery	86.84% ± 2.62	81.02% ± 4	91.59% ± 1.85	85.42% ± 4.07	96.23% ± 1.92	95.60% ± 1.24

3.1.3 HPLC Standardization.

The optimum HPLC operation specifications were developed from trial runs using β-carotene standard (Figure 3.2). Carotenoid retention time was reduced with increased tetrahydrofuran (THF) concentration. The appropriate mobile phase composition for best retention and run time was 90% MeOH : 10% THF pumped at a flow rate of 1.5 ml/min at ambient temperature. Re-equilibration after each sample run was found to be unnecessary.

It was also noted that there were some differences in the elution time of analytes even after standardizing the HPLC run condition. Preliminary runs using β-carotene standard showed that variation in retention times of samples analysed on the same day vary from run to run (Figure 3.3). However, the variation was not large and tended to stabilize in subsequent runs.

It was also noted that the experimental procedure used resulted in some degree of degradation of carotenoids. Some degradation products were noticed in β-carotene standard runs (Figure 3.3).

3.1.4 Carotenoid Standard curves

A Spectrophotometric β-carotene standard curve with a correlation coefficient of 0.997 was computed from β-carotene standard absorbance/concentration (Figure 3.4). HPLC β-carotene and β-Carotene-5,6-monoepoxide standard curves were plotted from serial concentrations of 0.005-0.05μg/μl. and 0.05-5μg/μl respectively (Figure 3.5 and 3.6).

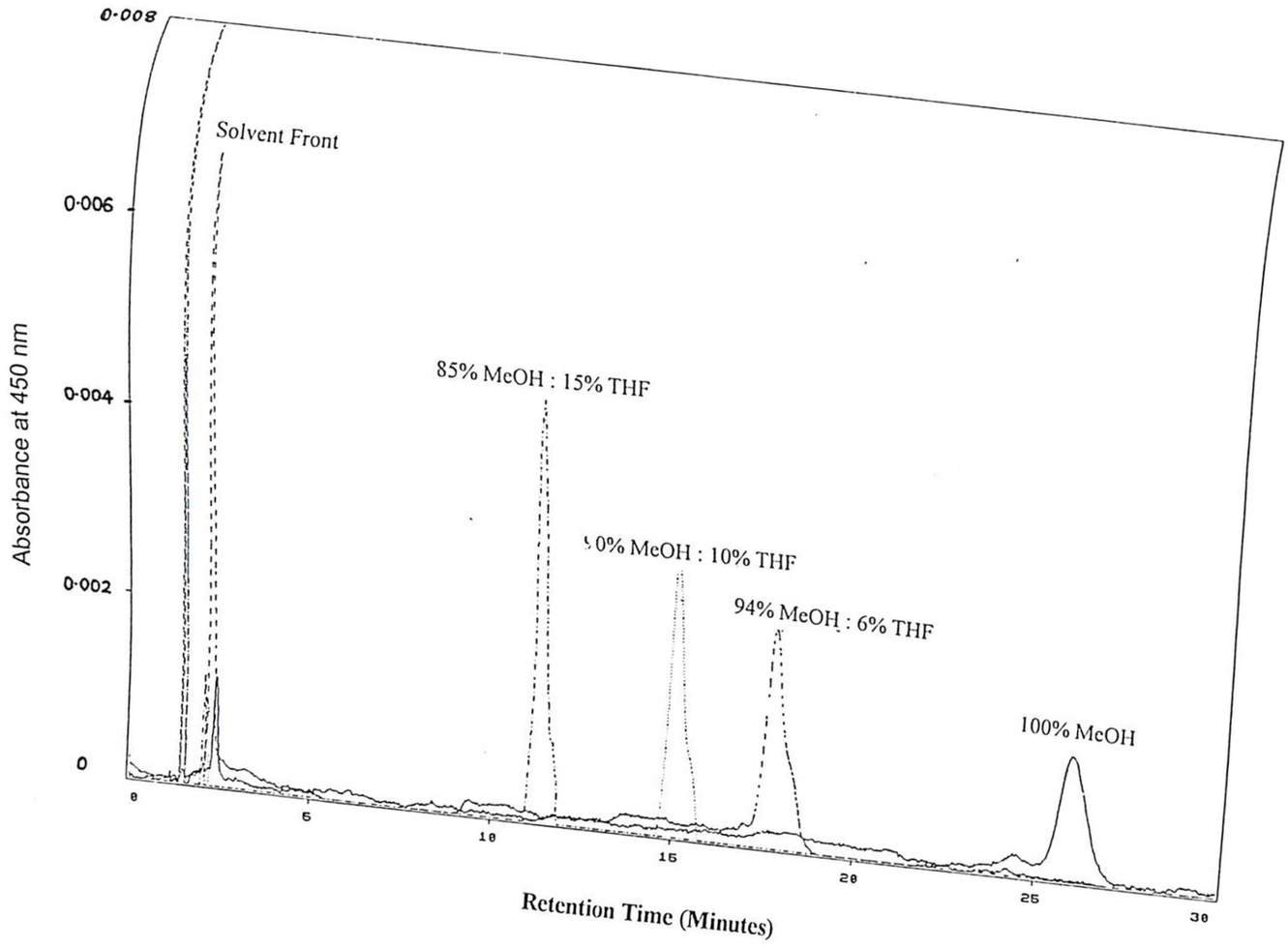

Figure 3.2: Profiles of HPLC System Standardization Runs using β-Carotene Standard in Different Mobile Phase Compositions (Flow Rate 1.5ml/min, Detection λ-450 nm).

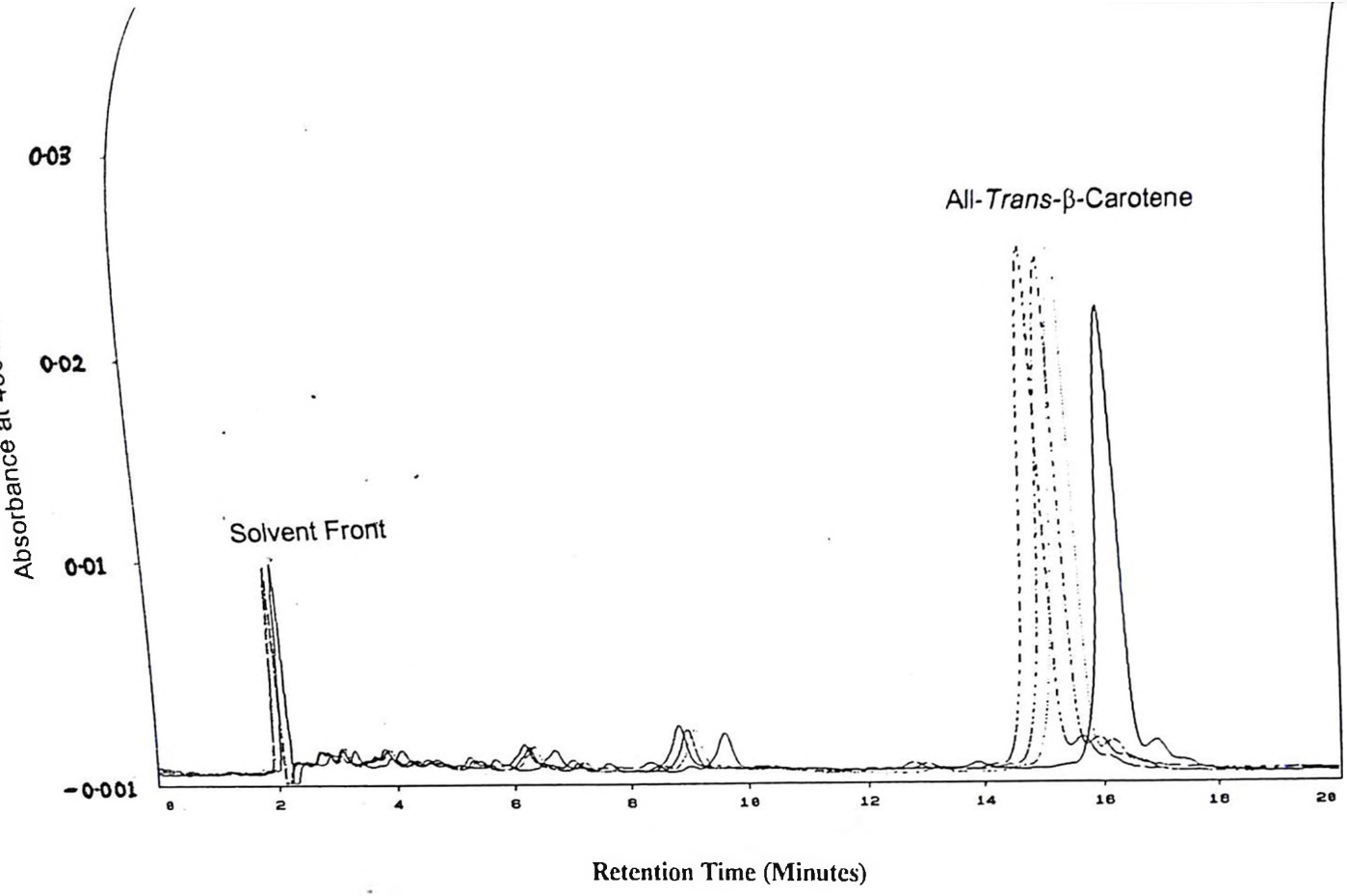

Figure 3.3: HPLC Profile of Preliminary Standard Runs Showing Variation and Stabilization of Elution and Retention Time of β-Carotene Standard (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

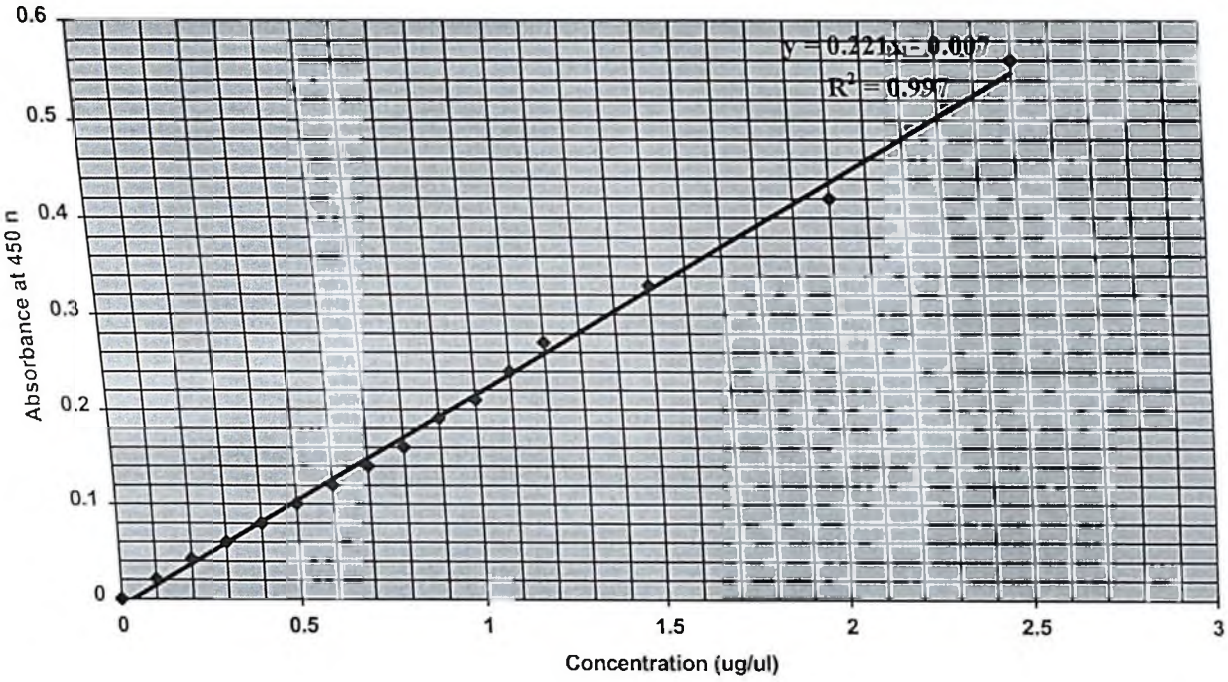

Figure 3.4: Spectrophotometric β-carotene Standard Curve in Petroleum Spirit 40-60 °C

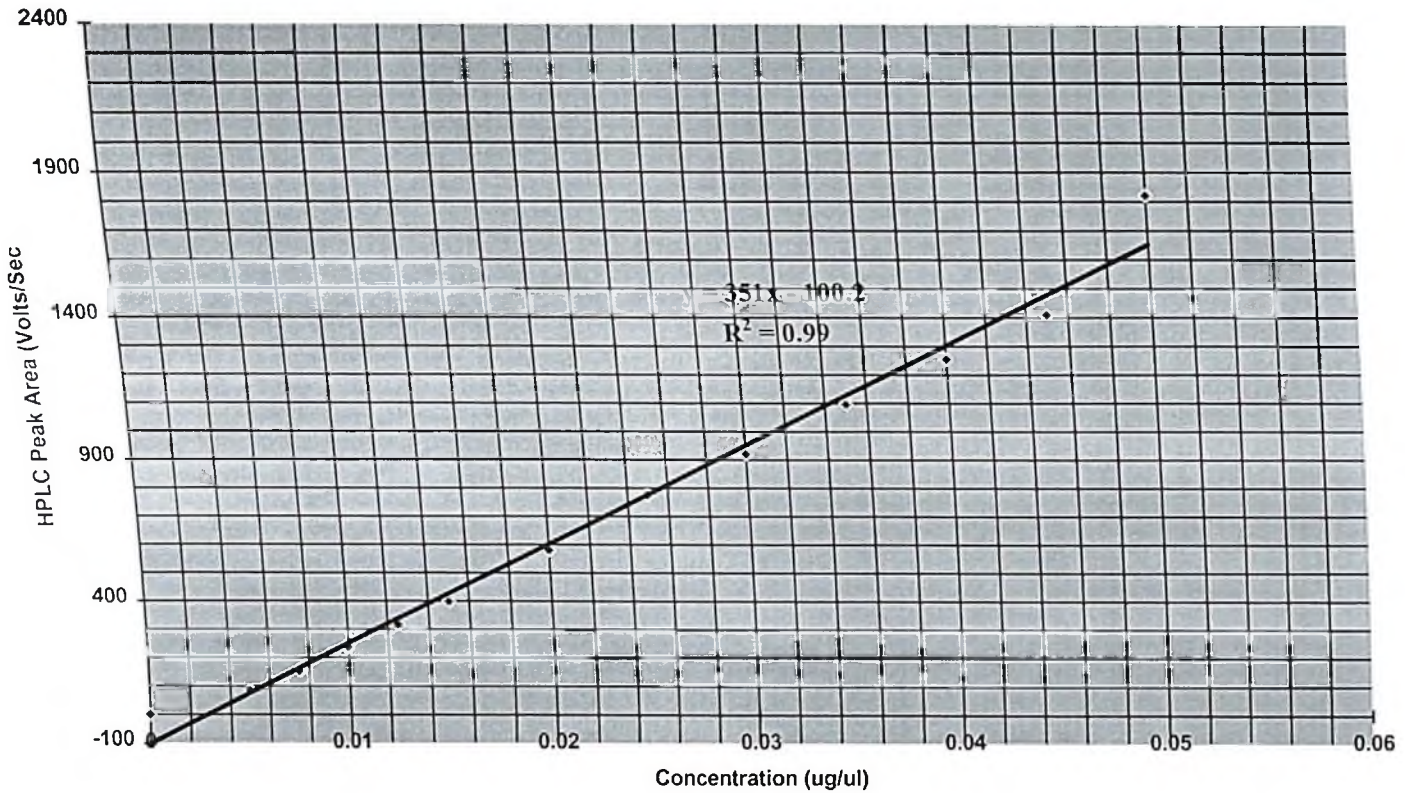

Figure 3.5: β-Carotene HPLC Standard Curve (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm)

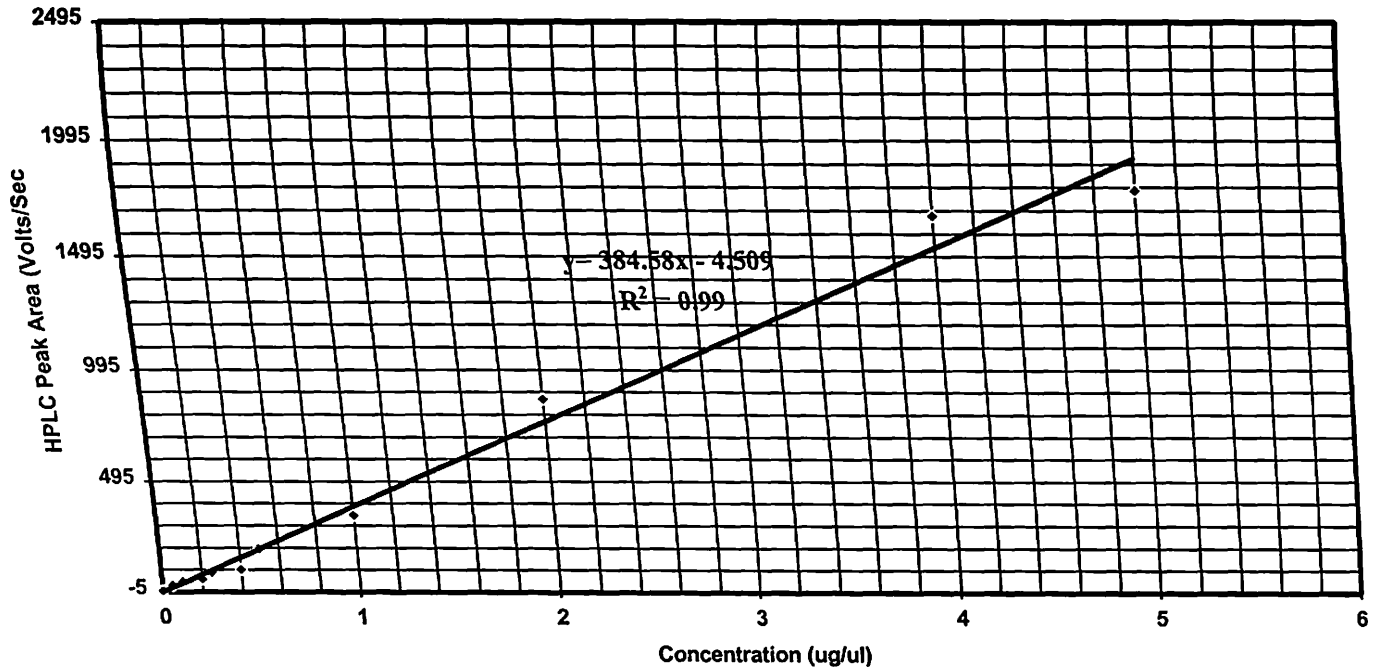

Figure 3.6: HPLC β-Carotene-5,6-Monoepoxide Standard Curve (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

3.2 General Survey

NARP HPLC studies indicated that 5 main carotenoids, represented by 5 HPLC peaks, were present in most cultivars in significant quantities (Figure 3.1 and 3.7). These carotenoids were referred to as P1, P2, I*P3, I*P2 and I*P1. The mean retention times of these carotenoids were 2.69, 3.75, 5.36, 8.61 and 14.60 minutes, respectively (Table 3.2). Three of these carotenoids, namely I*P3, I*P2 and I*P1 were positively identified. The identity of P1 and P2 was not established.

3.2.1 Identification of Carotenoid by NARP HPLC

Identification of carotenoid in sweetpotato extracts was based on consistent HPLC retention times and co-chromatography. The retention times of the sweetpotato carotenoids and pure standards were compared for similarity. Statistical comparison showed that the retention time of I*P3, I*P2 and I*P1 were significantly similar to those of β-Carotene-5,6,5',6'-Diepoxide , β-Carotene-5,6-Monoepoxide and all-*trans*-β-Carotene, respectively (Table 3.2 and 3.3, Appendix 3.1-3.3). It was therefore concluded that I*P3, I*P2 and I*P1 were β-Carotene-5,6,5',6'-Diepoxide , β-Carotene-5,6-Monoepoxide and all-*trans*-β-Carotene, respectively.

Japanese carotenoid extracts and 7 carotenoid standards were used in HPLC co-chromatography. These standards were α-Carotene, β-Carotene, β-Carotene-5,6-Monoepoxide, β-Carotene-5,6,5',6'-Diepoxide, β-Cryptoxanthin, Lycopene and ζ-Carotene. Ten main carotenoid peaks

were observed in co-chromatographic traces (Table 3.4 and Figure 3.8). Given that 7 carotenoid standards were used in co-chromatography and that 5 main carotenoids were present in sweetpotato extracts, 12 HPLC carotenoid peaks were expected in co-chromatography assuming none of the carotenoid standards were represented in the sweetpotato extracts. Because only 10 carotenoid peaks were discerned in co-chromatography, it meant that at least 2, (12 - 10) sweetpotato carotenoids co-eluted with the pure standards. Co-eluting species were considered to be identical. Therefore it was concluded that at least 2 of the 7 carotenoids used as standards were present in sweetpotato extracts.

Further comparison of HPLC profiles of carotenoid standards, sweetpotato extracts and co-chromatography indicated that 3 sweetpotato carotenoid peaks profiled at 5.36 (I*P3), 8.61 (I*P2) and 14.60 minutes (I*P1) matched with co-chromatographic peaks produced by β-carotene-5,6,5',6'-diepoxide, β-carotene-5,6-monoepoxide and all-*trans*-β-carotene, respectively (Figure 3.9). It was therefore concluded that the 3 sweetpotato carotenoids, I*P3, I*P2 and I*PI were β-carotene-5,6,5',6'-diepoxide, β-carotene-5,6-monoepoxide and β-carotene, respectively.

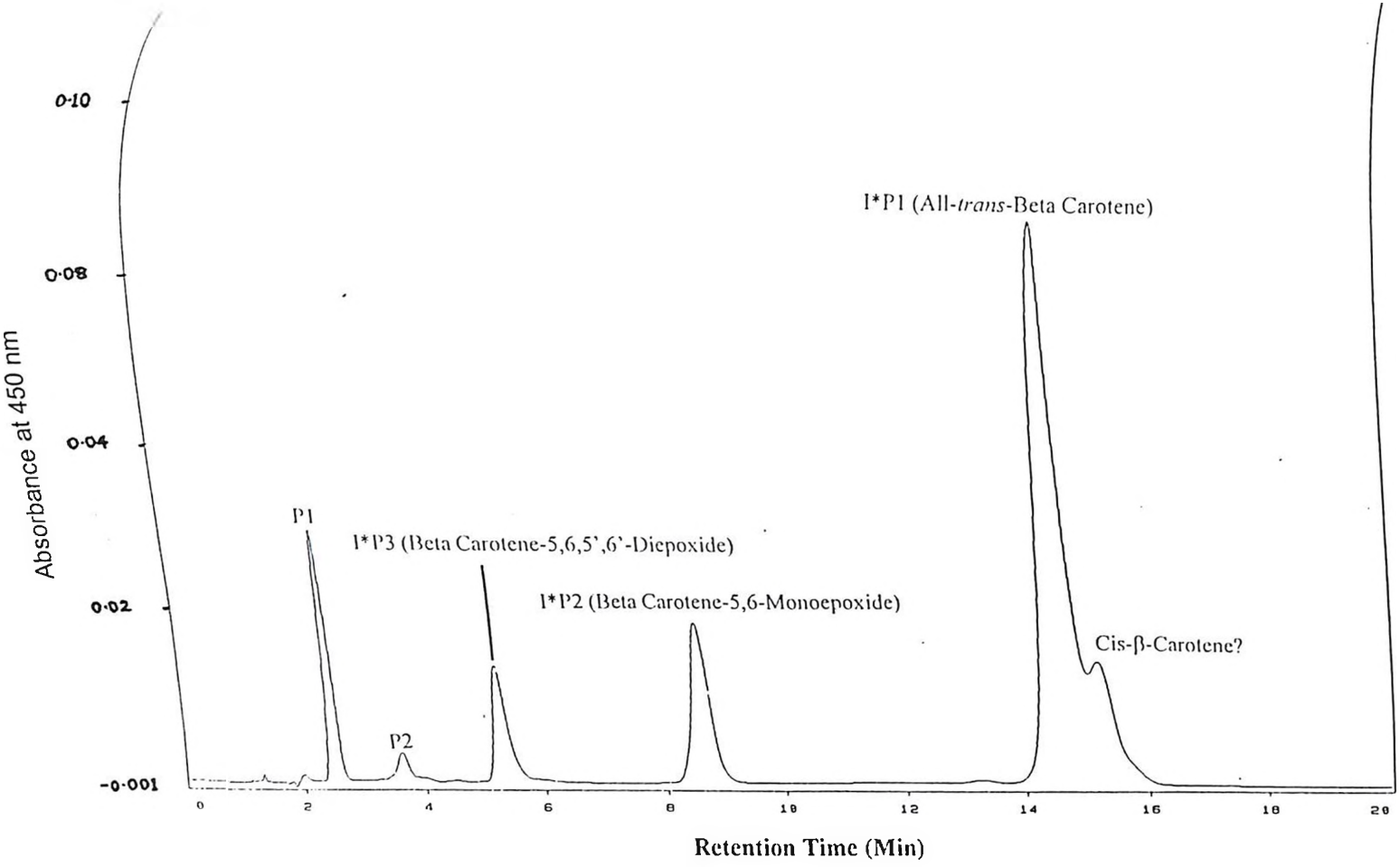

Figure 3.7: HPLC Profile of W-220 Showing Main Sweetpotato Carotenoids (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

Table 3.2: Retention Times of the Main Carotenoids in Sweetpotato Studied

Peak Identity	*HPLC Retention Time in Minutes ± SD (Sample Size)*
P1	2.69 ± 0.10 (28)
P2	3.75 ±0.15 (28)
I*P3	5.36 ± 0.22 (28)
I*P2	8.61 ± 0.40 (28)
I*P1	14.60 ± 0.87 (28)

3.3: HPLC Retention Time of Carotenoid Standards

Carotenoid Standard	*HPLC Retention Time in Minutes ± SD (Sample Size)*
β-carotene5,6,5',6'-diepoxide	5.28 ± 0.05 (10)
β-cryptoxanthin	6.3 ± 0.14 (10)
β-carotene-5,6-monoepoxide	9.19 ± 008 (10)
ζ- Carotene	12.42 ± 0.18 (10)
Lycopene	13.89 ± 0.20 (10)
α-carotene	14.36 ± 0.06 (10)
all *trans*-β-carotene	15.72 ± 0.49 (10)

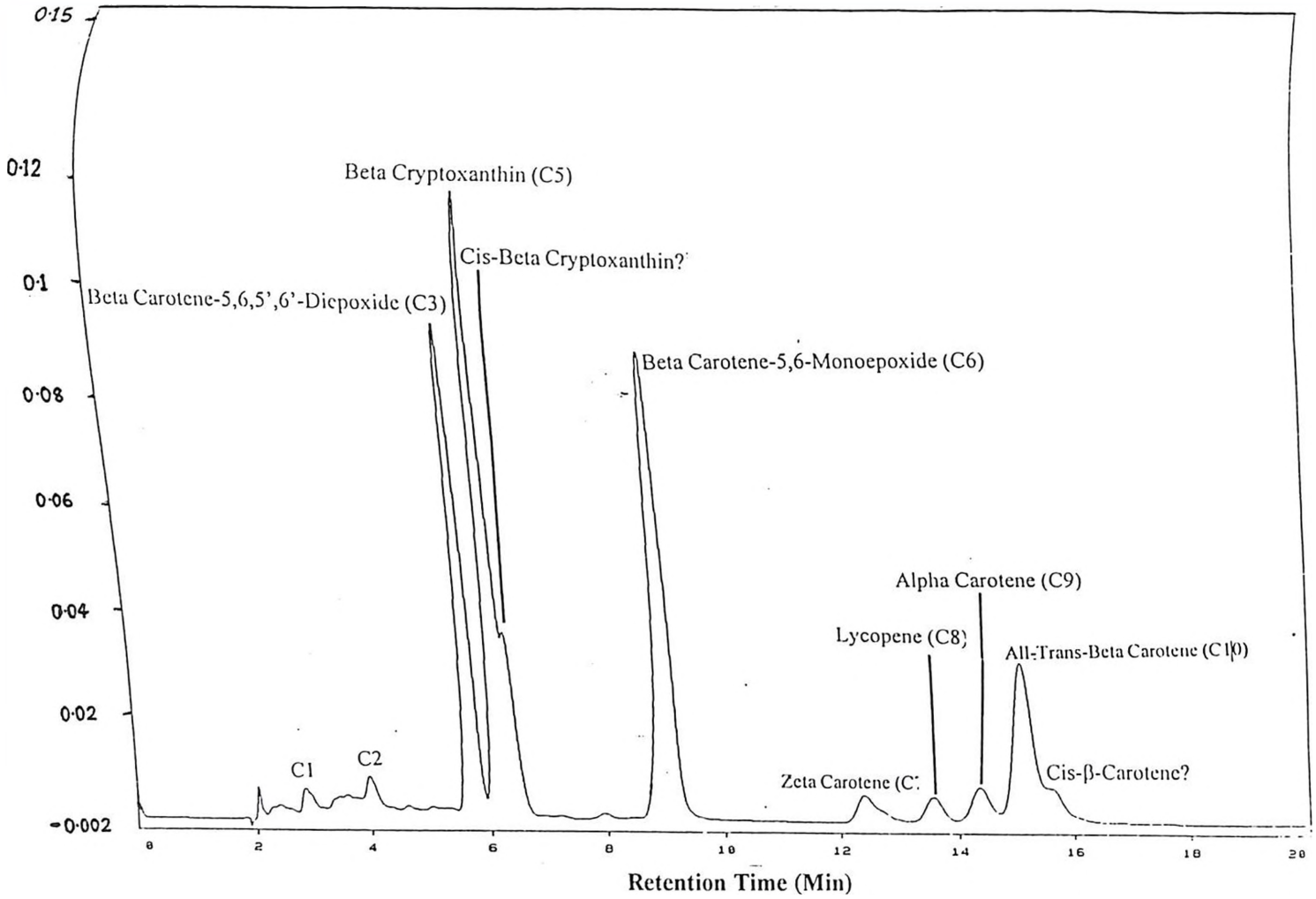

Figure 3.8: HPLC Profile of Co-chromatography of Carotenoid Standards and Japanese Extract (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm)

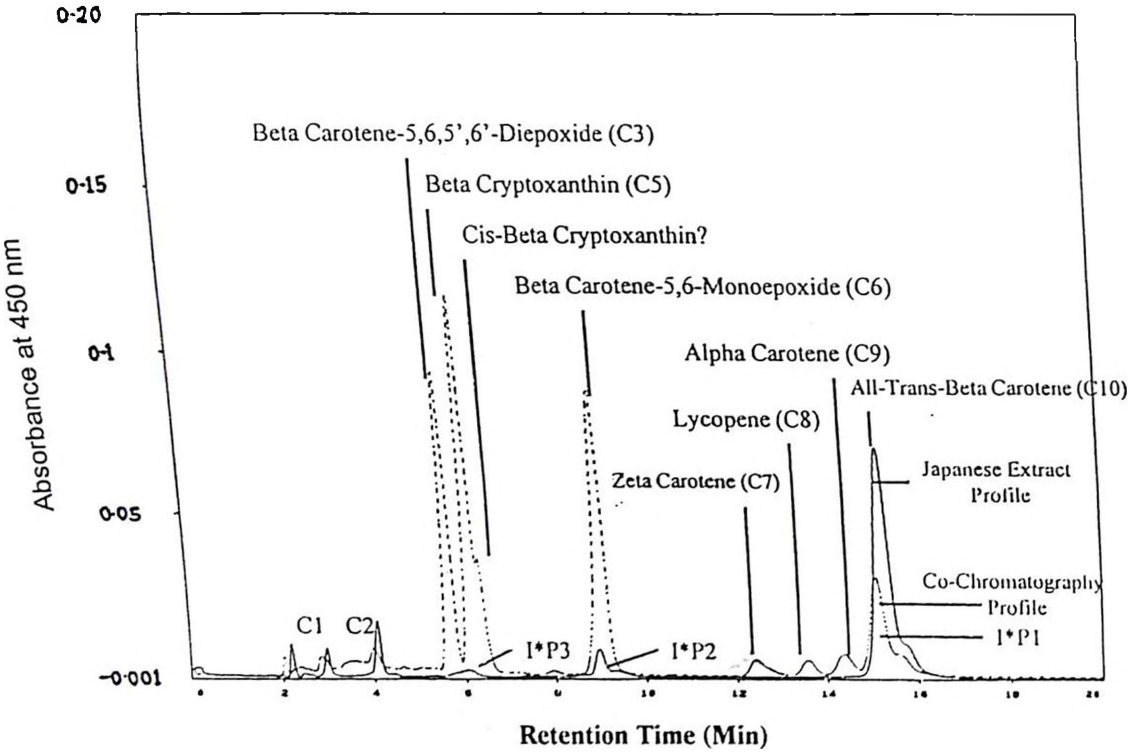

Figure 3.9: Comparison of HPLC Profile of Co-Chromatography and Japanese Extract Runs (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm)

Table 3.4: Retention Time and Identity of HPLC Co-Chromatographic Peaks

Peak Identity	*Retention Time (Minutes ± SD)*
Unknown (C1)	**2.89 ± 0.09 (5)**
Unknown (C2)	**4.05 ± 0.07 (5)**
β-carotene-5,6,5',6'-diepoxide (C3)	**5.83 ± 0.21 (5)**
Unknown (C4)	**6.12 ± 0.05 (5)**
β-cryptoxanthin (C5)	**6.30 ± 0.14 (5)**
β-carotene-5,6-monoepoxide (C6)	**9.00 ± 0.05 (5)**
ζ- Carotene (C7)	**12.57 ± 0.2 (5)**
Lycopene (C8)	**13.68 ± 0.14 (5)**
α-carotene (C9)	**14.43 ± 0.07 (5)**
all-trans-β-carotene (C10)	**15.21 ± 0.08 (5)**

3.2.2 *HPLC Photodiode Array Analyser Data.*

HPLC PDA results showed that an array of carotenoids were present in sweetpotato. HPLC PDA profiled ten main carotenoid species. Of these, only two, PH and PK, were found to possess wavelength of maximum absorbance (λmax) within 400-500 nm range where most coloured carotenoids fall. The λmax of these carotenoid species fall within yellow-orange colour spectrum range. The rest of the eluting species had lambda max of between 190-230nm (Table 3.5). The identities of these carotenoids were not established.

Certain salient distribution pattern of the main carotenoids species profiled by HPLC PDA in the selected cultivars was noted. Some carotenoid species were found to be common in all cultivars studied. These were PA, PC, PE, PF and PI (Table 3.5).

Other carotenoid species profiled were not common. These included PG and PH, which were present in only orange-fleshed Kakamega 4 and Japanese, PK that was found in all the cultivars except TIS 2534, PD and PB. Photodiode array detector's absorbance data suggested that PK is β-carotene or a closely related β-carotene derivative. This carotenoid species had a mean λmax of 455 nm which is within the β-carotene absorbance maximum in acetonitrile (Krinsky *et al.*, 1990). TIS 2534 is a typical white-fleshed cultivar.

The occurrence of other carotenoids did not exhibit any relations to the flesh colour or carotenoid richness. For example, PD was not detected in Japanese and KEMB 10, cultivars

with high and low carotenoid concentrations, respectively (Table 3.5). Species PB was not present in both low TIS 2534 and high carotenoid content Kakamega 4.

3.2.3 *Carotenoid Content of Sweetpotato Cultivars*

Carotenoid content of the sweetpotato cultivar studied indicated that there was a wide variation in total carotenoid content. A range of total carotenoid, β-carotene and β-carotene-5,6-monoepoxide concentrations were quantified among the 17 cultivars. Significant cultivar-dependent variation in total carotenoids and β-carotene was evident among the 17 cultivars investigated (Appendix 3.4 and 3.5). Total carotenoid content was found range from 6.91 µg/100g fwb in white fleshed Naveto to 8823.53 µg/100g in orange fleshed TIB 11 while β-carotene and β-carotene-5,6-monoepoxide varied from 0.02 - 7983.91 µg/100g and 0 - 208.94 µg/100g, respectively (Table 3.6). Large intra-cultivar variation in carotenoid content was noted in carotenoid rich cultivars like TIB 11, Japanese, and Kokei No.14? and W 220. The standard deviation was as high as 1371.66 µg/100g in Japanese.

Some general trends in carotenoid content were noted. Yellow- and orange-fleshed cultivars recorded higher total carotenoid, β-carotene and β-carotene-5,6-monoepoxide content than white and cream-fleshed cultivars. Orange fleshed cultivars had higher percentage of β-carotene to total carotenoids than white- and cream-fleshed cultivars (Table 3.6). Orange fleshed TIB 11 recorded as high as 90.84% β-carotene as compared to 0.14% in white fleshed Naveto.

Table 3.5: Mean λmax (nm) of the Main Carotenoids Profiled by HPLC PDA in 82.4% Acetonitrile: 11.8% Methylene Chloride: 5.9%Methanol

	Mean λmax in nm of Carotenoid Peaks									
Cultivars	PA	PB	PC	PD	PE	PF	PG	PH	PI	PK
TIS 2534	221.5	ND	221.5	116.7	221.5	221.5	ND	ND	221.5	ND
KEMB 10	221.5	221.5	221.5	ND	221.5	221.5	ND	ND	221.5	458.4
Kakamega 4	221.5	ND	ND	226.2	221.5	221.5	221.5	429.4	221.5	453.5
Japanese	221.5	221.5	226.2	ND	221.5	221.5	221.5	429.4	221.5	453.5

ND-Not detected

HPLC data showed that the unidentified carotenoid UN-P1 occurred in higher proportion in white/cream fleshed cultivars like Naveto, TIS 2534, KSP 11, LM88.002 and Ex-Diani than in yellow-orange fleshed cultivars (Figure 3.9 and Table 3.6). β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide and UN-P2 occurred without any consistent trend.

Sweetpotato cultivars studied had a range of provitamin A value that could supply variable vitamin A activity. The cultivars were grouped into 5 broad categories in terms of their provitamin A value (Table 3.7). Pertinent to the groupings was the recommended dietary allowance (RDA) for vitamin A. Recommended dietary allowance for vitamin A is dependent on sex, age group, physical activity, and health status of individuals (Simpson, 1990). For example, pregnant mothers need more vitamin A than un-pregnant mothers as do adults compared to children. The recommended dietary allowance for vitamin A for Infants, 1-10 year olds, 10-12 year olds and adults are 350, 400, 500 and 500-600μg of RE per day respectively (Simpson, 1990). Sweetpotato fresh root weight required to supply the RDA of vitamin A for every age groups were derived from the provitamin A value range of each group (Table 3.7).

Table 3.6: Total Carotenoid, β-Carotene, β-Carotene-5,6-Monoepoxide Content and Vitamin A Value of Some Sweetpotato Cultivars Grown at Kabete, Kenya

Cultivar	Flesh Colour	Total Carotenoid Content (μg/100g fwb ± SD)	β-Carotene Content (μg/100g fwb ± SD)	β-Carotene-5,6-Monoepoxide Content (μg/100g fwb ± SD)	% of β-Carotene to Total Carotenoids (% ± SD	Vitamin A Value (RE/100g fwb ± SD)
Naveto	White	6.91 ± 6.62	0.02 ± 0.01	1.52 ± 0.3	0.14 ± 0.13	0.13 ± 0.03
LM88.002	White	106.84 ± 10.89	5.06 ± 3.48	0.10 ± 0.04	4.48 ± 3.08	0.85 ± 0.58
KSP 11	White	159.63 ± 9.49	19.64 ± 1.51	0.03 ± 0.01	12.46 ± 0	3.28 ± 0.25
TIS 2534	White	138.26 ± 2.18	16.72 ± 1.66	0.06 ± 0.021	12.11 ± 1.18	2.79 ± 0.28
Ex-Diani	White	191.68 ± 24.32	19.14 ± 3.48	0.061 ± 0.05	10.13 ± 2.04	3.20 ± 0.59
Phillippine	Dark cream	158.5 ± 5.47	5.06 ± 1.74	0.27 ± 0.16	3.17 ± 1.09	0.87 ± 0.31
TIS 70357	Cream	248.87 ± 39.19	39.25 ± 6.91	0.20 ± 0.02	15.76 ± 1.01	6.56 ± 1.16
NG 7370	White	207.4 ± 32.66	20.14 ± 4.61	0.06 ± 0.01	9.94 ± 1.89	3.36 ± 0.77
Capadito	Pigmented	245.1 ± 18.85	35.73 ± 6.10	ND	14.95 ± 3.41	5.96 ± 1.02
KEMB 10	Cream	434.89 ± 24.24	125.4 ± 10.59	2.32 ± 0.17	39.6 ± 1.27	21.09 ± 1.76
Maria Angola	Pale orange	390.9 ± 28.38	110.64 ± 9.70	0.48 ± 0.06	28.4 ± 4.24	18.48 ± 1.62
Kakamega 4	Orange	2576.67 ± 152.39	1515.34 ± 140.4	68.04 ± 0.02	58.95 ± 6.69	258.23 ± 23.33
Zapallo	Pale orange	4296.13 ± 4.35	2906.99 ± 474.65	111 ± 19.25	67.67 ± 11.08	493.75 ± 80.17
Japanese	Intermediate orange	5467.57 ± 335.15	4565.11 ± 1371.66	90.21 ± 2.68	82.65 ± 20.68	768.37 ± 228.83
Kokei No.14?	Pale orange	7541.48 ± 718.42	6234.29 ± 92.16	98.48 ± 5.81	83.07 ± 6.42	1047.26 ± 15.81
W-220	Intermediate orange	8389.9 ± 445.76	6023.13 ± 464.85	208.94 ± 56.92	71.73 ± 1.92	1021.26 ± 82.07
TIB 11	Orange	8823.53 ± 654.20	7983.91 ± 339.06	90.96 ± 4.69	90.84 ± 80	1338.23 ± 56.89

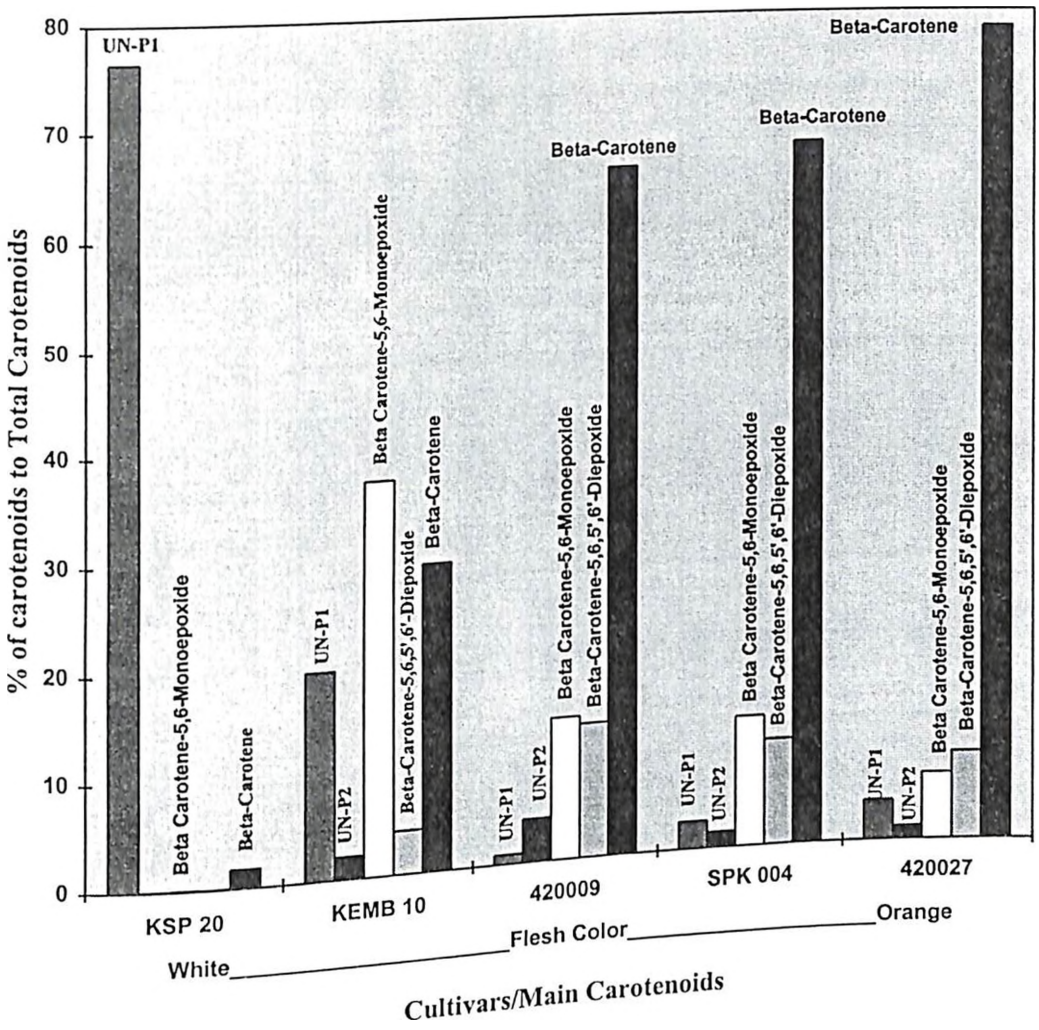

Figure 3.10: Proportion of the Main Carotenoids in Representative Cultivars with a Range of Flesh Colour

Table 3.7: Sweetpotato Fresh Root Weight Specifications of Different Categories of Cultivars Required to Supply Recommended Dietary Allowance of Vitamin A RDA for Different Age Groups

	Category A Less than 10 RE/100g fwb	Category B 10 - >100 RE/100g fwb	Category C 100- > 500 RE/100g fwb	Category D 500 - >1000 RE/100g fwb	Category E over 1000 RE/100g fwb
Age Groups	**Naveto, LM.88.002, KSP 11, KSP 20, Ex Diani, Philippine, TIS 70357, NG 7370, Capadito**	**KEMB 10 Maria Angola**	**Kakamega 4 Zapallo**	**Japanese**	**Kokei No. 14?, W-220, TIB 11**
Infants	**over 3.5 kg**	**350-3500g**	**70-350g**	**35-70g**	**<0 35g**
1-10 Years	**over 4 kg**	**400-4000g**	**80-400g**	**40-80g**	**<0 40g**
10-12 Years	**over 5 kg**	**500-5000g**	**100-500g**	**50-100g**	**<0 50g**
Adults	**over 6 kg**	**600-6000g**	**120-600g**	**60-120g**	**<60g**

3.3 Root Age

The effect of root age on carotenoid content was studied in KEMB 10, Kakamega 4, Zapallo and KSP 11 (Table 3.8). Root age did not have significant effect on the HPLC chromatographic elution pattern (Figure 3.11). The five main carotenoid peaks discerned in the general survey were present at all root ages. There was a general difference in peak area indicative of changing carotenoid concentration with root age.

Monthly analyses of carotenoid content revealed a significant effect of root age (Appendix 3.6). A highly significant cultivar-root age interaction occurred. It was noted that the time taken by cultivars to attain maximum carotenoid content varied. Whereas cultivars like Japanese and Kakamega 4 needed 20 weeks, KEMB 10, TIS 2534 and Zapallo required over 20 weeks for peak carotenoid accumulation (Table 3.8).

The change in carotenoid content with root age was not the same in all the cultivars. Variable percentage change in carotenoid content with root age among the cultivars was observed. It was also noted that except for TIS 2534 and Zapallo, the highest increase in carotenoid content with root age occurred early in root growth between 12 and 16 weeks (Table 3.9). Between this time there was a 61.09, 72.05 and 203.57% increase in carotenoid content in Japanese, KEMB 10 and Kakamega, respectively. In Zapallo and TIS 2534 the highest increment in carotenoid content occurred between 16 to 20 weeks. An anomalous change of carotenoid content with root age was observed in TIS 2534. Between 16 and 20 weeks, there was a 20% drop in carotenoid

content in this cultivar as compared to a steady rise of between 21 to 30% in the other cultivars (Table 3.9).

It was also apparent that the changes in carotenoid content of roots with root age was not related to the quantity of carotenoids in the roots. Whereas Japanese and Kakamega 4 that were relatively rich in total carotenoid with 5467 and 2576µg/100g, respectively, attained maximum carotenoid titre within 20 weeks, Zapallo which is also rich in carotenoids required up to 24 for weeks (Table 3.6 and 3.8). However, both KEMB 10 and TIS 2534 with lower quantities of carotenoids took longer than 20 weeks to attain maximum carotenoid content (Table 3.8). It was clear that 12 weeks old roots contained less carotenoids than older roots for all the cultivars.

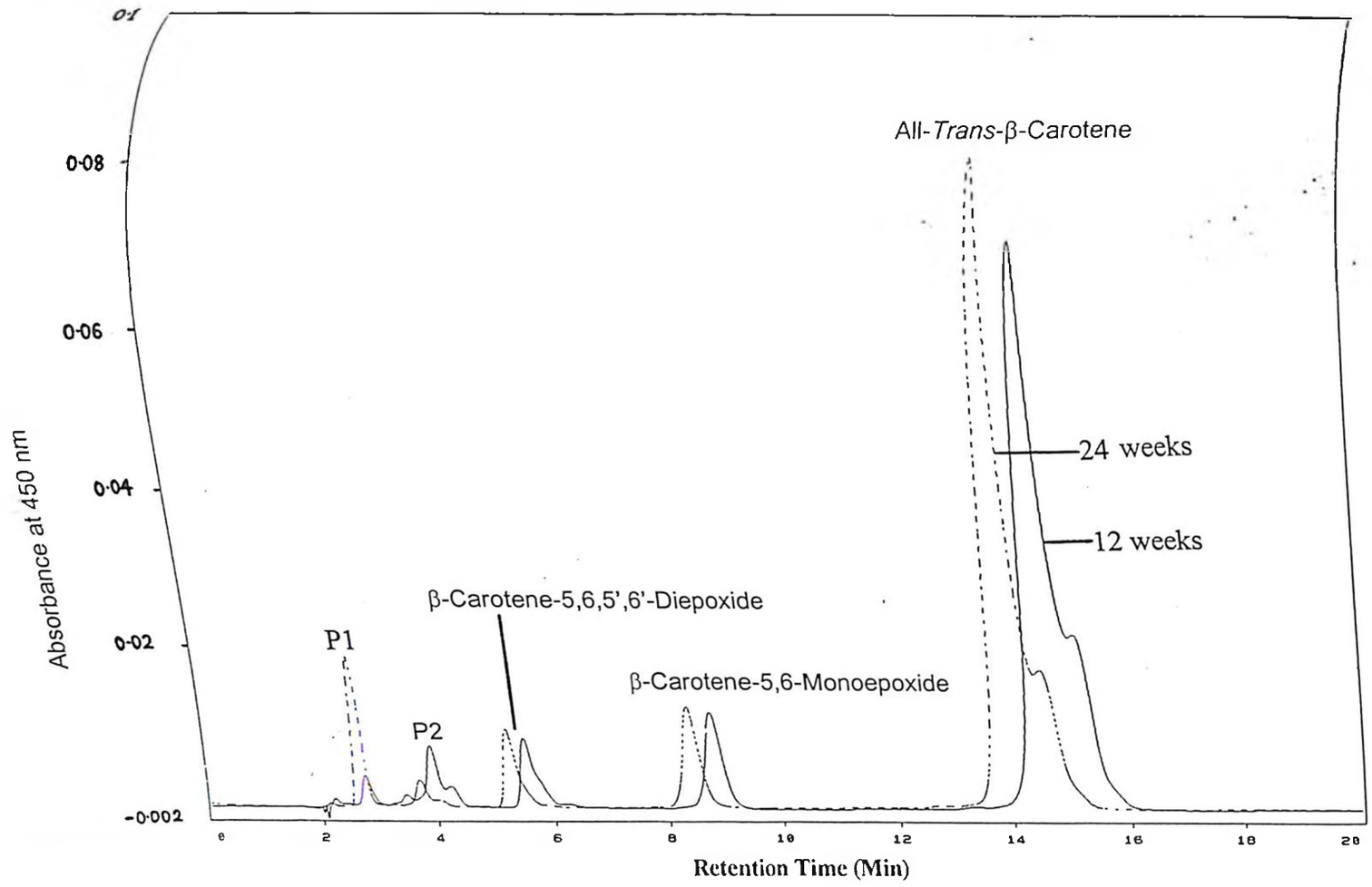

Figure 3.11: HPLC Profile of Carotenoid Extract of Zapallo at 12 and 24 Weeks Showing Main Carotenoids in Young and Mature Sweetpotato Roots (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

Table 3.8: Table of Means of Total Carotenoid Content (μg/100g, fwb) at Different Root Ages of Some Sweetpotato Cultivars Grown at Kabete

	Total Carotenoid Content in μg/100g, fwb			
	Age of Roots in Weeks			
Cultivar	**12**	**16**	**20**	**24**
KEMB 10	434.89 ± 24.24	748.24 ± 39.25	905.35 ± 37.71	1125.19 ± 431.00
Japanese	3394.04 ± 140.21	5467.57 ± 335.15	6913.40 ± 392.47	5166.10 ± 80.54
Kakamega	848.79 ± 161.09	2576.67 ± 152.39	3089.87 ± 380.94	3029.35 ± 76.20
Zapallo	3356.33 ± 65.31	4296.13 ± 4.35	5559.70 ± 130.42	6640.83 ± 614.08
TIS 2534	53.16 ± 13.06	80.0 ± 25.30	63.35 ± 9.33	159.63 ± 9.49

Table 3.9: Percentage Changes in Total Carotenoid Content in Sweetpotato Cultivars at Different Root Ages

	Percentage Change in Total Carotenoid Content (% ± SD, fwb)		
Cultivars	*12 to 16 Weeks*	*16 to 20 Weeks*	*20 to 24 Weeks*
KEMB 10	**72.05 ± 6.13**	21 ± 3.97	24.28 ± 3.31
Japanese	**61.09 ± 7.45**	26.44 ± 2.0	25.27 ± 4.86
Kakamega	**203.57 ± 22.67**	19.92 ± 5.61	-1.96 ± 1.2
Zapallo	**28 ± 3.74**	30 ± 3.04	19.45 ± 1.88
TIS 2534	**50.49 ± 8.93**	-20.81 ± 5.32	151.98 ± 16.89

3.4 Farming site

In this study, 4 cultivars grown at 3 farming sites were investigated (Table 3.10). HPLC profiles of each cultivar across farming site were similar (Figure 3.12). The number and identity of the main carotenoid peaks were identical in all the farming sites. Peak areas were however variable between sites. The variation in peak area was an indication of differences in carotenoid level between farming sites. Analysis of variance in a two factor completely randomized design (CRD) was used to assess these variation in total carotenoids and β-carotene among cultivars with location (Appendix 3.7 and 3.8). The analyses showed that there was a significant effect of farming location on total carotenoids. Calculated $F_{0.05}(2,4)$ was 43.68. This figure was found to be significant at P=0.05. Interaction between cultivar and location was also established. The $F_{0.001}(3,24)$ value was 38.61. This meant that total carotenoid content was dependent on farming site and cultivar. A different trend was found to be true of the relationship between β-carotene content and farming site. Farming site did not exert on effect on β-carotene content (Appendix 3.8). There was no interaction between cultivar and farming location on β-carotene content. Percentage of β-carotene to total carotenoids was also found to be unaffected by farming site.

Some general trends in the variation in total carotenoid content of different cultivars with farming sites were apparent. Generally lower carotenoid content was recorded in Kiboko than Kabete and Kisii. The only exception was the total carotenoid content of TIS 2534 that was found to be higher at Kiboko than Kisii, though lower than at Kabete.

There were cultivar dependent differences in the percentage changes in total carotenoid content among the farming sites studied (Table 3.10). There were also wide differences in the percentage changes in total carotenoid content with farming sites among the cultivars. While relatively low percentage change in total carotenoid content between sites was noted in a cultivars like Japanese, large variation occurred in LM88.002. The least percentage change of 0.57% was between KEMB 10 extracts sampled from Kabete and Kiboko, while the highest was 87.05% in LM88.002 sampled from Kabete and Kiboko.

Of all the cultivars studied for the effect of farming sites, Japanese exhibited the least changes in total carotenoid content from site to site. The highest percentage difference in this cultivar was 40% as compared to 59.82, 75.76 and 87.05% in TIS 2534, KEMB 10 and LM88.002, respectively (Table 3.10). The least and the highest overall mean percentage change in total carotenoid content of 26.58 and 61% was recorded in Japanese and LM88.002, respectively (Table 3.10).

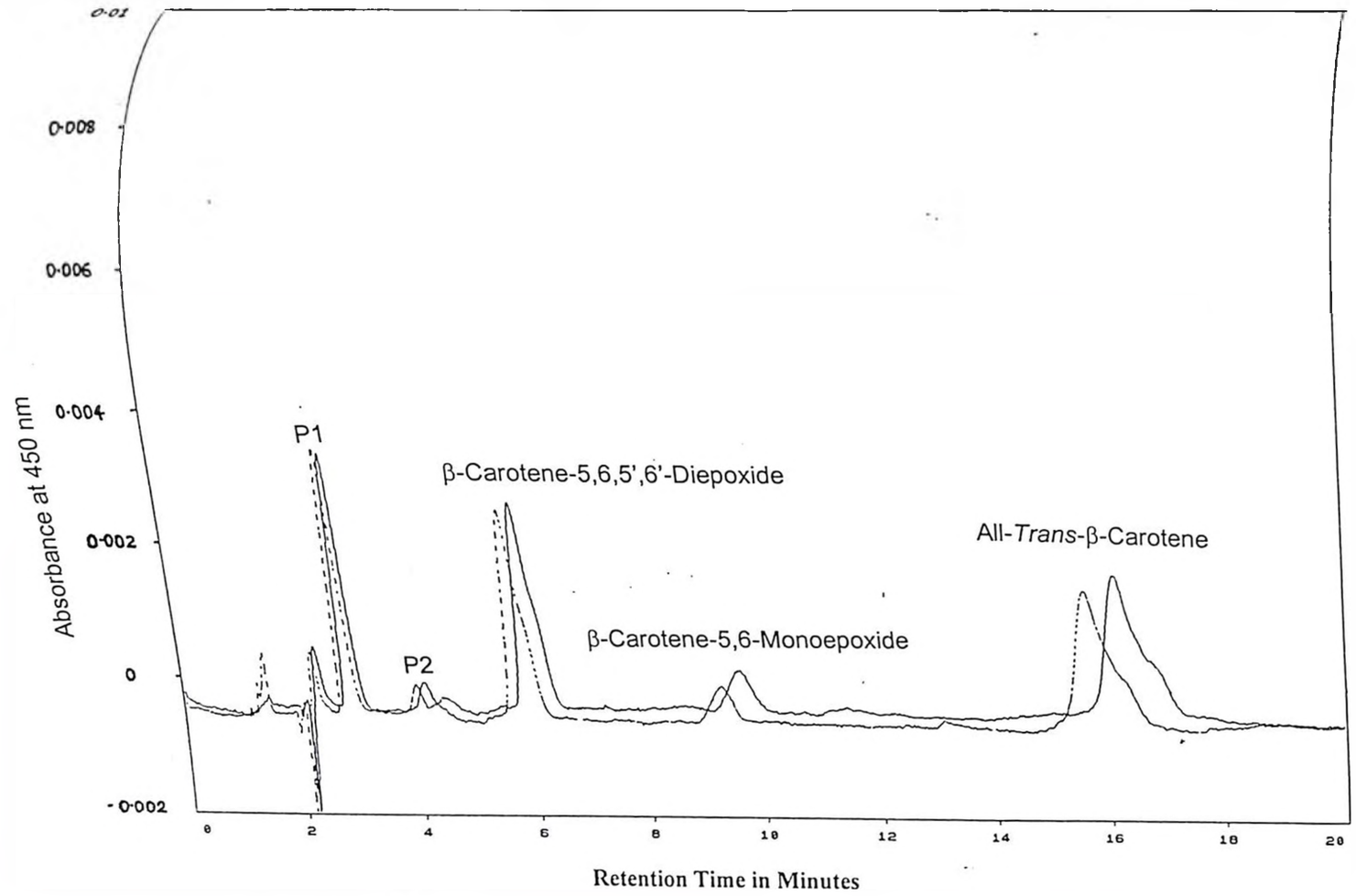

Figure 3.12: HPLC Profile of Main Carotenoids in Extracts of KEMB 10 Grown at Kabete and Kiboko (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

Table 3.10: Table of Means of Total Carotenoid and β-Carotene Content Across Farming Sites

		Carotenoid Content (μg/100g ± SD, fwb)			
		Japanese	***KEMB 10***	***TIS 2534***	***LM88.002***
Kabete	TC	5467.57 ± 335.57	434.90 ± 24.24	138.26 ± 2.18	106.84 ± 10.59
	BC	4565.11 ± 1371.66	98.57 ± 10.59	16.72 ± 1.66	5.06 ± 3.48
Kiboko	TC	5505.28 ± 397.27	248.87 ± 6.53	109.35 ± 42.83	13.83 ± 13.24
	BC	5460.03 ± 377.98	143.82 ± 14.88	4.55 ± 4	2.03 ± 3.49
Kisii	TC	7654.60 ± 284.68	437.41 ± 13.06	67.87 ± 55.55	121.92 ± 15.24

3.5 Boiling

KEMB 10, Kakamega 4 and Zapallo roots were analyzed for changes in carotenoid content after boiling (Table 3.11).. Effect of boiling was assessed on roots subjected to 3 different boiling duration of 30 min, 45 min, and 1 hour. A significant effect of boiling on total carotenoid was given by $F_{0.05}(3,16) = 16.3247$ which was significant at P=0.05 (Appendix 3.9).

There were certain general features noted in the change in total carotenoid content after

Table 3.12: Mean Total Carotenoid Content(μg/100g fwb) of Selected Sweetpotato Cultivars at Different Boiling Regimes

Cultivars	Mean Total Carotenoid Content (μg/100g fwb ± SD)			
	Raw	*Boil 30 Min.*	*Boil 45 Min.*	*Boil 1 Hr.*
KEMB 10	876.88 ± 30.98	575.22 ± 63.66	579.94 ± 38.98	372.55 ± 88.38
SPK 004	3063.91 ± 55.61	1244.53 ± 73.75	1711.16 ± 46.47	1734.73 ± 289.33
Zapallo	6850.35 ± 541.07	4803.17 ± 171.01	6325.98 ± 506.63	6438.91 ± 403.80

Table 3.13: Percentage Change in the Total Carotenoid Content of Some Sweetpotato Cultivars After Different Regimes of Boiling.

	Percentage Change in Total Carotenoid Content After Boiling (%±SD)				
Cultivars	Raw/B30	B30/B45	B45/B1Hr	Raw/B45	Raw/IHR
KEMB 10	-34.40 ± 3.84	0.82 ± 0.15	-35.76 ± 2.98	-33.86 ± 4.85	-57.519 ± 7.16
Kakamega 4	-59.38 ± 5.43	37.49 ± 2.69	1.38 ± 0.21	-44.15 ± 3.13	-43.39 ± 5.41
Zapallo	-29.88 ± 7.15	31.70 ± 5.28	1.79 ± 0..8	-7.65 ± 2.17	-6.01 ± 1.93

An apparent minimal increment in total carotenoid content in Kakamega 4 and Zapallo, and a significant reduction in KEMB 10 was noted in roots sampled after 45 minutes and 1 hour of boiling (Table 3.12). Comparison of raw and roots boiled for 45 minutes and 1 hour showed boiling caused a general reduction in total carotenoids in all the cultivars.

HPLC studies also showed that boiling caused a significant loss of β-carotene. Chromatographic profiles of cooked and uncooked samples showed that there was significant degradation of β-carotene after boiling (Figure 3.13). A general difference in the number and identity of carotenoids in raw and cooked roots was evident (Figure 3.13). Notably, cooked samples had a carotenoid species absent in uncooked samples (Appendix 3.10). This unidentified carotenoid (CB) represented by chromatographic Peak CB had a mean retention time of 3.03 minutes. Cooking was associated with very high level of CB. The relative amount of this carotenoid to total carotenoids was as high as 94.834% in cultivar Zapallo. Different boiling regimes had a consistent effect on the content of this carotenoid. Cooking of up to 45 min resulted in an increase in CB content. The proportion of this carotenoid increased with duration of boiling from 30 min to 45 min. However, its proportion and peak area was lower after 1 hour of cooking (Appendix 3.11)

There was poor HPLC peak separation of extracts from cooked samples (Figure 3.13). These results should therefore be treated with caution. Comparison of the results from spectrophotometric and HPLC methods used for quantification of carotenoids in this investigation, proved that there were no significant differences in data generated by these two techniques (Appendix 3.11). Completely different results noted in boiled samples calls for careful evaluation.

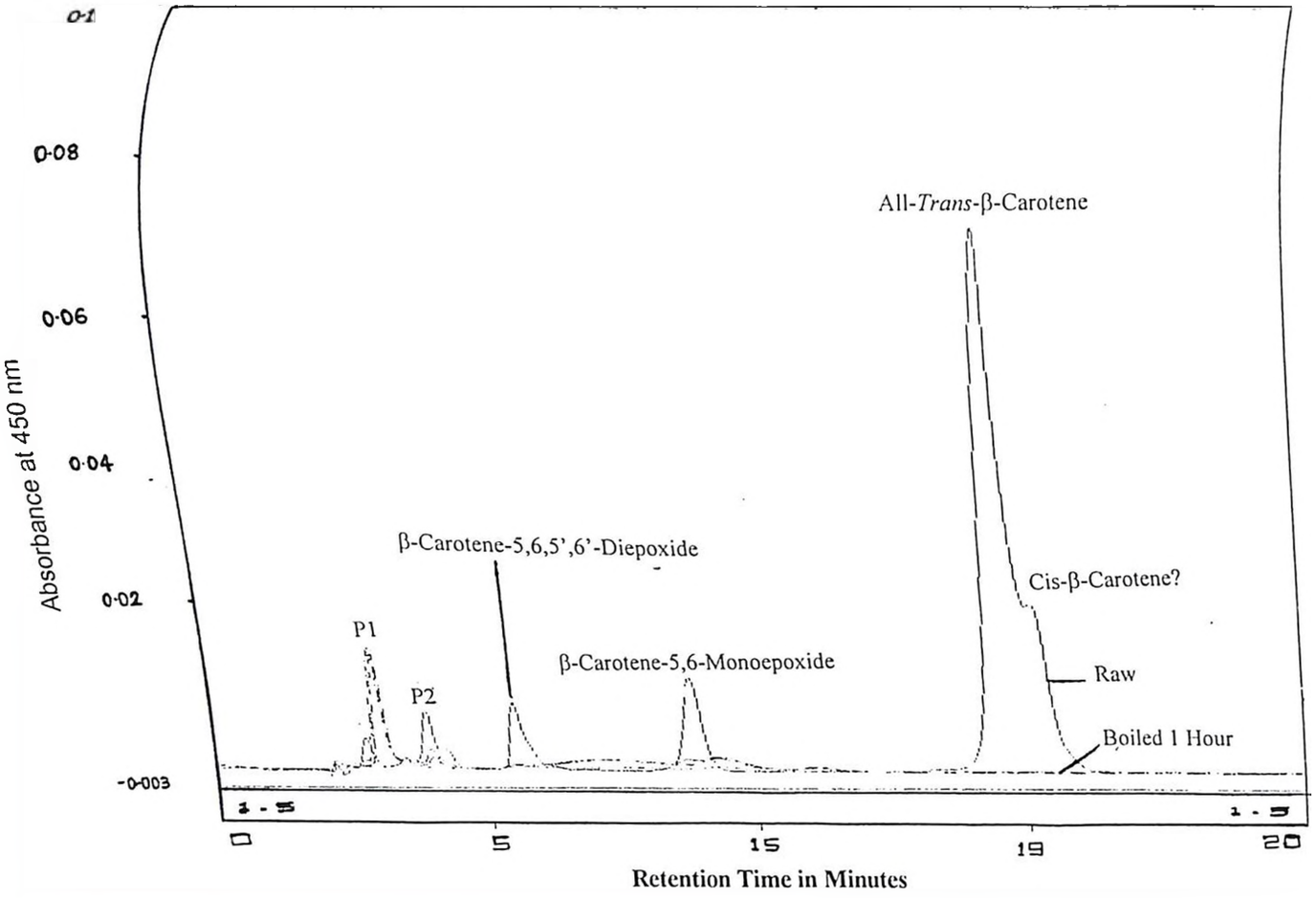

Figure 3.13: HPLC Profile of Boiled and Raw Zapallo (Mobile Phase-90%MeOH:10%THF, Flow Rate 1.5ml/min, Detection λ-450 nm).

CHAPTER 4

DISCUSSION

4.1. Techniques

HPLC standardization runs with β-carotene indicated that there were variations in retention times from run to run (Figure 3.3). Variation in retention time was also noted during carotenoids identification and sweetpotato samples run. The variations in retention times were attributed to one or a combination of the following factors:

a) Asynchronous injection-recorder signals: This was because the injection was manual. Any delay in initiating HPLC recorder after injecting the samples resulted in lower retention times. More precise results were obtained with sharp reflexes in synchronising sample injection and recorder signals.

b) Mechanical variation in HPLC system performance: These variations could be in the

(column) and the mobile phase. Any change in the characteristics of these phases would affect, among other things, retention times, absorbance (resulting in different peak areas and height), and the success of separation of analytes. Small errors in constituting the mobile phase and changes in the properties of the stationary phase could have therefore caused some degree of variation in the retention times noted.

d) Ambient temperature differences: Ambient temperature affects the performance of HPLC components and the interaction of analytes with the stationary and mobile phases (Craft, 1992). However, the variation in the ambient temperature must be significant for any effect to be apparent. Nevertheless, the effect of temperature could not be discounted.

The effect of the above factors was more pronounced in sweetpotato samples than in carotenoid standards runs (Table 3.2 and 3.3). This was because of, a) sweetpotato sample runs were carried out over a long period while standard runs were usually done in a single day, and b) larger samples were involved in sweetpotato than in carotenoid standard analyses. These explained the difference in the variance of the retention time of carotenoids in sweetpotato and carotenoid standards (Appendix 3.1 and 3.2).

The variation of retention times explained above caused the mismatch of chromatographic profiles of sweetpotato extracts used for the study of root age, farming site and cooking (Figure 3.11, 3.12 and 3.13). The similarities of the various carotenoid peaks were apparent, the mismatches notwithstanding. The similarity could be judged from the position (retention time) of the solvent front. Ideally, the retention time of the solvent should always be constant. The

similarity of the peaks would emerge if adjustments are made to compensate for the shifts of the solvent fronts.

In spite of all these, inferences made from retention time data on the identities of all-*trans*-β-carotene, β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide were conclusive (Appendix 3.1-3.3). The mean retention times of these carotenoids in sweetpotato extracts and pure standards were consistent (Table 3.2 and 3.3).

Co-chromatographic identification of all-*trans*-β-carotene, β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide was more conclusive. Key to identification by co-chromatography is the matching of chromatographic peaks of carotenoids suspected to be identical (Figure 3.8). All-*trans*-β-carotene, β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide in sweetpotato extracts and carotenoid standards mixture eluted as single compounds. Comparison of the chromatographic profiles of Japanese extract and co-chromatography ran on the same day produced conclusive matching of the all-*trans*-β-carotene, β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide peaks (Figure 3.9). Given the variations in retention times noted, the matching of the peaks was a significant proof of the identities of these carotenoids. However, more conclusive proof could be given by further analyses of HPLC carotenoids fractions. Characterization of carotenoids can be done by ultra violet-visible light absorption spectra (UV-VIS), infrared spectra (IR), mass spectra (MS), ^{1}H nuclear magnetic resonance spectra, circular dichroism spectra (CD), chemical analyses such as acylation, trimethylsilylation, methylation of allylic hydroxyl groups, reduction with $NaBH_4$ in the case of ketocarotenoids (Matsuno, 1992).

Some degree of degradation of carotenoids during the analytical procedures was suspected. HPLC run of supposedly pure β-carotene and β-cryptoxanthin samples suggested these standards were not composed of single compounds. Of particular interest were 'shoulders' that eluted immediately after these compounds (Figure 3.3 and 3.8). The trailing 'shoulders' could have been *cis* isomers of the carotenoids. This supposition was based on literature data especially on elution pattern and separation of *trans* and *cis* isomers by NARP HPLC (Craft, 1992; Khachik *et al.*, 1992). One of the weaknesses of NARP HPLC is its inability to effectively separate *cis* and *trans* isomers. *Trans* isomers usually elute before cis isomers, the latter eluting as a 'shoulder' of the former. *Trans-cis* isomerization could have occurred during transport, storage or analysis. It was likely that most of the isomerization occurred during analysis because the 'shoulders' was more pronounced in sweetpotato samples than in β-carotene standards (Figure 3.3 and 3.7). The 'noisy' baseline of β-carotene standard profile was indicative of a possible oxidation. *Trans-cis* isomerization and oxidation are promoted by heat treatment, light and acids (Fennema, 1996). Exposures of analytes to light and heat were inevitable, especially during Rotavapor® drying. To minimize exposure to degradation agents, Rotavapor® drying should be replaced with freeze-drying and carotenoid analyses should be done under subdued light.

4.2. General Survey.

HPLC PDA studies revealed that up to 10 carotenoids were present in the cultivars studied (Table 3.5). This suggested that quite an array of carotenoids occur in sweetpotato roots. It was also clear that a significant number of carotenoids occurred only trace amounts (Figure 3.7).

The presence of such an array of carotenoids in sweetpotato could be explained by the fact that plants are able to synthesise carotenoids *de novo* (Goodwin, 1993; Pfander, 1992). The carotenoid composition of plants is therefore enriched by the presence of minor or trace amounts of biosynthetic precursors along with derivatives of the main components (Krinsky, *et al.*, 1990).

Five main carotenoids were observed to predominate in the roots of the sweetpotato cultivars studied. Three of these carotenoids were positively identified. These were all-*trans*-β-carotene, β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide (Figure 3.7 and 3.10). This finding concurred with the observation that β-carotene and related carotenoids are preponderant in carotenogenic tissues like sweetpotato roots (Krinsky *et al.*, 1990,; Purcell and Walter, 1968). All suggested that quite an array of carotenoids occur in sweetpotato roots. It was also clear that a significant number of carotenoids occurred only in trace amounts (Figure 3.7). The presence of such an array of carotenoids in sweetpotato could be explained by the fact that plants are able to synthesise carotenoids *de novo* (Goodwin, 1993; Pfander, 1992). The carotenoid composition of plants is therefore enriched by the presence of minor or trace amounts of biosynthetic precursors along with derivatives of the main components (Krinsky, *et al.*, 1990). All-*trans*-β-carotene, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide are among a number of carotenoids that have been identified in sweetpotato. Other carotenoids so far isolated in sweetpotato include 15,15'-cis-β-carotene, 13-cis-β-carotene, 9-cis-β-carotene, neo-β-carotene, β-carotene epoxide, β-carotene furanoxide, tetrahydro-β-carotene, α-carotene, γ-carotene, ζ-carotene, hydroxy-ζ-carotene, β-ζ-carotene, neurosporene, phytoene, phytofluene, luteochrome, β-zeacarotene and neurosporene and several unspecified xanthophylls (Khachik and Beecher, 1992; Chandler and Schwartz, 1988; Almeida and Penteado, 1988; Martin, 1983; Picha 1985,

Martin, 1983; Purcell, 1962). Unconfirmed reports indicated that β-cryptoxanthin and lycopene were absent in 17 sweetpotato cultivars grown in Kenya. The existence of lutein could not be ruled out because of lack of an authentic standard. It should be noted that there was no report of the two epoxides especially, β-carotene-5,6,5',6'-diepoxide in the work of Tee (1991). This epoxide elute very close to β-cryptoxanthin in NARP HPLC. If Tee (1991) used NARP HPLC (no details of his work was available), then it is probable that his identification of β-cryptoxanthin was flawed. Moreover, no report exist on concurrent occurrence of β-cryptoxanthin and β-carotene-5,6,5',6'-diepoxide (Almeida and Penteado, 1988).

Identification of all-*trans*-β-carotene, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide in this investigation confirms earlier reports of occurrence of these carotenoids in sweetpotato (Ameny and Wilson 1997; Takahata *et al.*; 1993, Woolfe 1992; Almeida *et al.*, 1992; Almeida *et al.*, 1988; Chandler and Schwartz 1988). The occurrence of an unidentified carotenoid, denoted P1, in higher proportion in white/cream fleshed cultivars like KSP 20 and KEMB 10 is of great interest. This carotenoid species formed a significant proportion of the total carotenoids in these cultivars. The possible identities of this unidentified carotenoid could be postulated on basis of its elution pattern and literature data. During NAPR HPLC, xanthophylls being more polar, partition more preferentially in the polar mobile phase and therefore elute earlier than the less polar carotenes (Craft, 1992). Thus the early elution of P1 strongly suggests that it is a xanthophyll, possibly lutein or zeaxanthin (Craft, 1992; Ruddat and Will III, 1985).

Large variation was observed in carotenoid content among the 17 cultivars studied (Table 3.5-3.7). The large variation in carotenoid content was a reflection of the wide spectrum of root

flesh colour of sweetpotato. Root flesh colour ranged from white in NG 7370, to orange in TIB 11. These cultivars also recorded the lowest and the highest total carotenoid content, respectively. This finding was in line with the observation that carotenoids, especially β-carotene, are largely responsible for the yellow-orange sweetpotato flesh colour (Takahata *et al.*, 1993; 1992; Picha, 1985; Garcia *et al.*, 1970,). The depth of yellow-orange flesh colour is mainly a function of the concentration of β-carotene (Simon, 1997; Simmone *et al* 1993,; Kays, 1992). This observation was highlighted by the close relationship between β-carotene content and flesh colour noted among the 17 cultivars studied (Table 3.6). For example, white-fleshed Naveto contained 0.02μg/100g (fwb) while orange-fleshed TIB 11 contained 7983.91μg/100g (fwb) of β-carotene. Flesh colour value (Hunter colour parameters) and β-carotene content correlation could be used to evaluate β-carotene content (Ameny and Wilson, 1997).

The range of carotenoid content among the cultivars fell within the limits of recent studies. Most of the sweetpotato cultivars studied by other investigators contained between 0 - 9000μg/100 (fwb) of total carotenoids (Takahata *et al.*, 1993; Southgate; 1989, Chandler and Schwartz 1988). Some elite sweetpotato hybrids record exceptional carotenoid and vitamin A value, e.g., Arcadian with 3703 RE/100g, while some like early maturing H.8/168 record only 700 RE/100g (MacNair *et al.* 1956). The vitamin A value of orange-fleshed cultivars like Japanese, Kokei No. 14?, W-220 and TIB 11 compare well with the latter.

Large variation in vitamin A content was noted in some cultivars analysed from one site, e.g., Zapallo, Japanese and W-220. These large variations could be attributed to difficulty in assessing vitamin A value of foods. Such difficulty and variation in RE has been appreciated by many researchers, and by Food and Drug Agency (USA) (1997), who noted that vitamin A

quantification was accurate only 54% of the time because of difficulties in developing standard methodologies for analysing it. This could explain why large variations were only noted in carotenoid rich carotenoids. The variation could also be indicative of within-site differences in carotenoid content in cultivars. Such differences have been noted in sweetpotato (Woolfe, 1992). The cause(s) of such variation should be investigated.

The sweetpotato studied were categorized into 5 broad groups in terms of their vitamin A value. From the categories it was evident that a number of cultivars could supply the vitamin A RDA for humans. Orange-fleshed Category E cultivars that included Kokei No. 14?, W-220 and TIB 11 and white-fleshed Category cultivars were the richest and poorest in vitamin A value, respectively. Given that only a limited amount of sweetpotato can be eaten in one sitting, it was clear that only category D and E cultivars could supply the vitamin A RDA in one meal consisting of a maximum 120g (Table 3.7). It was also obvious that category A are not important suppliers of vitamin A RDA as unrealistic quantities of the cultivars have to be eaten to meet the limits. Consumption of over 3.5, 4, 5 and 6kg of sweetpotato per day by infants, 1-10, 10-12 years old and adults, respectively, is not possible. Consumption in one sitting is critical in infants who have limited consumption capacity. For infants category E cultivars should offer the best ingredient for baby food formula. The bracket of cultivars able to supply the vitamin A RDA could be enlarged if the consumption is spread over three to four meals. In such cases, even category C and B could supply vitamin A RDA, in addition to D and E. Absorption and utilization of vitamin A by the body could also be improved if sweetpotato is consumed fried or with a fatty menu. Fats enhance vitamin A uptake into the body (Krinsky *et al.*, 1990).

Quantitative and qualitative variations in carotenoids have been known for some time (Garcia *et al.*, 1970). A concerted effort has been made by sweetpotato breeders in the United States to increase the β-carotene content of new cultivars and lines (Simonne *et al.*, 1993). Progressive up-regulation of terpene synthesis pathway for increased carotenoids has resulted in a concurrent alteration in flavour chemistry that many consumers consider as undesirable. High carotene

Commercial utilization of carotenoid rich cultivars as sources of β-carotene concentrate can be considered. One kg of TIB 11 can yield about 0.08g of β-carotene. To produce 1kg of β-carotene, about 12500kg of TIB 11 i.e. needed. Given that the market prices for stabilized dispersible powders containing 5-10% of β-carotene is about $600, 1kg of 100%β-carotene can fetch about $12000 (Pfander, 1992). At this market rate the value of 1kg of TIB would be worth $0.96 (KSh 62.40). These figures appear economical, but for any entrepreneur to cut-even, processing costs should be settled. The high costs of chemicals, machines and technology of extraction, purification, formulation, packaging and storage may preclude commercial utilization. Cheap synthetic alternatives may also make such endeavour uncompetetive.

4.3 Effect of Root Age

Variation in carotenoid content with root age was noted over the 22 weeks of study. It was apparent that there is significant effect of root age on carotenoid content of sweetpotato. A highly significant cultivar-root age interaction was also established. This interaction was reflected in:

1. Variable time required by roots of different cultivars to attain maximum carotenoid content.
2. Variable percentage changes in carotenoid content with root age noted in different cultivars.
3. High increment in total carotenoid content between 12 and 16 weeks.

The implication of these observations was that carotenoid content and vitamin A value of sweetpotato generally increased with root age. There was for example nearly 3-fold increase in total carotenoid content in Kakamega 4 between 12 and 16 weeks. This early high increment in carotenoid content implied that sweetpotato roots concentrate carotenoids early in growth. The only exception was TIS 2534 in which the highest increase in carotenoids occurred between 20 and 24 weeks. This cultivar also contained the least carotenoids of all the cultivars considered. It would be valuable to do further studies on the possible relationship between the carotenoid content of cultivars and change in carotenoid content with their root age.

It was also evident that older roots contained higher carotenoid concentrations than younger roots. It would therefore be more worthwhile in terms of vitamin A gains to consume older roots. At the same time, the differences in vitamin A value of young and older roots depended on cultivars. The difference was higher in KEMB 10, TIS 2534 and Kakamega 4 than in Japanese and Zapallo. For example, more than two-fold benefit in vitamin A would be gained in consuming 24 rather than 12 weeks old Kakamega 4 and TIS 2534 (Table 3.9). Gains in consuming older KEMB 10, Zapallo and Japanese are lower but nevertheless significant. It should also be pointed out that it is not necessary to wait for 24 weeks to reap the highest vitamin A value when one is consuming Kakamega 4 and Japanese. These cultivars attain peak carotenoid content within 20 weeks.

Sweetpotato is usually harvested piece-meal as need arises (Woolfe, 1992). The effect of root age noted above may not bbe important as only large roots are gathered during piece-meal harvesting. More importantly, cultivars with early, high stable carotenoid production should be sought.

There are conflicting reports on the effects of root age on carotenoid content. While some researchers maintain that time of planting and harvesting of the tuber had no effect on its carotene content (Kimbrough *et al.*, 1946; Abdel-Kader, 1991), others contend that carotenoid concentration changes with root age (Simpson, 1990; Abubakar, 1981; Ezell *et al.*, 1952). Significant variation noted in this study was in agreement with the findings of the latter investigators. A general increment in carotenoid content with root age was reported by Edmond *et al.* (1950) and Abubakar (1981). Ezell *et al.*, (1952) reported variety-dependent differences in the rate of carotene formation. Such variety-dependent differences were manifested in varied time required by cultivars to attain peak carotenoid titre (Ezell et al., 1952).

A number of explanations have been forwarded for a possible association of carotenoid content with root age. Ezell and Wilcox (1958) hypothesised that the final carotenoid content was partially dependent on rate of root growth, the slower growing roots producing the higher carotenoid levels. In light of this hypothesis, more studies should be done to determine whether there is a difference in the rate of root growth, in the cultivars that exhibited different trends of carotenoid changes with root age. For example, studying rate of root growth in cultivars that required 20 and 24 weeks to attain maximum carotenoid content.

A possible explanation of the effect of root age could be drawn from a comprehensive study of the effect of root age on carotenogenesis that was carried in carrots by Bradley and Dyek (1968). They concluded that carotene synthesis shifted towards maximum β-carotene under cooler temperatures. If this could be held true in the case of sweetpotato, then shifts in carotenoid content with root age observed in this investigation could be attributed to pre-harvest temperature variation. Temperature study was not incorporated in this investigation. More detailed studies should therefore be carried out on the relationship of temperature and other environmental factors and carotenoid content.

Variations in carotenoid content with root age have been attributed to changes in the composition of roots during growth. Dry matter content is known to change during root growth (Bouwkamp and Ardle, 1988). Such changes may cause apparent changes in carotenoid content. It is therefore important investigate the changes in the composition of different components of sweetpotato roots during growth. Such a study would establish whether the reported changes in carotenoid content are relative or absolute.

4.4 Farming Site.

There was significant effect of farming site on the concentration of total carotenoids in the sweetpotato studied. Also noted was interaction of the effects of cultivars and farming sites in influencing total carotenoid content. Farming sites did not however affect β-carotene content.

The effects of farming site on total carotenoid content were highlighted in some general trends in the variation in total carotenoid content across farming sites. Generally lower total carotenoid content was observed in roots procured from Kiboko. Also noted were cultivar-dependent differences in percentage change in total carotenoid content from site to site. Some cultivars like TIS 2534 showed large differences in total carotenoid content while others as Japanese exhibited relatively lower differences. The observed difference in total carotenoid content across farming sites was inconsequential in terms of vitamin A value because β-carotene was not affected by farming sites.

HPLC studies showed that there were no significant differences in the number and identity of the main carotenoids found in the same cultivars from different farming sites (Figure 3.12). The main difference noted across farming sites was variation in total carotenoid content (Table 3.10). The variation in total carotenoid content across farming sites implied that there were certain carotenoids other than β-carotene, whose quantities were affected by farming site. These carotenoids must form a significant proportion of the carotenoids for their variation across farming sites to affect total carotenoid content. The NARP HPLC profiles indicated that, other than β-carotene, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide and unidentified carotenoid denoted P1, were the only carotenoids occurring in significant quantities in the sweetpotato studied. Further studies should be done to determine the effect of farming site on all the carotenoid species occurring in sweetpotato.

Significant variation in total carotenoid content observed across farming sites could be attributed to differences in environmental conditions evident among the studied locations. Environmental and biological stresses often limit sweetpotato production (Kays *et al.*, 1992). Different farming

sites presented different sets of environmental factors that might have exerted significant effect on carotenogenesis. Relevant in this investigation were soil moisture, temperature and soil fertility. The sites selected in this study represented contrasting environmental conditions. Notably, Kabete and Kisii were wetter than Kiboko. The effect of soil moisture was studied by Constantin *et al.*, (1974) who used sprinkler irrigation to compare the effect of available soil moisture levels on yield components of sweetpotato. They noted that as moisture levels were increased, there was a significant decrease in % dry matter, in total carotenoid pigments of fresh and processed roots, in firmness of canned roots, and in root protein content. The observation of Constantin *et al.*, (1974) was however not consistent with the findings of this investigation as more wet regions recorded higher total carotenoid content than the drier zones. Lower total carotenoid content was recorded in Kiboko that was drier than Kabete and Kisii. The contradiction of this finding with the works of Constantin *et al.*, (1974) mandates further studies on the relationship of moisture availability and carotenoid content.

Soil types were also noted to differ among the trial sites (Table 4.1). Soil studies show that soil nutrients have variable effect on carotenoid content. In their studies on the effect of varying phosphorus applications, Constantin *et al.*, (1977) showed that P applications (0-74 kg/ha) had no effect on dry matter or carotenoid content. However, K has been noted to improve the nutritional value of sweetpotato by inducing greater carotene accumulation in the root (Greig and Smith, 1961). Differences in carotenoid content across farming site could be attribute to variation of such nutrient that affects carotenogenesis. More thorough research should be done to conclusively establish the effect of various soil nutrients on carotenoid content and vitamin A value of roots.

The effect of farming site on carotenoid content could also be interpreted in the perspective of the limits of plant response and tolerance to environmental stress. There is a wide variation in the effect of environmental stress on plant structure and function. Some plant functions cannot be affected by mild environmental stress. For example, mild water stress of less than -0.5 MPa has little effect on cell biochemistry (Fitter, 1987). But as water stress increases from - 0.5 MPa, cell biochemistry is increasingly disturbed. Moderate to severe stress of below -1.5 MPa is associated with serious disruption of metabolism (Fitter, 1987). The effect of various levels of water stress on carotenogenesis should therefore investigated.

The good news from this investigation was that vitamin A value of sweetpotato roots is not affected by where the cultivation is undertaken, i.e., the same cultivar grown in contrasting environments would provide equal provitamin A activity.

4.5 Boiling Effects.

Boiling reduced the carotenoid content and vitamin A value of sweetpotato. There were certain general trends in the change of carotenoid content after different regimes of boiling. Notably, boiling roots for 30 minutes, 45 minutes and 1 hour resulted in reduction in total carotenoid content relative to the raw roots in all the cultivars studied (Table 3.12). The magnitude however, varied with cultivar. The mean reduction after 1 hour of boiling in cream-fleshed KEMB 10 (-57.52%) was higher than in orange-fleshed Zapallo (-6.01) and Kakamega (-43.39%). It has been noted by that carotenoids in cultivars less rich in carotenoids are more susceptible to degradation than in richer cultivars (Chandler and Schwartz, 1988). The

explanation of this phenomenon has not been established. A study to elucidate the observation and phenomenon would be relevant in predicting the changes in vitamin A value of roots with various concentrations of carotenoids after boiling.

Carotenoid content of roots boiled for 45 minutes was higher than in roots boiled for 30 minutes (B30/B45). The apparent increase in carotenoid content was higher in orange-fleshed Zapallo and Kakamega 4 than in KEMB 10. The apparent increase in carotenoid content in roots boiled for 45 minutes was not consistent with the general reduction in carotenoid content noted after boiling. However, this observation has been reported in a number of investigations (Ameny and Wilson, 1997; Bradbury and Holloway, 1988; Chandler and Schwartz, 1988). The apparent increase in carotenoid content upon heating has been attributed to leaching of solids and enhanced extraction (Bradbury and Holloway, 1988; Reddy and Sistrunk, 1980). Loss of soluble solids such as carbohydrates and mineral salts from the roots into boiling water causes relative increment in fresh weight carotenoid content. Boiling also extricates the hydrophilic matrix of roots and therefore enhances extraction by improving the access of solvents. The enhanced extraction would explain the high increment noted in orange fleshed Zapallo and Kakamega 4. Because these cultivars are richer in carotenoids than KEMB 10, a significant quantity of carotenoids remain in the residues after extraction. This fraction was recovered after boiling for 45 minutes. Boiling for 30 minutes apparently did not improve the recovery of carotenoids.

A different trend was observed after 1 hour of boiling. Apparent increment was only observed in Zapallo and Kakamega 4 after 1 hour as compared to 45 minutes of boiling. Loss in carotenoids was observed in KEMB 10. The loss in KEMB 10 highlighted the observation that

carotenoids in cultivars with low carotenoid content are more susceptible to degradation than those with high (Chandler and Schwartz, 1988). The apparent increment in Zapallo and Kakamega 4 (1.79% and 1.38%) were lower than in B30/B45 (37.49% and 31.70%, respectively). This suggested that the effect of the contributory factor reached asymptote after 45 minutes of boiling.

In spite of the above apparent increments, boiling generally caused a reduction in carotenoid content (Table 3.12). Comparison of raw and boiled roots indicated that up to 57.52% reduction in carotenoid content was noted. This is a significant change in terms of vitamin A value. The reduction noted in cream-fleshed KEMB 10 implied that even after 30 minutes of boiling the vitamin A value of this cultivar would be reduced to insignificant levels equivalent to category A cultivars. Boiling therefore completely discounts KEMB 10 and possibly other low carotenoid content cultivars as suppliers of vitamin A activity. Vitamin A activity could however be gained from carotenoid rich cultivars such as Zapallo and Kakamega 4. Boiling for 1 hour was associated with a 57.52% and 6.01% reduction in carotenoid content in Kakamega 4 and Zapallo, respectively. This translates to vitamin A value of approximately 100 and 450 RE/100g of boiled Kakamega and Zapallo, respectively. This means that after boiling Kakamega 4 would fall under category B, while Zapallo would remain in C. Boiled Kakamega 4 is therefore not a significant contributor to vitamin A nutrition. Less than 600g of boiled Zapallo would still be required to provide vitamin A RDA. Although this quantity is on the higher side for consumption, spreading it over 3 meals would make it manageable. It should be noted that Zapallo that was less affected by boiling contained higher concentration of carotenoids than Kakamega 4 (Table 3.6). It could therefore be generally observed from this

study that boiling of low carotenoid content cultivars make such cultivars insignificant contributors to vitamin A nutrition. Boiling generally reduce vitamin A value of sweetpotato.

Changes in carotenoid content and vitamin a value of cooked sweetpotato have been noted by many investigators. Almeida and Penteado (1988) verified a 26% and 56% loss of beta-carotene and beta-carotene-5,6-monoepoxide, respectively, after boiling roots in water for 10 minutes. In another study, Cascony *et al.* (1988) reported that a Brazillian cultivar containing 9mg/100g (fwb) of total carotenoids lost less than 10% total carotenoids and beta-carotene when place in water already boiling and cooked for 20 minutes. These reports indicated loss of carotenoids but of different magnitude. Cultivars have been noted to respond differently to cooking (Reddy and Sistrunk, 1980). This report was in line with cultivar-dependent changes in carotenoid content established in this study (Table 3.11 and Appendix 3.9).

The reduction in carotenoid content and vitamin A value could be explained by the fact that carotenoid are heat labile compounds and undergo oxidation and degradation upon exposure to heat, light, acids, peroxides, metals, and enzymes. Carotenoids are easily oxidised because of the large number of conjugated double bonds found in these compounds (Krinsky, 1990). The highly unsaturated carotenoid molecule is prone to isomerization and oxidation. Heat treatment, light and acids promote *trans-cis* isomerization. Light, enzymes, metals and co-oxidation with lipid hydroperoxides stimulate oxidative degradation. Carotenoids have different susceptibilities (Krinsky *et al.* 1990). The susceptibility of a particular pigment to oxidation is also highly dependent on its environment. Within tissues, the pigments are often compartmentalized and protected from oxidation. However, physical damage to the tissues increases their susceptibility oxidation. Breakdown is more rapid under high temperature and oxygen partial pressures.

Boiling water or steam has high heat transfer capacity and the surface temperature of the food being cooked reaches the temperature of the water and steam very quickly. The elevated temperature induces carotenoid degradation. The boiling conditions in this study caused carotenoid degradation. The degradation products of the highly conjugated and unsaturated structure of carotenoids are complex (Fennema, 1996). These products are largely uncharacterized, except for β-carotene (Figure 4.1). Because β-carotene formed a significant proportion of total carotenoids in most cultivars, especially yellow-orange fleshed varieties, carotenoid degradation products would consist mainly of β-carotene degradation compounds. Possible products produced as a result of boiling conditions used in this study could have been *cis* isomers, epoxide and lower molecular weight fragments associated with further oxidation. It was unlikely that higher temperature fragmentation products such as ionene, 4-methyl-acetophenone and toluene could have been produced because the temperatures reached were not austere (less than 100 °C).

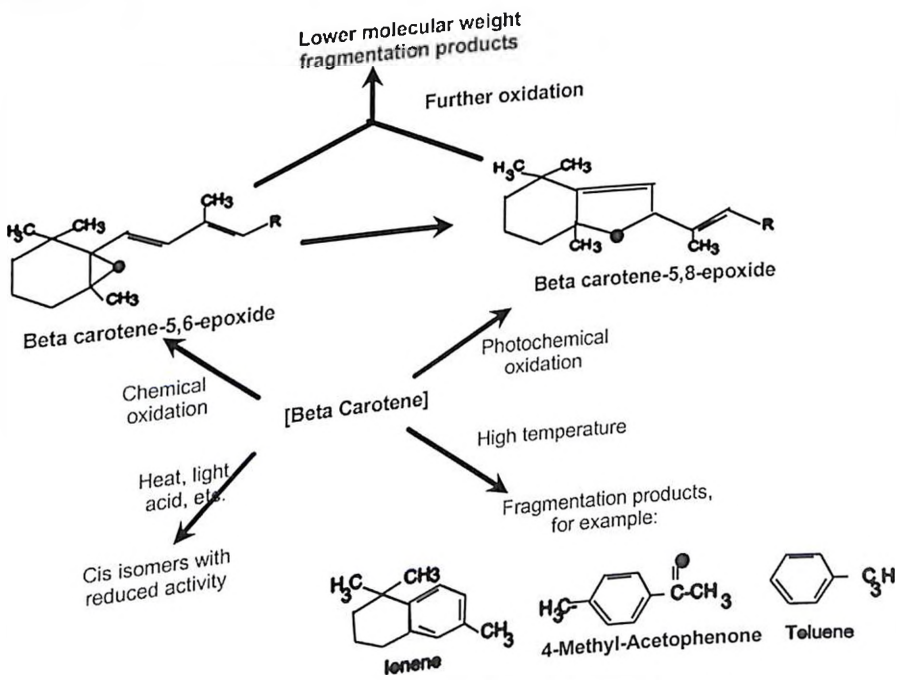

Figure 4.1: Some Degradation Reactions of β-Carotene

A complete analysis of all the compounds involved during boiling is important. More improved analytical methodologies are required for such a study. Variable wavelength (gradient) should be used in preference to fixed wavelength (isocratic) HPLC analyses as it affords a more comprehensive profiling of compounds in a complex mixture (Ruddat and Will III, 1985).

It was also noted that the proportion of main carotenoids varied during cooking (Appendix 3.5). The variation was cultivar-dependent. This can be explained by cultivar-dependent variation in carotenoid composition and differences in thermal stability of various carotenoids (Chen and Chen, 1993). Apart from the 5 main carotenoid common in all the cultivars, there were a number of carotenoids that were not (Table 3.5). It has also been observed that carotenoids differ in susceptibility to thermal degradation (Krinsky *et al.*, 1990). Chen and Chen (1993) found that epoxy-containing carotenoids are more susceptible to heat loss. ζ-carotene, lutein and violaxanthin are cited to be more labile than most other carotenoids. Sweetpotato cultivar-dependent variation in epoxy-containing β-carotene-5,6-monoepoxide and β-carotene-5,6,5',6'-diepoxide might have been reflected in the noted cultivar dependent change in carotenoid content during cooking (Table 3.5 and Figure 3.11).

Thermally mediated *cis*-isomerization may not necessarily lead to complete loss of vitamin A activity of carotenoid. In *vivo* studies with ferret model have shown that 9-*cis*-β-carotene has a good bioavailability and is a precursor of 9-*cis*-retinoic acid, which can be converted to vitamin A (Kays et al., 1992). If such metabolism occurs in human then boiling-associated *cis* isomerization did not cause complete loss of vitamin A value. Studies of the human metabolism of *cis*-isomers would clarify the vitamin A loss incurred as a result of cooking-induced *trans-cis* isomerization.

CHAPTER 5: RECOMMENDATION AND CONCLUSION

5.1 Recommendation

It was revealed that a number of carotenoids are present in sweetpotato roots. The identities of three of these carotenoids, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide and all-*trans*-β-carotene, were established. The identities of P1 and P2, two of the main carotenoids profiled in sweetpotato were not determined. These carotenoids, especially P1, should be identified as this carotenoid constituted a significant proportion of carotenoids in white to cream-fleshed cultivars. It should also be established whether P1 and P2 are natural carotenoid or products of degradation of the carotenoids found in sweetpotato. Detailed identification should also be done of the carotenoid species and other carotenoid products produced after boiling.

Harvesting age for maximum vitamin A activity is cultivar dependent. A detailed study of carotenogenesis in sweetpotato roots should be carried out to establish the what causes the variation in change in carotenoid content with root age. A Possible line of investigation rate of root growth and carotenoid content of roots. It is also of interest to establish the biological importance of carotenoids in sweetpotato roots with a view to understand this control mechanism. Any studies of the effect of root age should include other yield components of sweetpotato roots.

The significant effect of farming site should be investigated further to establish the factors that are responsible for the differences in total carotenoid content across farming sites. In particular responses of different cultivars to environmental factors such as rainfall, temperature, soil, and

altitude, worth studying. The effect of these factors on carotenogenesis should be established. Particular emphasis should be placed on the effect of, e.g. cell water status on cell biochemistry. Farming site had but did not have a significant effect on total carotenoid and β-carotene content, respectively. Further studies is needed to explain this observation.

Results from cooking studies is of great significance because even cultivars of high vitamin A value such category E cultivars would be of no dietary value if cooking is associated with significant degradation of β-carotene and other provitamin A carotenoids. HPLC studies implied that boiling was associated with complete degradation of β-carotene, an observation that did not hold as true for spectrophotometric studies. A more comprehensive cooking studies should therefore be undertaken to conclusively assess its effect on provitamin A value of foods. More informative and discriminative methodologies like gradient HPLC and HPLC-PDA analyses with should be used.

A white-cream fleshed cultivars that are preferred by consumers were lower in vitamin A value than yellow-orange cultivars most of which are disliked for their texture and taste. It could be of value to undertake breeding programmes to develop clones of high consumer acceptability and with high provitamin A activity. Further studies of high β-carotene cultivars would be important. This could go a long way in combating vitamin A deficiency.

5.2 CONCLUSION

A number of carotenoids are present in Sweetpotato cultivars grown in Kenya. Up to 10 carotenoids were established to occur in the cultivars studied. Five main carotenoids were noted to occur in significant amounts, the rest in traces. Of these 5, β-carotene-5,6-monoepoxide, β-carotene-5,6,5',6'-diepoxide and all-*trans*-β-carotene were positively identified.

Significant variation in carotenoid content exists among sweetpotato cultivars in Kenya. This variation can be appreciated from the range of flesh colour in different cultivars. Yellow-orange fleshed cultivars are richer in carotenoids and vitamin A value than cream-white cultivars. These cultivars were grouped into categories in terms vitamin A recommended daily allowance (RDA). Three categories encompassing yellow and orange-fleshed cultivars could supply vitamin A RDA. White and cream-fleshed category A and B cultivars were not significant contributors to vitamin A nutrition. Unfortunately, most of the cultivars preferred by farmers and consumers are white-cream fleshed with low provitamin A value.

Root age exerted significant effect on carotenoid content. A highly significant interaction between cultivar and root age. This interaction was manifested in the variable time required by cultivars to achieve peak carotenoid titre. The highest increase in carotenoids occurred between 12 and 16 weeks of growth in most cultivars. For sweetpotato cultivars with high provitamin A activity, effect of root age is of no consequence.

Farming site had no effect on β-Carotene. This means that in terms of nutritional gains farming site is of no importance. However, farming site was noted to have significant effect on total carotenoid content. This is not crucial as the content of main provitamin A carotenoid, β-carotene, was unaffected.

Boiling was generally reduced carotenoid content of sweetpotato. Loss of carotenoids was higher in cultivars with low carotenoid content. Boiling significantly reduced vitamin A KEMB 10 and Kakamega 4 but not Zapallo. Boiling should therefore be minimised for maximum provitamin A gains. Prolonged cooking of sweetpotato and other provitamin A rich products reduce vitamin A value of cooked products.

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Appendices

Appendix 3.1: F and T-Test Confirmation of the Identity of Sweetpotato IP*1Using pure β-Carotene Standard

	Sweetpotato β-Carotene (1)	β-Carotene Standard (2)
Mean Retention Time (Minutes)	14.93	15.72
Variance	1.32	0.24
Standard Deviation	1.15	0.49
F-TEST FOR THE HYPOTHESIS "VARIANCE 1 = VARIANCE 2"		
F Value	5.49	
Numerator Degrees of Freedom	9	
Denominator Degrees of Freedom		
Probability	0.018	
Results: Significant F, Reject the Hypothesis		
T-TEST FOR THE HYPOTHESIS "MEAN 1 = MEAN 2"		
Variance of the difference between the means:	0.1564	
Standard Deviation of the difference:	0.40	
t' Value	1.99	
Effective degrees of freedom:	12	
Probability of t':	0.06	
Result: Non-Significant t - Accept the Hypothesis.		
Confidence limits for the difference of the means (for $\alpha = 0.05$):0.786 ✂ 0.862 (-0.076 - 1.647)		

Appendix 3.2: Comparison of the Retention Times of Sweetpotato Carotenoid (IP*2) and β-Carotene-5,6-monoepoxide.

	Sweetpotato IP*1	β-Carotene-5,6-monoepoxide Standard (2)
Mean Retention Time (Minutes)	8.92	9.19
Variance	0.33	0.01
Standard Deviation	0.58	0.08
F-TEST FOR THE HYPOTHESIS "VARIANCE 1 = VARIANCE 2"		
F Value	49.96	
Numerator Degrees of Freedom	9	
Denominator Degrees of Freedom	9	
Probability	0	
Result: Significant F - Reject the Hypothesis		
T-TEST FOR THE HYPOTHESIS "MEAN 1 = MEAN 2"		
Variance of the difference between the means:	0.03	
Standard Deviation of the difference:	0.18	
t' Value	1.47	
Effective degrees of freedom:	9	
Probability of t':	0.16	
Result: Non-Significant t - Accept the Hypothesis Confidence limits: 270 ± 0.416 (-0.147 -0.686)		

Appendix 3.3: Comparison of the Retention Times of β-Carotene-5,6,5',6'-diepoxide and β-cryptoxanthin Standards.

	β-Carotene-5,6,5',6'-diepoxide (1)	β-cryptoxanthin (2)
Mean Retention Time (Minutes)	**5.28**	**6.3**
Variance	**0.06**	**0.02**
Standard Deviation	**0.24**	**0.14**
F-TEST FOR THE HYPOTHESIS "VARIANCE 1 = VARIANCE 2"		
F Value	**3.01**	
Numerator Degrees of Freedom	9	
Denominator Degrees of Freedom	9	
Probability	0.12	
Result: Non-Significant F - Accept the Hypothesis		
T-TEST FOR THE HYPOTHESIS "MEAN 1 = MEAN 2"		
Pooled s squared	0.04	
Variance of the difference between the means:	0.01	
Standard Deviation of the difference:	0.086	
t' Value	11.93	
Effective degrees of freedom:	18	
Probability of t':	0	
Result: Significant t - Reject the Hypothesis. Confidence limits for the difference of the means (for α=0.05): 1.024 □ 0.180, (0.844 - 1.205)		

Appendix 3.4: ANOVA Table of the Effect of Cultivar on Total Carotenoid Content

	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Between Cultivars	16	508475414.24	31779713.40	420.90**	0
Within Cultivars	34	2567205	75506.03		
Total	50	511042619.23			
Coefficient of variation 11.86%					
Chi-square = 119.616					
Number of Degrees of Freedom = 16					
Approximate significance = 0.000					

Appendix 3.5: ANOVA Table of the Effect of Cultivar on β-Carotene Content

	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
Between Cultivars	16	356898568.602	22306160.538	152.57**	0.00
Within Cultivars	49	70701.039	146197.089		
Total	50	361869269.641			
Coefficient of Variation = 21.96%					
Chi-square = 193.033					
Number of Degrees of Freedom = 16					
Approximate significance = 0.000					

Appendix 3.6: ANOVA Table of the effect of root age on Total Carotenoid Content of Sweetpotato Cultivars

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
2	**Cultivar (A)**	4	264663151.93	66165787.98	1246**	0
4	**Root Age (B)**	3	27307346.45	9102448.82	171.41**	0
6	**A*B**	12	20783938.06	1731994.84	32.62**	0
7	**Error**	40	2124103.12	53102.58		
	Total	59	314878539.56			
Coefficient of Variation: 8.55%						

Appendix 3.7: ANOVA Table of the Effect of Farming site on Total Carotenoid Content of 4 Sweetpotato Cultivars Grown at 3 Farming Sites. .

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
2	Location (A)	2	2602374.97	1301187.49	43.68**	0
4	Cultivar (B)	3	245306938.08	81768979.36	2745.10**	0
6	A*B	6	6899952.63	1149992.10	38.61**	0
7	Error	24	714895.08	29787.30		
	Total	35	255524160.76			
Coefficient of Variation: 10.20%						

Appendix 3.8: ANOVA Table of the Effect of Farming site on β-Carotene Content of 4 Sweetpotato Cultivars Grown at 3 Farming Sites

K Value	Source	Degrees of Freedom	Sum of Squares	Mean Square	F Value	Prob.
2	Farming Site (A)	2	661967.66	330983.82	1.813 ns	0.19
4	Cultivar (B)	3	186040828.2	6201.36	339.59 **	0
6	A*B	6	1952866.48	325477.75	1.78 ns	0.15
7	Error	24	4382729.10	182613.71		
	Total	35	19303891.44			
Coefficient of Variation: 31.52%						

Appendix 3.9: ANOVA Table of the Effect of Boiling on Total Carotenoid Content of 3 Cultivars Grown at Kabete, Kenya.

K value	Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F value	Prob.
2	**Cultivar (A)**	3	17926743	59754244.07	684.04 **	0
4	**Boiling (B)**	3	4278139.12	1426046.37	16.33 **	0
6	**AB**	9	4488515.99	498724	5.71 **	0.0013
7	**Error**	16	1397681.50	87355.09		
Coefficient of Variation: 13.57%						

Appendix 3.10: Mean Peak Areas (mV/sec) and % Main Carotenoid species observed after cooking

Cultivar	Condition	PK.CA (2.60Min)		PK.CB (3.04Min)		PK.CC (4.03Min)		PK.CD (4.80Min)		PK.CE (5.60Min)		PK.CF (8.76Min)	
KEMB 10	Boil 30 Min.	5065	9.82%	10684	20.71%	11573	22.44%	-	-	5385	10.43%	4940	09.58%
	Boil 45 Min.	-	-	146580	82.09%	12136	6.80%	1349	0.76%	-	-	-	-
	Boil 1 Hr.	-	-	109424	79.88%	21559	15.80%	-	-	-	-	-	-
SPK 004	Boil 30 Min.	6811	5.29%	94951	73.74%	20636	16.03%	-	-	-	-	-	-
	Boil 45 Min.	12695	5.06%	207635	82.74%	18229	7.26%	-	-	4899	1.95%	-	-
	Boil 1 Hr.	8891	5.27%	143717	85.23%	7134	4.23%	-	-	1541	0.91%	-	-
Japanese	Boil 30 Min.	-	-	191713	85.41%	14515	6.47%	3100	1.38%	4237	1.89%	5208	2.32%
	Boil 45 Min.	-	-	205870	84.96%	18419	7.60%	8375	3.46%	-	-	-	-
	Boil 1 Hr.	-	-	208445	78.87%	29849	11.29%	2489	0.94%	-	-	19879	7.52%

Appendix 3.11: Test for Difference Between HPLC and Spectrophotometric Carotenoid Content Determination

	Sample 1 Spectrophotometric BC Content	Sample 2 HPLC BC Content
Mean	2596.387	1925.88
Variance	11285997.017	6105502.82
Standard Deviation	3359.467	2470.936
F-TEST FOR THE HYPOTHESIS "VARIANCE 1 = VARIANCE 2"		
F Value	1.84	
Numerator Degrees of Freedom	14	
Denominator degrees of freedom	14	
Probability	0.2625	
Result: Non-Significant F - Accept the Hypothesis		
T-TEST FOR THE HYPOTHESIS "MEAN 1 = MEAN 2"		
Pooled s squared:	8695749.91	
Variance of the difference between the means	1159433.32	
Standard Deviation of the difference:	1076.77	
t Value:	0.6227	
Degrees of freedom:	28	
Probability of t:	0.537	
Result: Non-Significant t - Accept the Hypothesis		
Confidence limits (α=0.05) =670.5 ± 2205.66(-1535.16 - 2876.16)		