EFFECTS OF DEHYDRATION OF DARK-GREEN, LEAFY VEGETABLES ON BIOAVAILABILITY AND BIOCONVERSION OF SERUM BETA-CAROTENE AMONG PRESCHOOL CHILDREN

By

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A Thesis Submitted in Partial Fulfillment for the Requirements of the Award of the Degree of Doctor of Philosophy in the School of Pure and Applied Sciences, Kenyatta University

APRIL 2008

DECLARATION

This is my original work and has not been submitted for the award of degree in any other University

Mildred Pauline Oyugi-Nawiri

Signature _____ Date 28/4/08

The thesis has been submitted for examination and approval through my supervisors

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Signature _____ Date 28/04/08

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Signature _____ Date 28/4/8

Chemistry Department, Kenyatta University

DEDICATION

This work is dedicated to

My children Brolyn, Blaisy and Bailee,

My beloved Husband Festus

My Mother Patricia

and also to the loving memory of

My late Father Emmanuel A. Oyugi

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Its by God's Almighty's Grace that I have completed this study, **PRAISE BE TO HIM!**

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TERMS AND ABBREVIATIONS

Baseline	At the beginning of the 13 weeks of the study period
Control group	Study subjects who received recipes of white cabbage
DGLV's	Dark Green Leafy vegetables
Green group	A combination of the study subjects feeding on DGLV's
Intervention period	13 weeks of the study period
Negative	Subjects without parasitic infected stool
Positive	Subjects with parasitic infected stool
Post-intervention	At the end of the study period
RDA	Recommended daily allowances of 500 RE/day
RE	Retinol equivalents with respect to 10:1 conversion factor
Respondents	Women who filled out a structured questionnaire
S-BC	Serum beta-carotene
S-R	Serum retinol
Study group	Study subjects in the group receiving fresh vegetable or dehydrated vegetable's
Study subjects	Preschool children involved in the study
VAD	Vitamin A Deficiency
White group	A combination of the study subjects feeding on cabbage

Abstract

Vitamin A, an essential micronutrient has its deficiency remaining as a major public health problem in developing countries. The deficiency is caused by insufficient intake of foods rich in vitamin A or pro-vitamin A carotenoids and its prevalence contributes substantially to morbidity and mortality among children. Carotenoids, with β-carotene as the primary pro-vitamin A carotenoid in dark green leafy vegetables (DGLVs), are important for their various biological functions. The DGLVs are season dependent but can be preserved by dehydration to ensure their availability during the dry seasons to reduce cases of the deficiency. The β-carotene content and retinol (vitamin A) in serum of preschool children after consumption of dehydrated cowpeas and amaranthus leaves were quantified. Preschool children (study subjects) were involved in a 13-week intervention period. Extracts from vegetable and serum samples were analysed by High Performance Liquid Chromatography. Elution was performed isocratically with systems of methanol:dichloromethane:water (79:18:3, v:v:v), methanol:dichloromethane:water (83:15:2, v:v:v) and acetonitrile:water (85:15, v:v) for vegetable samples, serum β-carotene (S-BC) and serum retinol (S-R) analysis respectively. Fresh blanched vegetable leaves, contained high levels of the all-*trans* β-carotene; 779-827μg/g DM for cowpeas and 553-639μg/g DM for amaranthus. Although they reduced with dehydration and cooking, retentions for β-carotene were over 50% after dehydration and cooking. Thus, recipes provided sufficient amounts of retinol equivalents (RE)/day from both fresh and sun-dried vegetables to meet the recommended daily allowances for the study subjects. Serum beta-carotene concentrations were found to be within the normal range at baseline and increased significantly post-interventional for both study groups ($p<0.000$, $df = 110$, for fresh vegetable group and $p<0.000$, $df = 38$ for dehydrated vegetable group. There was a negative correlation between the baseline S-BC and change in S-BC for study subjects. However, the increase in S-BC of subjects in the fresh vegetable group was higher as compared to those of the sun-dried group. Although the control group for the fresh vegetable study group had an increase in S-BC, that of the dehydrated group had a decrease, but these changes were not statistically significant. The subjects in the fresh and dehydrated vegetable groups had marginally lower S-R concentrations at baseline but there was significant changes after intervention ($p<0.000$, $df = 110$, for fresh vegetable and $p<0.000$, $df = 38$ for dehydrated group). McNemars chi-square tests showed that at baseline, 55% and 70% of study subjects in the fresh and dehydrated vegetable study groups respectively had low S-R concentrations with the percentages reducing to 36% and 30% respectively post-intervention. Correlation analysis was negative between the baseline S-R and change in S-R for subjects in both the study and control groups. While data from individual subjects support the homeostatic regulation of vitamin A status, this study concludes that, intervention with the dehydrated vegetables improved the bio-availability of beta-carotene and bioconversion of beta-carotene to retinol. The findings contribute to the link between increased consumption of carotenoids from DGLVs and bio-availability of the same.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background and Statement of Problem

Vitamin A has been known as an essential micronutrient for almost 90 years although vitamin A deficiency (VAD) remains a major public health problem in developing countries (Underwood, 1994; Olson, 1999). Other major forms of malnutrition prevalent in developing countries are protein-energy malnutrition, iron deficiency anemia and endemic goiter. The VAD is caused by insufficient intake of foods rich in vitamin A or pro-vitamin A carotenoids to meet the requirements for growth, fighting infections, pregnancy and lactation (WHO/UNICEF, 2003). As a major public health and development problem in developing countries, deficiency of vitamin A, which occurs usually at serum retinol (S-R) concentration of less than or equal to 0.7 μmol/L deserves continuous attention and advocacy at all levels. Its prevalence contributes substantially to morbidity and mortality among young children due to maternal VAD resulting in low concentrations of vitamin A in breast milk, inadequate dietary intake of vitamin A during and after weaning, and prevalent illness (Miller *et al.,* 2002; Tanumihardjo, 2004; Caulfield *et al.,* 2004; WHO/UNICEF, 2003; West, 2002).

The incidence of VAD in children is particularly high in South and Southeast Asia and in Sub-Saharan Africa where it affects more than 100 million children residing in more than 100 countries (WHO/UNICEF, 2003; West, 2002). It is one of the leading causes of blindness especially among the children where more than 250,000 children become blind each year, and half of them die within a year of losing sight (WHO/UNICEF, 2003; FAO/ILSI, 1997). However, about 80% of blindness is avoidable being either preventable through improved vitamin A nutriture or treatable (FAO/ILSI, 1997). Improved vitamin A nutriture is expected to prevent approximately one to two million deaths annually among children aged one to four years (FAO/ILSI, 1997). Additionally, child deaths may be averted if improved vitamin A nutriture can be achieved during the latter half of infancy.

Before blindness occurs, a vitamin A deficient child faces a 25% greater risk of dying from a range of childhood ailments such as measles, malaria or diarrhea (Tanumihardjo *et al.*, 2004; Caufield *et al.*, 2004). Improved vitamin A nutriture therefore enhances the child's' chances of survival, reduces the severity of their illnesses, eases the strain on health systems and hospitals, and contributes to their well-being, their families and communities.

The fight against VAD to the at-risk populations is practiced and supported widely by both international and local organizations (Mejia and Arroyave, 1982; Beaton *et al.*, 1993; WHO, 1996; WHO/UNICEF, 2003). The approach is usually through the distributions of vitamin A supplements, fortifying certain foods commonly used by the at-risk and encouraging programs that increase availability of vitamin A-rich foods (Mulokozi *et al.*, 2004). Food fortification is a central strategy that is being introduced in more and more countries and holds great hope for the future. Oral administration and food fortification though, are expensive means. World Health Organization (WHO) and UNICEF have been actively promoting programs to control VAD through the encouragement of both exclusive and prolonged breastfeeding, the distribution of pharmaceutical vitamin A supplements to children and postpartum mothers, the fortification of food, and the implementation of programs aimed at increasing home gardening so as to increase the availability of fruits and vegetables (WHO/UNICEF, 2003).

The 1990 world summit for children proposed ways that will eventually eliminate VAD and its consequences, including blindness, by the year 2015 (WHO/UNICEF, 2003). The summit suggested that two national rounds of vitamin A supplementation be conducted six months apart with at least 70% coverage among children aged between 6 and 59 months, or carry out an effective nationwide food fortification program to achieve this goal (WHO/UNICEF, 2003). When little progress is made towards ending VAD, organizations, donors and leading technical experts usually stress on the importance of vitamin A supplementation as the only reliable and effective way to combat VAD while emphasizing the potential importance of food fortification (WHO)/UNICEF, 2003). This shift of focus has resulted in a number of developing countries able to provide at least one high dose vitamin A supplement to 70% or more of the under-fives; rising from only 11 nations in 1996, to 27 in 1998 and 43 in 1999 (WHO/UNICEF, 2003).

The UNICEF Global Database on Vitamin A supplementation coverage for Kenya indicated that 80% of children aged 6-59 months received at least one high dose of vitamin A capsule coverage in 1999 (WHO/UNICEF, 2003). It was estimated that by 2015, as many as a million child deaths worldwide may have been prevented as a result of this vitamin A supplementation (WHO/UNICEF, 2003). Despite the international support for supplement distribution, this exercise must be routinely repeated and is therefore costly and requires an appropriate health care infrastructure to ensure consistent broad coverage of the at-risk population.

Other interventions based on better use of local food sources of vitamin A, mainly from green and yellow vegetables and fruit seem more sustainable in the long term (Jalal *et al.,* 1998; Mulokozi *et al.,* 2004; de Pee and West, 1996). It is thus necessary to consider dietary habits and the natural food resources available in a region of a population group in order for prevention and control of VAD to be efficient (Mulokozi *et al.*, 2004). A lot of research is currently going on in this domain of dietary diversification aimed at increasing local production and consumption of carotene containing foods. This will contribute positively to cheaper means of improving bioavailability of beta-carotene and hence the vitamin A status of target groups. Most of the poverty-stricken populations do not afford pre-formed vitamin A rich foods such as meat, liver and eggs as these are expensive. Beta-carotene, the primary pro-vitamin A in fruits and vegetables and a diet rich in them may have an effect similar to a mega-dose of vitamin A supplement in improving vitamin A status thus their consumption can alter the vitamin A status of deficient populations (de Pee and West, 1996; Ribaya-Mercado *et al.*, 2000; Takyi 1999; Jalal *et al.*, 1998; Hussein and El-Tohamy, 1989; de Pee *et al.,* 1998; Reddy and Vijayaraghavan, 1995).

Nutritional surveys in Kenya have reported vitamin A adequacy of below 60% of the minimum daily requirements while the VAD prevalence is about 60.2% (Maundu *et al.*, 1999; Aguayo and Baker, 2005). The human body lacks the ability to synthesize vitamin A, thereby depending on dietary intake to provide adequate levels. Dietary sources of vitamin A include the pro-vitamin A carotenoids from dark green leafy vegetables (DGLVs) and fruits and preformed vitamin A primarily in the form of retinyl esters from animal products. While vitamin A-rich foods such as animal products and fortified

pharmaceutical supplements are available in developed countries, developing countries lack access to these foods and are therefore dependent on access to carotenoid-rich vegetables (Rodriquez-Amaya, 1997).

The availability of fruits and vegetables however, is season dependent, available during the short rain season and scarce during the long dry season when a lot of cases of VAD are reported. Home gardening of vegetables is a strategy that offers a potential for cost effective, long-term sustainability of plant foods, based however on the assumption that vitamin A from plant food sources is absorbed and utilized efficiently in the body (Jalal *et al.,* 1998; Mulokozi *et al.*, 2004). Since vegetables are highly perishable due to their high moisture content preservation ensures their availability during the dry seasons. Vegetables are preserved in various ways, the main ones being canning, freezing, fermentation and dehydration. However, dehydration is the main technique for the preservation of vegetables in developing countries. The dehydration of foodstuffs is accompanied by many changes, which affect their quality. The major changes encountered include microbial changes, enzymatic reactions, and physical (color, nutritive value, texture and flavor) and structural changes (Kordylas, 1990; Nyambaka, 1996). These changes are enhanced by the presence of light and oxygen, and depend on temperature, water activity and time. Among the techniques of processing food, drying and dehydration result in carotenoid losses (Mosha *et al.*, 1997; Omueti *et al.*, 1983; van het hof *et al.*, 1998; Mulokozi *et al.*, 2000; Mulokozi *et al.*, 2004; Nyambaka and Ryley, 2001). This is because dehydration of vegetables breaks down the cellular structure of the vegetables hence making beta-carotene more available for absorption. However, high amounts of pro-vitamin A carotenoids are maintained by solar dehydration as compared to sun drying (Nyambaka and Ryley, 2001; Mulokozi *et al.*, 2000; Mulokozi *et al.*, 2004). Different mechanisms have been proposed to explain the sequence of reactions involved in *trans*-β-carotene oxidative degradation (Marty and Berset, 1990; Kanasuwud and Crouzet, 1990; Handelman *et al.*, 1993).

Carotenoids are important natural pigments whose important role is coloration and various biological functions. They are a class of natural fat-soluble hydrocarbons (carotenes) and their oxygenated derivatives (xanthophylls) found principally in plants, algae, and photosynthetic bacteria, where they play a critical role in the photosynthetic

process. They also occur in some non-photosynthetic bacteria, yeasts, and molds, where they carry out a protective function against damage by light and oxygen (Palozza and Krinsky, 1992; Britton, 1995a; Britton, 1995b). They are responsible for many of the red (peppers and tomatoes), orange (carrots and citrus fruits) and yellow hues of plant leaves, fruits, and flowers, as well as the colors of some birds (flamingoes), insects, fish (salmon), and crustaceans (Pfander, 1992; Britton, 1995a; Britton, 1995b). Although animals appear to be incapable of synthesizing carotenoids, many animals incorporate carotenoids from their diet. In animals, carotenoids can be a source for vitamin A activity (Ong and Tee, 1992; Britton, 1995a).

The link between increased consumption of carotenoid-rich foods and vitamin A status is not clear because though the consumption of vitamin A-rich foods has been shown to increase serum retinol concentrations in children and alleviate signs of VAD, the results of similar studies have raised more questions (Wadhwa *et al.*, 1994; de Pee *et al.*, 1995). The intervention study by de Pee *et al.,* (1995) on lactating women showed that plant sources of beta-carotene were not effective in raising serum vitamin A concentrations compared with an equivalent beta-carotene supplement. The serum retinol concentration did not increase and these findings provided the impetus for the systematic investigation of carotenoid bioavailability (Jalal *et al.,* 1998; Ribaya-Marcado *et al.*, 2000). These studies showed that consumption of diets enriched with plant sources of beta-carotene increased serum retinol concentration in children who were marginally deficient in vitamin A. These and similar studies over the past decade such as Erdman, *et al,* (1988), Roodenburg *et al.*, (2000), Olmedilla *et al.,* (1994), Kostic *et al.*, (1995), Castenmiller and West, (1998), Castenmiller *et al.*, (1999), van het Hof *et al.,* (1999a,b), Boileau *et al.*, (1999), Cser *et al.,* (2004) and Tanumihardjo *et al.,* (2004) have led to the realization that the absorption and conversion of pro-vitamin A carotenoids by the human body are affected by many factors.

The bioavailability and bio-conversion of pro-vitamin A compounds to retinol is challenging in its contribution to improving vitamin A status, particularly in infants, preschool children, and pregnant and lactating women (de Pee and West, 1996). The knowledge on the bioavailability of pro-vitamin A carotenoids from DGLVs is insufficient and therefore the ability of such vegetables to supply daily vitamin A

requirement to the children require investigation. Bioavailability of carotenoids refers to the efficiency of absorption of an ingested compound and its bioactive metabolites for delivery to and for utilization in normal physiologic functions or for storage (Jackson, 1997; West and Eilander, 2001). This definition includes absorption of dietary carotenoids, their transfer from the food matrix to micelles, a process assumed to require initial partitioning into emulsified oil droplets; uptake from micelles by absorptive epithelial cells and; secretion of intact carotenoids or retinyl esters derived from cleavage of pro-vitamin A carotenoids into circulation via chylomicra (West and Eilander, 2001; Harrison and Hussain, 2001; Breithaupt *et al.*, 2002; Borel *et al.*, 1996; Failla, and Chitchumroonchokchai, 2005; Linder, 1991; Borel *et al.*, 2001). Alpha-carotene, beta-carotene and beta-cryptoxanthin are converted to retinol or vitamin A in the intestine and liver by the enzyme, 15-15'-beta-carotenoid dioxygenase. However both the amount of pro-vitamin A absorbed and converted into vitamin A, and other compounds in the body from the diets is largely unknown. The bio-availability of serum beta-carotene (S-BC) and S-R with consumption of sun dried and solar dehydrated indigenous DGLV therefore require investigation.

Progress in food chemistry has always depended on the progress in analysis of nutrients. There is a corpus of accepted techniques for extraction of these compounds from biological samples and for their qualitative and quantitative analysis. Chromatography, initially introduced for separation of plant pigments (including carotenoids), has quickly become indispensable for the separation of carotenoids and vitamin A compounds. The current appreciation of the crucial roles of retinoic acid (all-*trans* and 9-*cis* isomers) and the carotenoids, demand more sensitive and selective methods for analysis of these compounds in biological tissues and cell cultures. The early open-column methods have eventually been superseded by more efficient High Performance Liquid Chromatography (HPLC) techniques, with reversed-phase HPLC being the method of choice for analysis of retinoids and carotenoids in biological tissues.

This study was carried out among preschool children (the study subjects) from Kanzalu sub-location, Kangundo location, Machakos District. The area is in a semi-arid region where settlement and agriculture are limited by the lack of rainfall and of reliable sources of surface water. Machakos District experiences the most devastating drought

and famine events, leading to widespread acute and chronic malnutrition, leaving lasting effects on infants and young children deprived of adequate nutrition (Neumann *et. al.*, 1989-1992; Bwibo and Neumann, 2003; Anyango *et al.*, 1989). During a thirteen-week intervention period (the approximate duration of the typical seasonal decline in the availability of foods rich in pro-vitamin A carotenoids and vitamin A status is therefore in prevalence), study subjects were grouped into two study groups; one group was fed on fresh locally available green vegetables while the other was fed on dehydrated species of the same vegetables. The vegetables were dehydrated by direct sun drying being the most common method used by the local community. The control groups were fed on white cabbage, which has negligible levels of beta-carotene. Ultraviolet-Visible spectroscopy (UV-Vis) and HPLC techniques were used in the laboratory analysis of the all-*trans*-beta-carotene in raw and cooked vegetables, and the all-*trans*-beta-carotene and retinol in serum. The beta-carotene content in solar dried vegetables was also analyzed.

1.2 Hypothesis of the Study

That the bioavailability of beta-carotene and retinol in blood serum increases with consumption of dehydrated dark green leafy vegetables (DGLV).

1.3 Objectives of the Study

1.3.1 General Objective

The purpose of this study was to investigate *in vivo*, the effect of dehydration of indigenous DGLVs on the bio-availability of beta-carotene and its bio-conversion to retinol in serum of preschool children of Kanzalu Location, Machakos District.

1.3.2 Specific objectives

The specific objectives of the study were:-

i) To obtain baseline information on vitamin A deficiency and its awareness, vegetable availability and their consumption by preschool children in the study area.

ii) To determine the levels of beta-carotene in fresh *V.Unguiculata* and *amaranthus* leaves and their beta-carotene retention with dehydration and cooking.

iii) To assess baseline and post-intervention hemoglobin levels and parasitic infection among study subjects.

iv) To assess the post-interventional changes of serum beta-carotene and retinol with consumption of the dehydrated indigenous dark green leafy vegetables.

1.4 Scope and Limitations

This study investigated the beta-carotene content in *V.Unguiculata* and *amaranthus* leaves. The level of beta-carotene and amount of fibrousness in vegetable is influenced by the species variety of the vegetable, soil conditions, the stage of leaf maturity and the period between harvesting and analysis (Hui, 1992; Macrae *et al.,* 1993). Since these vegetables were bought from the market and not purposely grown for this study, these conditions could not be controlled. However, because the objective of the study was mainly concerned with the beta-carotene content from these vegetables, the conditions were not considered important for bioavailability studies and these factors were not likely to influence the results significantly. The interaction of beta-carotene with other carotenoids in these vegetables and their role on the degradation of beta-carotene can also have effect but was considered to be minimal.

The absorption of carotenoids is dependent on vitamin A status while consumption of beta-carotene–rich foods leads to an increase in serum retinol levels (Castenmiller and West, 1998; Jalal *et al.,* 1998; Takyi, 1999; Reddy and Vijayaraghavan, 1995). This study was limited by the fact that the study subjects had other food intakes at home and thus this study could not control these home intakes. However, food diaries were used to monitor intakes of food that would have effect on the intervention recipes.

1.5 Significance of the Study

Arid and semi arid areas have plenty of vegetables during the wet seasons but remain scarce in dry seasons when cases of vitamin A deficiency are prevalent among children. Preservation of these vegetables is necessary so that they can be availed during these dry periods. Sun drying is the popular dehydration method although solar drying is a better method as it retains higher levels of micronutrients.

Consumption of dark green leafy vegetables is known to improve the beta-carotene levels thus reducing vitamin A deficiency. It is important to determine the bio-availability of beta-carotene and its bioconversion to retinol with consumption of dehydrated DGLVs.

CHAPTER TWO

LITERATURE REVIEW

2.0 Introduction

This chapter reviews literature on the chemistry of carotenoids; the conversion of beta-carotene to vitamin A (retinol) and its bio-availability in human serum; the preservation of vegetables, with emphasis on dehydration being the technique used for preparing vegetable samples in this study; the analytical techniques used in analysis of carotenoids and retinoids, and lastly the study area in the context of the district in which it is located in Kenya are included.

2.1 Chemistry of Carotenoids

2.1.1 Carotenoids

Carotenoids are food colorings that are responsible for many of the colors of fruits and vegetables such as red (peppers and tomatoes), orange (carrots and citrus fruits), and some plant leaves, flowers, birds (flamingoes), insects, fish (salmon), and crustaceans (Pfander, 1992). They are the most widespread of all groups of naturally occurring pigments with about 100 million tons produced by nature per year (Britton, 1995a). In plants, they are found in the cells of non-green plastids, in chloroplasts and in chromoplasts. Chloroplasts are found in all photosynthetic tissues especially in leaves and unripe fruits, while chromoplasts are found in flowers, some roots and in some tubers. In the chloroplasts the carotenoids are masked by the green of chlorophyll where they occur in a definite pattern consisting of four major carotenoids, lutein, beta-carotene (β-carotene), violaxanthin and neoxanthin and other minor pigments.

Some 700 different carotenoids are known to occur naturally and new carotenoids continue to be identified (Beecher and Khackik, 1992; Furr *et al.*, 1992; Ong and Tee, 1992; Khachik *et al.*, 1992a; Khachik *et al.*, 1992b; Khachik *et al.*, 1997; Mercadante,

1999; Stahl and Sies, 1994; Stahl *et al.*, 1992). In human serum, about 14 carotenoids have been identified (Khachik *et al.*, 1995; Khachik *et al.*, 1997).

2.1.2 Classification

The chemical structure of carotenoids forms the basis of their identification (Britton, 1995a). They consist of eight isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the center of the molecule so that the two central methyl groups are in a 1, 6-position relationship and the remaining non-terminal methyl groups are in a 1, 5-position relationship. The most characteristic feature of carotenoid structure is the long system of alternating double and single bonds of the polyene chain that forms the central part of the molecule. The carotenoid system may be acyclic or contain different ring systems and may contain variable numbers of conjugated and isolated double bonds (Britton, 1995a). All carotenoids may be formally derived from the basic acyclic $C_{40}H_{56}$ structure, lycopene (Figure 2.1) containing a long central chain of conjugated double bonds, by double bond migration, hydrogenation, dehydrogenation, cyclization, oxidation, methyl migration or any other combination of these processes (Britton, 1995a).

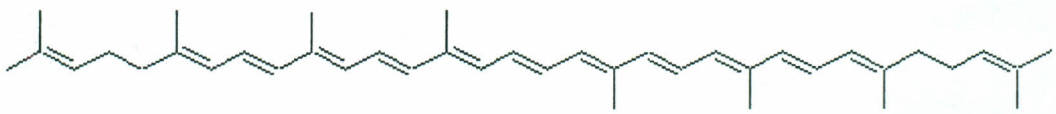


Figure 2.1: Structure of lycopene

Carotenoids with less than 40 carbon atoms are referred to as apo-carotenoids while those with more than 40 carbon atoms are homo-carotenoids. Carotenoids are classified as either hydrocarbon carotenoids or oxy-carotenoids. The hydrocarbon carotenoids are known as carotenes; examples being alpha (α)-carotene and beta (β)-carotene (the principal carotenoids in carrots). The oxygenated derivatives of hydrocarbons with hydroxy,

methoxy, aldehyde, oxo, carboxy, epoxy and ester groups as the main constituents are known as xanthophylls, examples being lutein, zeaxanthin, and β-cryptoxanthin (Gross, 1991; Britton, 1995a). Carotenoids can also be classified as pro-vitamin A or non-provitamin A compounds. Pro-vitamin A carotenoids serve as dietary sources of vitamin A because they can be enzymatically cleaved to yield either one (for example β-cryptoxanthin and α-carotene) or two (for example β-carotene) molecules of vitamin A.

In nomenclature, carotenoids are numbered from the ends to the center, with carbons of the main structure numbered 1 to 15 and the methyl groups, from 16 to 20. The symmetrical part is numbered 1' to 15' with the methyl groups numbered 16' to 20' (Figure 2.2). All specific names are based on the stem name carotene, which corresponds to the structure and numbering in Figure 2.2. Structures and names of important and characteristic carotenoids are given in Figure 2.3.

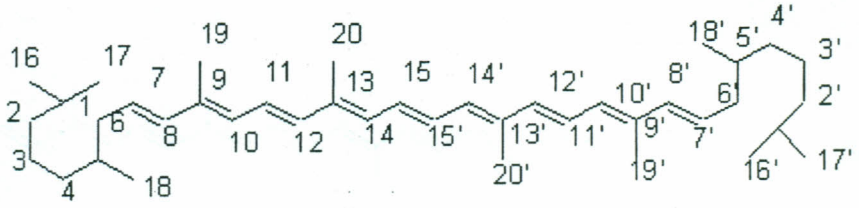
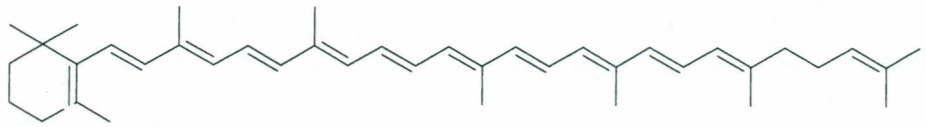
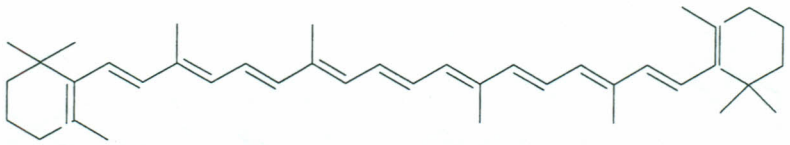


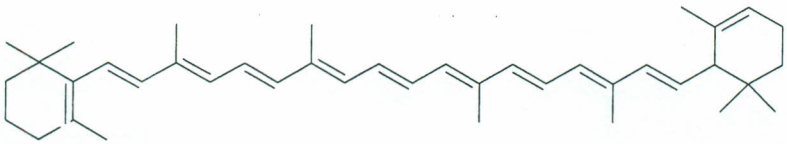
Figure 2.2: Numbering of the carotene stem



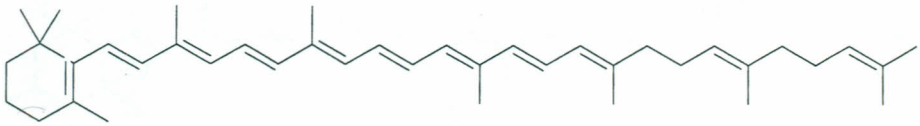
gamma-carotene



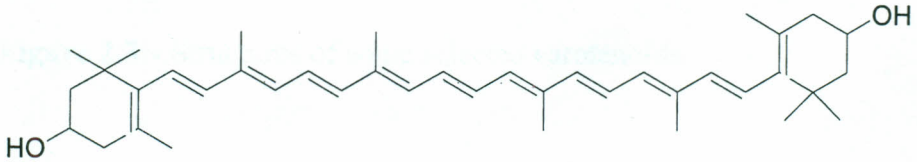
Beta-carotene



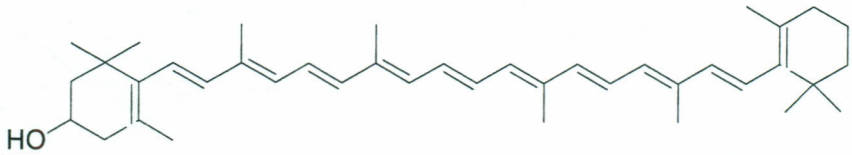
Alpha-carotene



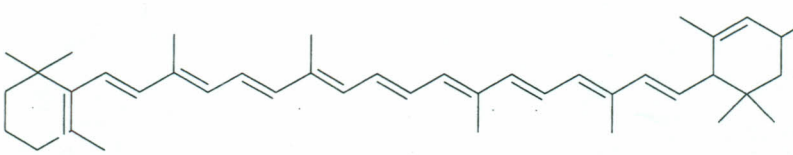
Beta-Zeacarotene



Zeaxanthin



Beta-cryptoxanthin



Alpha-cryptoxanthin

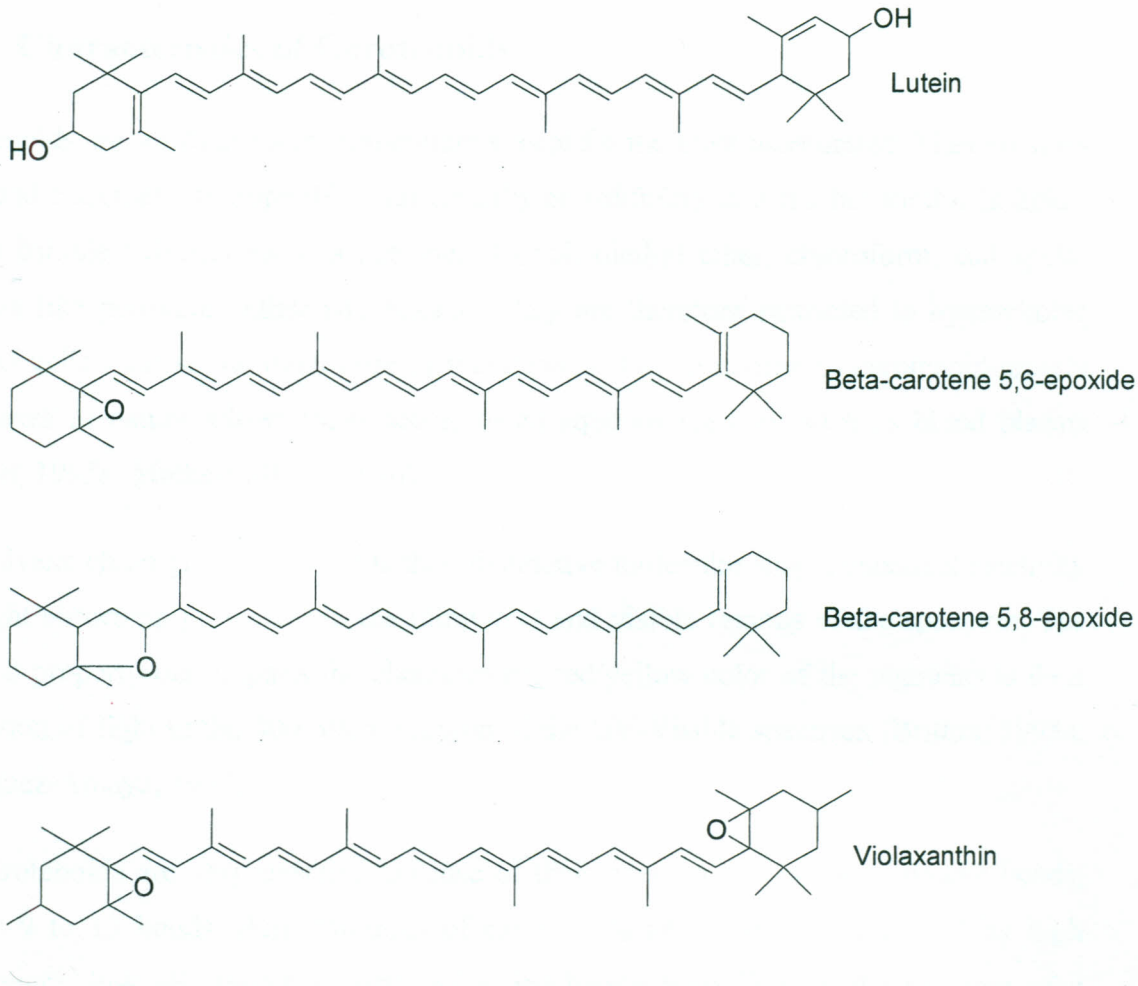


Figure 2.3:- Structures of some selected carotenoids

2.1.3 Characteristics of Carotenoids

Carotenoids are solids at room temperature except for the most unsaturated. They are non-polar and extremely hydrophobic with virtually no solubility in water but soluble in lipids, the fat-soluble solvents such as acetone, alcohol, diethyl ether, chloroform, and apolar solvents like petroleum ether and hexane. They are therefore restricted to hydrophobic areas in cells such as the inner core of membranes. The formation of carotenoid-protein complexes in nature allows them access to an aqueous medium such as blood plasma (Britton, 1995a; Mathews-Roth, 1990).

The polyene chain gives carotenoids their distinctive molecular shape, chemical reactivity and light absorbing properties in the ultraviolet and visible regions of the spectrum. The physical property that imparts the characteristic red/yellow color of the pigments is their absorption of light in the 400-500nm region of the UV-Visible spectrum (Britton, 1995a; Rodriquez-Amaya, 1997).

The carotenoids are very unstable because of their conjugated system of double bonds, usually 9 to 13 bonds. Pure solutions of carotenoids are destroyed or altered by high temperature, low pH, treatment with acids, irradiation with light in the presence of a catalyst such as iodine, oxygen and enzymes in the processes of isomerization and oxidation (Khachik *et al.*, 1992a; Britton, 1995a; Nyambaka and Ryley, 2001; Johnson, 1995; Kanasawud and Crouzet, 1990; Kennedy and Liebler, 1991). The all-*trans* isomer is stable and more predominant form in which carotenoids exist in nature, although both its degradation and *cis* isomers may form during food processing and storage (Rodriguez-Amaya, 1993; Rodriguez-Amaya, 1997; Nyambaka and Ryley, 2001; Mosha *et al.*, 1997; Jayarajan *et al.*, 1980; Dikshit *et al.*, 1988; Padmavati *et al.*, 1992; Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2004; Conning, 1991; Van het Hof, *et al.*, 1998; Gomez, 1981; Imungi and Potter, 1983; Khachik *et al.*, 1992d; Chandler and Schwartz, 1988; O'Neil and Schwartz, 1992).

The *cis-trans* isomerization gives rise to many geometric isomers with the total theoretically possible number of compounds in the whole class running into hundreds of thousands. The actual number of theoretical stereoisomers is limited by the presence of

methyl groups along the carotenoids chain that hinder the foundation of many *cis* isomers. The *Cis*-isomers have higher solubility and lower melting points than the all-*trans* isomers, with some exhibiting double melting points phenomena due to the *cis-trans* isomerization (Britton, 1995a; Nyambaka and Ryley, 2001). Pyrolyis occurs under intense heat with expulsion of low molecular weight molecules. When intense heat is applied, the structures are cleaved and molecular reactions occur involving the double bonds. Two types of thermal degradation products are formed; a volatile fraction of low molecular weight molecules which are vaporized and a non-volatile fraction from the larger fragments of the carotenoid molecules after cleaving off the volatile fraction (Glover, 1960; Johnson, 1995; Kanasawud and Crouzet, 1990).

2.1.4 Sources and Functions of Carotenoids

The structure of carotenoids determines their potential biological functions. Carotenoids are antioxidants with alternating single and double bonds in the polyene backbone that allows them to absorb energy from other molecules. Carotenoid interaction with biological membranes depends on the nature of the specific end groups of the carotenoid (Britton, 1995). Low levels of carotenoids have been associated with poor cognitive performance. Epidemiological studies have shown beneficial effects of carotenoids in the human that include their role as pro-vitamin A carotenoids, antioxidants, photo-protective agents, immuno-enhancers, better memory performance and modulators of transcriptional processes, cellular differentiation, embryogenesis, immune response, reproduction, haemopoiesis, human growth, protection against cancer and energy balance (Bartley and Scolnik, 1995; Olson, 1996; Landrum *et al.*, 1997; Olson, 1999; Basu *et al.*, 2001; Stahl *et al.*, 2002; Sharoni *et al.*, 2004; Bertram, 1999; Moeller *et al.*, 2000; Matthews-Roth, 1990; DiMascio *et al.*, 1991; Zhang *et al.*, 1991; Snodderly, 1995; Alvarez *et al.*, 1995; Schalch, 1992; van Poppel, 1996; Wolf, 2001; Nishino, 1998; Palozza and Krinsky, 1992; Bertram, 1991; Stahl *et al.*, 2000). These accessory pigments are also involved in energy transfer in photosynthesis, phototropism of simple and higher plant form, in photo-protective roles in bacteria and man, in plant growth regulation, reproduction regulation in fungi, in color

attraction in insect pollination in flowers, and in coloration of food for mankind (Olson, 1999; Pfander, 1992; Ong and Tee, 1992; Britton, 1995a; Gross, 1991).

2.1.4.1 Pro-vitamin A Carotenoids

Carotenoids capable of converting to vitamin A are known as pro-vitamin A carotenoids. The main condition for a carotenoid to be pro-vitamin A activity is that it must posses at least one un-substituted beta-ionine ring residue and a polyene side chain of not less than eleven carbons. The other end may have cylic or acyclic structure and the chain may be lengthened although chain lengthening decreases pro-vitamin A activity (Britton, 1995a, Rogriquez-Amaya, 1997). Carotenoids are found primarily in plant foods with the richest known sources of pro-vitamin A compounds being the palm oils. Animal products such as eggs are often considered to be a rich source of pro-vitamin A because of the rich color, but the major pigments here are lutein and zeaxanthin, with β-carotene making less than 7% of the total (Britton, 1995a). Carotenoids are a major source of vitamin A in populations in which VAD is a problem and the consumption of fruits and vegetables is promoted because of their content of pro-vitamin A carotenoids (de Pee *et al.,* 1998; Mulokozi *et al.*, 2000; Mulokozi *et al.*, 2004; Ribaya-Mercado *et al.*, 2000; Takyi, 1999; Gomez, 1981; Nyambaka and Ryley, 1995; Jalal *et al.*, 1998). Leafy vegetables that are dark green are really good sources because their carotenoid content in chloroplasts is roughly proportional to the concentration of chlorophyll (Britton, 1995a; Pfander, 1992). The carotenoids found in vegetables are: β-carotene, α-carotene, gamma carotene, β-zeacarotene, β-carotene 5,6-epoxide, β-carotene 5,8-epoxide, β-cryptoxanthin, cryptoxanthin-5,6-eppoxide, 3-hydroxy-α-carotene and cryptocapsin (Figure 2.3).

β-Carotene, found primarily in fruits and vegetables is the most common pro-vitamin A carotenoid in most diets providing a dual nutritional function; an antioxidant and a pro-vitamin A (Pfander, 1992; Khackik *et al.*, 1995; Jalal *et al.,* 1998; Nyambaka and Ryley, 2001; Mulokozi *et al.*, 2004; Olson, 1996; Gross, 1991; Ong and Tee, 1992). A single molecule of *trans*-β-carotene can theoretically provide two molecules of *trans* retinaldehyde in vivo (Barua and Olson, 2000; Wisps *et al.*, 2000; Lintig and Vogt, 2000;

Wolf, 1995). It has two β-ionine rings and is usually assigned the highest activity of 100% (Gross, 1991). Other pro-vitamin A carotenoids, including α-carotene, gamma-carotene, 5,6- and 5,8-monoepoxides of β-carotene, cryptoxanthin and its monoepoxides and the β-apocarotenals have an intact β-ionine ring at only one end of the molecule and hence have a lower vitamin A activity since they yield only one molecule of vitamin A when metabolized. *Cis* isomers though have a lower potency than the *trans* isomers (Rodriguez-Amaya, 1999). The vitamin A activity of some pro-vitamin A carotenoids and their *cis* isomers found in vegetables, expressed as a percentage of β-carotene is shown in Table 2.1.

Table 2.1: Relative vitamin A activity of pro-vitamin A carotenoids in vegetables

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

Source: Gross (1991)

Some of the indigenous common green leafy vegetables consumed in Kenya and their mean β-carotene levels are listed in Table 2.2 while the all-*trans*-β-carotene content has been determined in DGLV's as shown in Table 2.3.

Table 2.2: Mean beta-carotene content of some wild and cultivated green leafy vegetables from Kenya

Botanical name	Common name	Mean β-carotene content (μg/100g wet wt.)
Amaranthus Sp.	East African spinach	4,416
Amaranthus hybridus	East African spinach	7,415
Crotalaria brevidens	-	7,000
Gynandropsis gynandra	African herb	15,916
Vigna unguiculata	Cowpea	7,416
Cucurbita pepo	Pumpkin	8,375
Solanum nigrum	Nightshade	7,625
Curcurbita sp.	Pumpkin	8,291
Beta vulgaris	Swiss chard	6,125
Manihot utilissima	Cassava	14,437
Phaseolus vulgari	Bean	10,665
Brassica oleracea	Kale	8,312
Chorchoris olitorius	Bush okra	8,750
Solanum tuberrosum	Potato (Irish)	11,260
Colacasia esculenta	Cocoyam	9,700
Erucastrum arabica	-	7,625
Galinsoga perviflora	-	7,375

Source: Gomez (1981)

Table 2.3:- All-*trans*-β-carotene content and % retention of some dark green leafy vegetables

Product	All-trans-β-carotene (µg/g DM)		% Retention
	Range	Mean	
Kenyan samples			
Cowpea leaves			
Blanched	628-780	691	100
Solar dried	515-654	584	85
Sun-dried	411-568	499	72
UK samples			
Cowpea leaves			
Blanched	859-906	883	100
Freeze dried	545-778	700	79
Simulated solar dried	481-646	553	63
African herb			
Blanched	970-1110	990	100
Freeze-dried	817-840	827	84
Simulated solar-dried	775-820	782	79

Source: Nyambaka and Ryley (1995)

The all-*trans* isomer of β-carotene is the major source of dietary retinoids (vitamin A), due to its high pro-vitamin A activity (Khachik *et al.*, 1997; Gross, 1991; Britton, 1995a). Excessive intake of β-carotene is not known to induce vitamin A toxicity although negative feedback mechanisms in the body prevent the over-conversion of β-carotene to retinol. However, high levels of β-carotene in the diet induce a condition characterized by jaundice-like yellowing of the skin with the symptoms reversed when dietary intake is reduced (Groff *et al.*, 1995). Among its functions, β-carotene quenches singlet oxygen, induces gap junction communication and inhibits lipid peroxidation while high serum levels of β-carotene are correlated with low incidences of cancer in the mouth, lung breast, cervix, skin, and stomach (Ramaswamy *et al.,* 1990; Zhang *et al.,* 1991; Stich *et al.,* 1991; Stahelin *et al.,* 1991; Palan *et al.*, 1991). Beta-cryptoxanthin, a carotenoid which also exhibits pro-vitamin A activity is found richly in orange and red fruits and vegetables like sweet red peppers. This pro-vitamin A carotenoid is capable of quenching singlet oxygen (DiMascio *et al.,* 1989).

Vitamin A is the generic term for all β-ionine retinoid derivatives, a group of lipid-soluble compounds that have similar physiological functions and metabolic activities (Combs, 1992). Vitamin A is found exclusively in animal foods, with high levels of pre-formed vitamin A found in animal and fish livers and fish oils. The main forms are retinol, retinal (the aldehyde form), and retinoic acid. Retinol inter-converts between retinyl esters and retinal, and retinoic acid as the end product. Retinoic acid helps maintain the proper growth and differentiation of epithelial cells, whereas retinyl ester, retinol and retinal all maintain cellular differentiation, support reproduction and prevent blindness (Olson, 1996; Groff *et al.,* 1995; Combs, 1992).

The parent compound of vitamin A is the all-*trans* retinol, which has an isoprenoid structure and is found in animal tissue. Animal species are able to enzymetically convert particular plant carotenoids into vitamin A, thus its occurrence in animal foods is dietary (Olson, 1996; Combs, 1992). Vitamin A is a fat-soluble vitamin that is an essential nutrient for various physiological processes; with its best-defined role being in vision (Rando, 1994; Olson, 1991; Groff *et al.,* 1995). It is a functional constituent of rhodopsin,

a protein in the retina of the eye that absorbs light and triggers a series of biochemical reactions that ultimately initiate the nerve impulses that lead to vision (Groff *et al.,* 1995). It is involved in the activation of gene expression and, subsequently, the control of cell differentiation (in embryonic development) such that it affects the immune function, taste, hearing, appetite, skin renewal, bone development, and growth (Olson, 1996).

The deficiency and excess of vitamin A causes serious health consequences (Combs, 1992). Lack of vitamin A causes a rare condition called night blindness (poor dark adaptation) and also cause dry eyes, eye infections, skin problems and slow growth. Deficiency of retinol therefore results into an abnormal process known as xerophthalmia, meaning 'dry eye'. There have been reports on endemic xerophthalmia in India, Indonesia, South Asia, East Asia, Africa and Latin America (Sommer, 1997; WHO/UNICEF, 2003; FAO/ILSI, 1997; Beaton, 1993). Some conditions such as cystic fibrosis, diarrhea, continuing illness, long term injury, serious liver diseases, malabsorption problems and pancreas disease increase the need for vitamin A (Sommer, 1997; Tanumihardjo *et al.*, 2004; Caulfield *et al.*, 2004). Ingestion of too much preformed vitamin A (retinol) is toxic with symptoms of acute and chronic toxicity such as nausea, headache, blurred vision, cracking lips, dry and itchy skin, and bone and joint pain which disappear when over dosing stops. It also causes fetal resorption, spontaneous abortion, birth defects and permanent learning disabilities in children (Olson, 1996; Groff *et al.*, 1995; Combs, 1992).

Vitamin A deficiency (VAD) is a major public health issue, particularly in developing countries with an estimate of 500,000 preschool-age children, worldwide, becoming blind each year (WHO/UNICEF, 2003). Millions of others presumably suffer from night blindness; a common clinical sign of inadequate vitamin A intake and a further estimate of more than 100 million children worldwide suffer from vitamin A inadequacy without showing clinical signs of acute deficiency (WHO/UNICEF, 2003; West, 2002). The β-Carotene is known to be an effective dietary cure for VAD and an effective remedy for the symptoms of the disorder (Olson, 1996; WHO/UNICEF, 2003; FAO/ILSI, 1997).

2.1.4.2 Non-provitamin A Carotenoids

Non-pro-vitamin A carotenoids are not convertible to vitamin A but are fundamental in human health (Khachik *et al.*, 1997; DiMascio *et al.,* 1989; Zhang *et al.,* 1991; Khachik *et al.,* 1995; Schalch, 1992; Snodderly, 1995). Lycopene, the hydrocarbon carotenoid that gives tomatoes their red color, has its all-*trans* isomer typically quantified in serum, although 9-, 13- and 15-*cis* isomers are detectable and account for as much as 50% of the total lycopene (Sies *et al.,* 1992). Among its functions, it quenches singlet oxygen induces gap junction communication, inhibits lipid peroxidation, and displays chemo-preventive activity. High serum levels of lycopene have been inversely related to the risk of cancer in the pancreas and cervix (Zhang *et al.,* 1991; DiMascio *et al.,* 1989).

Lutein occurs in fruits and vegetables, although commercial sources are obtained from the extraction of marigold petals (Khachik *et al.,* 1995). It is a xanthophyll, which exists in the retina and functions in protecting photoreceptor cells from light-generated oxygen radicals, thus plays a key role in preventing advanced macular degeneration (Schalch, 1992; Snodderly, 1995). It possesses chemo-preventive activity, induces gap junction communication between cells and inhibits lipid peroxidation *in vitro* more effectively than β-carotene, α-carotene and lycopene (Zhang *et al.,* 1991). High levels of lutein in serum have been inversely correlated with lung cancer (Khachik *et al.,* 1995). In addition to lutein, zeaxanthin is a xanthophyll which also exists in the retina and confers protection against macular degeneration while Astaxanthin, a xanthophyll found in salmon, shrimp, and other seafood's, has potent antioxidant properties (Schalch, 1992; DiMascio *et al.*, 1991).

2.1.5 Oxidative Metabolism of Carotenoids

Animals are incapable of synthesizing carotenoids, but incorporate carotenoids from their diet. Many mammals accumulate carotenoids in their tissues with some altering them slightly by oxidative metabolism during digestion and absorption (Ong and Tee, 1992; Britton, 1995a; Failla, and Chitchumroonchokchai, 2005).

Oxygen is generally the source of oxidizing power in food systems and carotenoid containing foods are generally sensitive to oxidation (Macrae *et al.*, 1993; Britton, 1995a;

Kennedy and Liebler, 1991; Johnson, 1995; Rodriguez-Amaya, 1997). Auto-oxidation is the spontaneous chemical reaction between atmospheric oxygen and organic compounds especially the unsaturated ones at ambient temperatures. The auto oxidation process is initiated by presence of free radicals which are formed either enzymatically or chemically. The general mechanism is that of free radical reactions involving initiation, propagation and termination steps. A radical species $R^{\bullet}$ formed in the 'initiation" step reacts with oxygen to form peroxyl radicals (step 1) which undergoes 'propagation' steps (step 2) to form new radical species or adds to a double bond of a saturated molecule (**scheme 1**). The 'termination' step (step 3) occurs when two radical species react with each other to form non-radical adducts which include epoxides, apo-carotenals and other hydrocarbon derivatives of lower carbon chain (Kennedy and Liebler, 1991). The β-Carotene reacts chemically with peroxyl radicals to produce epoxide and apo-carotenal products.

$$RH \rightarrow R^{\bullet} + H^{\bullet}$$
$$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

Step 1

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$
$$ROO^{\bullet} + RH \rightarrow ROORH^{\bullet}$$

Step 2

$$R^{\bullet} + R^{\bullet} \rightarrow R - R$$
$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOOOR$$

Step 3

RH represents the unsaturated lipid or carotenoid molecule

Scheme 1: Showing initiation, propagation and termination steps of a carotenoid

In the presence of oxygen, light, humidity, heat, metal ions and enzymes carotenoid undergo the oxidation reaction. Various enzymes are involved in the *in-vivo* oxidation of carotenoids, lipoxygenase being the most common. By use of molecular oxygen, lipoxygenase catalyzes the oxidation of unsaturated fatty acids containing a *cis, cis*-1,4-

pentadiene system to a *cis, trans*-conjugated diene hydroperoxide, which in turn oxidizes the pigments found in the vegetable tissue (Gross, 1991; Borel *et al.*, 1996). Peroxidase and catalase enzymes found in vegetables cells also display carotenoid oxidative activity but use trace metals to catalyze the reaction between the substrate and oxygen to form radicals (Donnelly and Robinson, 1995).

The loss in β-carotene content in dehydrated products may be attributed largely to its oxidative degradation and is known to give many products where different mechanisms, have been proposed (Glover, 1960; Burton, 1989; Kanasawud and Crouzet, 1990; Marty and Berset, 1990; Wang *et al.*, 1991; Hui, 1992; Mordi *et al.*, 1993; Handelman *et al.*, 1993; Wang and Krinsky, 1998; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Barua and Olson, 2000; Olson and Hayaishi, 1965). Figures 2.4, 2.5 and 2.6 give some of these mechanisms that explain the sequence of reactions involved in *trans*-β-carotene oxidative degradation, some explaining the mechanisms by which carotenoids with vitamin A activity are converted to vitamin A and other metabolites.

In one scheme, the oxidation process involves an initial isomerization of the all-*trans* to the *cis* isomer followed by the formation of a di-radical, or both isomerization and formation of a di-radical occurring simultaneously and reversibly (Fig. 2.4). The involvement of the *cis-trans* isomerization in beta-carotene oxidation has been confirmed by HPLC analysis (Mordi *et al.*, 1993; Nyambaka and Ryley, 2001).

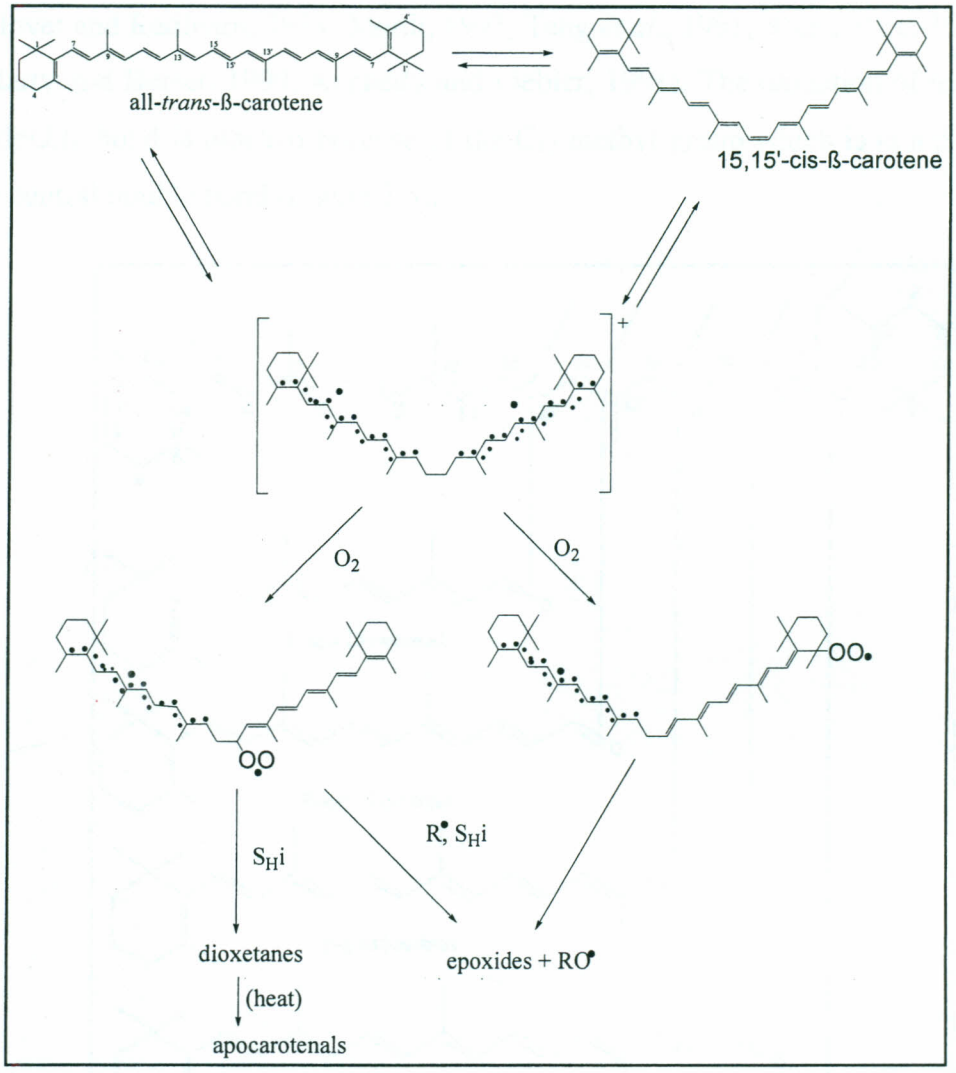


Figure 2.4:- Reaction sequence for the formation of epoxides and apocarotenals during oxidation of beta-carotene via isomerization.

Source: Mordi *et al.* (1993)

The cleavage of pro-vitamin A carotenoids to yield vitamin A occurs at random, assuming that the two ends of the conjugated system of double bonds are equally susceptible to attack. Due to resonance, the central double bond is more stable than the terminal ones thus less susceptible to attack (Zechmeister, 1962). Beta-carotene is eccentrically cleaved starting at either end of the conjugated system to produce a series of apo-carotenals and yielding only one molecule of retinal per molecule of beta-carotene cleaved (Glover,

1960; Glover and Redfearn, 1954; Mordi, 1993; Tang *et al.*, 1991; Wang *et al.,* 1991; Hui, 1992; Marty and Berset, 1990; Kennedy and Liebler, 1991). The oxidation of the central 15, 15' double bond is blocked because of the C_{13} methyl group which is at a β-position from the central double bond (Figure 2.5).

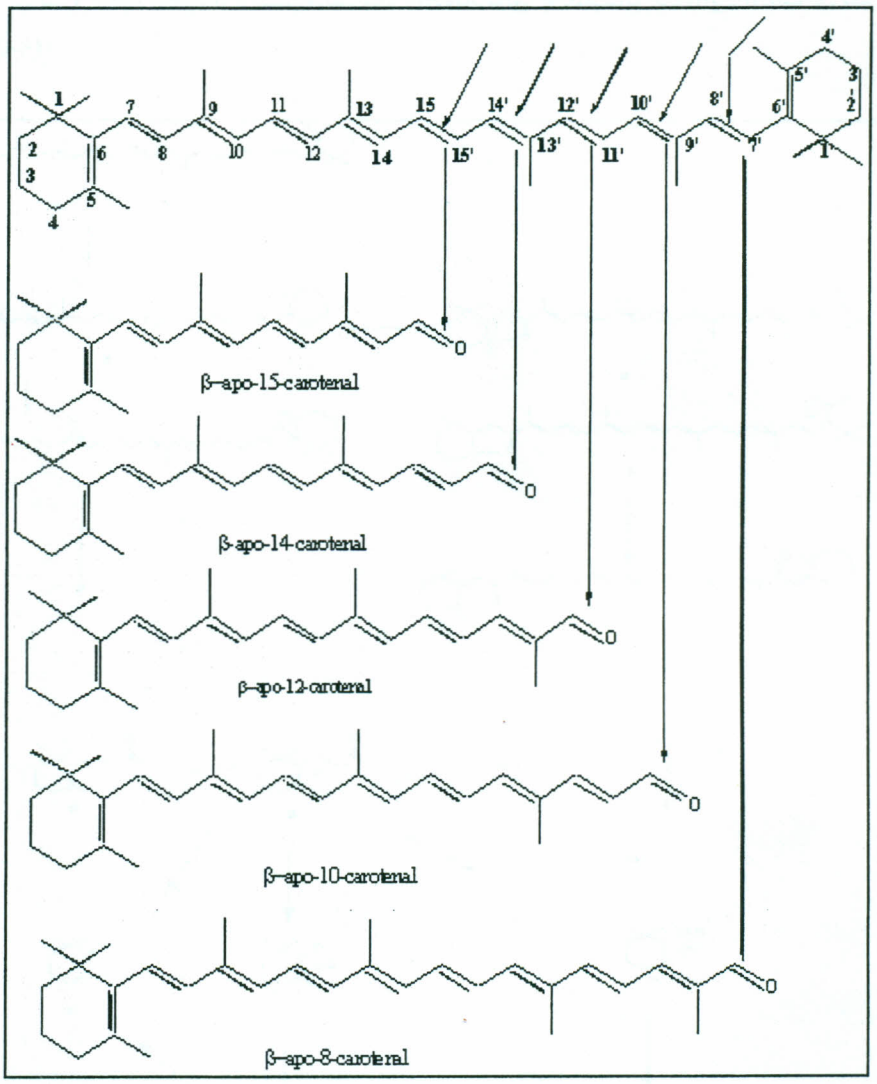


Figure 2.5:-Mechanism for β-carotene oxidation by oxygen attack at multiple sites.

Source: Glover (1960)

Auto-oxidation conditions of β-carotene has been postulated that the initial step in its breakdown occurs through the formation of epoxides (Fig 2.6). The terminal 5-6 double bond posses the highest electron density for easy attack and generates the formation of the 5, 6-epoxide as the initial product. The 5, 6-epoxide then decomposes to give the furanoid oxide (5, 8-epoxide) and other compounds, including volatile compounds (Kanasawud and Crouzet, 1990).

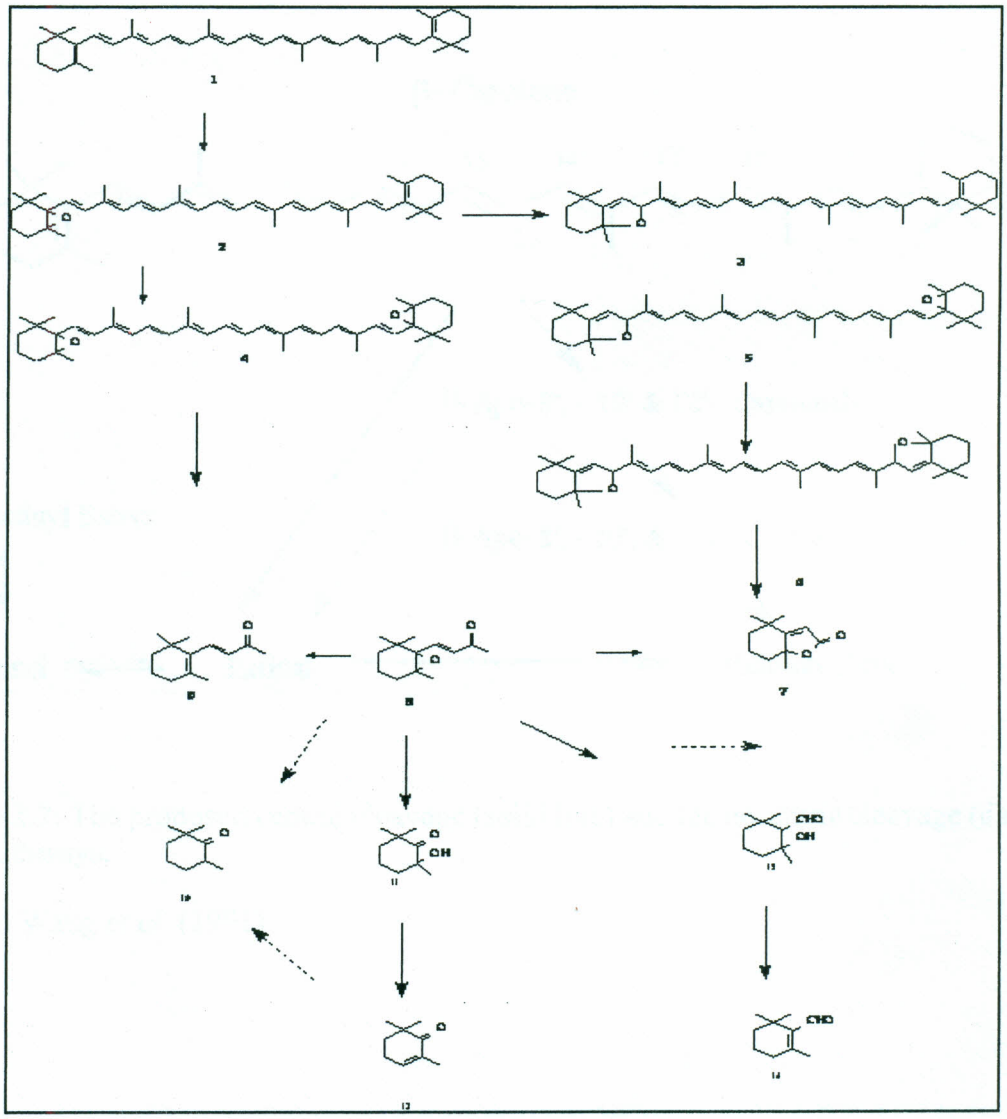


Figure 2.6 : - Reaction sequence for the formation of epoxides and carbonyl compounds during oxidation of beta-carotene: **1,** beta-carotene; **2,** 5,6-epoxy-betacarotene; **3,** 5,8-epoxy-beta-carotene; **4,** 5,6,5,6-diepoxy-beta-carotene; **5,** luteochrome; **6,** aurochrome; **7,** dihydroactinidiolide; **8,** 5,6-epoxy-beta-ionine; **9,** beta-ionine; **10,** 2,6,6-trimethylcyclohexanone; **11,** 2-hydroxy-2,6,6-trimethylcyclohexanone; **12,** 2-hydroxy-2,6,6-trimethylcyclohexane-1-carboxaldehyde; **13,** 2,6,6-trimethyl-2-cyclohexe-1-one; **14,** beta-cyclocitral. Source: Kanasawud and Crouzet, (1990)

More recently it has been proposed that within the intestine, β-carotene is cleaved to yield two molecules of vitamin A (retinol) by central cleavage thus to produce retinal through action of the enzyme β-carotene-15, 15'-dioxygenase (Barua and Olson, 2000a; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Wolf, 1995). The central and eccentric cleavage schemes are outlined (Fig 2.7).

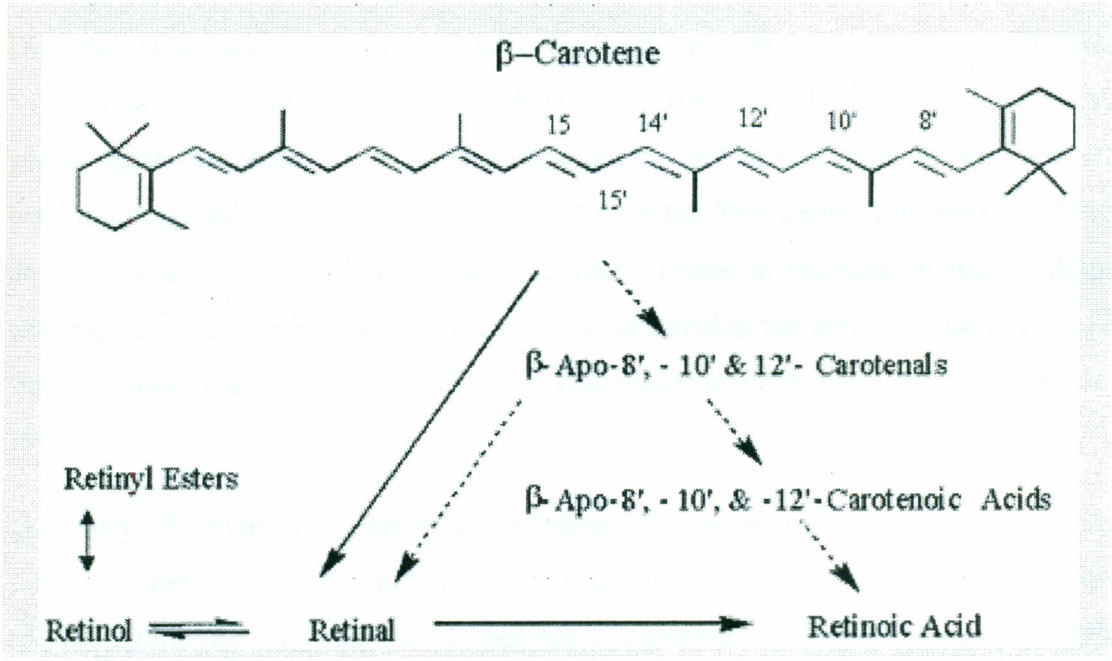


Figure 2.7: The proposed central cleavage (solid line) and the eccentric cleavage (dashed line) pathways.

Source: Wang *et al.* (1991)

2.1.5.1 Digestion, Absorption, Tissue Distribution and Excretion

Carotenoids are released from the food matrix during digestion, emulsified in the lipid phase of chyme, and solubilized in mixed micelles to be accessible for uptake by absorptive epithelial cells (Harrison and Hussain, 2001; Breithaupt *et al.*, 2002; Borel, et al., 1996; Failla and Chitchumroonchokchai, 2005; Linder, 1991; Borel *et al.,* 2001; Lakshman and Okoh, 1993; Ong, 1993).

Digestion is initiated in the oral cavity as the food is mechanically sheared and lubricated with saliva before entering the stomach (Linder, 1991). Hydrochloric acid, pepsin and gastric lipase are secreted into the gastric lumen and mixed with the ingested foods, resulting in partial release of the carotenoids from the food matrix into the emulsified oil droplets. Apolar carotenoids such as β-carotene reside in the core of the oil droplets, whereas polar carotenoids are preferentially distributed at the surface (Borel *et al.,* 1996; Harrison and Hussain, 2001; Failla, and Chitchumroonchokchai, 2005; Erdman *et al.*, 1993).

The entry of chyme into the small intestine is associated with release of pancreatic secretions and bile into the lumen. The acidity of chyme is neutralized by bicarbonate, and the hydrolytic enzymes further degrade components of the food matrix and release fat-soluble compounds partition into lipid droplets. Cholestrol esterase and pancreatic triglyceride lipase are capable of hydrolyzing polar carotenoid esters to free carotenoids while bile salts are required for the partition of the lipophilic products into mixed micelles (Linder, 1991; Breithaupt *et al.,* 2002; Harrison and Hussain, 2001; Failla, and Chitchumroonchokchai, 2005). Once formed, the mixed micelles diffuse across the unstirred water layer and deliver carotenoids and other fat-soluble compounds to the apical surface of the mucosal epithelium (Parker, 1996; Furr and Clark, 1997; Borel *et al.*, 2001; Borel *et al.,* 1996; Failla, and Chitchumroonchokchai, 2005). The formation of micelles is a necessary precondition for the absorption of carotenoids depicting the importance of dietary fat intake (Kayden and Traber, 1993; Mulokozi *et al.,* 2004; Takyi *et al.*, 1999; Roodenburg *et al.*, 2000; Erdman *et al.*, 1993). Once taken into the enterocyte, β-carotene can be converted to vitamin A by cleavage through central

cleavage of dietary β-carotene as the predominant mechanism of retinaldehyde formation (Barua and Olson, 2000b; Wyss *et al.* 2000; Lintig and Vogt, 2000; Lakshman and Okoh, 1993; Nagao, 2004; Nagao *et al.*, 1996).

Carotenoids and retinyl esters synthesized after cleavage of pro-vitamin *A* carotenoids are incorporated into nascent chylomicra in the golgi of enterocytes (Parker, 1996; Breithaupt *et al.*, 2002). Conversion of chylomicra to remnants is associated with uptake of the particles by liver where the carotenoids may be utilized, stored, or re-secreted into plasma in very low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Relatively high concentrations of pro-vitamin A carotenoids are commonly found in tissues expressing a high density of LDL receptors such as adipose tissue, liver, testis and ovary (Olson, 1999).

The different structural features of carotenoids account for the selective distribution in organ tissue, biological activity and pro-vitamin A potency, the *in vivo* conversion to vitamin A (Breithaupt, *et al.*, 2002). Due to the hydrophobic character, carotenoids are associated with lipid portions of human tissues, cells, and membranes. In general, 80-85% of carotenoids are distributed in adipose tissue, with smaller amounts found in the liver, muscle, adrenal glands and reproductive organs (Beecher and Khachik, 1992). Approximately 1% circulates in the serum on high and low density lipoproteins.

The major serum carotenoids are β-carotene, α-carotene, cryptoxanthin, lutein, zeaxanthin, and lycopene though smaller amounts of polyenes such as phytoene and phytofluene are also present (Beecher and Khachik, 1992). Since adipose tissue is the largest body pool for carotenoids, serum concentrations are fairly constant and slow to change during periods of low intake (Rock and Swendseid, *et al.,* 1992). Evidence for the existence of more than one body pool has been published (Micozzi *et al.,* 1992; Beecher and Khachik, 1992).

Serum levels reflect lifestyle choices and dietary habits within and between cultures (Khachik *et al.,* 1997). Variations can be attributed to different intakes, unequal abilities to absorb certain carotenoids, and different rates of metabolism and tissue uptake (Bendich and Olson, 1989). Decreased serum levels occur with alcohol consumption, the

use of oral contraceptives, smoking and prolonged exposure to UV light (White *et al.*, 1994; Forman *et al.*, 1995; Pamuk *et al.*, 1994).

Ingested carotenoids from foods may not be absorbed. It has been assumed that urinary losses of carotenoids are extremely low and in addition, very small quantities of endogenous carotenoids are lost by exfoliation of skin and low concentrations of carotenoids are identified in bile (Bowen *et al.*, 1993; Leo *et al.*, 1995; Harrison and Hussain, 2001). Fecal excretion represents the primary route of elimination from the body.

2.1.6 Bioavailability and Bioconversion of Carotenoids

The serum retinol concentration is the result of absorption of pro-vitamin A carotenoids, their conversion to retinol, and their subsequent distribution, storage, metabolism and clearance of retinol (Craft, 2001). Each of these aspects is being affected by several factors, which are difficult to control when estimating the conversion factor. β-Carotene has the highest activity usually assigned as 100% since a single molecule can theoretically provide two molecules of *trans* retinaldehyde in vivo (Barua and Olson, 2000; Wyss *et al.*, 2000; Lintig and Vogt, 2000). The assumption that 5-6 μg dietary β-carotene from fruits and vegetables provide one retinol equivalent (RE) was challenged by dee Pee, *et al.*, (1998) who suggested that amounts as high as 26 μg from DGLVs provide 1 RE. The larger amount of β-carotene reflects the fact that the bioavailability of carotenoids in most foods is significantly lower than that of dietary vitamin A. The conversion ratio of β-carotene to retinol of 12:1 was originally assigned though this ratio has recently been changed to 10:1 (FAO/WHO, 1988; NRC, 1990; Vuong *et al.*, 2002). However, Ribaya-Mercado *et al.*, (2000) used a 6:1 conversion ratio in DGLVs. Two systems are used to express the amounts of β-carotene and vitamin A in foods and supplements. The first system is based on retinol equivalents (RE), defined as 1 μg of all-*trans* retinol or 10 μg of all-*trans* β-carotene (NRC, 1990, Vuong *et al.*, 2002). The second system is based on International Units (IU), which is used to express potency in nutritional supplements containing vitamin A or β-carotene.

Bioavailability is the efficiency of absorption of an ingested compound and its bioactive metabolites for delivery to and utilization by target tissues (Jackson, 1997). The term bioavailability has also been defined as the fraction of an ingested nutrient available for utilization in normal physiological functions and storage (West and Eilander, 2001). Carotenoid bioavailability is based mainly on measurement of carotenoids in serum or plasma after ingestion (Castenmiller and West, 1998).

Bioconversion, on the other hand, is the proportion of bioavailable carotene converted to retinol (Castenmiller and West, 1998). Often, however, the term covers both the bioavailability and the bioconversion process. Provitamin A carotenoids are converted to retinol by the action of 15-15'-carotenoid dioxygenase. This central or eccentric cleavage process occurs primarily in the enterocytes, though enzyme activity is found in other tissues, such as the liver. A molecule of β-carotene, central cleavage would result in the formation of two molecules of retinal that could be reduced to retinol and then esterified, whereas eccentric cleavage would produce one molecule of a β-apo-carotenal that could be converted not only to retinal but also to β-apo-carotenoic acids and subsequently to retinoic acid (Wang *et al.*, 1991; Barua and Olson, 2000a; Wyss *et al.*, 2000; Lintig and Vogt, 2000).

Several factors have been identified that affect the dietary intake of vitamin A-rich foods (de Garine, 1990); chief among them being dietary beliefs, ecology and the economic status of the population. Exclusion of a food item rich in vitamin A activity from the diet can relate to dietary beliefs, although this effect is usually limited to certain sectors of the population. Certain foods are prescribed as preventive measures or as treatment for illness (de Garine, 1990) in some societies while others are often proscribed from the diet in response to alterations in physiological status such as menstruation, pregnancy and lactation, and illness. Cross-cultural differences in parental control and dietary beliefs influence the timing of the introduction of foods rich in vitamin A activity and the quantity ingested during the weaning period (David, 1990). This has important implications for the vitamin A status of infants and children, whose liver stores of vitamin A are more rapidly depleted since they eat relatively small quantities than adults.

Ecological factors such as climate, soil and water, and general environmental integrity all affect the availability of a food item. The availability of foods rich in vitamin A activity is often seasonal. The diversity of available edible species increases in the rainy season. When liver retinol stores are low or vitamin A status is compromised by disease, seasonal fluctuations usually lead to periods of greater risk of VAD (Ribaya-Mercado *et al.*, 2000). Dietary intake of foods rich in vitamin A activity is also determined by economic factors. Vitamin A intake is positively correlated with household income level, especially when pro-vitamin A and preformed vitamin A food sources are not readily available. Green leafy vegetables though have a lesser important economic role, they are often used during periods of food shortages and economic constraints.

2.1.6.1 Effectiveness of Plant Carotenoids

The study on carotenoids has attracted much scientific attention due to their chemistry and numerous functions (Rock and Swendseid, 1992; Xu *et al.*, 1992; Yong *et al.*, 1994; Bulux *et al.,* 1994; Nierenberg *et al.*, 1994; Kostic *et al.,* 1995; de Pee *et al.,* 1995; Fotouhi *et al.*, 1996; Devaraj *et al.,* 1996; Mulokozi, *et al.*, 2004; Parker, 1997; van Vliet *et al.*, 1996a,b; Rock and Swendseid, 1992; Paetau *et al.,* 1997; Clark *et al.*, 1998; O'Neill and Thurnham 1998; Van den Berg and Van Vliet, 1998; Takyi, 1999; Van het Hof *et al.*, 1999a; Tang, 1999; Van het Hof *et al.,* 1999b; Van het Hof, 2000a,b; Huang *et al.*, 2000; Ribaya-Mercado *et al.*, 2000). Most bioavailability studies were performed using β-carotene by both in *vivo* and in *vitro* modes. The ability of β-carotene rich foods to meet the vitamin A needs of those populations at risk is currently under scrutiny. Some studies on the bioavailability of pro-vitamin A carotenoids from foods give contradicting results, and therefore, a contradiction to their contribution in improving vitamin A status. Therefore the bioavailability of pro-vitamin A carotenoids from plant food sources remain uncertain.

The effectiveness of plant carotenoids in combating VAD or vitamin A inadequacy is questioned by Bulux *et al.,* (1994) in the study where an amount of cooked carrots to the daily diets of children aged 7–12 y and in the study by de Pee *et al.,* (1995), that used

lactating women with stir-fried, DGLVs. Both studies found no evidence of nutritional benefit of vitamin A after an increased consumption of DGLVs or carrots. de Pee *et al.*, (1998) similarly reported that fruits were more effective in improving vitamin A status than DGLVs in schoolchildren. However, they recommended further study on ways of improving bioavailability and bioconversion of dietary carotenoids, focusing on factors such as intestinal parasites, absorption inhibitors, and food matrixes. These findings were challenged by Takyi, (1999) in a study that determined if consumption of DGLVs would enhance the retinol status of preschool children to acceptable levels. There were 5 randomly divided groups, with children between the ages of 2.5-6 years. Stew was prepared for each group, each differing in the levels of fat and β-carotene provided. There were significant differences found in the percentages of children with deficient, low, and adequate levels of serum vitamin A before and after the feeding period. The percentage of children with adequate retinol status increased from 28.2% to 48.2% leading to a conclusion that consumption of DGLVs with fat (10 g/100 g) significantly enhanced serum retinol (Takyi, 1999). The study was successful in demonstrating that a food-based intervention could enhance vitamin A levels, implying that increased consumption of DGLVs would help prevent the incidence of VAD in developing countries.

In a 10 week study by Tang, (1998), 22 children were provided with green-yellow vegetables and light-colored vegetables per day for 5 days in a week while 19 children maintained their customary dietary intake. Serum retinol and carotenoid concentrations were measured by high performance liquid chromatography (HPLC) and results showed that carotenoid nutrition improved after consumption of green-yellow vegetables. Serum concentrations of retinol were sustained in the group fed with green-yellow vegetables but decreased in the group fed with light-colored vegetables. The study concluded that green-yellow vegetables could provide adequate vitamin A nutrition in the diet of kindergarten children and protect them from becoming vitamin A deficient during seasons when the pro-vitamin A food source is limited.

Van het Hof *et al.,* (1999a) also assessed the bioavailability of β-carotene and lutein from vegetables and the effect of increased vegetable consumption. In this study that took over four weeks, 22 healthy adult subjects consumed a high-vegetable diet, 22

consumed a low-vegetable diet, and 10 consumed a low-vegetable diet supplemented with pure β-carotene and lutein. The results showed that plasma concentrations of vitamin C and carotenoids (α-carotene, β-carotene, lutein, zeaxanthin, and β-cryptoxanthin) were significantly higher in the high-vegetable diet group than the low-vegetable diet group. Further study indicated that the bioavailability of β-carotene when provided as a pure compound was ten times higher than when it was present naturally in foods such as DGLVs (Van het Hof *et al.*, 2000).

2.1.6.2 Factors affecting bioavailability and bioconversion of carotenoids

For dietary carotenoids to be absorbed intestinally, they must be released from the food matrix and incorporated into mixed micelles (mixtures of bile salts and several types of lipids). Thus, the factors that interfere with this process will affect the overall bioavailability and bioconversion of the carotenoids ingested. A number of factors that have an effect on the amount of dietary carotenoid available for utilization in the body have been identified and the mnemonic "SLAMENGHI" is used to describe them (West and Castenmiller, 1998). This mnemonic stands for the factors; species of carotenoid, linkages at molecular level, amount of carotenoids, food matrix, effectors of absorption, nutrient status, genetics, host–related factors and interactions of carotenoids (Kostic *et al.*, 1995; Castenmiller and West, 1998; Castemiller *et al.*, 1999; van het Hof *et al.*, 1999b; Boileau *et al.*, 1999; Erdman, *et al.*, 1993; Olmedilla *et al.*, 1994; Herbst *et al.*, 1997; Van vliet *et al.*, 1996; Roodenburg *et al.*, 2000; Wingerath *et al.*, 1995). The review of some of these factors covers the bioavailability and bioconversion aspect.

(a) Species of carotenoids

The bioavailability of naturally occurring configuration of carotenoids in plant foods is usually the all-*trans* isomer. In general, *cis* isomers are more polar, less prone to crystallization, and more soluble in oils and hydrocarbon solvents. The *cis* isomeric

composition increases with food processing, particularly heating, and the quantity formed appears related to the severity and extent of the heat treatment. Most information on the relative bioavailability of species of carotenoids are based on ß-carotene (Rushin *et al.*, 1990; Van het Hof *et al.*, 2000; Ben-Amotz and Levy, 1996; Gaziano *et al.*, 1995; Jensen *et al.*, 1987; Tamai *et al.*, 1995; Stahl *et al.*, 1993; Stahl, *et al.*, 1995; Erdman *et al.*, 1998) although some data is available on non-provitamin A carotenoids (Stahl and Sies, 1992; Castenmiller *et al.*, 1999; Van het Hof, *et al.*, 1999a; van het Hof *et al.*, 1999b).

Rushin *et al.*, (1990) demonstrated that *cis* isomers had not formed after blood was drawn and that *cis* isomers of β-carotene were present at significant concentrations in the human circulation. The all-*trans* form is absorbed more readily in humans than the 9-*cis* form. When multiple doses of mixtures of the two were ingested, the proportion of the 9-*cis* form found in serum was less than one sixth of the amount of the carotenoid mixture (Ben-Amotz and Levy, 1996; Gaziano *et al.*, 1995; Jensen *et al.*, 1987; Tamai *et al.*, 1995). Similarly, after a single dose, the increase of all-*trans*-β-carotene in serum or in the chylomicron fraction of serum was much higher than for the 9-*cis* isomer (Stahl *et al.*, 1993; Stahl *et al.*, 1995), which indicates a strong preferential absorption or transport of the all-*trans* isomer in comparison with the 9-*cis* isomer. After a single oral dose, more than 95% of plasma ^{13}C-labeled all-*trans*-β-carotene and labeled retinol was derived from labeled 9-*cis*-β-carotene. This indicates that a significant portion of the labeled 9-*cis*-β-carotene dose was isomerized to labeled *trans*-β-carotene before entering the blood stream (You *et al.*, 1996).

For non-provitamin A carotenoids, lycopene uptake from processed and unprocessed tomato juice was studied in humans, and the *cis* isomer, was found to be better absorbed than the all-*trans* form (Stahl and Sies, 1992). This would be related to the better solubility of the *cis* form. The relative bioavailability of lutein from a diet supplemented with a variety of vegetables has been shown to be much greater than that of ß-carotene, thus 67 and 14%, respectively (Van het Hof *et al.*, 1999a). The same was found by Castenmiller *et al.*, (1999) for the relative bioavailability of lutein and ß-carotene from spinach thus 45 and 5.1%, respectively. The release of lutein into an aqueous environment is probably higher than that of ß-carotene because of its lower lipophilicity

compared with ß-carotene. In addition, the bioavailability of lutein appears to be lower from green leafy vegetables than from other vegetables, although the differences are less pronounced than those of ß-carotene (van het Hof *et al.*, 1999a).

In the bioconversion to vitamin A, all carotenoids containing at least one un-substituted β-ionone ring and a polyene side chain attached containing at least 11 carbon atoms are potential precursors of vitamin A, with β-carotene showing the highest vitamin A activity on a molar basis (Barua and Olson, 2000a; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Wolf, 1995).

(b) Molecular linkage

There is little information on bioavailability from the effect of carotenoid esters, which are common in fruits and vegetables. Herbst *et al.*, (1997) found that lutein diesters showed a trend toward greater bioavailability than free lutein did, which suggests that the human gut is efficient in cleaving esters of lutein, and therefore, esterified lutein in food may be equally or better bioavailable than free lutein. No β-cryptoxanthin or esters from other carotenoids were detected in chylomicrons or serum after consumption of tangerine juice (Wingerath *et al.,* 1995). Since esters are not absorbed by the enterocyte, it is not expected that molecular linkage would have an effect on bioconversion.

(c) Amount of carotenoids

The amount of carotenoids consumed in a meal and the duration of carotene supplementation are significant predictors in their bioavailability (Castenmiller and West, 1998; Shiau *et al.*, 1994; Prince and Frisoli, 1993). Shiau *et al.* (1994) reported that with a meal, only 35–71% of β-carotene was recovered in rectal effluent as compared with 83% without a meal, while Prince and Frisoli, (1993) found that administering β-carotene daily in three divided doses with meals increased the serum β-carotene concentration three times more than when the same total dose was administered once daily. Castenmiller and West, 1998 reported that when the amount of pro-vitamin A

ingested was small (<1 mg), most of it was converted to vitamin A in the intestines while with large amounts an increasing proportion of the absorbed carotenoid is found in the plasma and in the tissues. Data suggest that the concentration of α-carotene in plasma and chylomicrons and the concentration of lycopene in plasma reflect the content of these carotenoids in the meal or supplement (Carughi and Hooper, 1994; Gärtner *et al.*, 1996).

A study by FAO/WHO (1967) concluded that when a mixed meal was consumed 6 μg of β-carotene or 12 μg of other provitamin A carotenoids is absorbed and converted to 1 μg of retinol (FAO/WHO, 1967). However, in 1988 it was realized that retinol equivalence is related to dose level. When the β-carotene intake per meal is <1000 or >4000 μg, the amount of β-carotene equivalent to 1 μg of retinol was considered to be 4 and 10 μg, respectively (FAO/WHO, 1988). In rats, low intake of vitamin A increases cleavage activity as measured in vitro (Van Vliet *et al.*, 1996; Villard and Bates, 1986), and a high intake of β-carotene increases cleavage activity in the liver of rats. Based on the difference in liver vitamin A contents between β-carotene supplemented and unsupplemented rats, β-carotene conversion factors were estimated at 9:1 for rats fed high amounts of vitamin A and 4:1 for rats fed normal and low amounts of vitamin A. Intestinal β-carotene cleavage activity was higher in vitamin A deficient rats than in rats with a high intake of vitamin A or β-carotene. The addition of lutein to an incubation of β-carotene reduced retinal formation, whereas lycopene had no effect (Van Vliet *et al.*, 1996).

(d) Food Matrix

Disruption of the food matrix and release of carotenoids from their intracellular location constitute the first step in carotenoid absorption and hence its bioavailability. In green leaves, carotenoids exist within cell chloroplasts as pigment-protein complexes, which require disruption for the carotenoid to be released. In other vegetables and in fruits such as oranges, carotenoids are located in chromoplasts as lipid droplets from which they may be readily released.

The β-Carotene dissolved in oil is absorbed far more readily than β-carotene from foods. Compared with the serum response from β-carotene supplements, the bioavailability of β-carotene from various foods has been determined as follows: stir-fried vegetables, 7%, carrots, 18–26% and spinach, 7% (Castenmiller *et al.*, 1999; de Pee *et al.*, 1995; Brown *et al.*, 1989; Micozzi *et al.*, 1992; Törrönen *et al.*, 1996). For green vegetables, the leaf matrix seems to be an important factor that affects the accessibility of carotenoids, as it may hinder the release of the carotenoids during digestion (Erdman *et al.*, 1993; Van het Hof *et al.*, 1998).

Cooking however, assists in the release of carotenoids from the food matrix by disrupting cellular membranes and liberating nutrients so that carotenoids are absorbed more efficiently from cooked than from raw foods (Micozzi *et al.*, 1992; Van het Hof, *et al.*, 1998). The increased extractability from food matrix is associated with improved bioavailability of the carotenoids though when done excessively leads to oxidative destruction and isomerization of the carotenoid (Boileau *et al.*, 1999; Mosha *et al.*, 1997; Van het Hof *et al*, 1998; Rodriguez-Amaya, 1997). Other processing procedures such as mashing and juicing increase carotenoids bioavailability by destroying plant tissue structure thereby increasing surface area and interactions of hydrolytic enzymes and emulsifiers with food particles during the gastric and small intestinal phases of digestion (van het Hof *et al.*, 1998; van het Hof *et al.*, 2000a).

It has been reported that the absorption of carotenoids from carrots is greater than that from broccoli, which in turn is greater than that from spinach (Micozzi *et al.*, 1992; Castenmiller, *et al.*, 1999). To examine the effect of processing spinach and the dietary fiber on serum carotenoid concentrations a study was conducted where one group of subjects (n = 10) received a control diet over a three-week period, and other groups (n = 12 per group) a control diet supplemented with carotenoids or one of four spinach products; whole leaf spinach with an almost intact food matrix, minced spinach with the matrix partially disrupted, enzymatically liquefied spinach in which the matrix was further disrupted and the liquefied spinach (Castenmiller *et al.*, 1999). Consumption of spinach significantly increased serum concentrations of total β-carotene (all-*trans*-β–carotene, *cis*-β–carotene), lutein, α-carotene and retinol. Serum total β-carotene responses; changes in serum concentrations from the start to the end of the intervention

period, differed significantly between the whole leaf and liquefied spinach groups and between the minced and liquefied spinach groups. Addition of dietary fiber to the liquefied spinach had no effect on serum carotenoid responses. The study concluded that enzymatic disruption of the matrix (cell wall structure) enhanced the bioavailability of β-carotene from whole leaf and minced spinach.

A subsequent study in Indonesia (de Pee *et al.,* 1998) found that the serum response of β-carotene from fruits to be four times that from vegetables. In leaves, they are present in chloroplasts, whereas in fruits, and possibly also other parts of the plant, carotenoids are located in chromoplasts. This has led to the speculation (de Pee *et al.*, 1998) that chloroplasts may be less efficiently disrupted in the intestinal tract than chromoplasts. The low bioavailability of carotenoids from DGLVs may be attributed to their entrapment and complexing to proteins in chloroplasts and within cell structures. Such entrapment may not only be physical (matrix effect) but also molecular (effector effect). The α- and β-carotene in carrots exist as crystals up to 1000 μm in length. Although soluble in the intestinal contents, the transit time is probably insufficient for extensive solubilization to take place during this passage through the intestinal tract. Similarly, lycopene also exists in tomatoes in the crystalline form. In orange and yellow fruits (mango, papaya, etc) and in pumpkin and sweet potato, carotenoids are dissolved in oil droplets in chromoplasts and can be readily extracted during digestion. Cooking increases the bioavailability of carotenoids, possibly because of the softening or disruption of plant cell walls and the disruption of carotenoid-protein complexes (Erdman *et al.*, 1988). Stahl and Sies (1992) reported that lycopene concentrations in human serum increased from processed but not unprocessed tomato juice thus the bioavailability of lycopene was greater from tomato paste than from fresh tomatoes (Gartner *et al.*, 1997).

A study observed that the plasma response of lutein was significantly increased when spinach was consumed as chopped spinach instead of whole leaf spinach whereas the plasma response of β-carotene was not affected (Castenmiller *et al.*, 1999). The explanation was that either the different lipophilic character of the two carotenoids results in a greater release of lutein in response to homogenization or homogenization releases both carotenoids to the same extent but lutein inhibits β-carotene absorption. However,

the disruption of the matrix of spinach by enzymatic treatment and the nature and surface area of the food matrix has been found to enhance the plasma response of β-carotene but not that of lutein (Kostic *et al.,* 1995; van het Hof *et al.,* 1999a; Castenmiller *et al.*, 1999). The presence of dietary fiber in vegetables and fruits may explain in part the lower bioavailability of carotenoids from plant foods because fiber interferes with micelle formation and the partitioning bile salts and fat in the gel phase of dietary fiber (Boileau *et al.,* 1999).

The effect of the matrix on bioconversion is almost certainly limited to phenomena that take place in the lumen of the small intestine (Castenmiller and West, 1998). The reported effects of the matrix on the overall yield of retinol from carotenoids can be attributed to matrix effects on bioavailability. dee Pee *et al.*, (1998), calculated that 1 μg of retinol was provided by 26 μg of β-carotene from DGLVs and carrots and by 12 μg from yellow and orange fruits.

(e) Effectors of Absorption and Bioconversion

A variety of nutrients consumed together with carotenoids affect carotenoid absorption, metabolism, and/or bioconversion (West and Castenmiller, 1998; Olson, 2000). The presence of protein in the small intestine helps stabilize fat emulsions and enhances micelle formation and carotenoid uptake. A decrease in carotenoid bioavailability may be caused by interaction in the gastrointestinal tract with drugs or constituents of foods, such as sulfides and acids (Tang and Serfaty, 1995). Tang and Serfaty (1995) found that gastric pH plays a role when a single 120-mg dose of β-carotene increased plasma concentrations of β-carotene at normal gastric pH to a level twice as high as that at a gastric pH of 6.4.

Dietary fat has been found to increase carotenoid bioavailability by providing a depose for hydrophobic compounds released from the food matrix, stimulating the secretion of bile salts and pancreatic lipases required for micelle formation, and inducing chylomicron synthesis (Borel, 2003). The absorption and bioconversion of β-carotene is markedly reduced when the intake of fat is low (Jialal *et al.*, 1991; Prince and Frisoli, 1993).

Addition of a small quantity of fat to the diet greatly improves the absorption of vegetable carotenoids with optimal absorption requiring a minimum intake of 5 g of fat per day (Jayarajan *et al.,* 1980; Mulokozi *et al.*, 2004). A minimum amount of fat is therefore necessary for adequate micelle formation and for uptake of carotenoids although there is no dose-response relationship above the threshold value, but there is some increase in serum β-carotene response at a high-fat diet (Dimitrov *et al.*, 1988; Nierenberg *et al.*, 1991; Shiau *et al.,* 1994; Roodenburg *et. al.*, 2000).

Studies assessing the importance of dietary fat in comparison with its complete absence at the moment of ingestion indicate that absorption of β-carotene seems to be sub optimal because the increase in plasma concentration improved substantially when fat was added to the test meals (Mulokozi *et al.*, 2004; Takyi, 1999; Roodenburg *et al.,* 2000). Mulokozi *et al.*, (2004) measured β-carotene and α-carotene content and their *in vitro* bioavailability in green leafy vegetables and concluded that the bioavailability was significantly greater when vegetables are cooked with oil than without. A study carried out by Takyi, (1999) determined that the consumption of leafy vegetables by preschool children enhanced their serum vitamin A concentration to acceptable levels. The preschool children (n = 519) of 2.5 to six years old were randomly assigned to five feeding groups, differing essentially in the amount of fat and β-carotene, fed once per day, for 3 months. Serum retinol levels, among others were determined before and after study. Relative to the baseline serum retinol values, consumption of DGLVs (*Manihot sp.* and *Ceiba sp.*) with fat (10 g/100 g) significantly ($P < 0.05$) enhanced serum retinol and consequently, the percentage of children with adequate retinol status increased from 28.2 to 48.2% ($P < 0.05$).

Roodenburg *et al.* (2000) assessed the effect of the amount of dietary fat on plasma concentrations of vitamin E and carotenoids during two seven day periods using 4 groups of 14–15 volunteers who received daily a low-fat hot meal containing one of the four different supplements: vitamin E, α- plus ß-carotene, lutein esters, and placebo. The supplements were provided in a low- or high-fat spread supplied in random sequence during either of the 2 experimental periods. Plasma concentrations of vitamin E, α- and β-carotene, and lutein were significantly higher in the supplemented groups than in the placebo group. The amount of dietary fat consumed with the hot meal did not affect the

increases in plasma concentrations of vitamin E or α- and ß-carotene. The plasma lutein response was higher when lutein esters were consumed with the high-fat spread than with the low-fat spread. This led to conclusion that optimal uptake of vitamin E and α- and β-carotene requires a limited amount of fat whereas the amount of fat required for optimal intestinal uptake of lutein esters is higher.

The status of other nutrients, particularly iron deficiency, may also negatively affect serum retinol concentrations as shown in both human (Suharno *et. al.*, 1993; Tanumihardjo, *et al.*, 1996; Jalal *et al.*, 1998; de Pee *et al.*, 1998; Takyi, 1999; Rosales, *et al.*, 1999; Vuong *et al.*, 2002) and animal studies (Roodenburg *et al.*, 1996a; Roodenburg *et al.*, 1996b). Iron and zinc deficiencies are widespread and often co-exist with VAD. VAD is known to result in mild anemia and serum retinol levels have been positively correlated with the biochemical indices of iron status, including hemoglobin. β-carotene affects iron metabolism independently of the benefit of retinol by improving the iron absorption (Garcia-casal *et al.*, 2000) or by protecting erythrocytes from oxidative damage by free radicals (Garcia-casal *et al.*, 1998).While adequate protein and zinc intake assists maintenance of vitamin A, vitamin E as an antioxidant protect vitamin A from being oxidized (Nierenberg *et al.*, 1994). Dietary fiber, chlorophyll and non pro-vitamin carotenoids, which are commonly present in the diet, tend to reduce bioavailability of pro-vitamin A compounds while alcohol interferes with the conversion of beta-carotene to vitamin A (Deming *et al.*, 2000; Roodenburg *et al.*, 2000; Albanes *et al.*, 1997; Lecomte *et al.*, 1994; Forman *et al.*, 1995).

(f) Nutrient status of the host

The absorption of carotenoids is likely to be dependent on vitamin A status. Consumption of β-carotene–rich foods leads to an increase in serum retinol levels only when these are initially low (Castenmiller and west, 1998). Many studies have shown that consumption of carotene-rich plant foods can improve serum retinol concentrations (Jalal *et al.*, 1998; Takyi, 1999; Hussein and El-Tohamy, 1989; Reddy and Vijayaraghavan, 1995) although others, showed either no effect of plant foods on serum retinol (Micozzi *et al.* 1992; Bowen *et al.*, 1993; Bulux *et al.* 1994; de Pee *et al.*, 1995;

Yeum *et al.* 1996) or only small improvements that were much less than predicted on the basis of conventional carotenoid conversion factors (de Pee *et al.*, 1998). The lack of effect of plant carotene ingestion on serum retinol found by some investigators may have been because the subjects had a satisfactory vitamin A status (Ribaya-Mercado *et al.*, 2000). Moreover, in healthy individuals, serum retinol concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A are dangerously low (Castenmiller and West, 1998).

Ribaya-Mercado *et al.*, (2000), in attempting to understand the factors affecting strategies to improve the vitamin A status of populations, studied the effect of vitamin A status on the bioconversion of plant carotenoids to vitamin A. In this study, two separate, unrelated groups of 7–13 year-old children with poor or marginal serum retinol concentrations (0.32–0.93 μmol/L) were fed with controlled diets daily for 5 days per week for 12 weeks, after treatment with an anthelminthic drug. The children in one school (n = 27), were provided with a diet which contained retinol equivalents, mostly from orange fruit and vegetables and those in the second school (n = 25) had diets which contained no carotenes. Both mean serum β-carotene and serum retinol increased significantly (P = 0.0001) in the vegetable school. The study also found significant but lower increases in serum β-carotene and serum retinol in the school that had recipes containing no carotenes. The study concluded that bioconversion of plant carotenoids to vitamin A varies inversely with vitamin A status and that improvement in status after dietary interventions was strongly influenced by total body stores of vitamin A.

(g) Host related factors

The absorption of dietary carotenoids and their bioactive products is modulated by phenotypic characteristics of the host that affect processes associated with digestive and absorptive events. These include the composition and activity of luminal fluids and the morphological and functional integrity of the absorptive epithelium (Olson, 1999). Host-related factors may explain many of the differences observed in the serum response to ingestions of dietary carotenoids (Castenmiller and West, 1998; Gessler *et al.*, 1998; Hadi

et al., 2004). The serum response to beta-carotene is higher in women than in men though the reason is not known while age does not appear to be a factor (Olmedilla *et al.*, 1994). In several ways, men are more susceptible to develop VAD diseases than women.

Factors like respiratory infections and recurrent diarrhea, which is prevalent among children in developing countries, may severely affect the absorption and utilization of beta-carotene (Kücükbay *et al.*, 1997; Alvarez *et al.*, 1995; Stephensen *et al.*, 1994). Infections can also result in VAD cases through decreased absorption, increased utilization, and increased excretion of vitamin A and through reduced overall food intakes. Various diseases including anemia, malaria, diarrhea, measles, respiratory infections and parasitic infections are risk factors for VAD (Ahmed *et al.*, 1993; Tanumihardjo *et al.*, 1996; Gamble *et al.*, 2004; Filteau *et al.*, 1994; Quilan and Hayani, 1996; Friis *et al.*, 1996; Cser *et al.*, 2004)

Absorption of fat-soluble substances including carotenoids is impaired in a condition in which there is fat maldigestion and malabsorption such as intestinal parasitism (Alvarez *et al.*, 1995; Ahmed *et al.*, 1993). The metabolism of carotenoids by parasites residing in the intestinal lumen, the parasite-associated changes the numbers and maturation of absorptive cells along the villi, and the cytokine-mediated decreases in lipid absorption associated with parasite infection may all contribute to a decline in carotenoid absorption (Olson, 1999). Parasitic infestation remains one of the main problems of child development and a greater health hazard in developing countries (Watkins *et al.*, 1996; Kightligher *et al.*, 1995; Kightligher *et al.*, 1996). Protozoa or worms gaining entry into the body cause parasitic infestations. Most of these organisms cause infections by being ingested in the form of eggs or larvae, usually present on contaminated food or clothing, while others gain entry through skin abrasions (Anderson and May, 1991). Common parasitic infestations include amebiasis, giardiasis, hookworm, pinworm, threadworm, whipworm and tapeworm infestations. Antihelminthic drugs destroying the worms on contact or by paralyzing them, or by altering the permeability of their plasma membranes are usually prescribed for parasitic infestations. Most Antihelminthic drugs including albendazole, mebendazole and niclosamide are only active against specific parasites while some are toxic, thus before treatment, the parasites must be identified in feces, urine, blood, sputum, or tissues.

Studies have shown that absorption and utilization of beta-carotene are enhanced after de-worming of children infected with parasites (Jalal *et al.,* 1998) and that vitamin A supplementation of anemic subjects with a marginal vitamin A status can result in an increased hemoglobin level (Suharno *et al.,* 1993). However, the study by Takyi, (1999) that determined whether the consumption of leafy vegetables by preschool children enhanced their serum vitamin A concentration to acceptable levels found no significant differences among groups, ages or pre- versus post-anthropometric measurements, haemoglobin concentration, or levels of worm infestation. Friis *et al.* (1996) observed that a high intensity of parasite infection induces VAD while Tanumihardjo *et al.* (1996) found that VAD was commonly observed in children infected with parasites. Parasitic infections reduce vitamin A absorption by as much as 70% since the utilization of vitamin A is increased during parasitic infection (Mahalanabis *et al.,* 1979).

(h) Interactions between Carotenoids

Common carotenoids such as beta-carotene, alpha-carotene, lutein, zeaxanthin, lycopene and cryptoxanthin are commonly consumed in diets and are also prevalent in human plasma (Rock and Swendseid, 1992; Yong *et al.*, 1994; Khachik *et al.*, 1992c). The interactions among carotenoids at the intestinal level may reduce absorption of either of the carotenoids due to competition for absorption at the level of micellar incorporation, intestinal uptake, and lymphatic transport, or at more than one level (Kostic *et al.,* 1995).

Simultaneous ingestion of various carotenoids on the other hand may induce an antioxidant-sparing effect in the intestinal tract and thus result in increased levels of uptake of the protected carotenoids. A similar phenomenon may occur within the body, with respect to both sparing of antioxidant capacity and pro-vitamin A activity, and thus result in an enhanced status of carotenoids (Castenmiller and west, 1998). Possible sites for pre-absorption interactions between carotenoids include their competition for incorporation into micelles, uptake from the micelle by intestinal cells and incorporation into chylomicron (van den Berg, 1999). Absorption interactions between the different carotenoids are evident in several studies, although not all studies gave comparable

results (Micozzi *et al.,* 1992; Nierenberg *et al.,* 1994; Kostic *et al.,* 1995; Fotouhi *et al.,* 1996; van den Berg, 1999; Huang *et al.*, 2000; Tyssandier *et al.*, 2003).

Micozzi *et al.,* (1992) suggested an interaction between beta-carotene and lycopene when serial changes of four major plasma carotenoid fractions (alpha-carotene, beta-carotene, lutein/zeaxanthin, and lycopene) were determined in 30 men consuming defined daily doses of carotenoids from foods (broccoli, carrots, or tomato juice) or from purified beta-carotene in 12 or 30 mg capsules for six weeks. Beta-carotene increased in the capsule and carrot groups whereas alpha-carotene increased in the carrot group and lutein in the broccoli group. The study found lower lutein concentrations in recipients of beta-carotene capsules, which suggested an interaction between the two carotenoids. Tyssandier *et al.*, (2003) reported that the absorption of beta-carotene, lutein and lycopene from a single vegetable was greater when the food was administered alone than when it was co-administered with either a second carotenoid rich vegetable or the purified carotenoid from the second vegetable.

The β-carotene supplementation reduced the lycopene concentrations in LDL and serum significantly (Gaziano *et al.,* 1995; Prince and Frisoli 1993. In contrast, Wahlqvist *et al.*, (1994) found an increase in lycopene concentration after β-carotene supplementation, but only in men. Fotouhi *et al.*, (1996) showed no effect of β-carotene on serum levels of α-carotene, cryptoxanthin, lycopene, and lutein while at the same time Gartner *et al.,* (1996) showed that in the presence of high amounts of β-carotene, the uptake from the intestinal lumen into chylomicrons of lutein and zeaxanthin as compared with all-*trans*-β-carotene was preferred. While α-carotene concentrations were increased after β-carotene supplementation (dee Pee *et al.*, 1995; Micozzi *et al.*, 1992; Wahlqvist *et al.*, 1994), the ingestion of concurrent doses of β-carotene and canthaxanthin reduced the peak serum canthaxanthin concentration, but canthaxanthin did not inhibit the appearance of β-carotene in serum (White *et al.*, 1994).

2.2 Preservation of Vegetables

In developing countries, foods of plant origin particularly fruits and vegetables are the principle source of the micronutrients, especially the pro-vitamin A compounds. The African diet, rich in dark green leafy vegetables (DGLVs) is the most variable because it contains provitamin A carotenoids, vitamin C, protein, calcium and iron (Kordylas, 1990; Conning, 1991). However, the availability and consumption of fruits and vegetables is seasonal, with limited intake in arid and semi arid parts of a country and this affects the nutritional status of the people. During rainy seasons they are consumed fresh in large quantities but are scarce or not available during the dry seasons. Due to their high moisture content, they are highly perishable and can only be made available during the time of need by preservation.

Perishable foods get spoiled through autolysis, an enzymatic effect which comes after termination of plant growth or through microbial attack which is from invasion by bacteria and fungi (Conning, 1991). Food preservation processes are thus meant to minimize these spoilage processes, which are estimated to be between 40-50% in many developing countries (Jayaraman and Das-Gupta, 1992). Preservation converts perishable foods into stable products, which can be stored for longer periods. This changes them into new or more usable forms and makes them more convenient to prepare.

Preservation by exclusion of water is an important process since all active processes require water, which can be excluded by osmosis, freezing or drying. However, the main preservation methods for vegetables are, canning, freezing, fermentation and dehydration; which includes techniques that alter the storage conditions (Kordylas, 1990; Conning, 1991; Fontana, 1998; Earle and Earle, 1983; Rockland and Nishi, 1980; Levine and Slade, 1991). Dehydration is best suited for developing countries where facilities for other methods are poorly established.

2.2.1 Dehydration Modes and Effects on Carotenoid Content

The success of any drying operation depends on achieving a low water activity that does not allow microbiological growth to occur. Water activity which is the ratio of the vapor pressure above a complex mixture to that above pure water at constant temperature, is closely related to the actual water availability for microbiological growth, and hence food spoilage (Levine, and Slade, 1991; Earle and Earle, 1983). Water activity is important for characterizing the state of water in foods such as its availability for biological, physical and chemical changes. Most bacteria do not grow below water activity of 0.91, while yeasts do not grow below 0.88, although some organisms such as halophyic bacteria and osmophilic yeasts can grow to about 0.60 (Rockland and Nishi, 1980). The degree of water interaction with food components and its contribution to food texture is determined by both the moisture content and the water activity.

There are complex changes that occur in the food products during dehydration. These changes include simultaneous transfer of heat and mass in which heat is penetrated into the product and moisture is removed by evaporation into the surrounding medium. Vegetable cells contain large quantities of water (90-96% water content), which play a vital role in the evolution and reproduction cycle (Rockland and Nishi, 1980; Levine, and Slade, 1991; Earle and Earle, 1983). It has effects on the storage length and on the tissue reserve substances.

Dehydration is the oldest method of food preservation practiced by mankind. Natural sunlight is an important source of both energy and light and this has been used for drying of crops throughout the world for centuries. Kordylas (1990) states that dehydration is the best preservation method suited for developing countries where facilities for other methods are poorly established. Simple dehydration techniques are used in rural areas of many developing countries as a means of reducing waste of the highly perishable vegetables and ensuring their availability throughout the year (Kordylas, 1990; Mulokozi *et al.*, 2000). The dehydration that uses low heat during the drying cycle and the gentle airflow has between 3-5% loss of nutrients (Conning, 1991; Earle and Earle, 1983).

Dehydration involves the removal of moisture to prevent the growth and reproduction of microorganisms and to minimize many of the moisture mediated deteriorative reactions. Blanching is performed before dehydration because as it is considered necessary in controlling actions of enzymes and to reduce the initial concentration of organisms. It also partially cooks the plant tissues and renders the cell membranes more permeable to moisture transfer during drying, besides preserving odour, flavor, texture, color and ascorbic acid content.

Dehydrated products have less weight and volume making them easier to pack, store or even to transport them. However, drying and dehydration involve physical and structural changes including shrinkage of cells, loss of rehydration ability, and migration of solids, case hardening and loss of volatile aroma compounds which may have great effects on the sensory characteristics of the dried product (Fontana, 1998; Earle and Earle, 1983; Nyambaka, 1996).

Dehydration is an inexpensive low-level technology method that can adequately preserve DGLVs and make them available for consumption during dry seasons and in areas away from the source. The technology though has not been well adopted in Sub-Saharan African countries including Kenya, partly due to lack of information and promotional activities. Okalebo and Hankins (1997) reports that Kenya incurs post harvest losses of between 30-40% of her agricultural produce.

The basic types of drying processes recognized are sun drying and solar drying, atmospheric drying such as batch and continuous drying, sub-atmospheric dehydration such as freeze drying, and the low temperature/energy dehydration such as osmotic dehydration (Jayaraman and Gupta, 1992; Earle and Earle, 1983). Several drying methods and dryers are available for use in the dehydration of a variety of food products including leafy vegetables. The selection of a particular drying method or dryer depends on such factors as the nature of the raw material and its properties, the desired form and characteristics of the product, the operating conditions and costs (Jayaraman and Gupta, 1992).

Efforts to improve the sun drying procedure have led to the development of solar dryers. The solar dryers use the sun as the heat source, but are specially designed to increase the

temperature and air current to speed up the drying time. Shorter drying times reduce the risk of food spoilage or moulding. Dryer designs are classified based on either the mode of using solar radiation, in which case they are either direct or indirect dryers, or by the mode of airflow, as natural or artificial convection dryers. Drying chambers are meant to minimize problems experienced in open-air methods and effectively utilize solar energy. Two types of dryers are discussed; the direct dryer and the indirect dryer.

(a) Open-Air Solar Dehydration

The use of open sun drying continues to be a common method for preservation of agricultural products in developing countries (Maundu *et al.*, 1999; Prasad, 2005; Bassey, 1992). Traditional sun dryers consist simply of any suitable drying surface exposed to solar radiation such as a bare ground, and or an aluminum sheet lay on the ground or raised above the ground. The food is thinly spread and the rate of drying depends on the availability of radiation and the temperature of the underneath surface.

Although sun drying is simple and cheap, and faces many drawbacks including possible contamination by dirt or rodents, infestations by insects, exposure to weather elements, animals, human beings, causing spoilage and loss, and uncontrolled drying conditions, it is still widely practiced (Bassey, 1992; Bolin and Salunkhe, 1982). Despite these limitations, various foods such as fruits, vegetables, meat, fish grain and spices have been successfully dried by this method. The quality of these products may generally be acceptable although improvements are possible. The bioavailability of sun-dehydrated products has also not been exclusively investigated.

The study by Omueti *et al,* (1983) in which fresh leaves, sun-dried leaves, blanched refrigerated leaves and blanched frozen leaves of Amaranthus *spp.*, were analysed for their total carotenoid contents for over 4 weeks period of storage shows that extractable total carotenoids of the fresh vegetables increased with blanching and sun drying. Slight losses occurred in the blanched vegetables during storage in the refrigerator and freezer but the carotenoid content of the sun-dried materials was unaffected by storage at room temperature. The study concludes that sun-dried and freezer-stored vegetables retained

more of the visual and culinary properties than those stored in the refrigerator (Omueti *et al.*, 1983).

(b) Direct Solar Dehydration

The direct solar drying method is a modification of the open-air drying in which air is heated to a higher temperature than the ambient temperature. A simple model of direct dryer consists of an enclosed chamber with a transparent cover and the food trays placed directly beneath the cover. Solar energy, absorbed by both the food and the internal mass of the dryer elevates the temperatures through the greenhouse effect. The heated air is less dense than the ambient air and therefore escapes through the outlets at the upper part while ambient air enters through the inlet vents located at the lower sides of the chamber, thus establishing a continuous flow of air through the dryer (Bolin and Salunkhe, 1982).

The effectiveness of such a dryer depends on maximizing temperatures in the chamber. High temperatures reduce the relative humidity of the air and thus enable it to pick more moisture. They can be attained by insulting the sides and the base of the chamber to reduce loses of heat, and proper sealing of the cover to prevent uncontrolled movement of air. However, high temperatures cause rapid removal of moisture which results in case-hardening, the movement and deposition of food components, such as salts and protein, on the surface as water difficult, a situation which increases the temperature of the food and results in reduced quality. The other conditions that affect the rate of drying are air velocity and relative humidity. In a natural convection dryer, the airspeed depends on the extent of pressure drop created. The rate of evaporation of water depends on the partial pressure of the water vapour in the air around the drying food and in the surface of the food. The more dry the air, the faster the rate of evaporation. High relative humidity prolongs drying, resulting in food spoilage before dehydration is achieved (Bolin and Salunkhe, 1982).

Nyambaka and Ryley (2001) used a solar dryer when analysing pro-vitamin A carotenes in dehydrated and stored cowpeas. The findings (**Table 2.5**) concluded that solar-

dehydration was a promising means of preserving vegetables since the solar dehydrated vegetables retain high amounts of pro-vitamin A carotenoids. These findings are supported by Joshi (2004) that states that beta-carotene losses could be minimized by almost 50% by drying vegetables in a solar dryer. The alpha-carotene, beta-carotene and total provitamin A carotenoids and the effect of traditional processing practices on the retention of these provitamins were studied using amaranth, cowpea, peanut, pumpkin and sweet potato leaves by Mosha *et al.* (1997). Results in this study indicated that the content of total carotenoids, beta-carotene and alpha-carotene were in the range of 26.79-44.74 mg, 4.16-19.12 mg, and 0.99-10.26 mg per 100 g of dry vegetables, respectively. The traditional processing practices of sun drying and storage in ventilated containers resulted in a significant ($p < 0.05$) decrease in the concentration of total carotenoids, beta-carotene and alpha-carotene for all the vegetables while conventional blanching and cooking resulted in a significant ($p < 0.05$) increase in the concentration of carotenoids in the cowpea, peanut and pumpkin leaves while in amaranth and sweet potato greens, thermal processing resulted in a significant ($p < 0.05$) decrease in the concentration of these nutrients (Mosha *et al.*, 1997).

Mulokozi *et al.* (2000) assessed the suitability of using an improved solar drying technology among rural women and reports that women preferred the technology, being more hygienic and foods retained their original color, flavor and nutritive value. Vegetable dried in improved driers retained 20-37% more carotene than did traditionally dried vegetables (Table 2.6).

Table 2.4:- Pro-vitamin A and the vitamin A content[1] of dehydrated and stored cowpea leaves

Product	Pro-vitamin A content (μg/g DM)[2]						Vitamin A content (μg/g DM)
	All-trans-β-	9-Cis-	13-cis-	5,6-E-	5,8-E-	All-*trans*-α-	
F-dried-0	776	95	39	Nd	nd	50	860
F-dried-16	148	16	7	7	18	12	173
S.s-dried-0	646	91	39	nd	5	52	731
S.s-dried-16	167	17	8	8	14	8	190
S-dried-0	599	78	30	nd	7	26	660
S-dried-5	252	27	16	7	12	10	293
Sun-dried-0	520	68	36	6	18	18	585
Sun-dried-5	250	30	20	7	16	7	286

F-dried = freeze dried; S.s-dried = simulated solar dried; S-dried = solar dried. 0 = immediately after dehydration; 5 = 5 months storage in laminated bags at room temperature; 16 = 16 months storage in vacuum tins at 5°C, nd = not detected; DM = dry matter; [1]Expressed as all *trans*-equivalent; [2]Mean of three samples from the same batch.

Source: Nyambaka and Ryley (2001).

Table 2.5: Beta-carotene content of green leafy vegetables (mcg/g) blanched then processed[1]

	Not dried	Sun-dried	Solar-dried
Ngwiba	554	308(56%)	499(90%)
Cowpeas	526	296(65%)	462(88%)
Mganani	917	484(53%)	776(85%)
Pumpkin	592	287(49%)	426(72%)
Sweet potatoes	715	389(54%)	470(66%)
Amaranthus	677	368(54%)	449(66%)
Maimba	588	305(52%)	330(56%)

[1]values in parenthesis are % retentions based on the not-dried vegetable

Source: Mulokozi *et al.* (2000)

(c) Indirect Solar Dehydration

Some foodstuffs are affected when dried in direct sunlight, either by bleaching or losing some of the nutrients, especially vitamins. In direct drying methods, the use of black polythene covers instead of transparent ones minimizes the effect of direct sunlight coming into contact with the foods (Bolin and Salunkhe, 1982). Indirect solar dryers normally consist of three main parts; an air heater, a drying chamber, and a device to induce airflow, usually a chimney. The air used for drying is heated in a solar collector and then circulated through the foodstuff. The heated air accelerates the drying rate in two ways: by transferring some of its heat to the product being dried, thus raising its vapour pressure causing faster moisture loss; and by increasing its water holding capacity as the temperature of air mass increases.

The airspeed in natural convection solar dryers is normally low, affecting the performance of such dryers. Various technological improvements, such as the use of the chimney, varying the amount of air entering the chamber and using slanted heating chambers have been made to improve airspeed and thus the efficiency of these dryers (Mulokozi *et al.*, 2000). Changing the cross-sectional area of the heater inlet to influence the temperature airspeed relationship by creating an additional pressure drop can regulate the amount of air flowing in the heater. Reducing the inlet cross-section area decreases the airspeed and increases the temperature of the air (Bolin and Salunkhe, 1982). This lowers the relative humidity of the air at the heater outlet, enabling the drying power of air to increase and thus increases the rate of drying. However, bioavailability of carotene in these products has not been investigated hence the need for this study.

2.3 Instruments of Analysis

2.3.1 Questionnaire

A questionnaire is a type of statistical survey handed out in paper form usually to a specific demographic population to gather information (Willett, 1998). Questionnaires are an inexpensive way to gather data from a potentially large number of respondents and can be

used to correlate performance with a test system. Questionnaire for dietary assessment are used to estimate the nutrient or food intake of an individual or a group and also aid an analyst to make decisions on various issues including selection of the food items to be used in an intervention (Willett, 1998; Drewnowski and Hann, 1999). This is self-reported by individuals since direct observation of intake by trained observers is impractical. These have been used in dietary intervention trials, particularly when the goal is to measure dietary intake rather than an estimate of usual intake and includes the ability to more accurately quantify intake of food groups and to describe intake patterns as well as to explore attitudes, knowledge and awareness (Cynthia, *et al.,* 2003; Perez-Rodrigo, *et al.,* 2003; Willett, 1998; Drewnowski and Hann, 1999).

Dietary risk is among the categories of nutrition risk, but since it is the basis for eligibility for participation in an intervention study therefore dietary information is particularly important (Bartlett, *et al.,* 2000). Dietary intake data are collected with the main purposes: (1) for determining dietary risk for eligibility purposes (2) as a starting place for nutrition education, and (3) for tailoring food packages. Strategies addressed to improve acceptance for a food group should be considered when designing questionnaires aimed at promoting adequate consumption of the foods among target population. Food preferences, food consumption and practices as well as nutrition-related information are assessed by means of a questionnaire (Cynthia *et al.,* 2003; Perez-Rodrigo *et al.,*_2003) and remain the principal method for assessing diet since it may aid in capturing dietary intake over time. Questionnaire format and participant motivation among other issues can influence the ability to gather reliable and valid self-reported dietary intake data (Bartlett *et al., 2000).*

2.3.2 Hemoglobin Analysis

Hemoglobin (Hb) is the iron-containing oxygen-transport metalloprotein in the red cells of the blood in mammals and other animals (Campbell, 1999). Its decreased levels, with or without an absolute decrease of red blood cells, leads to symptoms of anemia. Anemia has many different causes, although iron deficiency and its resultant iron deficiency anemia are the most common causes. Anemia therefore is a condition characterized by a reduction in the

volume of the red blood cells and consequently, a decrease in the concentration of the hemoglobin (Hb) in the blood. The levels of anemia can be classified as severe (Hb, <7.0 g/dl), moderate (Hb, 7.0- 9.9 g/dl), or mild (Hb, 10.0 to 10.9 g/dl), for pregnant women and children(WHO, 1996). Hemoglobin levels are amongst the most commonly performed blood tests, usually as part of a full blood count or complete blood count with results reported in g/L, g/dL or mol/L. It has been noted that there exists a correlation of anemia, parasitic infestation and vitamin A status (Suharno *et. al.*, 1993; Tanumihardjo *et al.*, 1996; Jalal *et al.*, 1998; de Pee *et al.*, 1998; Takyi, 1999).

A hemoglobin analyzer is used for Hb analysis as part of a full blood count to measure an individual's blood so as to obtain the anemia condition. The Hemoglobin Analyzer system consists of a battery-operated photometer and a disposable microcuvette, coated with a dried reagent that serves as the blood-collection device. It can be safely operated in field studies in temperatures of between 15-40°C. The analyzer measures light absorption and presents the results on a display. The microcuvette is a plastic disposable unit that serves both as a reagent vessel and a measuring device. It contains a yellow reagent (sodium azide) in dry form. The humidity sensitive microcuvette is designed to draw up the exact amount of blood required using a disposable hemoque safety lancet which is an automated, disposable incision device used to obtain blood samples from the fingertip. The device is shaped to fit easily on the skin surface, thus minimizing skin indentation. When the trigger is pressed, a surgical blade with an angle set for maximum blood flow, quickly protrudes from the device and then automatically retracts.

2.3.3 Chromatographic Analysis of Carotenoids and Retinoids

Chromatography, introduced by Tswett for separation of plant pigments (including carotenoids), has become an indispensable analytical method for separation of carotenoids and vitamin A compounds. The early open-column chromatography (OCC) methods have been superseded by the more efficient High Performance Liquid Chromatography (HPLC) technique, with reversed-phase HPLC (RP-HPLC) stationary phase coupled with spectrophotometric and fluorometric detection as the current method (Rock and Swendseid,

1992; Khachik *et al.*, 1997; Rock *et al.*, 1998; Tučková and Kaštel, 1999; Castenmiller *et al.*, 1999; Takyi, 1999; van het Hof *et al.*, 1999a,b; Nyambaka and Ryley, 2001; Méndez *et al.*, 2000; Craft *et al.*, 1990; Hudon *et al.*, 2003; Mulokozi, 2004; Seo *et al.*, 2005). This is due to the advantages of HPLC which include its high speed, resolution, sensitivity, accuracy and its automated systems. Gas chromatography has little use for analysis of carotenoids and retinoids because of the thermal instability and limited volatility. Other techniques, such as mass spectroscopy (coupled with HPLC), immunoassays, supercritical fluid chromatography, and capillary electrophoresis, though, may also be useful in certain applications (Pfander and Niggli, 1995; Shi, *et al.*, 1995; Song, *et al.*, 2000).

The chromatographic procedures for analysis of carotenoids and retinoids follow basic steps upon which various modifications have been made (Rodriguez-Amaya, 1999). The general procedure usually consist of sampling, extraction, partition to a solvent compatible with the subsequent chromatographic step, saponification and washing, concentration or evaporation of solvent, chromatographic separation, identification and finally quantification.

(a) Extraction

The purpose of extraction is to release the compounds from the matrix into an extraction solvent without altering them (Rodriguez-Amaya, 1999). The procedure is adapted to suit the matrix being analyzed using solvents with low boiling points to avoid prolonged heating during evaporation. Acetone has widely been used as an extraction solvent although tetrahydrofuran (THF) is also a popular extraction solvent. For biological samples, which normally contain large amounts of water, extraction is performed using a water-miscible organic solvent (e.g acetone, methanol, ethanol or mixtures) to allow better solvent penetration; with dried samples are preferably re-hydrated before extraction. During extraction sample(s) may be homogenized in a blender with the solvent for 1-2 minutes with addition of a neutralizing agent (such as magnesium carbonate) to neutralize the acids liberated during tissue disintegration in order to prevent isomerization and degradation. Solid samples are then filtered and the residue re-extracted with fresh solvent until the sample residue is colorless (Rodriguez-Amaya, 1999).

In biochemical assessment, extraction of blood serum is done using an organic solvent such as petroleum ether or hexane after samples are treated with ethanol or methanol or perchloric acid to precipitate protein (Britton, 1995a; Craft, 2001).

(b) Partitioning

The extract usually contains a substantial amount of water, which is removed by partitioning to hexane, petroleum ether, diethyl ether, or dichloromethane or a mixture of these solvents. Extracts with large amounts of xanthophylls are preferably partitioned with solvent mixtures rather than pure hexane. The water miscible solvent is removed by washing it out severally with water. Partitioning is an important step in open column methods, where a low mobile phase polarity is required, which then is increased in the separation process. In HPLC methods, the extract may be evaporated to dryness and re-dissolved in the mobile phase or a solvent compatible with the mobile phase (Rodriguez-Amaya, 1999).

(c) Saponification and Washing

Basic saponification has long been an integrated part of both vitamin A (retinol) and carotenoid analysis (Tee and Lim, 1991; Tee and Lim, 1992; Tee, 1992; Kimura *et al.*, 1990; Granado *et al.*, 2001; Rizzolo and Polesello, 1992; Riso and Porrini, 1997). In carotenoid analyses, saponification is carried out with the dual aim of eliminating chlorophylls that interfere in spectrophotometric assay of carotenoids and xanthophylls and of hydrolyzing carotenoid esters and removing unwanted lipids (Kimura *et al.*, 1990; Granado *et al.*, 2001; Lesellier *et al.*, 1993). This step may be carried out directly in the homogenized matrix but is also frequently performed after extraction with an organic solvent. For more fibrous vegetables, in which pigments are not directly accessible, saponification is often done after extraction to reduce the saponification time and hence the possibility of degradation of carotenoids.

Saponification is usually performed with methanolic sodium or potassium hydroxide (5-30%) which is added to the pigment extract, and the mixture stirred in open container under an atmosphere of nitrogen or under reflux for a period (Kimura, *et al.*, 1990; Granado *et al.*, 2001; Riso and Porrini, 1997; Lesellier *et al.*, 1993; Lessin *et al.*, 1997; Britton, 1995a). This can therefore be performed either cold (overnight in the dark) or hot (Larsen and Christensen, 2005). The alkali is then washed off and the residue dried with anhydrous sodium sulfate. A decrease in the carotenoid content has been observed during saponification varying from a few percent to 100% depending on the concentration and structure of carotenoids and on the saponification procedure (Kimura *et al.*, 1990; Granado *et al.*, 2001; Riso and Porrini, 1997) although this was not observed in other studies (Nyambaka and Ryley, 1995). Recently a gentle saponification method using a strongly basic resin (Ambersep 900 OH) for the quantitative determination of carotenoids was developed (Larsen and Christensen, 2005).

Saponification is usually not required for blood samples since over 95% of the vitamin A normally found in blood is bound to its carrier, a retinol-binding protein, although some studies have employed this step during analysis (dee Pee, *et al.*, 1998).

The extraction mixture after saponification, washing and drying is concentrated before separation by open column chromatography or HPLC. Concentration is achieved by evaporating the solution to dryness to be taken up in the mobile phase or appropriate solvent for HPLC or to a point to be used in OCC.

2.3.3.1 Chromatographic Separations

Separation methods are an important part of analysis with chromatography having developed into the premier analytical technique for separating and purifying carotenoids (Britton, 1995a; Snyder *et al.*, 1997; Meyer, 1994; Bidlingmeyer, 1992). The IUPAC defines chromatography as a physical method of separation in which the components to be separated are distributed between two phases, one phase held immobile (stationary) and the other phase is mobile moving in a definite direction (Britton, 1995; Snyder *et al.*, 1997; Meyer,

1994). As the mobile phase passes over and through the stationary phase, the components of the mixture ideally equilibrate or differentially partition between the two phases resulting in different migration rates through the system. At any given time an analyte molecule is either in the mobile phase moving along at its velocity, or in the stationary phase and not moving at all in the downstream direction. The sorption-desorption process occurs many times as the molecule moves through the bed and the time required to do so depends mainly on the proportion of time it is sorbed and held immobile. A separation is effected if the various components emerge from the bed at different times (retention times).

Different techniques in chromatography are sub-classified by naming the mobile phase followed by the stationary phase, and thus gas-solid chromatography (GSC), gas-liquid chromatography (GLC), liquid–liquid chromatography (LLC) and liquid-solid chromatography (LSC) are some of the techniques. Recently supercritical fluids have been used as mobile phases and these techniques have been named supercritical fluid chromatography (SFC) (Britton, 1995b). The chromatographic separation techniques used in carotenoids and retinoids analysis are discussed below.

(a) Thin–layer Chromatography

Thin–layer Chromatography (TLC), although efficient in for the separation and identification of compounds, is not suitable for quantitative analysis due to the danger of degradation and isomerization on the highly exposed plate. The adsorbents commonly used are the basic oxides and carbonates, which are normally mixed with binders such as calcium sulphate, kieselguhr and starch. The mobile phase is normally a non-polar solvent. The retardation factor of the separated carotenoid is compared with that of the standard and for further identification and quantification, the adsorbent with separated carotenoid is scrapped from the plate directly into the eluent, filtered before analysis.

(b) Column Chromatography

Column chromatography, also known as open-column chromatography (OCC) is a gravity flow chromatography, that employs a large diameter column (> 1cm internal diameter) packed at ambient pressures with relatively large adsorbent particles (>37μm) and has little other instrumentation except a fraction collector. The result of this is a loosely packed (low resolution) column with low-pressure requirements. Common column adsorbents include calcium oxide, calcium carbonate, calcium hydroxide, magnesium carbonate, magnesium oxide, zinc carbonate and aluminum oxide.

Column chromatography is a classical method for separating carotenoids for quantitative analysis but is also useful in isolating and purifying carotenoids to be utilized as standards for HPLC. The types of carotenoids to be separated, the polarity and the number and type of the double bonds in the molecule determine which adsorbent to be used. For example, cyclic carotenes with a double bond in the ring are adsorbed less firmly than linear carotenoids, and β-carotene with a longer chromophore is more strongly adsorbed than α-carotene. Vegetable carotenoids such as of α-carotene for pro-vitamin A value have been separated on magnesium oxide-Hyflo super cel columns (Rodrigue-Amaya, 1999). *Cis-trans* isomers can be separated on calcium hydroxide and zinc carbonate using solvents of increasing polarity, such as acetone in petroleum ether. Although OCC methods successfully separate pro-vitamin A carotenoids, they have shortfalls such as being lengthy procedures, non-reproducible, difficulty in quantification and may result in degradation of the carotenoids (Britton, 1995; Rodrigue-Amaya, 1997).

(c) High-Performance Liquid Chromatography

High-Performance Liquid Chromatography (HPLC) is a column chromatographic method in which column packing materials have been tightly packed (>6000psi) with small and uniformly sized particles (≤ 10μm) which results in high-resolution separations. The tightly packed column demands high pressure and requires a high-pressure pump and a specialized injection device that isolates the sample (at ambient pressure) from the high-pressure flow

(Synder *et al.*, 1997). An online detector is used for continuous monitoring of the eluents. These components are connected with low volume tubing and connectors in order to minimize the dispersion of the sample as it travels through the system.

The delivery of the mobile phase into the HPLC column can be done isocratically or by gradient elution. Isocratic elution refers to the technique of using constant solvent composition throughout the chromatographic analysis while gradient elution is when the mobile phase is changed from a 'weak` to a 'strong' solvent during the analysis of complex mixtures (Barua and Olson, 1998; Larsen and Christenen, 2005). However, gradient elution requires a more sophisticated (and expensive) type of pumping system. Isocratic separation is rapid, can be performed with simple equipment and results in a stable baseline and more reproducible retention times. It has been sufficiently used for the determination of pro-vitamin A carotenoids or the principle carotenoids of food samples (Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2004; Rodriguez-Amaya, 1999; Seo *et al.*, 2005; Rock and Swendseid, 1992; Rock *et al.*, 1998).

The mobile phase properties to be considered are its physical and chemical properties and its effect on the chromatographic procedures (Gidding, 1991; Snyder *et al.*, 1997; Sadek, 2002; Craft and Soare, 1992). The UV-cut off is used as a rapid way of assessing the gross characteristics of a solvent to check if it is the appropriate choice based on the systems operating wavelength. It is defined as the wavelength at which the absorbance of the solvent in a 1cm cell is equal to unity. The mathematical relationship between the absorbance, the incidence beam intensity and the transmitted beam intensity at a specified wavelength is represented by Beers law (Equation 1).

$$\log\left(\frac{1}{T}\right) = \log\left(\frac{I_o}{I}\right) = A = \varepsilon bc \qquad [1]$$

Where;

T = transmittance

I_o = incidence beam intensity

I = transmitted beam intensity

A = absorbance

ε = molar absorptivity (l/mol.cm)

b = cell path (cm)

c = concentration of the compound in solution (mol/L).

When impurities are present in any solvent, the overall absorbance for the solvent at a given wavelength is the sum of the absorption contributors of each component (Equation 2).

$$A(\lambda) = \varepsilon(\lambda)_{solvent}.bc_{solvent} + \varepsilon(\lambda)_{impurity_a} bc_{impurity_a} + ...\varepsilon(\lambda)_{impurity_n} bc_{impurity_n} \qquad [2]$$

Therefore alongside the UV-cut off, the absorbance versus wavelength spectrum that a solvent generates is important (Sadek, 2002). The primary solvents for reversed phase HPLC are acetonitrile and methanol although some modifications can be made to obtain the desired retention time, increase solubility and improve resolution (Craft, 1992; Craft and Soare, 1992). Acetonitrile has the advantage of its lower viscosity and better selectivity for xanthophylls although methanol being more available, cheaper and less toxic has been reported for its higher recoveries (Epler *et al.*, 1992). Addition of tri-ethylamine to acetonitrile-based solvents was found to enhance the recovery of xanthophylls (Hart and Scott, 1995). Mobile phase modifiers (MPM's) may be added into the mobile phase in

concentrations of less than 5% though the consideration of their absorbance versus wavelength is important (Sadek, 2002).

The HPLC column can either be of normal phase or reversed phase. Normal phase columns are made of similar adsorbents as in open-column chromatography, with the common adsorbents being magnesium oxide and calcium hydroxide for carotenes, and porous silica based columns such as Zorbax SIL, Porasil and LiChrosphere for xanthophylls. Mobile phase solvents for normal phase columns are usually made of non-polar solvents such as hexane or petroleum ether containing varying concentrations of a polar solvent.

Reversed phase (RP) columns are normally made of octyl-(C_8) or octadecyl- (C_{18}) material chemically bonded to silica gel, such as Bondapack C_{18}, Partisil ODS, Vydac ODS and Oermaphase ODS. The mobile phase solvents for these columns consist of polar solvents such as alcohol (methanol) or acetonitrile bases mixture with water or buffers. RP-HPLC systems are more widely used than normal phase systems in the analysis of carotenoids and retinoids in biological samples (Khachik *et al.*, 1997; Jalal, *et al.,* 1998; Nyambaka and Ryley, 2001; Méndez *et al.,* 2000; Breithaupt and Bamedi, 2001; Hudon *et al.*, 2003; Mulokozi, *et al.*, 2004; Seo *et al.*, 2005; Larsen and Christensen, 2005; Rock and Swendseid, 1992; Rock, *et al.*, 1998; Tucková and Kaštel, 1999; Castenmiller *et al.*, 1999; Takyi, 1999; van het Hof *et al.*, 1999; Craft *et al.*, 1990). With this rapid, reproducible and highly sensitive technique, separation, identification and estimation are achieved simultaneously.

Nyambaka and Ryley (2001) reported the pro-vitamin A active compounds that were produced during sun and solar dehydration and storage of dark green leafy vegetables. The HPLC system used consisted of a RP column chromspher PAH for epoxides and Vydack TP-201 for isomers, each of 5μm particle size. The separations were achieved using a mobile phase consisting of acetonitrile:dichloromethane:water (38:9:2) for epoxides while methanol:dichloromethane:water (79:15:6) was used for mixtures of α- and β- carotene isomers. Changes in the biosynthesis of individual carotenoid pigments have been investigated during fruit ripening by Méndez *et al.,* (2000) using RP C_{18} Spherisorb ODS-2 (5μm, 0.46 cm × 25 cm) column. Breithaupt and Bamedi, (2001) determined the amount of

carotenoids and carotenoid esters in vegetables and fruits on a C_{30} RP material (250 x 4.6mm, 5µm). Hudon *et al.*, (2003) performed carotenoid pigments elution isocratically on a Zorbax SB- C_{18} RP column (4.6mm i.d x 250mm) with a mixture of acetonitrile:methanol: dichloromethane (41:50:9).

Recently the efficiency of different vegetable preparations in contributing to the daily safe intake level of vitamin A for children was evaluated (Mulokozi *et al.*, 2004). The carotenes were determined by isocratic RP-HPLC using methanol:methyyl-t-butyl ether:water (56:40:4) as mobile phase on a C_{30} polymeric column. Seo *et al.*, (2005) used a Prodigy 5µm C_{18} ODS 3 100A pore (250mm x 4.6mm i.d) column to analyse carotenoids in pumpkin with a solvent system consisting of acetonitrile:THF:methanol-ammonium sulfate (85:5:5:5), while Larsen and Christensen, (2005) analyzed carotenoids in green vegetables on a LiChrospher 100 RP C_{18} column (5µm; 244 x 4mm i.d) with a mobile phases of methanol:water (80%:20%) solvent A and 100% EtOAc solvent B. Similar studies include that of Rock and Swendseid, (1992) who analysed Plasma β-carotene on a RP supelcosil L C_{18} HPLC column using a mobile phase of acetonitrile:methanol:methylene chloride (70:10:20) and Rock, *et al.*, (1998) who determined plasma carotenoid concentration on a Suplex PKB-100 RP-column (4.6 i.d. × 250 mm) and a solvent system consisting of 10:40:40:10 methanol:acetonitrile:isopropanol:water.

An isocratic HPLC method for analysing of vitamins A, E, and β-carotene in animal blood plasma were performed in a study by Tucková and Kaštel (1999) on a RP-C_{18} column and a mobile phase consisting of methanol:water (94.3: 5.7). Jalal *et al.* (1998) used similar solvents with composition in proportions of 95:5 (v/v). Castenmiller *et al.* (1999) used a RP-Vydac 218 TP 54 C_{18} column and a mobile phase consisting of a mixture of methanol:THF (98:2) to analyze serum carotenoid concentration. Takyi, (1999) determined serum retinol levels using a Vydac 218 TP C_{18} column with methanol as the mobile phase. The bioavailability of β-carotene and lutein in plasma were assessed by van het Hof, *et al.* (1999a) on a RP- HPLC Vydac column using a step gradient of methanol:ammonium acetate (19:1) solvent A and methanol:THF (19:1) solvent B. Craft, *et al.*, (1990) performed HPLC separation of retinol isocratically using a mobile phase of acetonitrile:dioxane:methanol

(83:13:4) containing ammonium acetate and triethylamine on a Spherisorb column (ODS 2; 3 µm, 150 x 4.6 mm).

2.3.3.2 Identification and Quantification

Several methods are used for the identification of carotenoids. HPLC identification of carotenoids is based on the retention times as well as co-chromatography with standards. Quantification is carried out by means of internal or external calibration for which the concentrations of the standards are also spectrophotometrically determined since they have varying purity (Britton, 1995b; Rodriguez-Amaya, 1999; Craft *et al.*, 1990; Szpylka and De vries, 2005).

The first step in determining the overall purity is the determination of the spectrophotometric purity. The absorbance at a specific wavelength of a solution containing a known amount of the standard is taken and the spectrophotometric purity is calculated by using the Beer's Law. The accuracy of the spectrophotometric purity is compromised if the solution contains other components that also absorb at the same wavelength; therefore the presence of interfering components is determined by chromatographic analysis. The second step is the determination of the chromatographic purity. The chromatographic purity is calculated as the ratio of the standard peak relative to all peaks in the chromatogram, because the total area of all the peaks represents the total spectrophotometric absorbance. The true purity of the standard can then be calculated as the product of the spectroscopic and the chromatographic purities (Britton, 1995).

In the calibration procedure, standards are prepared of varying concentration and injected into the column to obtain a linear curve preferably with a correlation coefficient greater than 0.9 (Khachik *et al.*, 1992a; Khachik *et al.*, 1992b; Britton, 1995). Hence quantitative determination is attained by use of comparison of the peak area or peak height of the authentic standards with those of the samples.

The structure of carotenoids can also be identified by spectroscopic techniques such as infra-red and proton magnetic resonance. Infra-red has the ability to detect hydroxyl, carbonyl, allenic, acetylenic and aromatic groups, while the proton magnetic resonance determines the number and structural environment of both the methyl protons and protons in the carotenoid molecule. The absolute configuration of carotenoids can be obtained by other methods such as mass spectroscopy, optical rotatory dispersion and circular dichroism (Dachtler *et al.*, 2001; Dueker *et al.*, 2000; Parker *et al.*, 1993; Shi *et al.*, 1995). However, the standard method of carrying out the quantitative determination of carotenoids is by spectrophotometry (Britton, 1995b).

2.3.4 Spectrophotometric Analysis

The purpose of a ultraviolet-visible (UV-Vis) spectrophotometry is to provide a beam of monochromatic radiation to illuminate a sample so as to measure the ratio of incident and transmitted radiation of a compound. When a beam of radiation strikes any object it can be absorbed, transmitted, scattered, reflected or it can excite fluorescence (Wikipedia contributors, 2006). The processes concerned in absorption spectrometry are absorption and transmission with the conditions under which the sample is examined chosen to keep reflection, scatter and fluorescence to a minimum. In the UV-Vis regions of the electromagnetic spectrum, the bands observed are usually not specific enough to allow a positive identification of an unknown sample, although this data may be used to confirm its nature and for quantitative analysis of a sample in solution. This is based on the relationship which exists between the color of a substance and its electronic structure. A molecule or ion will exhibit absorption in the UV-Vis region when radiation causes an electronic transition within its structure. Thus, the absorption of light by a sample in the UV-Vis region is accompanied by a change in the electronic state of the molecules.

The characteristic energy of a transition and hence the wavelength (λ) of absorption is a property of a group of atoms thus, conjugation, (the greater the length of a conjugated system in a molecule, the nearer the λ max is to the visible region) and the presence of chromophores and auxochromes. In general a compound will absorb in the visible region if it

contains at least five conjugated chromophoric and auxochromic groups. A chromophore (literally color-bearing) group is a functional group, not conjugated with another group, which exhibits a characteristic absorption spectrum in the UV-Vis region. Auxochromes groups intensify the color of a molecule but generally do not absorb significantly in the 200-800nm region, but will affect the spectrum of the chromophore to which it is attached (e.g – OH groups in xanthophylls). Steric hindrance and isomerisation of linear polyenes show differences in their UV-Vis spectra.

The Beer-Lambert Law states that the concentration of a substance in solution is directly proportional to the 'absorbance', A, of the solution. The law is only true for monochromatic light (light of a single wavelength or narrow band of wavelengths), and provided that the physical or chemical state of the substance does not change with concentration. When monochromatic radiation passes through a homogeneous solution in a cell, the intensity of the emitted radiation depends upon the cell thickness (l) and the concentration (C) of the solution.

Carotenoids in solution obey the Beer-Lambert law thus their absorbance is directly proportional to the concentration. Therefore, absorbance spectroscopy can be used for quantification of retinoids and carotenoids, either of pure solutions or of biological extracts (Britton, 1995) using their absorption coefficients, $A_{1cm}^{1\%}$ (absorbance at a given wavelength of a 1% solution in 1 cm light-path spectrophotometer cuvette). Carotenoids absorb light due to their conjugated polyene systems, with light absorption in regions of the visible or ultra-violet spectrum where few other biological compounds absorb (wavelength, λ of 400-450 nm for carotenoids and λ of 325–380 nm for retinoids) and with particularly high molar extinction coefficients, ε (Britton, 1995a; Furr *et. al.*, 1994; Barua and Furr, 1998a; Barua and Furr, 1998b; Barua *et al*, 2000b).

Although the main absorption bands for most carotenoids fall within the 400-500 nm region, there is considerable variation in the shape of the spectrum for different carotenoids (Britton, 1995a; Rodriguez-Amaya and Kimura, 2004). The spectrum for most carotenoids has vibrational fine structure, thus they show just not a single absorption band but three more or less distinct peaks. This is based on the solvent used and the structural features of the

molecule (length of polyene chain, position of the end double bond in the chain or ring, the taking out of conjugation of one double bond in the ring or its elimination by epoxidation) thus influencing the position of the maxima of the spectral peaks. The nature of the specific end groups influences their polarity (Britton, 1995; Rodriguez-Amaya and Kimura, 2004).

2.4 Precautions in Carotenoids Analysis

The complication in the analysis of retinoids and carotenoids arise from their instability to light, heat, oxygen and acids that cause oxidation. Precautionary measures taken to avoid formation of artifacts and quantitative losses include completion of analysis within a short time, exclusion of oxygen, protection from light, avoiding high temperatures, avoiding contact with acid and use of high purity solvents, free from harmful impurities (Britton, 1995a; Schiedt and Liaaen-Jensen, 1995). To prevent the oxidation during saponification, extraction and chromatography processes, antioxidants are usually added to samples during grinding, saponification or to solvents. Butylated hydroxytoluene (BHT) is the most commonly used antioxidant and has no adverse effects on the results. Other antioxidants used include ethoxyquin, pyrogallol, ascorbic acid, sodium ascorbate and hydroquinone.

The use of oxygen-free nitrogen to create an inert atmosphere may be employed. This is frequently used when evaporating solvents usually at elevated temperatures, from test solutions to reduce the volume or to enable the use of another solvent. The use of a stream of nitrogen not only excluded oxygen from the warm solution, but also speeds up the evaporation process. Evaporation is done at reduced pressure, at a temperature below 40°C and solvent evaporation (to near dryness) finished with nitrogen or argon (Tonucci *et al.*, 1995).

Carotenoids may decompose, dehydrate or isomerizes in the presence of acids. Most are stable under alkali conditions. A neutralizing agent may be added during extraction to neutralize acids liberated from the matrix. Another precaution required is protection of samples from acids and metal ions, which may be present in the solutions used. Suitable solvents should therefore be used or a purification procedure carried out on the solvents.

Reagent-grade, UV/vis-grade or HPLC grade solvents are required. Technical grade solvents should be purified, dried and freshly distilled before use.

Carotenoids and retinoids undergo structural photo-transformations particularly when exposed to light in the ultra-violet region (Britton, 1995). The effect of light is usually reduced using suitable blinds or tinted glass, or using aluminum foil to wrap containers or chromatography columns. Solutions in carotenoid and retinoid analysis should be stored in the dark at about -20°C, if possible under nitrogen. Containers should be cleaned and well sealed. Standard solutions are not stable over long periods and their concentration and purity should be checked before use. To prevent oxidation and polymerization, samples should be immediately analyzed or stored frozen in the dark at -70°C (Furr *et al.*, 1992).

Particulate materials in HPLC solvents block the solvent reservoir, inlet filters, score pistons, wear pistons, seals and plug the column inlet frits (Bidlingmeyer, 1992; Meyer, 1994; Snyder *et al.*, 1997) and must therefore be removed from the solvent prior to use. Molecular oxygen has a non-negligible absorbance in the UV region and it is therefore prudent to degas all solvents prior to use (Sadek, 2002).

2.5 The study area

The study area is Kanzalu Sub-location of Kangundo Division, Machakos District, a region described as a land that is degraded and reports frequent crop failures and water shortages. Land degradation and water shortage threatens the health and livelihoods of the people in the region. The lack of food has been variously attributed to overpopulation and environmental degradation, to colonization and development, or to insufficient development and thus constitutes an endangered region (Neumann *et al.*, 1989; Bwibo and Neumann, 2003).

The region is situated on a predominantly semi-arid, eastward-facing slope, which becomes progressively lower and drier to the East. It is part of Kenya's Eastern Foreland Plateau, an eroded basement complex broken by residual hill masses and occasionally overlain by tertiary volcanic (Bernard *et al.*, 1989). The area forms an environmental gradient of

decreasing altitude (from 2,100 m to 440 m), increasing temperatures, and decreasing moisture (from 1,270 to 381 mm average annual rainfall) from west to east. Elevation controls the quantity of rainfall at the regional scale, whereas topography strongly influences rainfall distribution at the local scale. Rainfall is low and unreliable, except in the hill regions, and has a bimodal pattern (Bernard *et al.*, 1989). The soils are all generally of low fertility, and many places are highly erodable. A rough estimate of the agricultural quality of the region's soils indicates that less than 20 per cent of the area has well-drained, deep, friable red and brown clays of good fertility; while more than 60 per cent of the region has very erodable, relatively shallow, sticky, red, black, and brown clays of variable fertility, on steep slopes; and less than 20 per cent has poorly drained, shallow, stony soils of low fertility (Bernard *et al.*, 1989). The dominant vegetation here is dry bush with trees, and, in the higher areas, savanna with scattered trees. The hills were once forested, but now most of the desirable agricultural land had been cleared, leaving patches and corridors of forest along ranges, rivers, ravines, and hilltops, as well as dry forest in large expanses of grazing land (Silberfein, 1984).

The population of the district is approximately 2 million people, with a vast majority (90%) living in rural villages where they rely on a both subsistence and commercial agriculture, as well as some wage labour (Ondiege, 1992). A survey of sources of income in rural households in Machakos District reveals that agriculture accounted for 50 per cent of income, off-farm enterprises for 17 per cent, salaries and wages for 24 per cent, and other sources for 9 per cent (Ondiege, 1992). Bernard, *et al.*, 1989 asserts that of the population density has exceeded the number of people that can be supported by subsistence or commercial agriculture under present technology. Rural settlement patterns in the region reflect the productive potential of the agro-ecological zones. The higher-potential upland areas are much more densely populated than the dry lowlands. Highlands have population densities ranging from 24 to 113 people/Km^2 while lowland densities range from 6 to 22 people/Km^2 though some local highland densities are as high as 240 people/Km^2 (Ondiege, 1992). Landholdings range from 0 to 1000's of hectares (ha), but 30 per cent are less than 0.9 ha in size (Ondiege, 1992).

Most households, with an average of eight persons keep both cattle and goats. The most common food crops are maize, beans, cowpeas, pigeon peas, pumpkins, sweet potatoes, green gram, and bananas with cabbages, tomatoes, onions, red peppers, and greens usually limited to river flood plains or poorly drained valley sites. In the drier parts of the region most farmers grow maize, beans (on moister sites), cowpeas, pigeon peas, and sometimes greengram and cotton. Sweet potatoes, pumpkins, and bananas are found in the field of wetter areas, along the base of terrace walls, in deep pits (1 m^3) with fruit trees, or in home gardens.

The main limiting factors for settlement and agriculture are lack of rainfall and reliable sources of surface water. The drought and famine of 1984-1985 was the most devastating to people of Machakos District, particularly children and the elderly, who died, from acute and chronic malnutrition, while leaving lasting effects on infants and young children (Neumann, *et al.*, 1989).

The differential vulnerability of the people is as result of food shortage (less than 66 per cent food self-sufficiency on farm), food poverty (insufficient income to purchase food and/or inability to acquire food through kin and other social networks), and food deprivation (food shortage serious enough to cause wasting, stunting, and vulnerability to disease, or to force migration) (Anyango *et al.*, 1989; Bwibo and Neumann, 2003). Machakos District has a relatively higher proportion of semi-arid land with high cases of malnourished children arising from the proportion of the population that was both food short and food poor. There is still a high and growing vulnerability to severe malnutrition among children owing to rising population densities and inadequate diversification of household access to food among the poor (Anyango *et al.*, 1989).

CHAPTER THREE

METHODOLOGY

3.1 Research design

The study determined the baseline information on the vitamin A awareness, availability and consumption of the indigenous vegetables by children using a questionnaire. The questionnaire was administered to the parents and caretakers, who also consented to the study. The other baseline procedures carried out include stool examination for parasitic infestation and consequent de-worming, and hemoglobin levels examination. The intervention using dehydrated indigenous vegetables recipes was undertaken for thirteen weeks where blood samples were obtained for beta carotene and retinol analysis. Laboratory analysis were also done on vegetable samples for beta-carotene levels when fresh, dehydrated and cooked.

3.2 Sample Population

The study was conducted in pre-schools in Kanzalu location, Machakos District, which is a semi-arid District in Kenya. Permission to carry out this study was sort from the Ministry of Education, Nairobi, Kenya and the same conveyed to the District Commissioner Machakos and the District Officer, Kangundo. The preschools were identified through the District Officer, Kangundo Division, District Educational Officer (DEO) and Zonal Educational Officers (ZEO). Four preschools were randomly selected from the zone based on their accessibility and number of children. One hundred and sixty eight preschool children aged 2.5-6 years were the study subjects.

3.2.1 Exclusion and Inclusion criterion

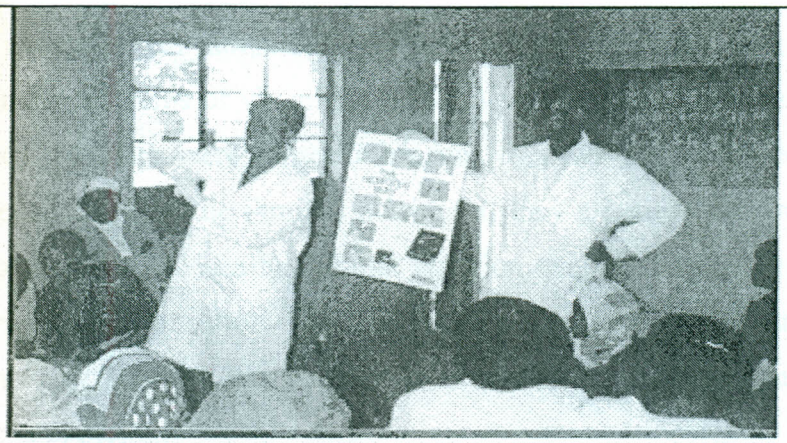
Children whose parents did not sign the consent form were excluded from the study. Parents with children who had chronic illness or/and those who had been recently discharged from hospital were advised not to sign consent and therefore the children excluded from the study.

Children who participated in the study provided their anthropometric information as well as their biological samples (stool and blood).

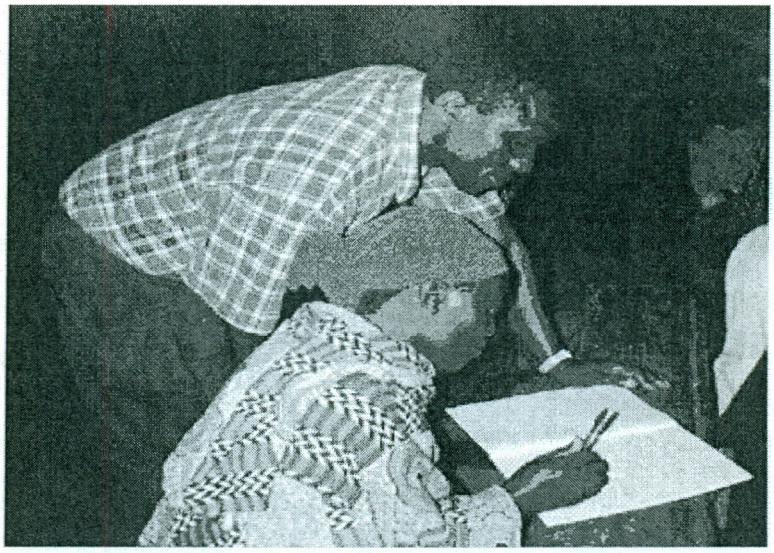
3.3 Baseline studies

3.3.1 Meetings

Prior to intervention, awareness meetings were held with parents in each of the pre-schools in which the ZEO, school head teachers, class teachers and the research team attended. The meetings were used to inform and explain the purpose of the study and demonstrate the blood collection procedures to be used. A procedure being explained to parents is captured in Figure 3.1(1). The presence of parents was particularly important in order to seek consent for their children to participate in the study. Parents who agreed signed an informed consent form (Figure 3.1(2).



(1)



(2)

Figure 3.1: Parents during a meeting where a procedure is explained (1) and a parent signing an informed consent form (2).

More meetings with mothers during the intervention period were held at the pre-schools to explain the problems of vitamin A deficiency (VAD) and the role of vitamin A-rich foods in nutrition. Mothers were targeted because they are better placed to improve their young children's as well as the family's nutrition if they are more informed on the food preparation and consumption. It was hoped that nutrition education could bring change, especially that involving family nutrition. The present study focused on the entire community members, though study subjects were used so as to control the study more effectively during the intervention period.

3.3.2 Questionnaire Administration

A designed questionnaire (Appendix 3.1) was administered to mothers in all pre-schools by members of the research team, who had been trained on its interpretation in both English and the local language (Kamba). The administration of the questionnaire was done before intervention.

The questionnaire collected demographic data including, the overall family size, and number of pre-school children per family, mothers' educational level and the social-economic status of the family. Information on the knowledge of vitamin A including its sources as well as information on locally available vegetables, their preservation methods and of consumption both by the entire family and preschool children was also collected in the questionnaire.

The information obtained helped in choosing the type of DGLVs used during the intervention period based on the local preference feeding habits for the children.

3.3.3 Stool Examination

Stool examinations were performed both before and after the intervention period. Fresh morning stool sample was collected from each study subject in a clean, wide-mouth specimen

container, which was distributed by fieldworker a day before collection. All stool samples were examined for the presence of parasite eggs under direct light microscopy by smear technique (in physiological saline).

The subjects who tested positive were subsequently treated with Antihelminthic drug for intestinal infestations (Albendazole syrup, 10 mls of 400 mg/child; SmithKline Beecham Pharmaceuticals). This was done two weeks before the intervention period to rid the study subjects off intestinal parasites which would affect the bio-availability and bio-conversion of beta-carotene. Treatment was again repeated at the end of intervention for subjects who tested positive.

3.3.4 Hemoglobin Examination

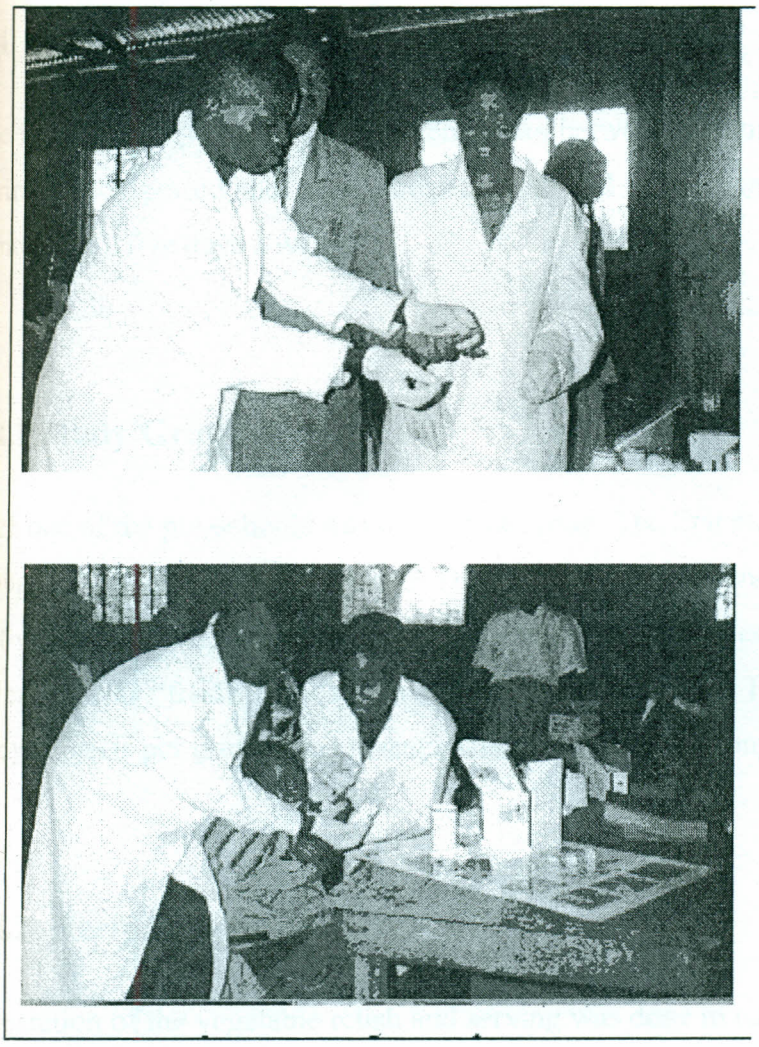
The blood hemoglobin (Hb) of the study subjects was measured at baseline and post-intervention and was based on partial total blood count for the study subjects using a hemoque analyzer. Hemocue measurement was performed using a portable battery-operated Hemocue equipment (Model B-Hemoglobin, Angelholm, Sweden) that displayed results within seconds and with minimum blood handling. The equipment was calibrated on each day of analysis before any measurements were taken by measuring a control microcuvette. The microcuvette holder was cleaned before use with cotton swap soaked in soapy water and dried prior to re-inserting it in the photometer with a sample. The Hemoque microcuvettes are sensitive to humidity; therefore the microcuvette container was usually resealed immediately after taking out the microcuvette for blood sampling.

The subject's fingertip skin was thoroughly cleaned using spirited pads and allowed to dry before being punctured so as not to cause hemolysis. Using a rolling movement, the finger was lightly pressed from the top knuckle towards the tip to stimulate the flow of blood. Maintaining a gentle pressure at the fingertip, the skin was punctured by placing the lancet blade-slot surface against the finger area. When the trigger was pressed, the tip of the blade ejected through the blade slot, producing a micro-incision in the skin, and immediately retracted into the device.

The first few drops of blood to appear were wiped away using sterile gauze to stimulate a spontaneous blood flow. The hemoque microcuvette was then applied to the middle of the blood drop where it automatically filled by capillary action. Any surplus blood was wiped off both sides of the microcuvette using sterile gauze. The fingertip was then wiped off any excess blood flow and the punctured site bandaged.

The microcuvette was placed in the cleaned microcuvette holder and gently pushed into the photometer for analysis. Blood hemoglobin results were displayed after 15-20 seconds. At the end of each blood collection and hemoglobin measurements, all materials used during the testing (gloves, hemoque microcuvettes, lancets, spirit swaps) were placed in a container and discarded into a pit latrine. Hemoglobin examination was performed both at baseline and post-intervention.

Some parents had Hb examination performed on them before the same was done on study subjects, this being a motivation for the study subjects. A parent undergoing a hemoglobin examination procedure is captured in Figure 3.2(1) while that of a study subject is in Figure 3.2(2).



(1)

(2)

Figure 3.2: A parent undergoing an Hb blood sampling procedure (1) and a study subject undergoing a blood sampling procedure (2)

3.4 Intervention Period

The intervention period lasted thirteen weeks between September to November, 2003 during which period the study subjects were fed on a vegetable diet once per day (lunch time), five days a week.

3.4.1 Study Groups

Each one of the pre-schools was coded as a group. The first study group coded "fresh" was the group that received recipes of fresh DGLVs while the second group coded, "sun-dried" was that which received recipes of the dehydrated DGLVs. The control groups were assigned one for each of the "fresh" and "sun-dried" groups respectively. This was based on the number of study subjects per group so as to statistically evaluate the group changes.

3.4.2 Recipe and Serving

Preparation of the vegetable relish and serving was done in each preschool in order to obviate the possibility that it could be exchanged between preschools or consumed by others. Study subjects at one of the feeding times during the intervention period are captured in Figure 3.3.

Figure 3.3: Study subjects at a feeding session during intervention

Two local indigenous DGLVs were selected for vegetable recipes based on information on their preference from the questionnaire. These were cowpea leaves (*vigna unguiculata*) and East African Spinach (*Amaranthus sp.*), which are also common vegetables consumed in Kenya (Gomez, 1981). The vegetables were purchased from a local market (Tala market).

Subjects in the "fresh" group, received meals of mixed cooked fresh cowpeas and Amaranthus leaves, while those in the "sun-dried" group, received cooked dehydrated recipe of the same vegetables. Subjects in the control group fed on cabbage, a low carotene containing vegetable. After completion of sun-drying procedure, the sun-dried vegetables for intervention were packed free of nitrogen. The fresh and sun-dried vegetables were delivered in the morning on a daily basis to each preschool with total amounts based on the number of subjects per group.

The cooks in all preschools were instructed on the preparation, cooking and serving of the recipes. The cooking procedure was basically adapted from that used by the local community where vegetables are boiled for 10-15 minutes and fried with fat. No spices were included except tomatoes and onions that were added to flavor the food and then salted to taste. Every 80-90 g wet weight of cooked vegetable recipe required an average of 8 g fat, 10g each of tomatoes and onions.

The served recipe per subject contained on average 80-90 g (wet weight) of DGLVs to meet the recommended daily allowance (RDA) of 500 retinol equivalents (RE) for children aged between 1-10 years old. These vegetables were served with carbohydrate from maize meal (commonly known as *ugali*)

The study subjects were encouraged to consume all the served vegetable. A food diary for food consumed at home was completed by parents daily and collected by teachers. This information was used to check for any unusually high intakes of carotenoids or preformed vitamin A. During the intervention period parents were allowed to visit the schools freely and were encouraged to ask questions they deemed necessary.

3.5 Samples and Sample Preparation

The samples in this study were DGLVs, white cabbage, stool, whole blood and serum. Vegetable samples were analyzed for all-*trans*-beta-carotene. Stool was analyzed for parasitic infestation; whole blood was examined for hemoglobin; while serum samples were analyzed for both all-*trans*-beta-carotene and retinol. Sample preparations for the individual samples are described in the sections that follow.

3.5.1 Vegetable Samples

A statistical sampling procedure was used to obtain analytical samples for laboratory analysis from the gross sample. Here, the gross sample was obtained from ten different vegetable

bundles put together, each weighing an average of 250 g. This was continuously reduced into fewer bundles by dividing into two bundles and then further putting together half of the vegetables from each bundle. Reduction was repeated until an analytical sample of about 250 g was obtained. Fresh leaves were thoroughly washed under tap water, destalked and all edible parts removed as is in normal kitchen practice. Cowpea leaves were chopped into small cuts of approximately 1-2 cm in width and placed in a sieve before blanching by immersing in hot water for three minutes. Amaranthus was blanched for one minute.

The blanched samples for laboratory analysis were divided into three parts. The first small portion was analyzed as fresh, while the second and third parts were solar dried and sun dried respectively.

Open sun drying of vegetables (both for laboratory analysis and for intervention) was performed using a perforated wire mesh tray where blanched vegetables were spread in a thin layer and exposed to dry in the open sun.

In the indirect solar dehydration, a solar dryer was constructed using locally available materials at the School of Pure and Applied Science Workshop, Kenyatta University, Nairobi. The main framework of the dryer was made of plywood supported at the edges with timber. The dryer was made sloping at an angle of 8° to aid the flow of air, and to receive solar rays by natural convection. It was painted black in the inside to conserve more heat and also covered at the top with a black polythene paper so that direct sunrays do not reach the vegetable samples. When using the dryer it was placed in an open area away from buildings and positioned facing the direction of solar rays. The air temperature inside the dryer was allowed to warm for about 30 minutes before loading the trays.

The blanched vegetables were evenly and loosely spread on the dryer tray and on the perforated wire mesh tray used for sun-drying. Since blanched leaves have a tendency to adhere to each other, spreading on both sun drying and solar drying trays was done such that lumping of leaves together was minimized by ensuring a thin layer thickness. In the course of drying, the vegetables were turned about occasionally to ensure even removal of moisture and accelerate drying. The drying parameters were dependent very much on the prevailing weather conditions with the ambient day temperatures ranging between 21-28° C. The study period

was generally dry and sunny with mean ambient air temperature of about 25°C and relative humidity of 41%. The maximum ambient air speed experienced during such dry season is 2.5 m/s (Nyambaka, 1996). The vegetables were let to dry to a moisture content of less than 10%, a period that took between five to eight hours (Nyambaka, 1996, Gomez, 1981). The time taken to dry a sample by open-sun drying was 2-3 hours less than that of a sample in the dryer. This is because the samples in sun drying were on a perforated tray raised above the ground and exposed to uncontrolled free moving air, which readily picked up moisture. The direct solar energy also increased internal temperatures and created pressure changes, leading to faster removal of moisture.

After completion of the drying procedure, the samples were packed in laminated plastic bags and immediately sealed. Samples for laboratory analysis were stored under nitrogen and kept at room temperature until analyzed while sun-dried vegetables for intervention recipes were packed free of nitrogen.

3.5.2 Blood Samples

A clinical laboratory technician performed bleeding of the study subjects. About 3 ml whole blood was drawn from the study subject's forearm by venipuncture into no-additive vacuttainers (Becton Dickinson, Franklin Lakes, NJ). The samples were protected from light by covering with aluminum foil and placed at -20°C on icepacks in a cool box while in the field before separation of serum within one hour.

Serum was obtained by centrifuging whole blood at 800 RPM for 10 minutes at room temperature. The serum was separated, transferred to Cryo Tubes (Cryo Tubes Nunc Inc, Rochester, NY), and kept at -20°C in ice packs before being transferred to Kenya Medical Research Institute (KEMRI) laboratories within one hour where they were kept at –80°C until analysis. The serum obtained was used for analysis of both all-*trans*-beta-carotene and all-*trans*-retinol.

3.6 Chemicals and Reagents

The solvents used during HPLC analysis were either of HPLC grade or analar grade; the latter were double distilled before use.

Dichloromethane (DCM, HPLC grade), Sodium chloride, Ethyl acetate, Methanol, Sodium sulphate and Ascorbic acid were obtained from Merck Eurolab; Acetone from Baker/Acros; Butylated Hydroxytoluene (BHT) from Sigma Chemical Co, St Louis; All-*trans*-beta-carotene (Type IV) and all-*trans* retinol standards, and potassium hydroxide from Fluka Chemicals, Switzerland, tert-butyl methyl ether from Rathburn chemicals, acetonitrile (HPLC-grade) from Aldrich chemical Co.

Hexane, acetone, ethanol, and tetrahydrofuran (THF) were supplied by Kobian Kenya Limited and Universitat Siegen. Distilled de-ionized water was prepared using a Distiller, de-ionizer (Model, EYELA STILL ACE SA-2100A, Tokyo Rikakikai Co. Ltd, Japan).

3.7 Equipment and Accessories

The two equipments used in this study were a high performance liquid chromatography (HPLC) and a ultraviolet-visible spectrophotometer (UV-Vis spec). Two models of HPLC were used one during analysis of samples in Kenya (HPLC-1) and another during sample analysis in Germany (HPLC-2). The specifications of the systems and their accessories are given in Table 3.1.

Table 3.1: Equipment specifications and accessories

Equipment 1-HPLC		
Part/accessory	**HPLC-1**	**HPLC-2**
High pressure pump	LC-10AS pump, liquid chromatography, Shimadzu	L-6200 an intelligent Pump, Merck-Hitachi
Reversed phase column	C_{18}, 250 x 4.6 mm internal diameter, Vydac TP-201, 5µm particle size, Chrompack	Eurospher 100 C_{18}, Knauer, 250 x 4.6 mm internal diameter, 5µm particle size and Prontosil 200 C_{30}, 250 x 4.6 mm internal diameter, 3µm particle size
HPLC oven	CTO-10A, Shimadzu.	L-5025 Column Thermostat, Merck
Injection loop	20-µl injection loop	20-µL Rheodyne injection valve (model 7725)
Detector/ lamps	Tungsten (W) lamp (SPD-10AV, Shimadzu) Deuterium (D) lamp (SPD-10AV, Shimadzu) UV-VIS detector (SPD-10AV, Shimadzu).	UV/VIS, 783 Programmable Absorbance Detector, Hoechst-Applied Biosciences.
Recorder	C-R6A chromatopac, Shimadzu	Varian STAR version 4.01
Equipment 2-UV-VIS SPEC		
UV-Visible spectrophotometer		Cary 50 Scan equipped with UV varian version 2.0 software

3.8 Experimental Procedures

All glassware was thoroughly cleaned with soap and tap water, rinsed with distilled water and either dried in an oven at 110°C, or left to dry overnight. The glassware was always rinsed three times with acetone and air blown to dry before being used for the preparation of solutions.

3.8.1 Solutions

A stock solution containing 100 µg/ml all-*trans*-β-carotene was prepared by dissolving 0.0100g of the all-*trans*-beta-carotene standard in hexane containing 0.1% BHT (w/v) and making to the mark in a 100ml volumetric flask. The stock solution when prepared was ultrasonically degassed (Ultrasonic degasser model; Sibata ultrasonic cleaner, SU-6TH, Sonorex Digital Super 10P, Bandelin) for 20 seconds to homogenize the solution before using it to prepare working standards. The stock solution was kept in the freezer (-20°C) under nitrogen wrapped with aluminium foil. The solution was used for three weeks before a fresh one was prepared. Working standard solutions were prepared from the stock solution by pipetting a suitable volume into 50 ml volumetric flasks and diluting to the mark with a mixture of methanol and dichloromethane (DCM) in the ratio 9:1 (v/v).

A stock solution containing of all-*trans*-retinol was prepared by dissolving 25 mg of the all-*trans*-retinol standard in hexane and volume made to 25 ml in a volumetric flask. The working solutions were always prepared fresh and their concentration confirmed with a UV-Vis spectrophotometer. The solutions were used for calibration, with one solution injected after every five replicate runs of samples.

The HPLC equipments were calibrated using freshly prepared working standards with the absorbance set at 450 nm for beta-carotene and 325 nm for retinol. Five different standard solution of increasing concentrations were injected into the HPLC column. Plotting peak area counts of the five standard solutions against the spectrophotometrically determined concentrations generated calibration curves for all-*trans*-beta-carotene and retinol.

3.8.2 Concentration and purity of standard solutions

The concentration of the working standards was calculated using Equation 3 where the molar absorpitivity value of 2592 for beta-carotene was used (Britton, 1995).

$$\rho = \frac{A_{\lambda_{II}} \times 10^4}{\varepsilon} \qquad [3]$$

Where

ρ = concentration (μg/ml)

A= absorbance

λ = wavelength of maximum absorptivity (nm)

ε = molar absorptivity (L/mol.cm)

A UV-Vis spectrum of the working standard solutions was also used to check for purity, using bands II and III and Equation 4.

$$Purity = \frac{A_{\lambda_{II}}}{A_{\lambda_{III}}} \qquad [4]$$

Where

A= absorbance

II , III = 2nd and 3rd bands respectively

The purity spectra of all-*trans*-beta-carotene standard solutions was checked as a ratio of bands II and III obtained from UV-Vis spectrum of the freshly prepared solution with standard spectra run in the 300-550 nm range. The purity check was performed to ascertain that the solutions contained the carotenoid in the all-*trans* configuration. Solutions were regarded as pure and majorly in the all-*trans* configuration with acceptable limits of purity to be used as reference if the band ratio values falling within 1.14-1.18 range and a maximum wavelength absorption for all-*trans*-beta-carotene being 450 nm (Rodgriguez–Amaya and Kimura, 2004, Britton, 1995b). Retinol standard is stable and therefore it was not necessary to check its purity.

3.8.3 Extraction solvents

The extraction solvent for vegetable samples was prepared by mixing acetone and hexane in the ratio of 3:2 (v:v) and adding 0.1% BHT. For the extraction of beta-carotene and retinol from serum samples, pure hexane was used.

3.8.4 HPLC mobile phase solvents

Different mobile phase solvents were prepared for elution of vegetable and serum extracts. Fresh mobile phase when prepared was filtered and ultrasonically degassed for one hour before use. The UV-Vis spectrum for the mobile phases was checked to rule out their possibility to absorb in the 400-500 nm regions.

A C_{18} column was used with a mobile phase consisting of methanol:DCM:water in the ratio 79:18:3 (v:v:v) while for the C_{30} column, a non-aqueous mobile phase consisted of methanol:tert-butyl methyl ether (MeOH:tBME) in the ratio 72:25 (v:v). Both mobile phases contained 0.1% BHT as an antioxidant.

The mobile phase for the isocratic elution of serum extracts for beta-carotene analysis consisted of a ternary mixture of methanol:DCM:water in ratios of 83:15:2 (v:v:v)

containing 0.1% BHT. The mobile phase for the isocratic elution of serum extracts for retinol analysis consisted of an aqueous binary mixture of acetonitrile:water in ratios of 85:15 (v:v).

3.9 Extraction procedures

All extractions and HPLC procedures were performed under subdued light and samples protected from light by wrapping the glassware with aluminum foil. Extractions were completed on the same day and extracts injected into the HPLC column to reduce exposure time of the sample extracts.

3.9.1 Beta-carotene extraction from vegetables

Extractions and analysis were done in triplicates. Twenty five grams of the vegetable sample were homogenized by blending with 50ml of distilled de-ionized water containing 0.5% ascorbic acid for five minutes. Five grams of the resultant mixture was extracted with 50 ml of the extraction solvent by ultrasonically agitating (Ultrasonic agitator Model KS 250 Basic IKA Labortechnik AS 1.9, HPLC-1; Sonorex Digital Super 10P, Bandelin, HPLC-2) at moderate speed for 10 minutes. The mixture was carefully decanted into a separating funnel and the residue re-extracted until it was colorless. Three extractions were found to be sufficient. For dry samples, 0.5g of ground dehydrated vegetable samples were re-hydrated with 2 ml of distilled water before the extracted as above.

The extracts were pooled and saponified with 25 ml of saturated solution of methanolic potassium hydroxide (10%v/v), shaken thoroughly for 30 seconds and allowed to stand for 30 minutes. The mixture was then washed to remove the water miscible solvent (acetone) using two portions of 100 ml sodium chloride (10%v/v) followed by four similar portions of distilled water, discarding the aqueous layer. A clear yellow hexane extract with no trace of the green chlorophyll confirmed that saponification was complete. Excess water was then dried over anhydrous sodium sulphate. Hexane was evaporated off under reduced pressure to

near dryness in a rotatory evaporator (Solvent rotary evaporator model, Heidolph) at 30°C with the vacuum broken with a stream of nitrogen. The extract was re-constituted in a mixture of methanol:DCM (9:1, v/v) containing 0.01% BHT in a 50 ml volumetric flask and volume made to the mark for HPLC analysis.

3.9.2 Beta-carotene extraction from serum

Frozen serum samples were let to thaw for 20 minutes before 200-µl aliquots were pipetted into serum vials using a micropipette (Model Nichiryo-200-1000-µl) and diluted with 200-µl double distilled de-ionised water. Samples were then de-proteinated by vortex mixing (Vortex mixture model Automatic Labo-mixture NS-8, Pasolina Type: Sibata test tube mixture TTM-1) for 30 seconds with 400-µl ethanol containing BHT (0.0599 g/ml).

The sample was extracted with 3 ml hexane, vortex mixed and centrifuged at 800 RPM at 5°C for 15 minutes (Centrifuge Type H-2000C KOKUSAN, Japan). The extraction was repeated twice and the resultant supernatant was combined and evaporated under a stream of nitrogen at 30°C. The residue was re-dissolved in 150-µl DCM:methanol (4:1), vortex mixed and ultrasonically sonicated for 10 seconds before injection into the HPLC column.

3.9.3 Retinol extraction from serum

Frozen serum samples were let to thaw for 20 minutes before 300-µl aliquots of serum were pipetted into serum vials using a micropipette and diluted with 300-µl double distilled de-ionised water. Samples were then de-proteinated by vortex mixing for 30 seconds with 600-µl ethanol containing BHT (0.0599g/ml) as an antioxidant. Extraction was repeated twice with 2 ml hexane and the combined supernatant was evaporated under a stream of nitrogen at 30°C. The residue was dissolved in 70-µl ethyl acetate and vortex mixed for 10 seconds. The sample was diluted with 200-µl of the mobile phase and ultrasonically agitated for 10 seconds before injection into the column.

3.10 HPLC separations and quantification

Sample extracts were checked for their UV-Vis maximum absorbance before injection into the HPLC columns to avoid column overloading. Sample extracts were injected into the column with absorbance unit between 0.4 AU – 1.2 AU (Britton, 1995a). The extracts were passed through a single use membrane filter (0.45μm pore size) before injection into the HPLC column. Carotenoid separation on HPLC systems was performed by isocratic elution of the mobile phases. Samples were introduced into the injection loops (20 μl) using a micro-syringe (Injector syringe model, Hamilton injector syringe 1750 GASTIGHT) onto reversed phase columns. The injection syringe was rinsed with acetone three times both before and after every injection. After the days work, the column was cleaned with and kept in methanol which was allowed to pass through the column for 1 hour (HPLC-1) or the mobile phase was left to run at very low flow rate of 0.1 ml/min overnight (HPLC-2).

A volume of 20-μl of vegetable extracts was injected into the HPLC colum. The elution took 40 minutes at a mobile flow rate of 0.8 ml/min on the C_{18} column and 1.5 ml/min on the C_{30} column. For serum extracts, a volume of 20-μl of was injected into the HPLC column (HPLC-1) for an isocratic elution. The mobile phase flow rate of 0.8 ml/min and 1.5ml/min and a 15 minute run time was done for beta-carotene and retinol respectively.

Beta-carotene was monitored using a UV-Vis detector (tungsten lamp) at 450 nm at a sensitivity of 0.0500 absorbance units' full scale (*aufs*). All-*trans*-beta-carotene in samples was identified by comparing the retention time with that of the standard solution and by spiking sample extracts with standard solution.

Retinol was monitored using a UV Vis detector (Deuterium, lamp) and detected at a wavelength of 325 nm using sensitivity of 0.0500 *aufs*. Retinol was identified by comparing the retention time with that of the standard and from an extraction of retinol from a retinol (vitamin A) tablet.

Chromatograms were integrated with respective recorder/software. Peak areas were used for quantification of all-*trans*-beta-carotene and all-*trans*-retinol using Equation 5. The concentration was expressed in μg/g dry weight for vegetables and μmol/L for serum. The retinol equivalents were calculated based on a 1:10 conversion factor while percent contribution of all-*trans*-beta-carotene to the recommended daily allowance (RDA) was calculated based on a RDA value of 500 RE.

$$\mu g/g(DM) = \frac{Pa._{(s)} \times Con._{(std)} \times Vol._{(s)}}{Pa._{(std)} \times W_{(s)}} \qquad [5]$$

Where

C = Concentration (μg/g(DM) or μmol/L)

$Pa._{(s)}$ = Peak area of analyte (mm^2)

$Pa._{(std)}$ = Peak area of standard (mm^2)

$Conc._{(std)}$ = Concentration of standard solution(μg/ml or μmol/L)

$Vol._{(s)}$ = volume of sample solution (ml or μL)

$W_{(s)}$ = weight in g of vegetable sample (or volume in μl of serum extract)

3.11 Data analysis

Two sample Student t-tests were performed to compare the means of within and between groups based on the assumption of equal variances where two by two (2 x 2) factor ANOVA indicated there were no significant differences in the variances for between and within groups. One-tailed t-tests were performed on data within groups for comparison of baseline and post-intervention means. The tests were also performed between study groups and their control groups post-intervention while, two-tailed t-tests were performed on data between groups at baseline. Significance was defined as $p<0.05$ with the probability values (p) and degrees of freedom (df) given where significance was realised. The individual one tailed or two-tailed t-test is mentioned during the output of the result.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Descriptive Results

4.1.1 Demographic Information

A questionnaire was administered to two hundred and seventy two (272) mothers who responded during the initial meetings that targeted preschool caretakers and their demographic information is tabulated in Table 4.1.

Table 4.1: Percentage response to various socio-economic items

Item	Percentage (%)(n=272)
(a) Age of respondents	
Below 25	20.6
26-35	47.8
36 and above	31.6
(b) Basic education status	
Yes	97.1
No	2.9
(c) No. of children	
1 to 2	38.3
3 to 5	46.1
6 and above	15.6
(d) Women group participation	
Yes	84.6
No	15.4
(e) Monthly income	
Below 5000	95.2
5001 and above	4.8

Respondents of the targeted study subjects were of varied ages, ranging from below 25 years to over 36 years. However, about half the population of respondents (47.8%) was in the 26-

35 years, the middle age bracket. Primary education (up to standard 7 or 8) was considered as being the minimal level of education (basic education) for any simple knowledge of nutrition. Majority of the respondents (97.1%) had basic education. The implication here is that the understanding and interpretation of the questionnaire was therefore manageable. A high percentage (84.6%) of them was involved in women groups as compared to those who were not in any women group. The organized women groups are an important unit for introducing a technology such as that of solar drying and nutrition education. This study to a wider perspective targeted the entire community in terms of nutrition and introducing the technique for vegetable preservation so as to alleviate vitamin A deficiency (VAD).

Over 80% respondents had less than 5 children, with 46.1% having 3-5 children and 38.3% with one or two children. The family monthly income was below five thousand shillings (Ksh. 5000) for 95.2% of the families. This in relation to the family size signifies poor economic living standards implying that most families are peasant farmers.

4.1.2 Vegetable Availability and Preservation

The dark green leafy vegetables (DGLVs) that are commonly found in the study area are given in Table 4.2. The extent of whether the vegetable is common or not is given as percentage commonality based on the response. Also shown is the percentage of the respondents who preserve each vegetable by sun drying.

Table 4.2: The commonly available DGLVs in the study area, their percentage commonality and seasonal availability and preservation by sun drying

Vegetable names				Seasonal availability		
Local	Common	Botanical	Commonality (%)	Wet season (%)	Dry season (%)	Preservation by sun drying (%)
Kikowe	-	*Flueggea virosa*	30.51	75.37	14.60	0.00
Ikovisi	African herb Nightshade	*Gynandropsis gynandra*	36.03	77.57	20.46	11.22
Kitulu	-	*Solanum nigrum*	41.18	90.44	12.52	3.57
Uuoa	Spinach*	-	58.46	95.59	2.44	2.52
-	Pumpkin	-	94.85	100	93.01	3.49
Ulenge	E.A Spinach	*Cucurbita pepo*	97.79	73.90	16.80	50.75
Telele	Cowpea	*Amaranthus sp*	98.90	75.73	27.76	70.26
Nthoko	Sukuma wiki*	*V. unguiculata*	100	100	36.03	76.84
Sukuma		*Brassica oleracea*	100	100	95.59	26.10

* non-indigenous vegetables

Nine types of DGLVs were found to be commonly available in the study area, although two of the common DGLVs (*Brassica oleracea* and spinach) were not indigenous. The most common indigenous DGLVs were V. *unguiculata*, *Amaranthus* sp and *Cucurbita pepo* leaves as indicated by 100%, 98.9% and 97.79% of the respondents respectively. The other DGLVs found in the study area were however fairly common indicated by 30-58% of respondents.

All the commonly available DGLVs were shown to be available during the wet seasons as indicated by over 70% of the respondents but the availability decreased considerably during the dry seasons with a significantly lower percentage (less than 36%) of respondents indicating DGLVs availability during the dry seasons. *V. unguiculata, uuoa* and *Solanum nigrum* leaves were common in the wet season, with over 90% of respondents indicating their availability during this season. The most common (%commonality) indigenous vegetables, V. *unguiculata*, *Amaranthus* sp and *Cucurbita pepo* leaves in the study area were common in the wet season alongside *Flueggea virosa* and *Gynandropsis gynandra.* The drop in the mothers' response over their availability in the dry season is not unusual as the availability of fruits and vegetables, is season dependent (Mulokozi *et al.*, 2004). The implication is that the area serves as a potential source of DGLVs during the wet seasons. This therefore if well managed in terms of preservation would minimize VAD to both mothers and children, a group that is most vulnerable (Underwood, 1994; Olson, 1999; WHO/UNICEF, 2003; Miller *et al.,* 2002; Tanumihardjo, 2004; Caulfield *et al.,* 2004; WHO/UNICEF, 2003; West, 2002).

The respondents indicated that all the DGLVs would be preserved by open-air sun drying except *Flueggea virosa.* However, for those DGLVs preserved, V. *unguiculata*, *Amaranthus* sp and *Cucurbita pepo* leaves were shown to be the most commonly preserved vegetables with over 50% of the respondents preserving them. These responses were important as they formed a guideline in the selection of DGLVs and acceptable drying procedures for the DGLVs used as recipes in this study.

Further information on the vegetable preservation and storage is summarized in Table 4.3.

Table 4.3: Percentage respondents on the information related to vegetable preservation and storage

Information	*Respondents* (%)
Preservation by sun-drying	
Preserve (n=81)	29.80
Don't preserve (n=191)	70.20
Person responsible for Preserving	
Mother (n=81)	100.00
Other	0.00
Age of mothers (n=81)	
Up to 45 years	28.33
46 and above	50.00
Education status (n=81)	
Had basic education	97.53
No basic education	2.47
Storage period of preserved vegetables	
Less than 1 week (n=49)	60.49
1-4 weeks (n=2)	2.47
Over 1 month (n=30)	37.04

In terms of general vegetable preservation by sun-drying, about 30% of the respondents indicated that they preserved DGLVs by open air sun-drying. This implies that preservation of vegetables was not a common practice as compared to 70% of respondents who did not preserve DGLVs in the study area. Open air sun drying is a common method for preservation of agricultural products in developing countries. The method however, can be improved by the use of solar dryers (Maundu *et al.*, 1999; Prasad, 2005; Jayaraman and Gupta, 1992; Kordylas, 1990; Mulokozi *et al.*, 2000). The open sun drying procedure is simple and cheap but may face drawbacks including possible contamination by dirt or rodents, infestations by insects, exposure to weather elements, animals, human beings, causing spoilage and loss, and uncontrolled drying conditions (Bassey, 1992; Bolin and Salunkhe, 1982).

All the respondents (n=81) who preserve vegetables indicated that women performed the preservation procedures. This is expected since women are better placed to improve the family's nutrition if they are more informed on the food production, preparation and consumption (Mulokozi *et al.*, 2004). However, the older mothers are engaged in the preservation more than the younger mothers. It was noted however, that for both young and old mothers, basic education would be a contributing factor since a high percentage, 97.5% of those who preserved vegetables had attained the minimum basic education.

Vegetables when preserved are mostly stored for up to one week as indicated by 60% respondents although 37% stored the dried vegetables for more than 1 month after preservation. The risk involved however, in long storage periods is the degradation of carotenoids in the DGLVs. Carotenoids are very unstable because of their conjugated system of double bonds and are easily degraded during food processing and storage (Rodriguez-Amaya, 1993; Rodriguez-Amaya, 1997; Nyambaka and Ryley, 2001; Mosha *et al.*, 1997; Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2004; Conning, 1991; Van het Hof *et al.*, 1998; Gomez, 1981; Chandler and Schwartz, 1988; O`Neil and Schwartz, 1992).

4.1.3 Consumption Preference of Vegetables to Pre-school Children

Table 4.4 gives a summary on the consumption preferences of DGLVs to preschool children. The demographic information of mothers who fed preschool children on DGLVs is also given. The percentages of the age are based on the number of persons per age bracket of those who preserved DGLVs (n=81, Table 4.3). The DGLVs fed to preschool children when fresh and after dehydration are indicated as a percentage response of the mothers.

It can be ascertained that all respondents indicated that preschool children are fed on the available fresh DGLVs while 22.4% (n=61) of the respondents indicated that preschool children were fed on preserved vegetables. The low response to the feeding of children on dehydrated DGLVs is pegged to seasonal availability of these vegetables (Jalal *et al.,* 1998; Mulokozi *et al.*, 2004) and the availability of the non-indigenous vegetables such as *Brassica oleracea* and spinach throughout the seasons. The common fresh DGLVs were indicated to be *V. unguiculata Amaranthus sp* and *Cucurbita pepo*. The preferred sun dried DGLVs fed to preschool children were *Cucurbita pepo* (100%), cowpeas (68.2%) and amaranthus (56.4%) leaves respectively. Preschool children are therefore fed preferably on fresh DGLVs although dehydrated DGLVs were acceptable.

Basic education was a contributing factor to feeding preschool children on dehydrated DGLVs, since 96.7% (n=59) of women with basic education indicated they fed preschool children on preserved vegetables. The findings established acceptance of both fresh and dehydrated cowpea and amaranthus vegetables among the targeted study group. Food processing has effects on the sensory characteristics of products (Arya *et al.*, 1979; Fontana, 1998; Nyambaka and Ryley, 2001). Hence, it was important to conform to the local preferences of these vegetables to preschool children so as to alleviate dietary risk (Bartlett *et al.*, 2000; Cynthia *et al.*, 2003; Perez-Rodrigo *et al.*, 2003).

Table 4.4: Percentage respondents on consumption preference of DGLVs to preschool children and the respondent's bio data

	Respondents (%)	
Feeding preschool children on:		
Fresh vegetables	99.6 (n=271)	
Preserved vegetables	22.4 (n=61)	
Type of DGLV	Fresh	Sun-dried
Flueggea virosa	23.8	0
Gynandropsis gynandra	12.2	80
Solanum nigrum	1.5	1.6
Uuoa	17.7	4.9
Spinach	98.6	16.4
Cucurbita pepo	100	100
Amaranthus sp	100	56.4
V. unguiculata	99.3	68.2
Brassica oleracea	100	75.4
Respondents Information (%)		
Basic education	96.7	
No basic education	3.3	
Age bracket		
Up to 45 years	75.0	
46 years and above	25.0	

4.1.4 Vitamin A Deficiency Awareness

Results on vitamin A deficiency (VAD) awareness Table 4.5 (a), medical attention Table 4.5 (b) and regular VAD check-up Table 4.5 (c) are shown. The demographic information of the respondents is included in the same tables to measure their impact. Various sub-categories of the total 272 respondents have been considered as sub-sets of the total respondents in the tables as shown by the value of "n" varying in different categories of the questionnaire.

Table 4.5 (a): Percentage and number of respondents on VAD awareness and related socio-economic information

Item tested	n	% Respondents
VAD awareness	272	
Knowledge of VAD	223	82
Lack of VAD knowledge	49	18
Age bracket (for those with VAD knowledge)	223	
Upto 45 years	207	92.8
Above 45 years	16	7.2
Education level (for those with VAD knowledge)	223	
Attained basic education	219	98.2
No basic education	4	1.8
Medical attention	272	
Sought attention	111	40.8
Didn't seek attention	161	59.2

Out of 272 respondents, 223 (82%) were aware of VAD and the majority (92%) were aged below 45 years, which was the age bracket with the majority population. Most of those who were aware of VAD had attained basic education (98.2%) implying that VAD awareness can be attributed to education.

Table 4.5(b): Percentage and number of respondents on medical attention and related socio-economic information

Questionnaire item	n	% Respondents
Medical attention	272	
Sort attention	111	40.8
Didn't seek attention	161	59.2
Age bracket (for those who Sought medical attention)	111	
Upto 45 years	105	94.6
Over 45 years	6	5.4
Educational level (for those who Sought medical) attention	111	
Basic education	108	97.3
No basic education	3	2.7
Income (for those who Sought medical attention)	111	
Below Ksh. 5000	106	95.5
Over Ksh. 5000	5	4.5

In spite of the majority of the respondents being aware of VAD, those who did not seek medical advice in event of this deficiency were more (59.2%) compared to those who did seek medical attention (40.8%). The respondents who were aware of VAD were able to

define it and give signs that accompany the deficiency. More of the younger respondents sort medical attention as opposed to the older ones. Education level was also a contributory factor to this as over 90% of those who sort medical attention had basic education. The family monthly income was noted to be low suggesting that it could be a drawback to seeking medical attention.

Table 4.5(c): Percentage and number of respondents on VAD check-up and related socio-economic information

Questionnaire item	n	% Respondents
Performed check-up (for those with VAD knowledge)	223	
Regularly	80	35.9
No check up	143	64.1
Education (for those who performed regular check up)	80	
Basic	78	97.5
No basic	2	2.5
Education (for those who did not perform check up)	143	
Basic	141	98.6
No basic	2	1.4
Age for regular (for those who performed regular check up)	80	
Upto 45 years	74	92.5
Over 45 years	6	7.5
Age for no regular (for those who did not perform check up)	143	
Upto 45 years	133	93
Over 45 years	10	7

Regular check up on VAD status was poor among respondents despite the knowledge about the same. A higher percentage (64%) of mothers who were aware of VAD did not perform a regular checkup of VAD on the preschool children. This was an unfortunate observation since 97.5% and 98.6% of those who performed a regular check and those who did not respectively had achieved basic education. The same were younger mothers although this finding is pegged to the overall group size (n=272).

4.2 Performance of the Analytical Method

4.2.1 HPLC Calibration and peak identification

Working standard solutions of different known concentrations of all-*trans*-beta-carotene and all-*trans*-retinol were prepared from their stock solutions and used for HPLC calibration. A typical calibration curve shown in Figure 4.1 was derived for the determination of beta-carotene by plotting the chromatographic peak areas as a function of the concentration of the standards.

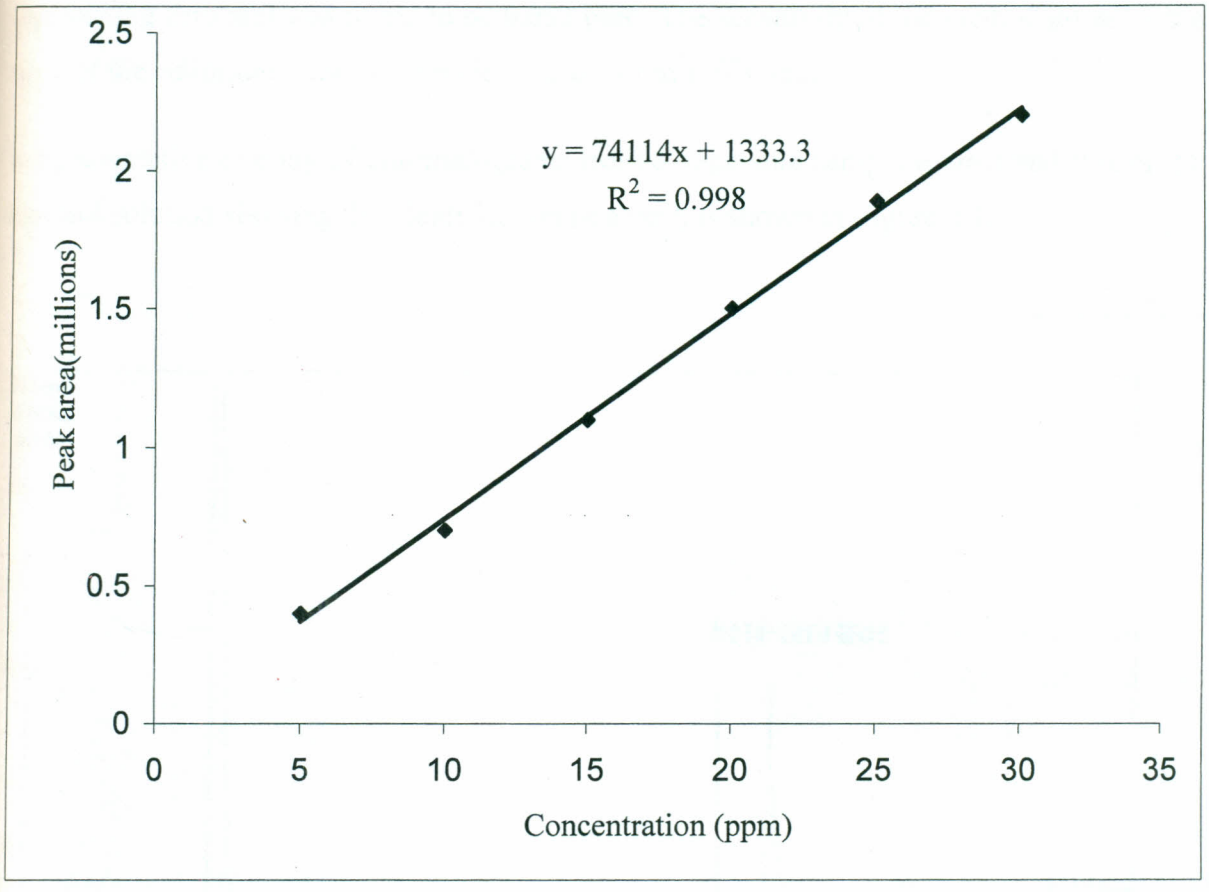


Figure 4.1: Calibration curve for beta-carotene standard by HPLC

Similar curve was generated for retinol standard and the plot were found to approximate a straight line within the concentration ranges determined as is typical and desirable (Britton, 1995a). However, because of indeterminate errors in the measuring process, not all the data fall exactly on the line. Thus regression analysis provided a means for objectively obtaining the line giving pearsons's correlation coefficient values of 0.9981 and 0.9987 for beta-carotene and retinol respectively. This indicated that there was a linear relationship between the chromatographic peak area and the beta-carotene concentration. The linearity also indicates that the detectors of the HPLC equipments were responding positively to different concentrations of analytes (Khachik *et al.*, 1992a). The detection limit, the lowest concentration which gives a signal equal to the blank signal plus three standard deviations of the blank was determined using the regression equations which had the y-intercept

approaching zero and was found to be 0.057 ppm. The sensitivity of the method given by the slope of the calibration line was obtained as 0.079 $m^2(10^6)$/ ppm.

A representative overlay of chromatograms from a vegetable sample extract and that of the standard solution showing the identification of a peak is shown in Figure 4.2.

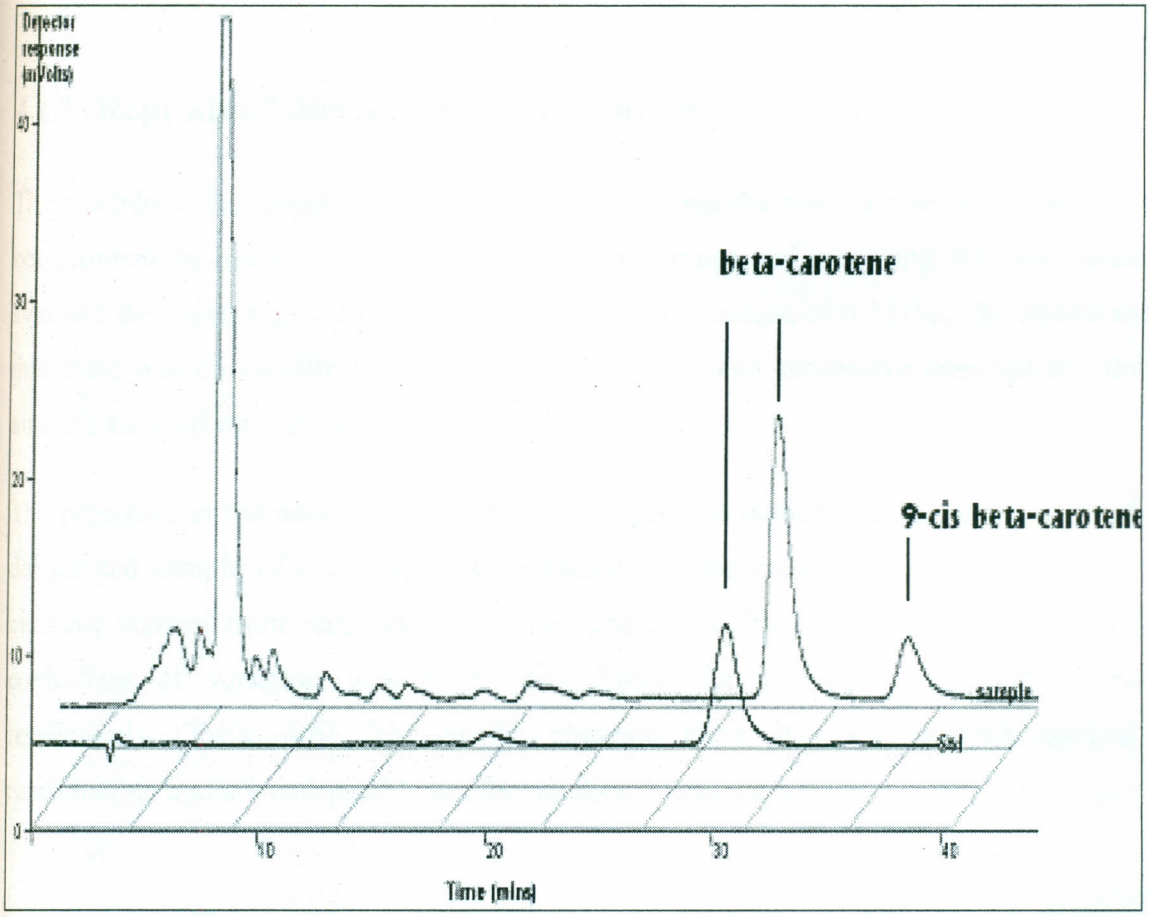


Figure 4.2: Overlay of chromatograms of a vegetable extract and standard solution indicating the beta-carotene peak. Conditions; PRONTOSIL 200 C_{30}, 250 x 4.6 mm i.d, 3µm particle size. Mobile phase; Methanol:tBME (72:25, v:v), flow rate 1.3 ml/min.

Beta-carotene in the vegetable samples eluted as a sharp peak with varying peak area depending on the concentration of beta-carotene in the individual samples. Other compounds were noted by the appearance of consistent peaks in the chromatograms at different retention times during the analysis. Two of the peaks were identified on the basis of their observed retention times thus, beta-carotene relative to the standard and its 9-*cis* isomer by comparison with similar studies (Nyambaka and Ryley, 2001).

4.2.2 Reproducibility and precision studies

The reproducibility study was carried out to determine the precision in the determination of bet-carotene by injecting a 10 ppm standard five times and recording the peak areas. The standard deviation was 0.0019 with a coefficient of variation of 0.121%. The results showed that there was no significant difference (p=0.05) between successive injection and thus the analysis method was reproducible.

The precision in the analysis of vegetables sample was determined by taking five, 0.5 g of dehydrated sample of each vegetable extracted and analyzed in duplicate. The mean beta-carotene content from each was determined and the coefficient of variation calculated. The coefficient of variation was 1.07% and 2.42% for *V. unguiculata* and *amaranthus* respectively (Table 4.6). The results obtained show that the analysis method was reproducible and are comparable to other studies (Manuche, 2003).

Table 4.6 Precision variables of HPLC procedure on vegetable analysis

Sample	Range(µg/g)	Mean(n=5)	CV (%)
V. Unguiculata	641.64-659.21	653.73 ±7.02	1. 07
Amaranthus	689.54-732.08	712.38 ± 17.28	2.42

4.2.3 Recovery studies

To determine the efficiency of the extraction and analysis procedure, a recovery study was done by adding known amounts of beta-carotene standard to finely ground vegetable sample before extraction. Samples were drawn from the batch of each of the two vegetables. The content of beta-carotene in these samples was pre-determined and the added amount was approximately equal to the beta-carotene content in the vegetable sample. The peak area was expected to be twice that of the original samples so that comparison could be made. The mixture was subjected to the extraction and quantification procedure. The percent recovery was calculated and Table 4.7 shows the results obtained.

Table 4.7: Percent recovery studies of beta-carotene determination in vegetables

Sample	Amount added	Amount recovered	% recovery
Amaranthus	160.0	151.65	94.82
V.Unguiculata	270.0	248.40	92.0

The results indicate that the extraction process was satisfactory and no significant losses occurred at the various stages of the extraction and analysis. The purpose of extraction was to release the compounds from the matrix into an extraction solvent without altering them (Rodriguez-Amaya, 1999). A decrease in the carotenoid content has been observed during saponification varying from a few percent to 100% depending on the concentration and structure of carotenoids and on the saponification procedure (Kimura *et al.*, 1990; Granado *et al.*, 2001; Riso and Porrini, 1997) although this was not observed in other studies (Nyambaka and Ryley, 1995). Therefore, these results indicate that saponification was not a problem in the analysis of vegetable samples.

4.3 Analysis of beta-carotene in vegetables

4.3.1 Moisture Content

The percent moisture content in the fresh and dehydrated samples was determined and used to obtain the dry weight in each sample. The results are given in Table 4.8.

Table 4.8: Moisture content of fresh and dry vegetable samples

Vegetable sample	% Moisture	
	Fresh ± SD	Dry ± SD
Amaranthus	96.22±0.01	6.05±0.18
V. unguiculata	94.14±0.01	5.18±0.05

The moisture content was 94.14 % and 96.22 % for the fresh cowpeas (*V. unguiculata)* and *Amaranthus* leaves respectively. The moisture content of most fresh tissue foods is usually very high, above 70% which makes them very susceptible to microbial spoilage and hence limiting their storage stability (Macrae *et al.,* 1993). The total moisture content for the vegetables is taken as the sum of free water that is loosely held outside the tissue matrix and the bound water held within the tissue matrix. Usually, the free bound water is lost during the dehydration process. The moisture content of the dehydrated samples; 5.18 % and 6.06 % for the *V. unguiculata* and *Amaranthus* leaves respectively was as a result of the bound water that constitutes the moisture content of dry sample (Arya *et al.*, 1979). The moisture content however varies with different vegetable varieties for the same period of drying. The dehydrated vegetables were therefore safe from microbial growth. Beta-carotene was considered to be stable in the dehydrated DGLVs since the compound is known to be stable at moisture levels of less than 10% a level where the optimum water activity lies (Nyambaka and Ryley, 2000).

4.3.2 Beta-carotene content in vegetables

The mean concentrations of all-*trans*-beta-carotene in the fresh and dehydrated vegetables are given in Table 4.9.

Table 4.9: Concentration (μg/g DM) and retention (%) of all-*trans-beta*-carotene on dehydration of cowpea and amaranthus leaves

	Concentration (μg /g DM)		Retention
Vegetable	Mean ± Sd[1] (CV, %)	Range	(%)
V. unguiculata			
Fresh	806 ± 21 (2.6)	779-827	100.0
Solar -dried	579 ± 23 (4.0)	550-603	73.4
Sun-dried	553 ± 29 (5.2)	533-601	70.1
Amaranthus			
Fresh	599 ± 34 (5.7)	553-639	100.0
Sun-dried	402 ± 45 (11.2)	350-466	66.2
Solar-dried	412 ± 23 (5.6)	386-441	64.6

[1]mean of triplicate analysis from 6 different samples

Fresh cowpea leaves, contained mean concentrations of 806 μg/g, DM in the range of 779-827 μg/g DM. This reduced on dehydration to mean concentrations of 553 μg/g, and 579 μg/g, DM for sun dried and solar dried respectively. The retentions were 70.1% and 73.4% for sun-dried and solar dried cowpeas respectively. The mean concentration of 599 μg/g DM in fresh amaranthus was in the range of 553-639 μg/g DM. The vegetable incurred reduction of beta-carotene content to mean levels of 412 μg/g DM and 402 μg/g DM and thus retentions of 64.6% and 66.2% for solar dried and sun dried respectively. The high retention of beta-carotene in the vegetable leaves indicate a minimal effect of UV radiation from the

sun during dehydration conditions (Michels, 1979; WHO, 1979; Burroughs, 2001; Drake, 2000).

The implication of these results is that despite the losses by both solar drying and sun drying, dehydration is a promising means of preserving vegetables in developing countries as it retains high amounts of pro-vitamin A carotenoid (Nyambaka and Ryley, 1995, Mulokozi *et al.*, 2000; Maundu *et al.*, 1999; Prasad, 2005; Bassey, 1992). It has been reported that beta-carotene losses could be minimized by almost 50% by drying vegetables in a solar dryer and that the technology retains more carotene than traditionally dried vegetables (Joshi, 2004; Mulokozi *et al.*, 2000). Despite the losses observed, the technique is inexpensive low-level technology method that can adequately preserve DGLVs and make them available for consumption during seasons of low availability and in areas away from the source as well as minimize post harvest losses (Okalebo and Hankins, 1997).

The amount of beta-carotene determined in this study in both fresh and dehydrated cowpea and amaranthus leaves are comparable to those reported in earlier studies. Cowpeas and amaranthus leaves have been analyzed when fresh for their beta-carotene content in other studies (Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2004; Gomez, 1981; Mulokozi *et al.*, 2000). The concentration of beta-carotene reported for cowpeas leaves were 7,416 μg/100 g wet weight (Gomez, 1981), 526 μg/g, DM (Mulokozi *et al.*, 2000), 726 μg/g, DM (Mulokozi *et al.*, 2004), 691 μg/g, DM for Kenyan samples (Nyambaka and Ryley, 1995) and 883 μg/g, DM for UK samples (Nyambaka and Ryley, 1995). The concentrations in amaranthus leaves have been reported as 677 μg/g, DM (Mulokozi *et al.*, 2000) and 557 μg/g, DM (Mulokozi *et al.*, 2004). Nyambaka and Ryley, (1995) reported a mean of 584 μg/g, DM while Mulokozi *et al.*, (2000) reported 462 μg/g, DM for solar dehydrated cowpea leaves and 449 μg/g, DM for solar dehydrated amaranthus leaves. Mulokozi *et al.* (2000) reported sun dried cowpea leaves to contain 296 μg/g, DM beta-carotene.

Decreases in the concentration of beta-carotene with both traditional processing practices of sun drying and of solar drying in amaranth and cowpea have been reported (Mosha *et al.*, 1997; Gomez, 1981; Nyambaka and Ryley, 1995). Nyambaka and Ryley (1995) reported the percent retention of all-*trans*-beta-carotene in these vegetables to range between 57-79%

while Mosha *et al.*, (1997) reported significant decrease in the concentration of beta-carotene for vegetables due to sun drying. However, Omueti *et al.* (1983) showed that extractable total carotenoids of the fresh vegetables increased with blanching and sun drying.

The differences in the amount of all-*trans*-β-carotene retained or lost is attributed to factors such as differences in varieties, degree of ripeness, time of harvest, and growing and storage conditions as well as to the oxidative degradation of beta-carotene during thermal processing by blanching and drying processes (Glover 1960; Mordi *et. al.*, 1993; Wang *et al.*, 1991; Marty and Berset, 1990; Rodriguez-Amaya, 1997; Nyambaka and Ryley, 2001; Britton, 1995a; Crouzet and Kanasawud, 1992; Gomez, 1981; Omueti *et al.*, 1983; Gross, 1991, Nyambaka and Ryley, 1995; Rockland and Nishi, 1980; Levine and Slade, 1991; Burton, 1989; Kanasawud and Crouzet, 1990; Hui, 1992; Handelman *et al.*, 1993; Wang and Krinsky, 1998; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Barua and Olson, 2000; Olson and Hayaishi, 1965; Lessin *et al.*, 1997; Chandler and Schwartz, 1988; Mosha *et al.*, 1997).

4.3.3 Beta-carotene content in vegetable recipes

The recipes given to the study subjects was analysed for beta-carotene and the mean concentrations of all-*trans*-beta-carotene is given in Table 4.10.

Table 4.10: Concentration (µg/g DM) and retention (%) of all-*trans-beta*-carotene in cooked and uncooked vegetables

Vegetables	All-*trans*-beta-carotene (µg /g DM) Mean ±Sd (CV, (%) n=3	Range	Retention (%)
Cowpea leaves			
Fresh uncooked	806 ± 21 (2.6)	779-827	100.0
Fresh cooked	612 ± 34 (5.6)	583-649	77.6
Solar-dried cooked	499 ± 12 (2.4)	488-520	62.3
Sun-dried Cooked	474 ± 48 (10.1)	391-509	61.0
Amaranthus leaves			
Fresh uncooked	599 ± 34 (5.7)	553-639	100.0
Fresh cooked	476 ± 46 (9.7)	428-526	76.5
Sun-dried cooked	395 ± 42 (10.6)	331-439	59.0
Solar-dried cooked	367 ± 45 (12.3)	301-426	57.0
Cabbage leaves			
Fresh uncooked	105 ± 13 (12.3)	94-127	100.0
Cooked	85 ± 10 (11.8)	69-105	79.5

It can be seen from table 4.10 that cooking and drying of the vegetables resulted in loss of the all-*trans*-beta-carotene in the fresh and dehydrated vegetables. The mean concentrations of cowpea leaves reduced from 806 μg/g DM for the uncooked cowpeas to 612, 499 and 474 μg/g DM for the fresh cooked, solar dried cooked and sun dried cooked respectively. This translated into retentions of 61% and 62.3% for sun-dried and solar dried cowpeas respectively. Amaranthus leaves had its mean concentration reduce from 599 μg/g DM for fresh uncooked to 476, 395 and 367 μg/g DM for the fresh and cooked, solar dried and cooked and sun dried cooked respectively. This translates into over 50% of beta-carotene still being retained in the vegetables after cooking. However, some of the loss is already attributed to the oxidative degradation of beta-carotene during dehydration and storage (Glover 1960; Mordi *et. al.*, 1993; Wang *et al.*, 1991; Marty and Berset, 1990; Rodriguez-Amaya, 1997; Nyambaka and Ryley, 2001; Britton, 1995a; Kanasawud and Crouzet, 1992). Oxidative degradation and *cis/trans* isomerization occur as direct result of the thermal processing during blanching and drying processes as well as during cooking. The two processes have effect on beta-carotene content (Rivzi, 1995; Rockland and Nishi, 1980; Levine and Slade, 1991; Burton, 1989; Hui, 1992; Handelman *et al.,* 1993; Wang and Krinsky, 1998; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Barua and Olson, 2000; Lessin *et al.*, 1997; Chandler and Schwartz, 1988 and Mosha *et al.*, 1997).

The values obtained in this study are within literature values for studies that reported loss of beta-carotene as a result of cooking green leafy vegetables (Jayarajan *et al.*, 1980; Dikshit *et al.*, 1988; Padmavati *et al.*, 1992; Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2004). Mulokozi *et al.* (2004) found that preparation methods caused losses of either 14-51% or 6-34% based on various cooking methods. Other studies have reported losses of all-*trans*-β-carotene during cooking of green leafy vegetables to be in the range of 24-50% (Jayarajan *et al.*, 1980), 51-83% (Dikshit *et al.*, 1988) and 18-61% (Padmavati *et al.*, 1992).

In some other studies an increase in total content of carotenoids has been reported after cooking vegetables (Mosha *et al.*, 1997; Gomez, 1981; Imungi and Potter, 1983; Dietz *et al.*, 1988). The increase was attributed to increased tissue breakdown and accessibility of the carotenes to the extracting solvent, possibly as a result of disruption of carotenoid-protein complexes. Benhura and Chitsiku (1997), reported an increase of 31% beta-carotene after

boiling green leaves for 20 minutes but prolonged boiling up to 60 minutes resulted in a 65% loss. However, a study by Khachik *et al.* (1992d) reported the content of carotenes under mild cooking conditions remained unchanged.

In spite of the reduction in carotene content due to cooking, heat processing has the potential of increasing the bioavailability of carotenoids as it disrupts or softens plant cell walls and breaks carotenoid-protein complexes (Hussein and El-Tohamy, 1990; van het Hof *et al.*, 1998). In green leaves, carotenoids exist within cell chloroplasts as pigment-protein complexes, which require disruption for the carotenoid to be released for their bioavailability. Though, the leaf matrix may affect the accessibility of carotenoids, by hindering the release of the carotenoids during digestion, cooking assists in the release of carotenoids from the food matrix by disrupting cellular membranes and liberating nutrients for efficient absorption (Micozzi *et al.*, 1992; Van het Hof *et al.*, 1998; Castenmiller *et al.*, 1999; Erdman, 1988, Stahl and Sies, 1992; Erdman *et al.*, 1993).

The variations in the standard deviation is wide due to samples obtained from different batches. This made the coefficient of variation of some samples to go above the 10% level although some degree of homogenity is implied (Rivzi, 1995; Rockland and Nishi, 1980; Levine and Slade, 1991). The differences in the amount of all-*trans*-β-carotene retained in cooked vegetables is possibly due to differences in the degree of susceptibility of leaves to heat treatment as a result of differences in varieties, degree of maturity, time of harvest, and growing and storage conditions (Gomez, 1981; Omueti *et al.*, 1983; Gross, 1991; Nyambaka and Ryley, 1995). Despite cooking the vegetables, the concentration of beta-carotene was high to serve as a good source of pro-vitamin A and would hence be used to alleviate VAD (Mulokozi *et al.*, 2004).

4.3.4 Retinol equivalents and Contribution to RDA

The retinol equivalents (RE) in vegetables and its percentage contribution to the recommended daily allowances (RDA) for children aged 2-6 years is given in Table 4.11. The RE was calculated based on a 10:1 conversion for beta-carotene:retinol as per National

research council (1990) and Vuong (2002). The percent contribution to the RDA is based on assumption of 100% bioconversion of beta-carotene in the mucosa for an RDA that is set at 500 µg for children aged 2-6 years.

Table 4.11: RE (per g and per day) and RDA contribution (%) of cooked recipes

	Cowpea		Amaranthus		Mixed recipe*		Cabbage
	F	SD	F	SD	F	SD	F
RE/g	61.2	47.4	47.6	39.5	-	-	8.5
RE/day(80-90g)	408.20	316.15	158.50	131.53	546.70	437.68	
(%)RDA	81.64	63.23	31.70	26.30	103.34	87.54	17.80

KEY: F; Fresh, SD; sun dried

* cowpea and amaranthus leaves

The study subjects were feeding on 80-90 g wet weight of cooked mixed vegetable recipe containing an average of 8 g fat, tomatoes and onions but different amounts of carotenoids based on whether they were in the fresh, sun dried or control group. The recipe was served once a day, five days a week for thirteen weeks. The fresh vegetable recipes provided a total of 546.70 RE/day. This is as a result of the contribution of 408.20 RE/day and 158.50 RE/day from cowpea and amaranthus leaves respectively. The sun-dried recipe provided a total of 437.68RE/day, a contribution of 316.15 RE/day and 131.53 RE/day from cowpea and amaranthus leaves respectively. While the individual cowpea and amaranthus vegetables would respectively provide 81.64% and 31.70% for fresh recipe, 63.23% and 26.30% for sun dried recipe, the mixed recipe provided comparably high percentage contribution of 103.34% and 87.54% to the RDA. This implies that both the fresh and sun dried recipes provided

sufficient amounts of all-*trans*-beta-carotene (NRC, 1989). This RE/day hence ensured a minimum and sufficient intake of the RDA for beta-carotene during the intervention period. Cowpeas leaves and amaranthus have been shown to contribute 23-46% and 45-54% RE per edible portion by *in vitro* methods depending on the method of cooking (Mulokozi *et al.*, 2004).

There have been variations in recipes of plant sources from other studies ranging from the minimum RDA to an amount that is approximately 5 times the RDA. The amounts of RE/day provided are comparable to other vegetable recipes such as 500 RE/day (Vuong *et al.*, 2002), 684 RE/day (de Pee *et al.*, 1998), 750 RE/day (Jalal *et al.*, 1998) and 400 RE/day (Takyi, 1999). Ribaya-Mercado *et al.* (2000) however, provided recipes containing 2192 RE/day from plant sources, being approximately four and five times the fresh and sun-dried recipes respectively used in this study. Although there were losses of beta-carotene due to cooking these did not lead to substantial loss of retinol equivalents.

4.4 Intervention Studies

4.4.1 Anthropometric characteristics

The characteristic mean age and mean weight at baseline and post intervention of the study subjects is given in Table 4.12. In the table, study subjects who fed on fresh DGLVs are coded "F-group", while those on the dehydrated DGLVs are "SD-group". Those who fed on cabbage are the control group, one each for the F-group and the SD-group.

Table 4.12:- Mean age and weight of subjects in the study and control groups[1]

	Age (Years±SD)	Weight (Kg±SD)	
		Baseline	Post-intervention
F-group (n=56)	4.34 ± 0.96 (3.00-6.00)	15.575 ± 2.35 (11.50-22.50)	16.286 ± 2.078 (12.00-22.00)
Control (n=51)	5.00 ± 1.11 (3.00-6.00)	16.25 ± 2.36 (11.00-21.00)	16.569 ± 2.249 (11.00-20.50)
SD-group (n=20)	4.95 ± 1.15 (2.30-6.50)	15.55 ± 3.06 (10.00-21.00)	15.5 ± 2.57 (11.00-21.00)
Control (n=25)	4.78 ± 0.99 (3.50-6.00)	16.52 ± 2.64 (12.00-22.50)	16.816 ± 2.382 (12.50-22.00)

[1] ranges in parenthesis

Out of 168 study subjects involved in this study, there was a 10% fall out leaving 152 subjects (90%), for whom data is discussed. The drop of 10% was mainly due to refusal by the subject to have the blood samples drawn since obtaining blood samples, other than done by parents consent, required the consent of the study subject. The subjects were aged between 2.3-6.0 years and of comparable weights. They were grouped into two study groups each with its control group based on the number of subjects in the study group.

The mean age of the study subjects in the fresh vegetable study group (n =56) was 4.34 ± 0.96 y (3.0-6.0 y) and of no statistical difference with those in the control group (n =51) aged 5.00 ± 1.11 y (3.0-6.0 y). The subject groups had no statistical difference in their weights thus 15.575 ± 2.35 Kg (11.50-22.50 Kg) and 16.25 ± 2.36 Kg (11.00-21.00 Kg) respectively at baseline. There were no significant differences in the mean weight of subjects between the study and control group post-interventional. However, those in the study group had a

significant increase in the mean weight to a value of 16.286 ± 2.078 Kg (12.00-22.00 Kg) (p= 0.046, df = 110) post-intervention.

The mean age of the subjects in the sun-dried group (n =20) was 4.95 ± 1.15 y (2.30-6.50 y) with no statistical difference to those in the control group (n =25) who had a mean age of 4.78 ± 0.99 y (3.5-6.0 y). The subjects had weights that were not significantly different of 15.55 ± 3.06 Kg (10.00-21.00 Kg) and 16.52 ± 2.64 Kg (12.00-22.50 Kg) respectively at baseline. There were no significant increases in weight in both the study and control groups although the subjects in the sun dried study group had a significant increase in weight post-intervention (p=0.041, df = 43).

The age and weight of the subjects in the two study groups (F-group and SD-group) had no statistical difference. This information implies homogeneity of the study subjects in carrying out the statistical comparisons of the bioavailability studies.

4.4.2 Prevalence of Intestinal Parasitic Infection

The examination of stool was performed both before and after the intervention period. The percent prevalence's of parasitic infestation of the study subjects in their respective groups is given in Table 4.13.

Table 4.13: The percentage prevalence of parasitic infestation at baseline and post-intervention for the subjects

Group	Subjects infected (%)	
	Baseline	Post-intervention
Fresh vegetable (n=56)	46	9
Control (n=51)	57	6
Sun-dried vegetable (n=20)	65	5
Control (n=25)	40	8

At baseline, 46% of the subjects in the fresh vegetable study group and 57% of those in their control group were infected with at least one parasite among the parasites *Ascaris lumbricoides* and hookworm. There were significant reductions in the percent infected at post-intervention ($p<0.05$). In the dehydrated vegetable group 65% and 40% for the study and control group respectively were infected. These percentages reduced significantly to less than 10% post-intervention after the subjects were treated with an antihelminthic drug. This indicates the effectiveness of the drug. This also implies that the subjects were majorly free from infection during the intervention period. Host-related factors such as intestinal parasitism cause differences in the serum response to ingestions of dietary carotenoids (Castemniller and west, 1998; Kücükbay *et al.,* 1997; Jalal *et al.,* 1998; Friis *et al.,* 1996; Tanumihardjo *et al.,* 1996; Mahalanabi *et al.,* 1979).

4.4.3 Hemoglobin Levels

The overall mean of Hemoglobin (Hb) levels for study groups and their respective control groups are presented in Table 4.14.

Table 4.14: Mean hemoglobin levels of study and control subjects at baseline and post-interventional [1]

Period/ %change	Hemoglobin concentration (g/L)	
	Fresh vegetable group (n=56)	Control group (n=51)
Baseline	11.538 ± 1.099 (7.90-13.30)	12.367 ± 1.208 (9.70-14.80)
Post-intervention	12.221 ± 1.179 (7.50-13.90)	12.312 ± 1.163 (9.4-14.70)
Change (%)	(+) 5.92	(-) 0.44
	Sun-dried vegetable group (n=20)	Control group (n=25)
Baseline	11.635 ± 1.066 (8.9-13.00)	12.280 ± 1.144 (10.70-14.20)
Post-intervention	12.175 ± 1.153 (9.8-14.20)	12.240 ± 1.087 (10.30-14.00)
Change (%)	(+) 4.64	(-) 0.33

[1] ranges in parenthesis

The mean hemoglobin concentration for study subjects in the fresh vegetable study group was low at baseline, 11.538 ± 1.099 g/l (range: 7.9-13.30). There was high percentage of subjects with marginal Hb (82%) and this contributed to the overall low mean Hb at baseline for the study group. Two-tailed t-tests indicated that the mean was significantly lower ($p<0.001$, $df=105$) than the mean for the control group, which was 12.367 ± 1.208 g/l (range: 9.7-14.8). At post intervention the mean reflected a 5.92% increase, giving a mean of 12.221 ± 1.179 g/l (range: 7.5-13.9) for subjects in the fresh vegetable group. This translated to 57% increase in subjects with sufficient Hb levels post intervention. This was as a result of a highly significant reduction from 82% to 25% post-intervention for subjects with low Hb

(p=0.001, df=110). The control group had a 0.44% reduction in the mean Hb levels and this reduction was not statistically significant.

The Hb for the study subjects in the dehydrated vegetable group at baseline was 11.635 ± 1.066 g/l (range: 8.9-13.00) which was not significantly different from those of the subjects in the control group having hemoglobin concentration of 12.280 ± 1.144 g/l (range: 10.7-14.2). The increase for the study group reflected a 4.64% change while the control group had a 0.33% reduction. These post interventional changes were not however significant for both the study and control group. McNemars chi-square test indicated that at baseline, 55% of study subjects in the study group had haemoglobin concentrations that were anaemic (WHO, 1993). These reduced from 55% to 35% post-intervention, while for the control group the figures were 40% and 36% at baseline and post-intervention, respectively.

The levels of haemoglobin against the WHO levels of 12 g/l are presented in Figures 4.3a and b.

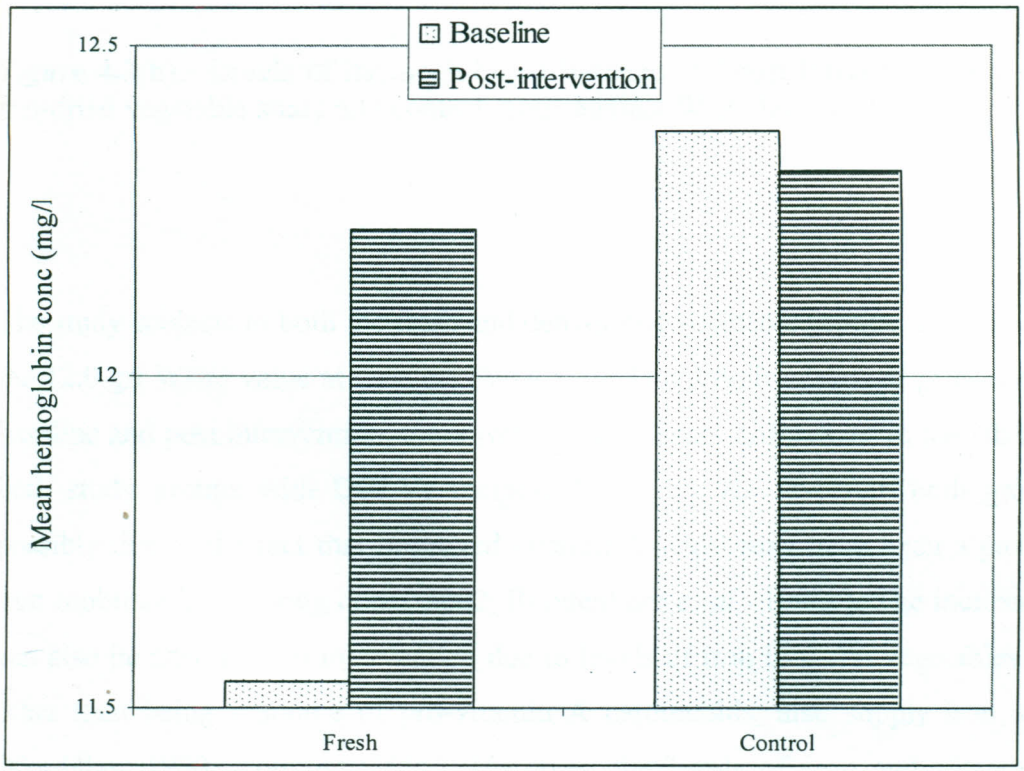


Figure 4.3(a):- Levels of haemoglobin at baseline and post-intervention for subjects in the fresh vegetable study and control group against WHO level of 12 mg/L

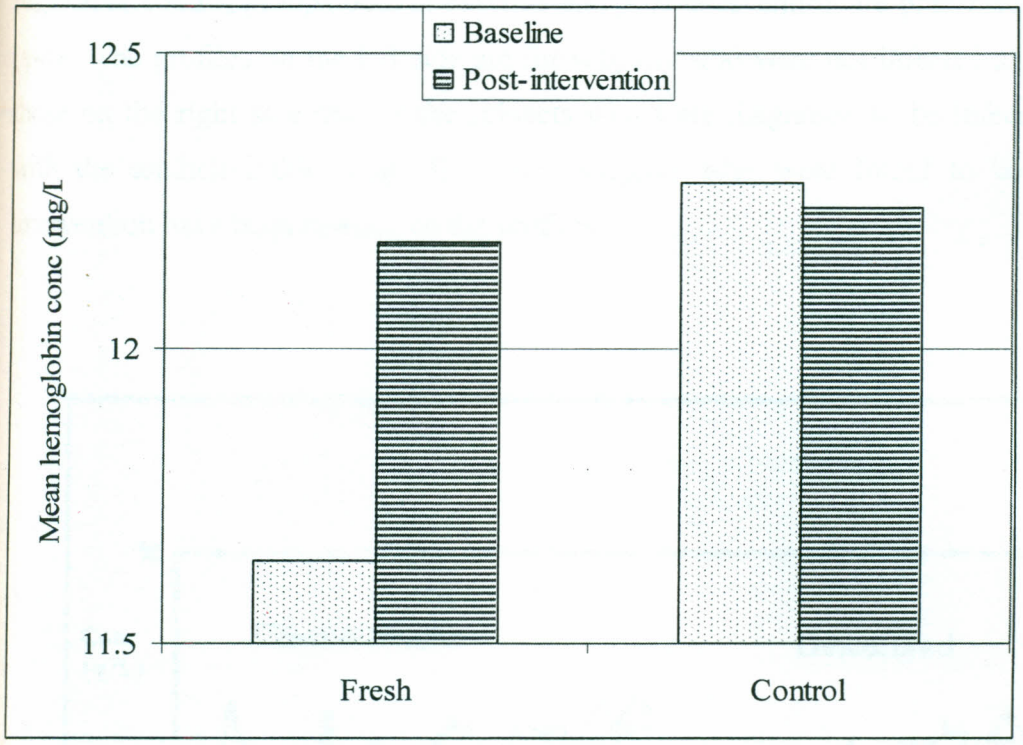


Figure 4.3(b):- Levels of haemoglobin at baseline and post-intervention for subjects in the sun-dried vegetable study and control group against WHO level of 12 mg/L

The study subjects in both the fresh and dehydrated vegetable groups had mean values below the 12.0 g/l WHO value at baseline (WHO, 1993) but both improved post-interventional. At baseline and post intervention, there were no significant differences in the Hb levels between both study groups with DGLVs recipes. The mean Hb levels in both groups increased possibly due to the fact that improved vitamin A status may have been a positive effect on iron mobilization (Vuong *et al.*, 2002; Roodenburg *et al.,* 1996b). The increase in Hb levels can also be attributed to intervention due to levels of iron in green vegetables since DGLVs other than being a source of pro-vitamin A carotenoids, also supply iron and vitamin C (Kordylas, 1990; Conning, 1991). However, the levels of iron and vitamin C were not determined in these foods.

The individual profiles of hemoglobin (Hb) for study subjects for the study groups and their respective control groups both at baseline and post-intervention are presented in Figures 4.4 (a-d). The profiles on the left side are for subjects who were not infected at baseline while those on the right side are for the subjects who were diagnosed to be infected and treated with the antihelminthic drug. The study subjects who were found to be infected post intervention have been marked on the profiles.

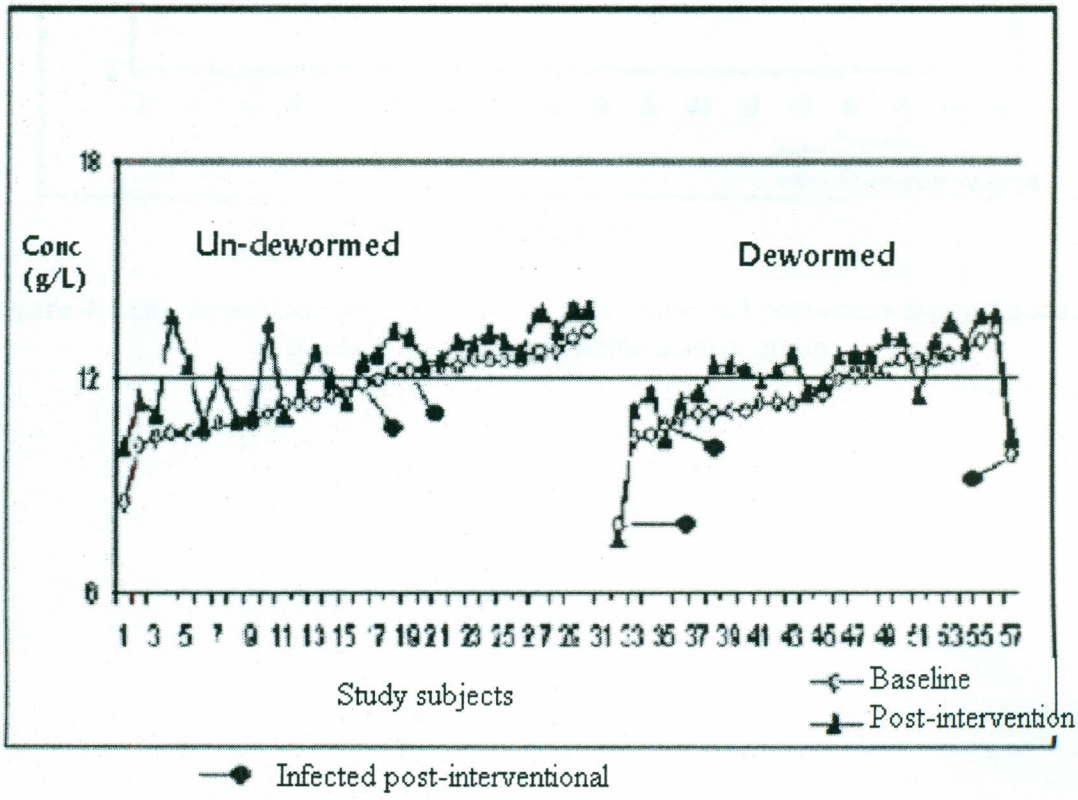


Figure 4.4 (a): Blood haemoglobin profiles at baseline and post-intervention for study subjects in the fresh vegetable study group

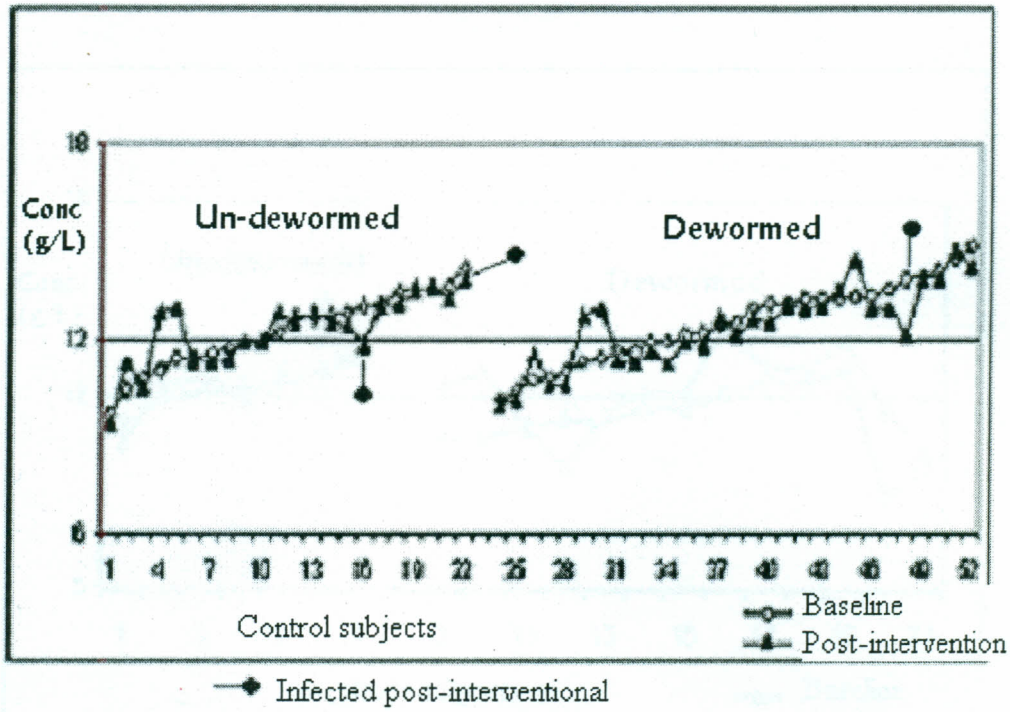


Figure 4.4 (b): Blood haemoglobin profiles at baseline and post-intervention for study subjects in the fresh vegetable control group

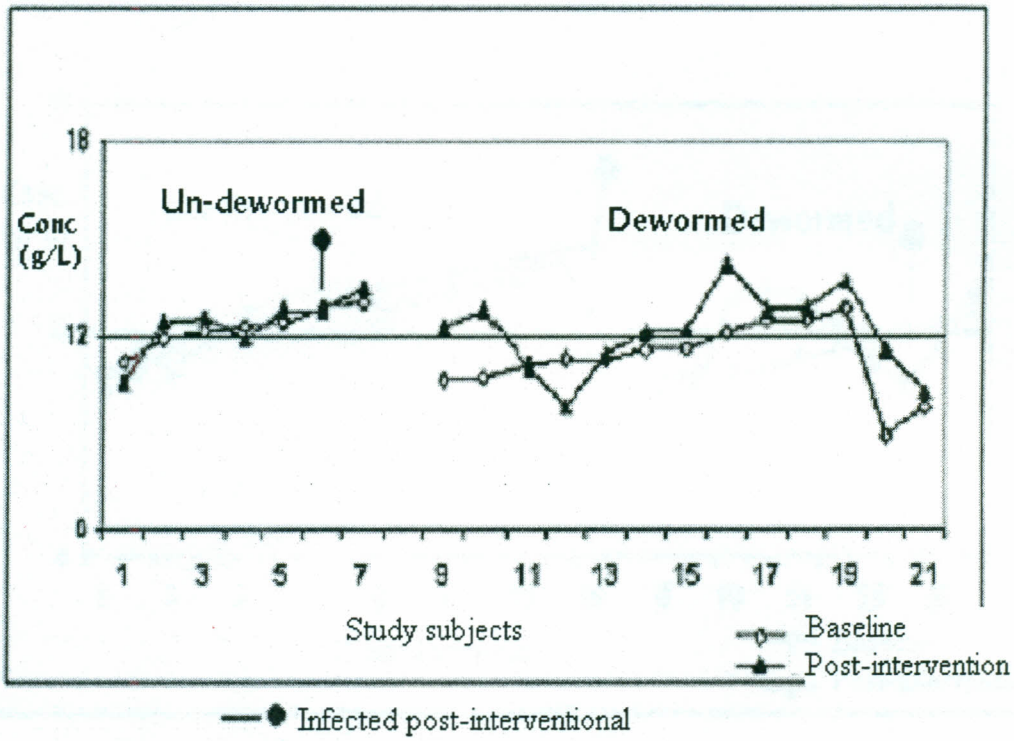


Figure 4.4 (c): Blood haemoglobin profiles at baseline and post-intervention for study subjects in the sun-dried vegetable study group

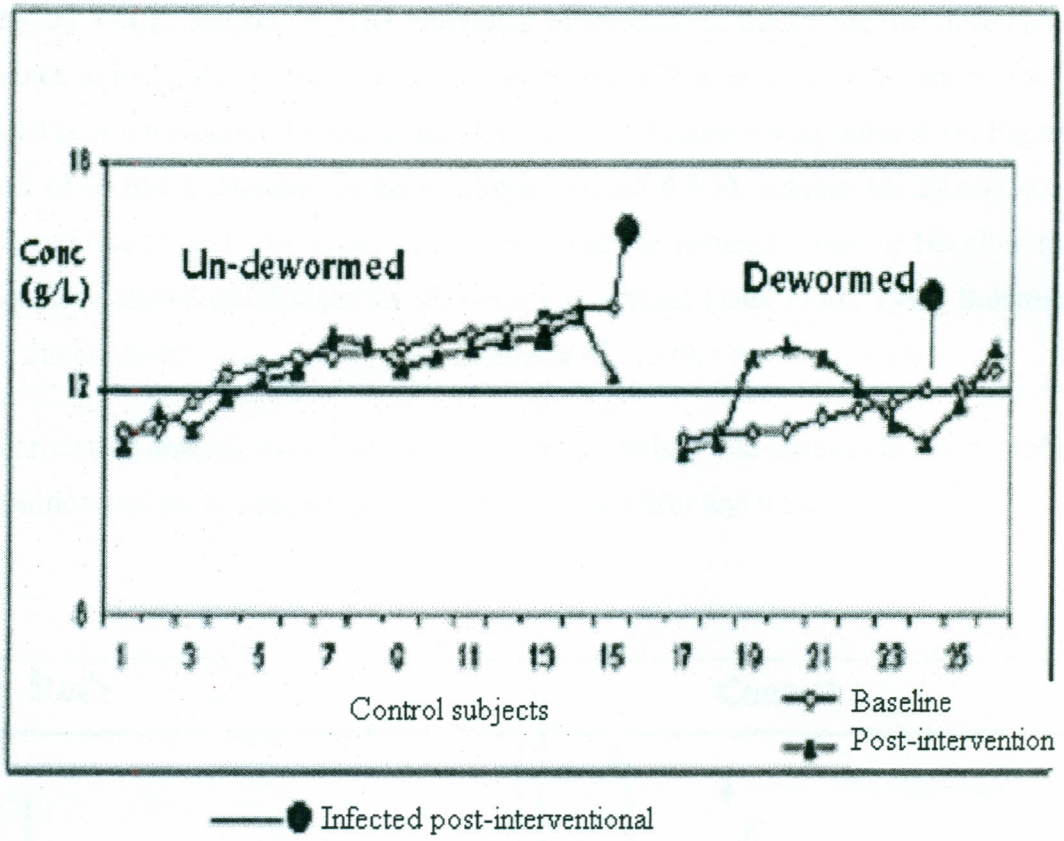


Figure 4.4 (d): Blood haemoglobin profiles at baseline and post-intervention for study subjects in the sun-dried vegetable control group

The inter-individual variations show that while some individuals improved their Hb levels, some decreased while others had no noticeable changes. These trends were observed for various individuals despite their groupings. McNemars chi-square tests showed that at baseline, 82% and 55% of study subjects in the fresh vegetable group and the dehydrated group respectively had haemoglobin concentrations that were lower than the WHO levels (WHO, 1993).

Some subjects were found to be infected post-intervention (Table 4.13) despite having had negative stool at baseline as labelled in figures 4.4(a), subjects 16 and 19; Figure 4.4(b), subject 16 and 22, Figure 4.4(c) subject 6; Figure 4.4(d), subject 15. Others who were de-wormed were re-infected as follows Figure 4.4(a), subjects 32 and 57; Figure 4.4(b), subject

42, Figure 4.4(d), subject 24. The individual differences in the trends therefore can also be explained as being due to the extent of intestinal parasitism since all the subjects found to be infected post intervention had no noticeable increase (Figure 4.4(a), subject 16; Figure 4.4(c) subject 6) or had a decrease in haemoglobin (Figure 4.4(b), subject 16, 22 and 42, Figure 4.4(d), subject 15 and 24). Parasitic infection leads to reduced levels of Hb although other studies have shown no changes in Hb despite infections (Jalal *et al.,* 1998; Suharno *et al.,* 1993; Tanumihardjo *et al.,* 1996; Mahalanabis *et al.,* 1979, Friis *et al.,* 1996).

The correlation analysis was done between baseline values and changes in haemoglobin post-intervention and the results are presented in Figures 4.5(a) and 4.5(b).

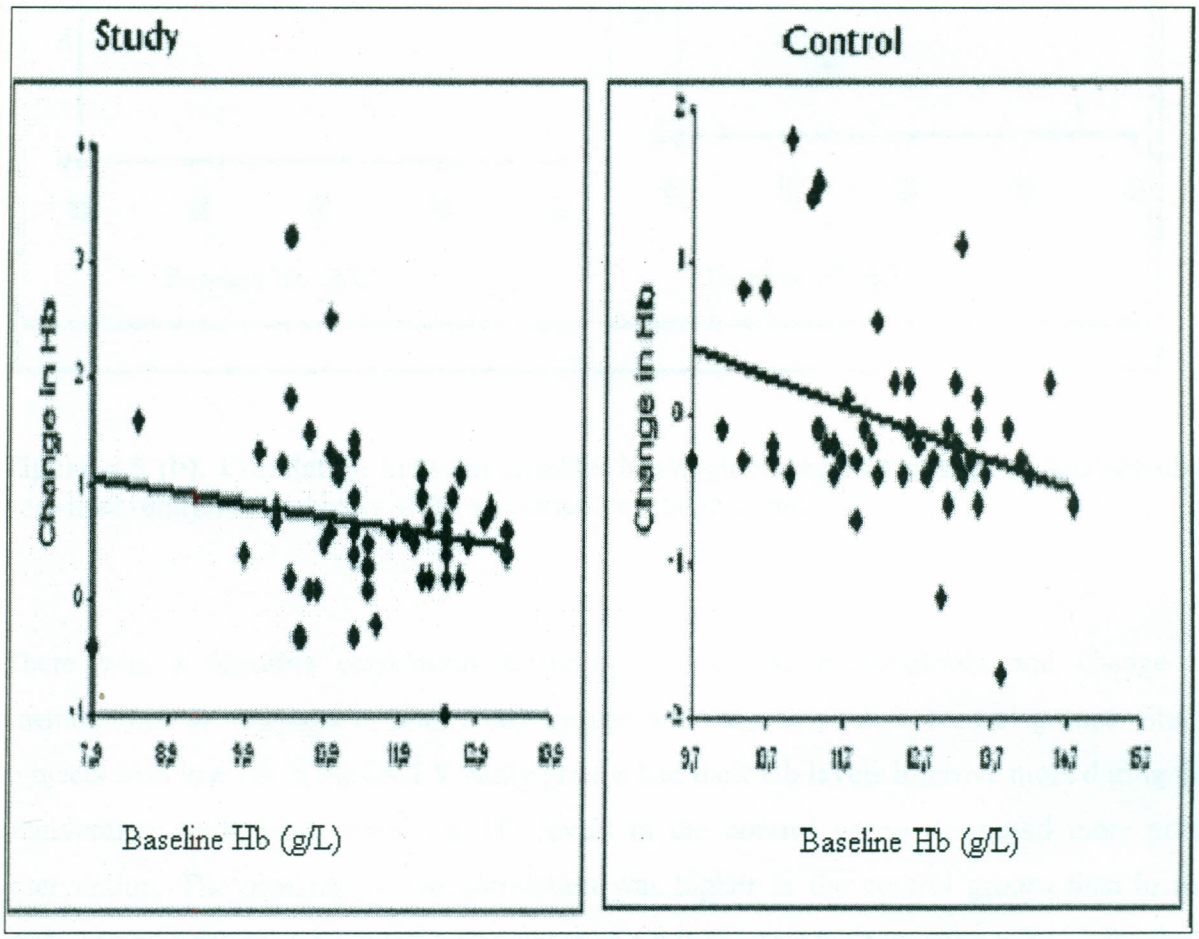


Figure 4.5 (a): Correlation between baseline haemoglobin against change in haemoglobin post-intervention for subjects in the fresh vegetable group

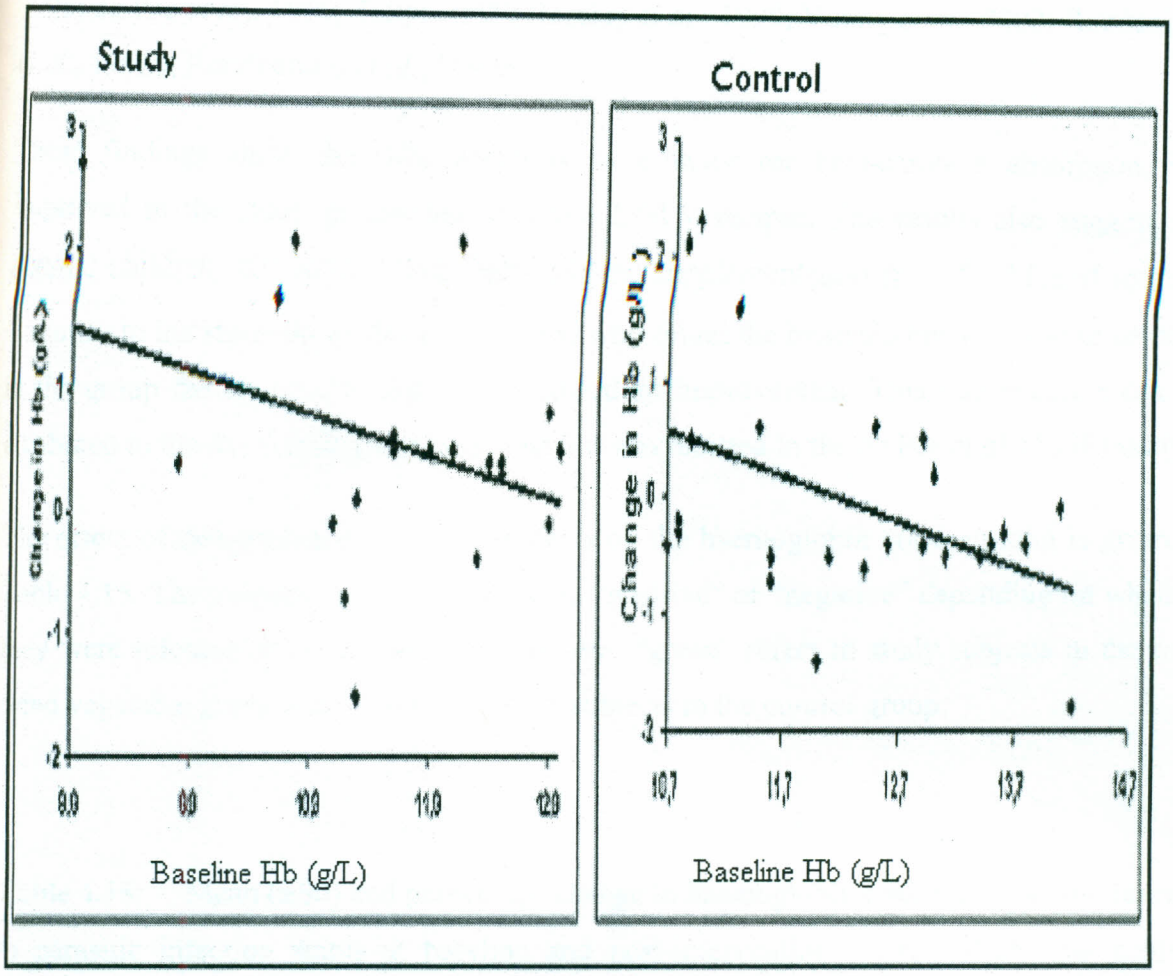


Figure 4.5 (b): Correlation between baseline haemoglobin against change in haemoglobin post-intervention for subjects in the sun-dried vegetable group

There was a negative correlation between the baseline haemoglobin and change in haemoglobin for subjects in both study groups and their respective control groups. Study subjects with low Hb in the DGLV study groups had their Hb levels improve more during the intervention while those with low Hb levels in the control group decreased more post-intervention. The intensity of the correlation was higher in the control groups than in the study groups with pearsons correlation value of r = 0.333 (control) versus r = 0.183 for fresh vegetable subjects and r = 0.473 (control) versus r = 0.374 for subjects in the sun dried groups. This can be attributed to the DGLVs that provided sufficient iron to improve the iron

absorption hence Hb levels (Suharno *et. al.*, 1993; Tanumihardjo *et al.*, 1996; Jalal *et al.*, 1998; de Pee *et al.*, 1998; Takyi, 1999; Rosales *et al.*, 1999; Vuong *et al.*, 2002; Roodenburg *et al.*, 1996a; Roodenburg *et al.*, 1996b).

These findings show that Hb, which is an effector for beta-carotene absorption, was improved in the study groups that received DGLV recipes. The results also suggest that anemic children will benefit from beta-carotene supplementation from food-based sources. Contrary to the study by de Pee *et al.* (1998) who found the bioavailability of iron to be poor in the group fed on DGLV, this study showed an improvement. This improvement can be attributed to the de-worming that was done but was omitted in the de Pee *et al.* (1998) study.

The effect of deworming of infected subjects on the haemoglobin concentration is given in Table 4.15. The subjects were categorized as "positive" or "negative" depending on whether they were infected or not at baseline. The term "green" refers to study subjects in the sun-dried vegetable group and "white" is for the subjects in the control group.

Table 4.15: Mean (±Sd) and percentage change in haemoglobin concentration with respect to parasitic infection status at baseline and post-intervention in both study and control categories

Category	Mean hemoglobin (g/L)[1]		
	Baseline	Post-interventional	% Change
Negative			
-Green	11.711 ± 1.030 (8.50-13.30)	12.376 ± 1.032 (10.1-13.9)	5.68
-White	12.478 ± 1.146 (9.70-14.20)	12.354 ± 1.040 (9.40-14.00)	(-) 0.99
Positive			
-Green	11.423 ± 1.128 (7.90-13.30)	12.051 ± 1.271(7.50-14.20)	5.50
-White	12.205 ± 1.212 (10.10-14.80)	12.226 ± 1.222 (10.00-14.70)	0.17

[1]ranges in parenthesis

The "negative green" subjects had hemoglobin levels of 11.711 ± 1.030 g/L (range; 8.5-13.3 g/L) at baseline. These subjects had a 5.68% increase to 12.376 ± 1.032 g/L (range; 10.1-13.9g/L) post-intervention. The change was found to be significant (p= 0.004, df= 76) implying that intervention with DGLV improved the Hb levels. This is supported by the Hb levels for subjects in the control group who had Hb of levels 12.478 ± 1.146 g/L (range; 9.7-14.2 g/L) at baseline and had a reduction of 0.99% to levels of 12.354 ± 1.040 g/L (range; 9.4-14 g/L) post-intervention. This decrease though was not statistically significant. The subjects who were infected at baseline and treated with an antihelminthic drug (positive green subjects), had their mean value of Hb improve significantly (p= 0.012, df= 76) from baseline levels of 11.423 ± 1.128 g/L (range; 7.9-13.3g/L) to levels of 12.051 ± 1.271 g/L (range; 7.5-14.2g/L), a change that had 5.50% increase. Subjects in the control group (positive-white study subjects), also had the Hb levels increase from 12.205 ± 1.212 g/L (range; 10.1-14.8g/L) to 12.226 ± 1.222 g/L (10-14.7 g/L) an increase that was less than 1%, although this increase was not significant. The results show the effectiveness of DGLVs in improving the Hb levels since freeing subjects from infection and feeding on DGLV had about 6% post-intervention change in Hb, a change that was similar to subjects who were free of infection at baseline.

Correlation analysis between baseline hemoglobin and change in hemoglobin post-intervention was negative. The subjects with lower hemoglobin levels at baseline indicated more intense changes than those with higher Hb. This correlation was stronger for subjects that had negative stool at baseline unlike those who were positive. This explains the higher levels of Hb of 12.376 ± 1.032 /L for subjects in the negative green group as compared to 12.051 ± 1.271 /L for subjects in the positive green group post-intervention. The results on the association between low Hb and helminthic infestations, was found to be similar to the results of other studies (Curtale *et al.*, 1998; Hawdon and Hotez, 1996). Curtale *et al.*, (1998) found parasitic infection among children to be significantly associated with low levels of hemoglobin ($p < 0.0001$) and Hawdon and Hotez (1996) showed that hookworms cause severe anemia.

4.4.4 Bioavailability and Bioconversion Studies

4.4.4.1 Serum Beta-carotene

Beta-carotene in serum from study subjects was analysed by HPLC. The mean serum beta-carotene (S-BC) concentrations for the study groups and their respective control groups are presented in Table 4.16.

Table 4.16: The baseline and post-intervention serum beta-carotene mean concentration of study and control groups for the fresh and sun-dried categories

Period/ %Change	Serum beta-carotene concentration (μmol/L)[1]	
	Fresh vegetable group, n=56	Control group, n=51
Baseline	0.134 ± 0.148 (0.03-0.90)	0.138 ± 0.109 (0.05-0.48)
Post-intervention	0.473 ± 0.212 (0.09-0.92)	0.151 ± 0.112 (0.05-0.64)
Change (%)	252.99	9.42
	Sun-dried vegetable group, n=20	Control group, n=25
Baseline	0.163 ± 0.105 (0.05-0.48)	0.137 ± 0.068 (0.05-0.26)
Post-intervention	0.380 ± 0.219 (0.09-0.79)	0.111 ± 0.050 (0.05-0.22)
Change (%)	133.13	(-) 18.98

[1] ranges in parenthesis

The baseline S-BC concentration were 0.134 ± 0.148 μmol/L (range: 0.03-0.9) and 0.138 ± 0.109 μmol/L (range: 0.05-0.48) for subjects in the fresh vegetable and in its control groups respectively. The subjects in the sun-dried vegetable group had S-BC levels of 0.163 ± 0.105 μmol/L (range: 0.05-0.48) while subjects in the control group had 0.137 ± 0.068 μmol/L (range: 0.05-0.26). Two-tailed t-test showed that the means for the study groups as compared to that of the respective control group was not statistically different at baseline. These levels

of S-BC at baseline were within the normal range of 0.093–0.465 μmol/L for children in the subjects age group (Combs, 1992).

In the post-intervention analysis, subjects in the fresh vegetable study group as well as their control group had an increase in the mean S-BC. The increases reflected a 252.99% change giving 0.473 ± 0.212 μmol/L (range: 0.09-0.92) from 0.134 (range: 0.03-0.90) for the fresh vegetable study group against 9.42% for its control group. One-tailed t-test indicated the increase in subjects of the fresh vegetable study groups to be highly significant ($p<0.000$, $df = 110$) unlike that of the control group. The overall effect was that the control group had significantly lower ($p<0.000$, $df = 105$) mean S-BC of 0.151 ± 0.112 μmol/L (range: 0.05-0.64) post-intervention. Contrary to the change for subjects in the control group, a study by Ribaya-Mercado *et al.,* (2000) noted a highly significant ($P = 0.0001$) increase in the mean S-BC in subjects who fed on low carotene-containing vegetables.

Study subjects in the sun-dried group had an increase that reflected a 133% change against a decrease of 18.98% for its control group. One-tailed t-test indicated a highly significant increase ($p<0.000$, $df = 38$) of mean S-BC, 0.380 ± 0.219 μmol/l (range: 0.09-0.79) from 0.163 ± 0.105 (range; 0.05-0.48) in the study group while the control group decreased though not significantly. This led to a statistical difference in the mean S-BC concentrations between the groups post intervention with the control group having a significantly lower ($p<0.000$, $df = 43$) mean S-BC, 0.111 ± 0.050 μmol/L (range: 0.05-0.22) in comparison to 0.380 ± 0.219μmol/L.

The results suggest that beta-carotene in both fresh and sun-dried *V. unguiculata* and *amaranthus* vegetables contributed positively towards its bioavailability among the study subjects. Though the increase in both groups feeding on DGLVs post-intervention was significant, the increase in S-BC for study subjects in the fresh vegetable group was higher as compared to that of the sun-dried vegetable group. The greater increase in S-BC concentrations after intervention in the fresh vegetable group (252%) than in the dehydrated vegetable group (133%) is pegged to the discrepancy in the amount of beta-carotene in the fresh vegetables, a factor that is a significant predictor in bioavailability of S-BC (Castenmiller and West, 1998; Shiau *et al.*, 1994; Prince and Frisoli, 1993). It cannot be ruled

out that home diets had some contribution to the bioavailability of S-BC although the foods listed in home diaries (Appendix 2) were majorly non-pro-vitamin A carotenoids. The loss in β-carotene content in dehydrated products is attributed largely to its oxidative degradation as well as to cooking (Glover, 1960; Burton, 1989; Kanasawud and Crouzet, 1990; Marty and Berset, 1990; Wang *et al.*, 1991; Hui, 1992; Mordi *et al.*, 1993; Handelman *et al.,* 1993; Wang and Krinsky, 1998; Wyss *et al.*, 2000; Lintig and Vogt, 2000; Barua and Olson, 2000; Olson and Hayaishi, 1965). Cooking however, is inevitable and it assists to release carotenoids to be absorbed more efficiently. Heat increases the bioavailability of carotenoid as it disrupts or softens plant cell walls and break carotenoid-protein complexes to release carotenoids (Micozzi *et al.*, 1992; Van het Hof *et al.,* 1998; Castenmiller *et al.,* 1999; Erdman, 1988; Stahl and Sies, 1992; Hussein and El-Tohamy, 1990).

The ability of beta-carotene rich DGLV and other vegetable matrixes to meet the vitamin A needs of at risk populations (both children and adults) is under scrutiny. While some findings indicate the potential of DGLVs in improving vitamin A status, others contradict the results (Takyi, 1999, Tang *et al.*, 1998; Van het Hof *et al.,* 1999; Micozzi *et al.*, 1992; Castenmiller *et al.,* 1999; Bulux *et al.,* 1994; de Pee *et al.,* 1995; de Pee *et al.*, 1998; Wadhwa *et al.,* 1994; Jalal *et al.,* 1998; Ribaya-Mercado *et al.,* 2000; Vuong *et al.*, 2002; Bulux *et al.*, 1994; Mulokozi *et al.,* 2004). Bulux *et al.* (1994) reports a three fold increase in plasma beta-carotene concentrations in the group supplemented with purified beta-carotene, while concentrations remained unchanged with carrot supplementation. Beta-carotene supplementation in lactating women showed a significant increase in the concentration of plasma beta-carotene in the group given an enriched wafer but no change in the group supplemented with DGLVs (de Pee *et al.*, 1995).

The processing of vegetables increases the bioavailability of carotenoids, although other findings indicate otherwise (Castenmiller *et al.*, 1999; Stahl and Sies, 1992; de Pee *et al.,* 1998; Van het Hof, 2000; Micozzi *et al.*, 1992). Castenmiller *et al.*, (1999) reports that, consuming spinach when processed, the plasma response of beta-carotene was unaffected. However, Stahl and Sies (1992) had reported that bioavailability of lycopene was greater from tomato paste than from fresh tomatoes, signifying the importance of processing. A study by de Pee *et al.* (1998), concludes that the bioavailability of beta-carotene from fruits is

four times that from vegetables. This is because chloroplasts in vegetables are less efficiently disrupted in the intestinal tract than chromoplasts in fruits. Carotenoids are dissolved in oil droplets in chromoplasts and can be readily extracted during digestion (de Pee *et al.,* 1998). Based on the effect of different vegetable matrixes, the absorption of carotenoids from carrots is greater than that from broccoli, which in turn is greater than that from spinach (Micozzi *et al.*, 1992; Castenmiller *et al.,* 1999) after enzymatic disruption of the matrix (cell wall structure). However, the bioavailability of beta-carotene when provided as a pure compound is ten times higher than when it was provided naturally in foods such as DGLVs (Van het Hof, 2000).

The results of this study support those who suggest that the bioavailability of beta-carotene increase with consumption of DGLVs (Wadhwa *et al.,* 1994; Jalal *et al.,* 1998; Ribaya-Mercado *et al.,* 2000; Vuong *et al.*, 2002; Bulux *et al.,* 1994; Mulokozi *et al.,* 2004) while contrast with other findings (de Pee *et al.,* 1995; de Pee *et al.,* 1998). The differences may be due to the different amounts of the carotenoid and therefore these gave different effectiveness in bioavailability studies. This is coupled with the "SLAMENGHI" factors that have been found to affect the bioavailability of beta-carotene (Kostic *et al.*, 1995; Castenmiller and West, 1998; Castenmiller *et al.*, 1999; van het Hof *et al.,* 1999b; Boileau *et al.*, 1999; Erdman *et al.,* 1993; Olmedilla *et al.,* 1994; Herbst *et al.*, 1997; Van vliet *et al.*, 1996; Roodenburg *et al.*, 2000; Wingerath *et al.*, 1995).

The individual profiles of serum beta-carotene (S-BC) for study subjects for the study groups and their respective control groups both at baseline and post-interventional are presented in Figures 4.6 (a-d). The profiles on the left side are for subjects who were not infected at baseline while those on the right side are for the subjects who were diagonised to be infected and treated with the antihelminthic drug.

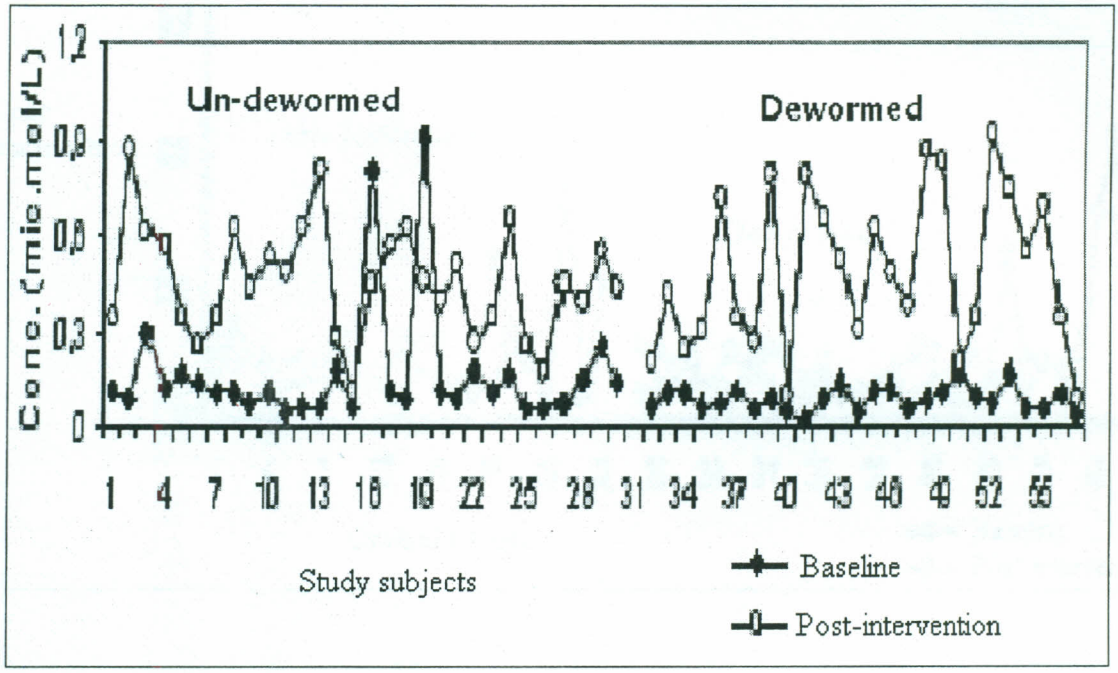


Figure 4.6a: Serum beta-carotene profile at baseline and post-intervention for individual study subjects in the fresh vegetable study group

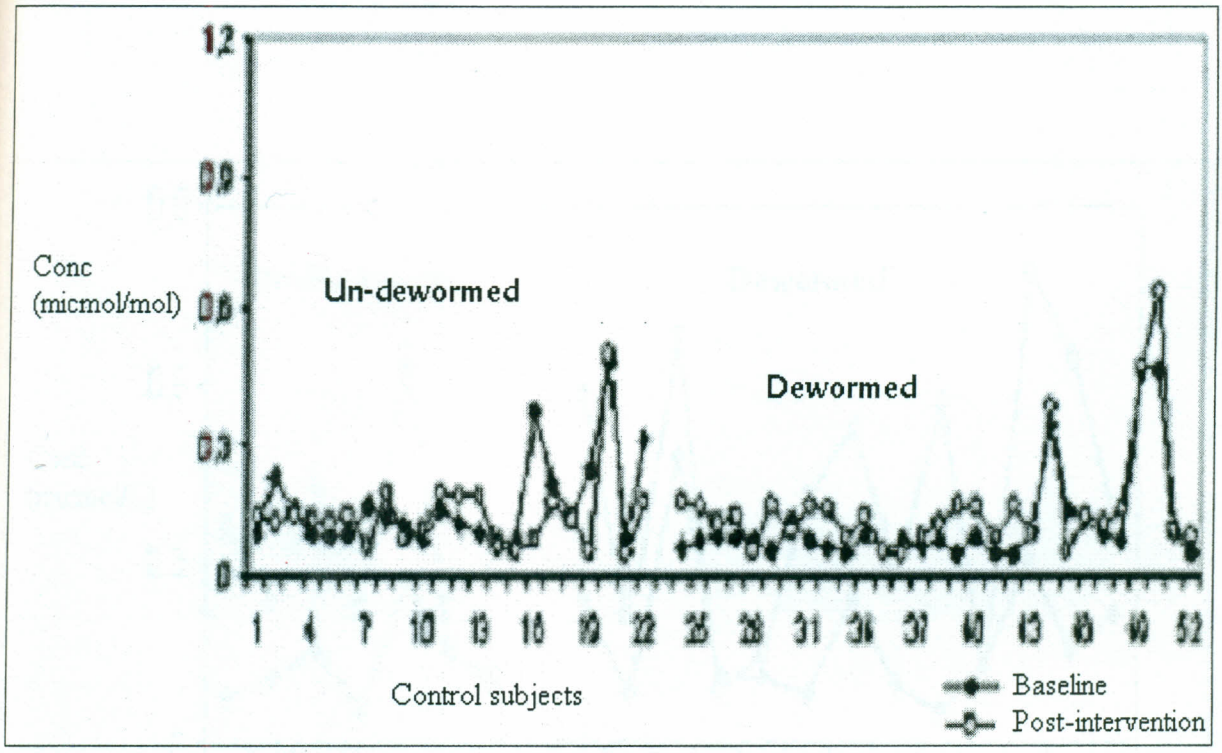


Figure 4.6b: Serum beta-carotene profile at baseline and post-intervention for individual study subjects in the fresh vegetable control group

The profiles indicate inter-individual variations. There was a random inter-individual pattern across the group as individual variations led to various changes. A majority of study subjects in the study groups showed improvements in S-BC content. Others showed un-noticeable improvements while a minor percentage showed a decrease. In the fresh DGLV group, 89% had major noticeable increase, 7% had un-noticeable increases while 4% had their S-BC decrease over the intervention period. These changes are attributed to infection post intervention as well as re-infection. Subjects whose stool tested positive post-intervention (Figure 4.4 a and b) had their S-BC noticeably decrease post-intervention (Figure 4.6(a), subjects 16 and 19; Figure 4.6(b), subject 16 and 22). Subjects in the dehydrated vegetable group had their trends as shown in Figure 4.6 c and d.

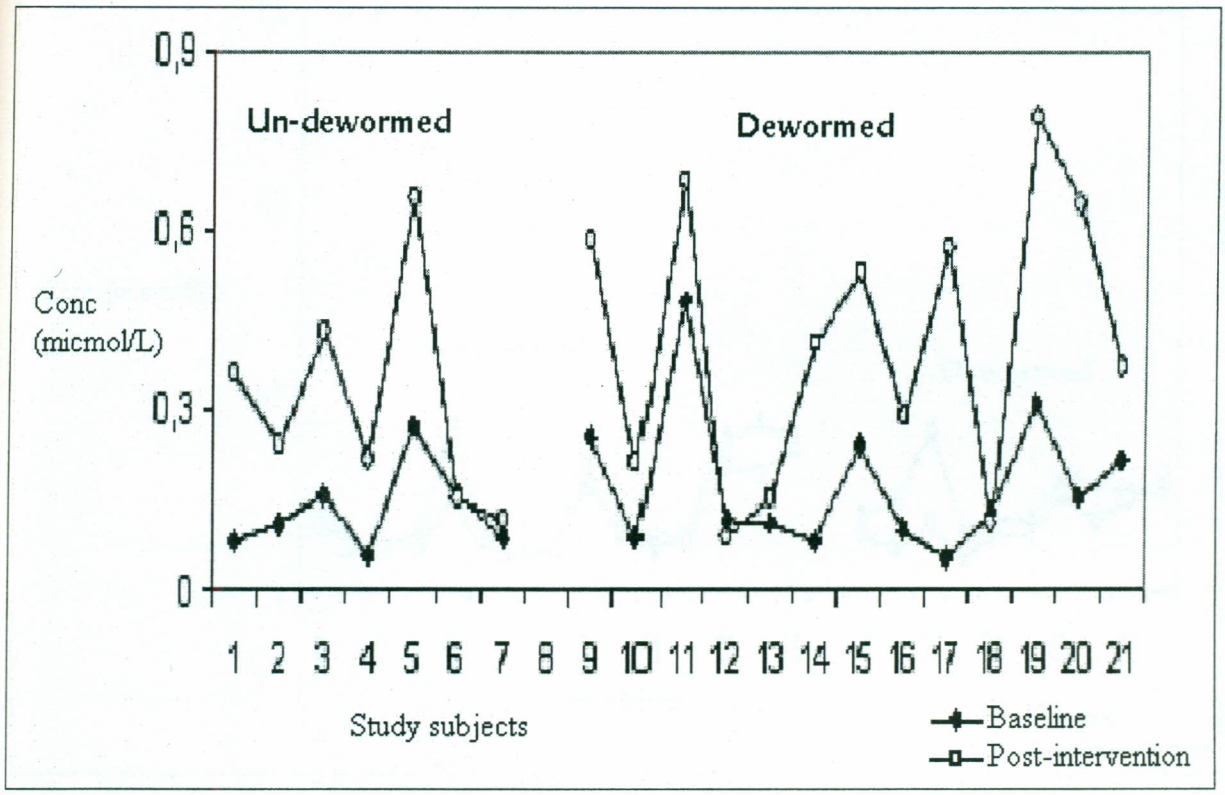


Figure 4.6c: Serum beta-carotene profile at baseline and post-intervention for individual study subjects in the sun-dried vegetable study group

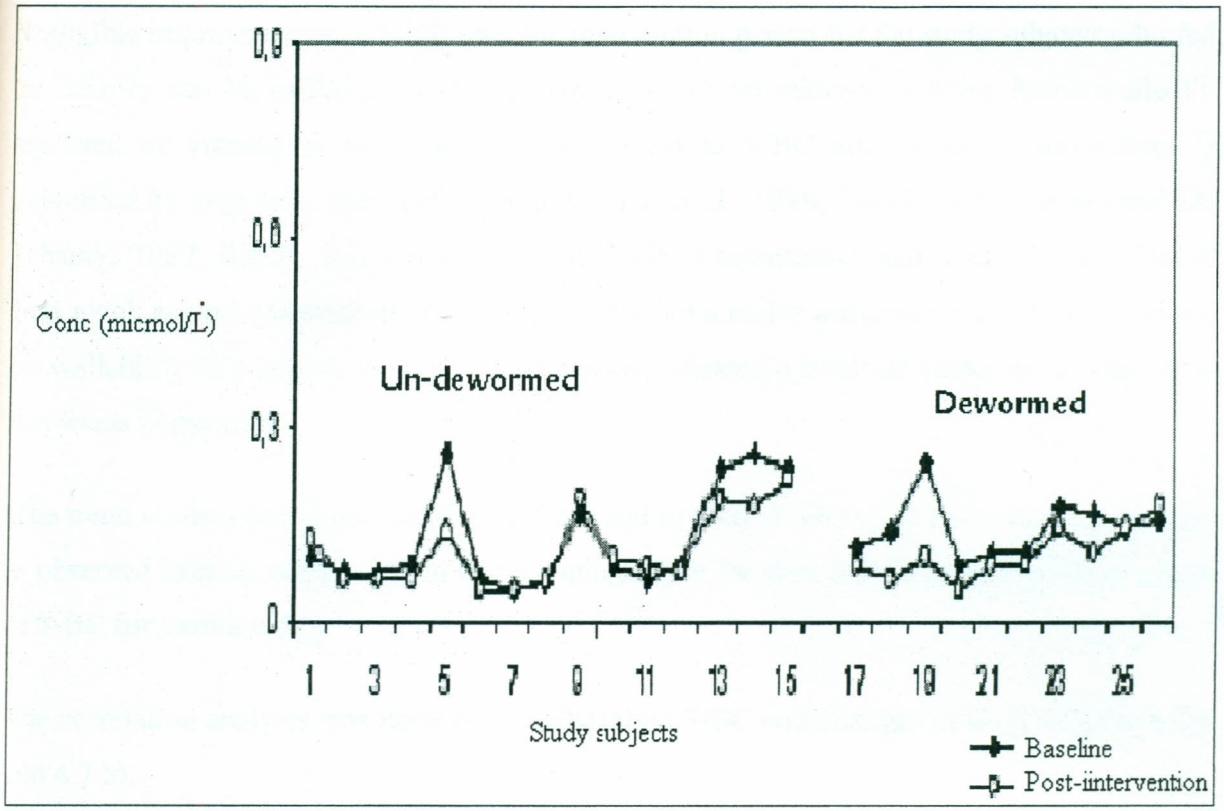


Figure 4.6d: Serum beta-carotene profile at baseline and post-intervention for individual study subjects in the sun-dried vegetable control group

The profiles similarly had inter-individual variations with a random inter-individual pattern across the group as individual had various changes. The distributions were 75%, 15% and 10% for increase, un-noticeable increase and decrease in S-BC respectively. The high percentage of subjects who improved in their S-BC levels is as a result of deworming. Studies have shown that absorption and utilization of beta-carotene are enhanced after de-worming of children infected with parasites (Jalal *et al.,* 1998; Tanumihardjo *et al.,* 1996; Mahalanabis *et al.,* 1979). A majority of study subjects in the study groups showed improvements in S-BC levels except subjects 6 and 7 (Figure 4.7b) who had no noticeable

changes while subjects 12 and 13 had some decrease. These changes can partly be attributed to infection post intervention since subjects whose stool tested positive post-intervention (Figure 4.5 c and d) had their S-BC decrease post-intervention (Figure 4.7(c) subject 6; Figure 4.7(d), subject 15 and 24).

Negligible improvements in S-BC over the intervention period for the study subjects who fed on DGLVs can be explained as being due to S-BC concentrations being homeostatically regulated by vitamin A status since improvement in S-BC after dietary interventions is influenced by total body stores of vitamin A (Jalal *et al.,* 1998; Takyi, 1999; Hussein and El-Tohamy, 1989; Reddy and Vijayaraghavan, 1995; Castemniller and West, 1998). Home diets much as was a limitation of this study, were not a major influence in contributing to the bioavailability of beta-carotene. The food diaries indicated a minimal intake of provitamin A rich foods (Appendix 2).

The trend on the control groups (Figure 4.6 b and d) were observed to have minimal changes as observed from an almost one to one mapping of the baseline and post interventional levels of S-BC for individuals.

The correlation analysis was done between baseline S-BC and changes in S-BC (Figure 4.7 a and 4.7 b).

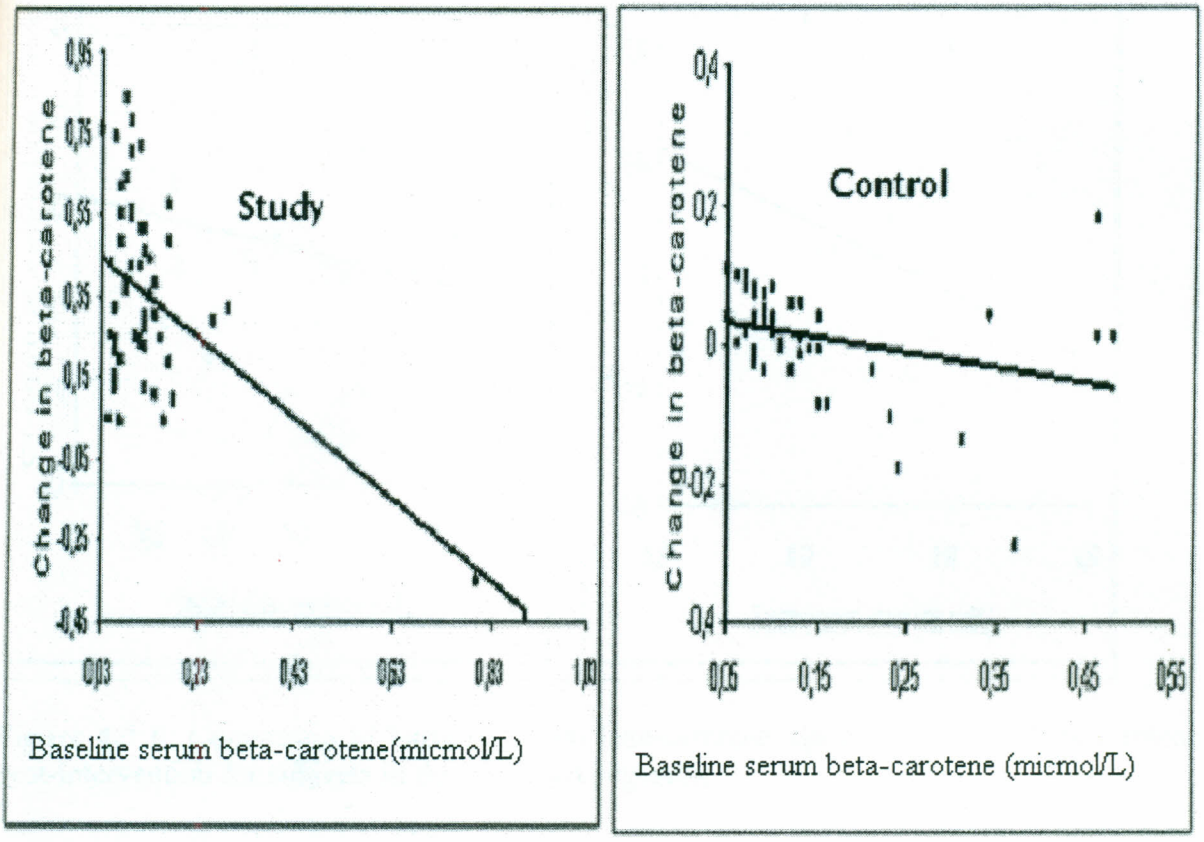


Figure 4.7 a: Correlation of baseline serum beta-carotene against change in beta-carotene post-intervention for subjects in the fresh vegetable group

The results indicate a negative correlation between the baseline S-BC and change in S-BC for subjects in both the study and control group, thus, subjects with low S-BC improved more during the intervention. The intensity of the correlation was higher in the fresh vegetable study group than for subjects in the control group with pearsons correlation value of r = 0.571 versus r = 0.315, this supporting the effectiveness of DGLVs in improving S-BC levels (Takyi 1999; Jalal *et al.*, 1998; Hussein and El-Tohamy, 1989; de Pee *et al.*, 1998; Reddy and Vijayaraghavan, 1995; Ribaya-Mercado *et al.*, 2000).

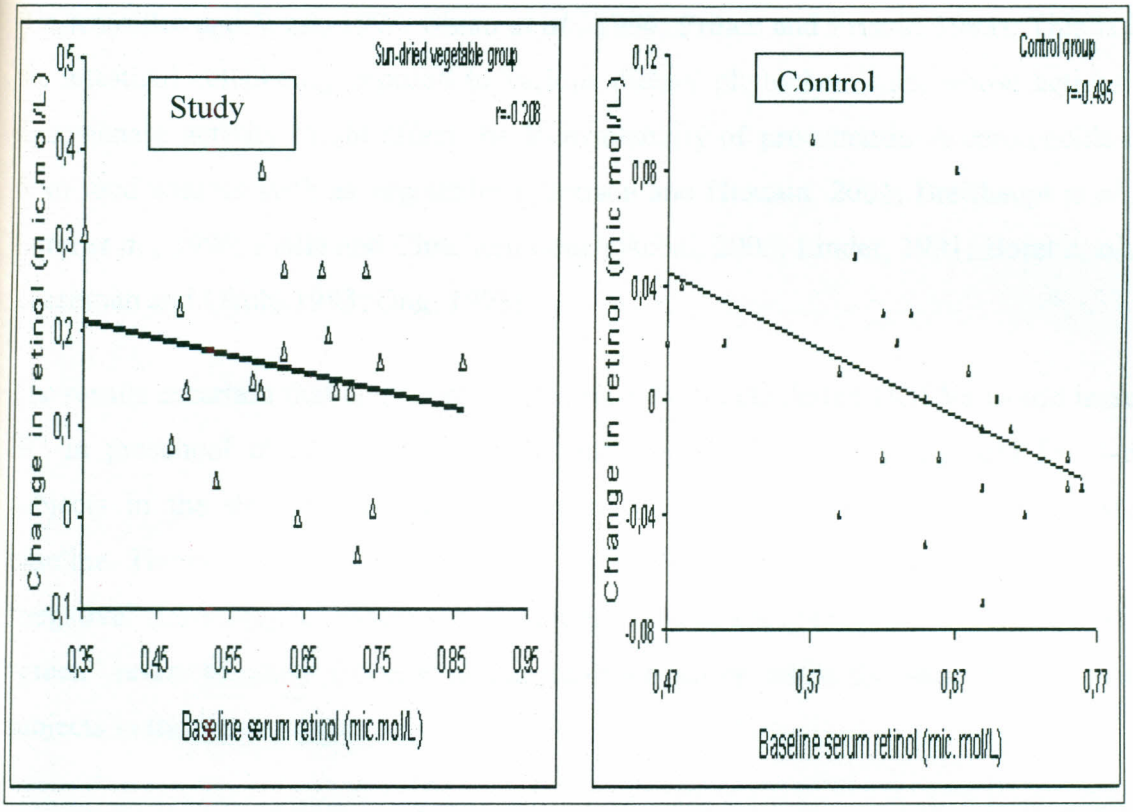


Figure 4.7 b: Correlation of baseline serum beta-carotene against change in beta-carotene post-intervention for subjects in the sun-dried vegetable

There was a negative correlation between the baseline S-BC and change in S-BC for subjects in both the study and control group thus subjects with low S-BC in the study group improved more during the intervention while those in the control group decreased more in their S-BC post-intervention this supporting the effectiveness of DGLVs in improving S-BC levels (Takyi 1999; Jalal *et al.*, 1998; Hussein and El-Tohamy, 1989; de Pee *et al.*, 1998; Reddy and Vijayaraghavan, 1995; Ribaya-Mercado *et al.*, 2000). The intensity of the correlation for subjects in the study and control group had pearsons correlation values of r = 0.208 and r = 0.495 respectively. The higher intensity of the increase in S-BC for subjects in the control group can be pegged to bioconversion of S-BC thus decreasing the levels for its bioavailability. This reduction is due to the low carotene containing recipe during the intervention coupled with deworming. The amount of carotenoids consumed in a meal and the duration of carotene supplementation are significant predictors in their bioavailability

(Castenmiller and West, 1998; Shiau *et al.*, 1994; Prince and Frisoli, 1993). This is besides the intestinal cells being exposed to various dietary phytochemicals, whose actions on the dioxygenase activity might affect the bioavailability of pro-vitamin A carotenoids derived from food sources such as vegetables (Harrison and Hussain, 2001; Breithaupt *et al.*, 2002; Borel *et al.*, 1996; Failla and Chitchumroonchokchai, 2005; Linder, 1991; Borel *et al.*, 2001; Lakshman and Okoh, 1993; Ong, 1993).

The results ascertain that consumption of diets rich in dehydrated DGLVs would increase S-BC in preschool children. This was further monitored by analyzing data for S-BC for subjects in the dehydrated vegetable group based on their parasitic infection status at baseline. The results are given in Table 4.17. The subjects were categorized as "positive" or "negative" depending on whether they were infected or not at baseline respectively. The term "green" refers to study subjects in the sun-dried group while the term "white" is for the subjects in the control group.

Table 4.17: Beta-carotene concentration (µmol/L) and percentage change in study and control parasite positive subjects and negative subjects at baseline and post-intervention

Categories	Serum-carotene (µmol/L)[1]		
	Baseline	Post-intervention	% Change
Positive			
-Green	0.120 ± 0.084 (0.03-0.48)	0.465 ± 0.244 (0.09-0.92)	287.50[2]
-White	0.126 ± 0.097 (0.05-0.46)	0.146 ± 0.113 (0.05-0.64)	15.87
Negative			
-Green	0.164 ± 0.176 (0.05-0.90)	0.431 ± 0.184 (0.11-0.87)	162.80[2]
-White	0.151 ± 0.096 (0.05-0.48)	0.129 ± 0.079 (0.05-0.49)	(-) 14.57

[1]Ranges in parenthesis

[2]Change was significant

Study subjects who had parasites in stool before deworming at baseline and fed on DGLVs during the intervention (positive –green), had their mean S-BC increase from 0.120 μmol/L (range; 0.03-0.48 μmol/L) to 0.465 μmol/L (range; 0.09-0.92 μmol/L). The post-intervention mean value of S-BC shows an improvement that is significant from that of the baseline ($p< 0.001$, $df= 76$). Similarly, study subjects who had no parasites in stool at baseline and fed on DGLV (negative –green) had an increase in S-BC from 0.164 μmol/L (range; 0.05-0.9 μmol/L) to 0.431 μmol/L (range; 0.11-0.87 μmol/L). This post-interventional increase was also statistically significant ($p< 0.001$, $df= 76$). The positive-white study subjects also had the S-BC increase from 0.126 μmol/L (range; 0.05-0.46 μmol/L) to 0.146 μmol/L (range; 0.05-0.64 μmol/L), though this was not a statistically significant increase.

These increases noted in the two study groups (positive green and negative green) imply that pro-vitamin A carotenoids from DGLVs would improve the S-BC levels of beta-carotene more so when subjects are freed of parasitic infection. The improvements noted could also be explained as being due to decreased intestinal parasitic load as a result of de-worming, which improved liver stores of beta-carotene (Castemniller and West, 1998; Kücükbay *et al.*, 1997; Jalal *et al.*, 1998; Friis *et al.*, 1996; Tanumihardjo *et al.*, 1996; Mahalanabis, 1979). It was found that the 'positive green' had a much higher percentage change post-interventional as compared to the negative green group (287% versus 162%). This can be explained by the fact that those who had parasites had lower liver stores and once dewormed had levels increased. This is supported further by the trend in the negative white group, whose concentrations decreased by about 15% although the drop was not significant.

Correlation analysis were done with charts similar to Figures 4.8a and b that showed a significant negative correlation between baseline S-BC and change in S-BC post-intervention for all groupings. The subjects with lower S-BC levels at baseline had higher changes than those with higher levels at baseline. The correlation was stronger for negative subjects unlike the positive subjects. This finding supports the effectiveness of DGLVs in improving the S-BC status of preschool children (Wadhwa *et al.*, 1994; Jalal *et al.*, 1998; Ribaya-Mercado *et al.*, 2000; Vuong *et al.*, 2002; Bulux *et al.*, 1994; Mulokozi *et al.*, 2004).

4.4.4.2 Serum retinol

The mean serum retinol (S-R) concentrations for the study groups and their respective control groups are presented in Table 4.18.

Table 4.18: Baseline and post-intervention serum retinol concentration (μmol/L) and change of the study subjects in the fresh and sun dried vegetable study and control groups

Period/change	Serum retinol (μmol/L)[1]	
	Fresh vegetable group; n=56	Control group; n=51
Baseline	0.637± 0.064 (0.48-0.75)	0.632 ± 0.130 (0.31-1.00)
Post-intervention	0.821± 0.153 (0.49-1.08)	0.669± 0.102(0.52-1.02)
Change (%)	(+) 28.89	(+) 5.85
	Sun-dried vegetable group; n=20	Control group; n=25
Baseline	0.626 ± 0.120 (0.35-0.86)	0.644 ± 0.078 (0.47-0.76)
Post-intervention	0.788± 0.144 (0.55-1.03)	0.645± 0.068 (0.49-0.75)
Change (%)	(+) 25.88	(+) 0.16

[1] ranges in parenthesis

The subjects in the fresh vegetable study group and their control group had statistically similar mean S-R concentration of 0.637 ± 0.064 μmol/L (range: 0.48-0.75 μmol/L) and 0.632 ± 0.130 μmol/L (range: 0.31-1.00 μmol/L) respectively at baseline. Similarly, the group mean S-R was statistically similar for the dehydrated group and its control group, thus, 0.626 ± 0.120 μmol/L (range: 0.35-0.86 μmol/L) and 0.644 ± 0.078 μmol/L (range: 0.47-0.76 μmol/L) respectively. These S-R concentrations in both study groups and the control groups were marginally lower than 0.7 μmol/L, the recommended level of serum retinol in children (Combs, 1992). The low S-R concentrations indicate that VAD is a nutritional problem in the study area, a common case in developing countries, (Lee and Lim, 1990,

Olson, 1999). The high percentage of subjects with marginal S-R (55% and 70% of study subjects in the fresh and dehydrated vegetable study groups respectively) can be ascertained to have contributed to the overall low serum retinol at baseline for the study groups.

At post-intervention, mean S-R increased in both the study and control groups reflecting a 28.89% increase for the fresh vegetable group against 5.85% for its control group. For the sun-dried group this increase reflected a 25.88% increase against 0.16% for its control group. One-tailed t-tests indicated that the increases were statistically significant within the study groups unlike in the control groups ($p<0.000$, $df = 110$, for fresh vegetable and $p<0.000$, $df = 38$ for dehydrated group). One-tailed t-tests indicated the control group to have significantly lower ($p<0.000$, $df = 105$) mean S-R of 0.669 ± 0.102 μmol/L (range: 0.52-1.02 μmol/L) as compared to 0.821 ± 0.153 μmol/L (range: 0.49-1.08 μmol/l) for the fresh vegetable study group post-intervention. For the dehydrated vegetable group the control group had statistically lower ($p<0.000$, $df = 43$) mean S-R of 0.645 ± 0.068 μmol/L (range: 0.49-0.75 μmol/l) as compared to 0.788 ± 0.144 μmol/L (range: 0.55-1.03 μmol/L) of its study group. This led to a statistical difference in the mean S-R concentrations between the study groups and their respective control groups post intervention results. The increases in the control groups however, were not significant. These increases in S-R for control groups are attributed to de-worming and to the foods the subjects took at home (Vuong *et al.*, 2002).

The implication here is that sun-dried vegetables positively contribute to the bioconversion of beta-carotene to retinol. The findings consequently give evidence with the increase in S-R (0.637 μmol/L to 0.821 μmol/L and 0.626 μmol/L to 0.788 μmol/L). The post-intervention changes for the study group and control groups are shown in Figure 4.8.

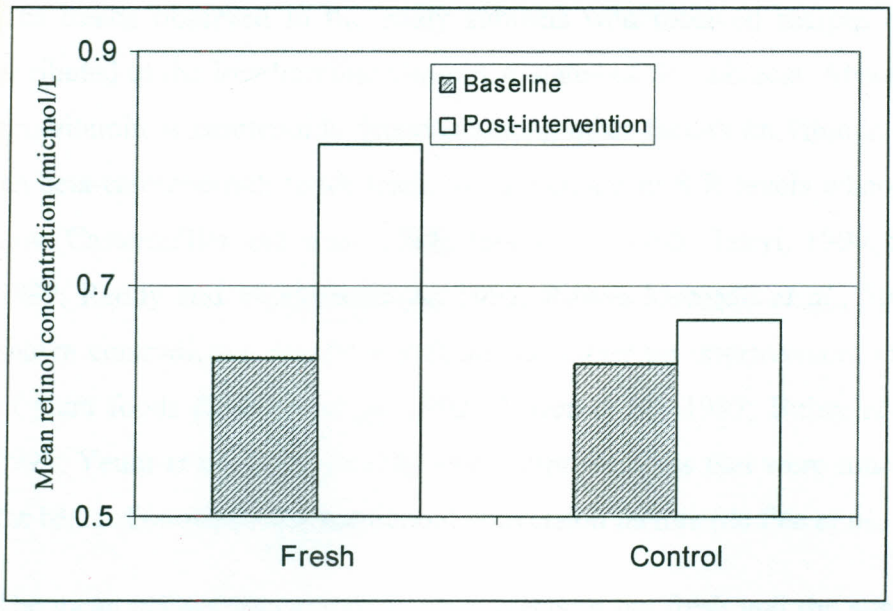


Figure 4.8a: Mean changes of serum retinol in the study subjects against WHO level of 0.7 micmol/L for subjects in fresh vegetable study and control group

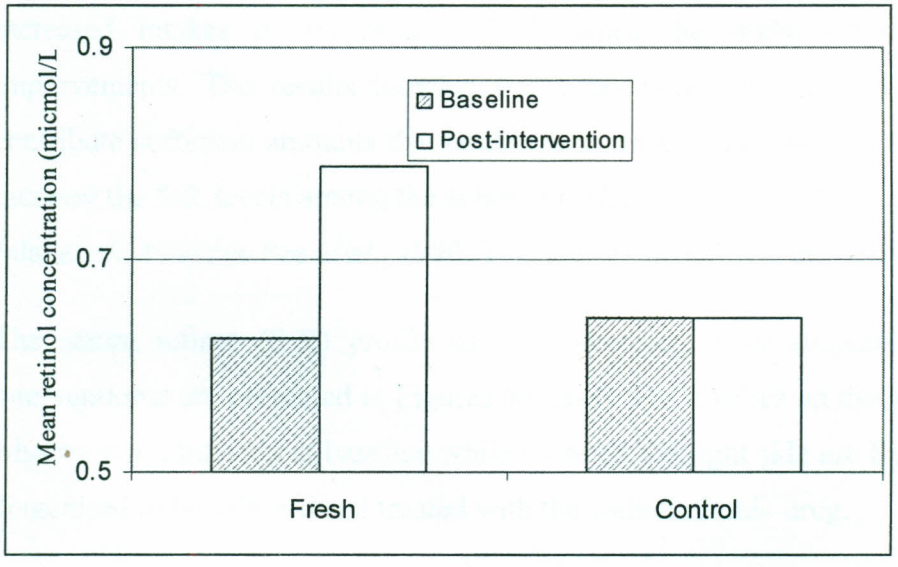


Figure 4.8b: Mean changes of serum retinol in the study subjects against WHO level of 0.7 micmol/L for subjects in sun-dried vegetable study and control group

The trends observed in the study subjects who received recipes of DGLV's can also be attributed to the low baseline vitamin A status of the subjects. Absorption and conversion of pro-vitamin A carotenoids depends among other factors on vitamin A status so that feeding on beta-carotene-rich foods leads to an increase in S-R levels when vitamin A was initially low (Castemniller and west, 1998; Jalal *et al.,* 1998; Takyi, 1999; Hussein and El-Tohamy 1989; Reddy and Vijayaraghavan 1995; Ribaya-Mercado *et al.*, 2000). Other studies have shown contradicting results that there was either no improvement in S-R after consumption of plant foods (Micozzi *et al.* 1992; Bowen *et al.*, 1993; Bulux *et al.* 1994; de Pee *et al.*, 1995; Yeum *et al.* 1996) or only small improvements that were much less than predicted on the basis of conventional carotenoid conversion factors (de Pee *et al.*, 1998).

The mean retinol concentration for subjects in the fresh and the sun-dried vegetable groups were not statistically different at baseline but both groups had significant changes post-interventional (Figure 4.9). The post-interventional serum retinol concentrations were however not significantly different between the two groups signifying the effectiveness of dehydrated DGLVs in improving the bioconversion of S-BC to retinol. The results show that it is possible to obtain a substantial improvement in the vitamin A status of children by increased intakes of sun-dried DGLVs since the study subjects showed significant improvements. The results indicate that beta-carotene content in the sun dried DGLVs contribute sufficient amounts that could aid in the bioconversion of beta-carotene and hence increase the S-R levels among the subjects (Suharno *et. al.*, 1993; Tanumihardjo *et al.*, 1996; Jalal *et al.*, 1998; de Pee *et al.*, 1998; Takyi, 1999; Rosales *et al.*, 1999; Vuong *et al.*, 2002).

The serum retinol (S-R) profile of the individual study subjects at baseline and post-interventional are presented in Figures 4.9 (a-d). The profiles on the left side are for subjects who were not infected at baseline while those on the right side are for the subjects who were diagonised to be infected and treated with the antihelminthic drug.

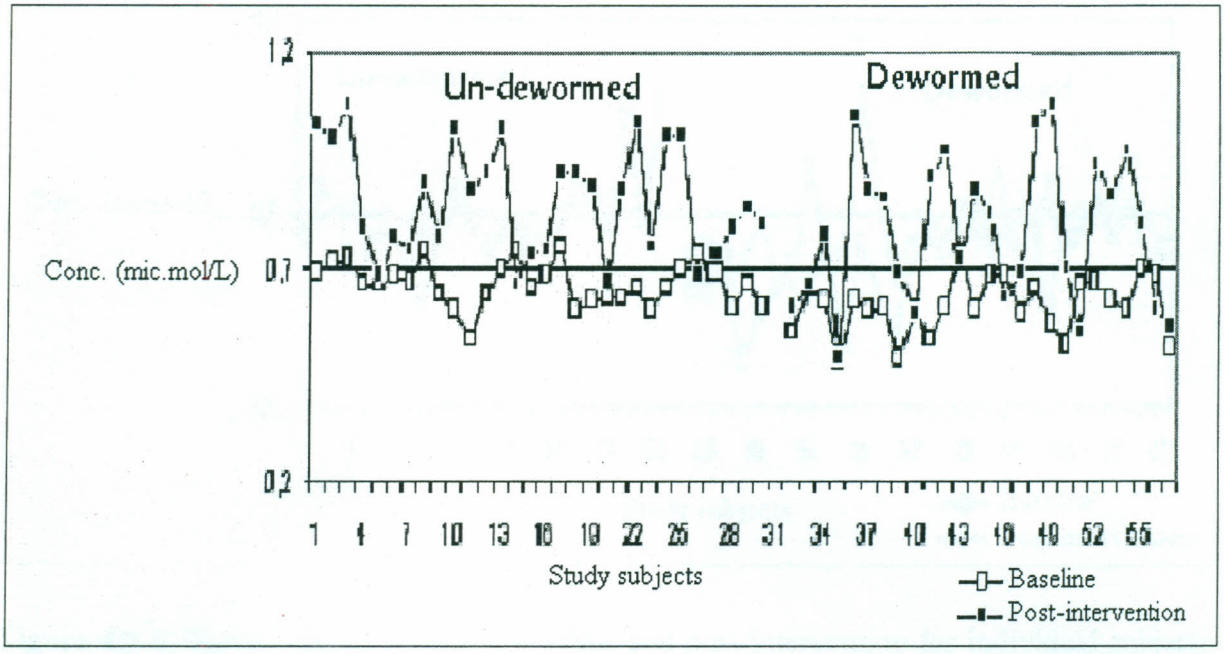


Figure 4.9 a: Serum retinol profile at baseline and post-intervention for individual subjects in the fresh vegetable study group

The individual profile indicates that there was no general pattern since there were inter-individual variations in the group. McNemars chi-square tests showed that at baseline, 55% of study subjects had S-R concentrations that were <0.70 μmol/L, the recommended level of S-R in children under six years this reducing to 36% post-intervention. The higher percentage of subjects who improved in the S-R levels (64%) implies the improvement in vitamin A status after dietary interventions (Castenmiller and west, 1998). This may also be coupled with the fact that the subjects were free of parasitic infection. However, it was noted that subjects 32, 35 and 57 were re-infected and this may explain the reduction or minimal changes in this subject's' S-R post-intervention. For the control group, the profile is as shown in figure 4.9 b.

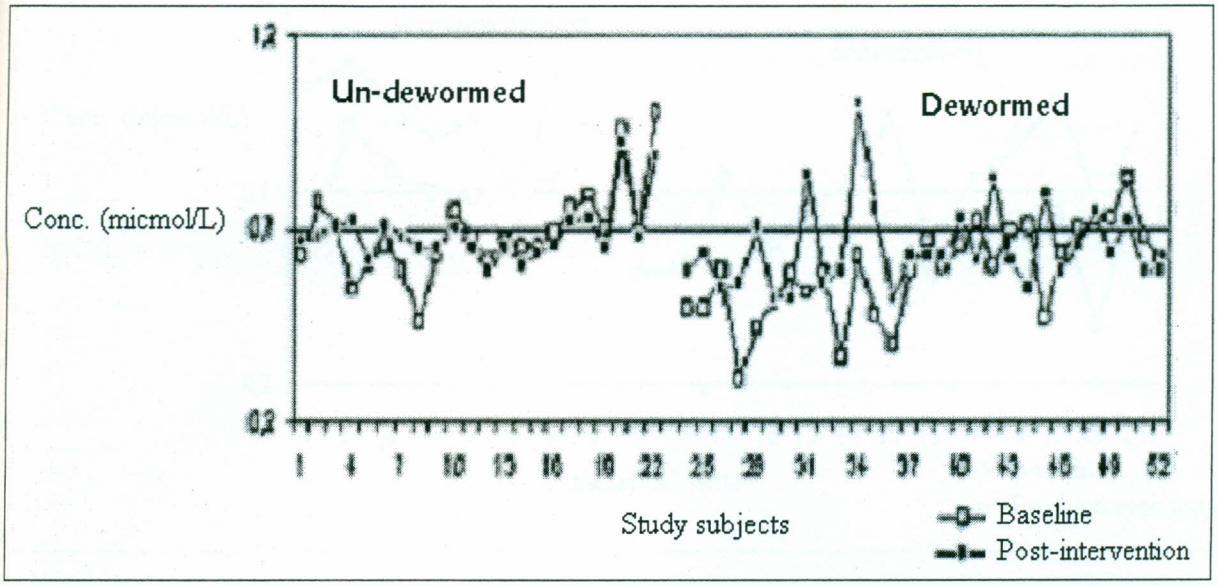


Figure 4.9 b: Serum retinol profile at baseline and post-intervention for individual subjects in the fresh vegetable control group

The individual profiles for the fresh vegetable control group was similar to that of the study group where there were inter-individual variations. McNemars chi-square tests showed that at baseline, 73% of study subjects had S-R concentrations that were <0.70 μmol/L, the recommended level of S-R in children under six years this reducing to 67% post-intervention. The percent change was not significant and was observed from a one to one mapping of the baseline and post-intervention levels of S-R. Subjects 16 and 19 who were found to be infected post-intervention had reduction in their S-R levels. Failure to improve in S-R in some study subjects can be explained besides the subjects consuming low-carotene recipes and also due to serum concentrations being homeostatically regulated by vitamin A status. In marginally nourished individuals, serum VA is maintained within a narrow range and may not show consistent responsive to an increase in vitamin A consumption to closely reflect recent dietary intake (Olson, 1999, Vuong *et al.,* 2002). Subjects in the dehydrated vegetable group are shown in Figure 4.9c.

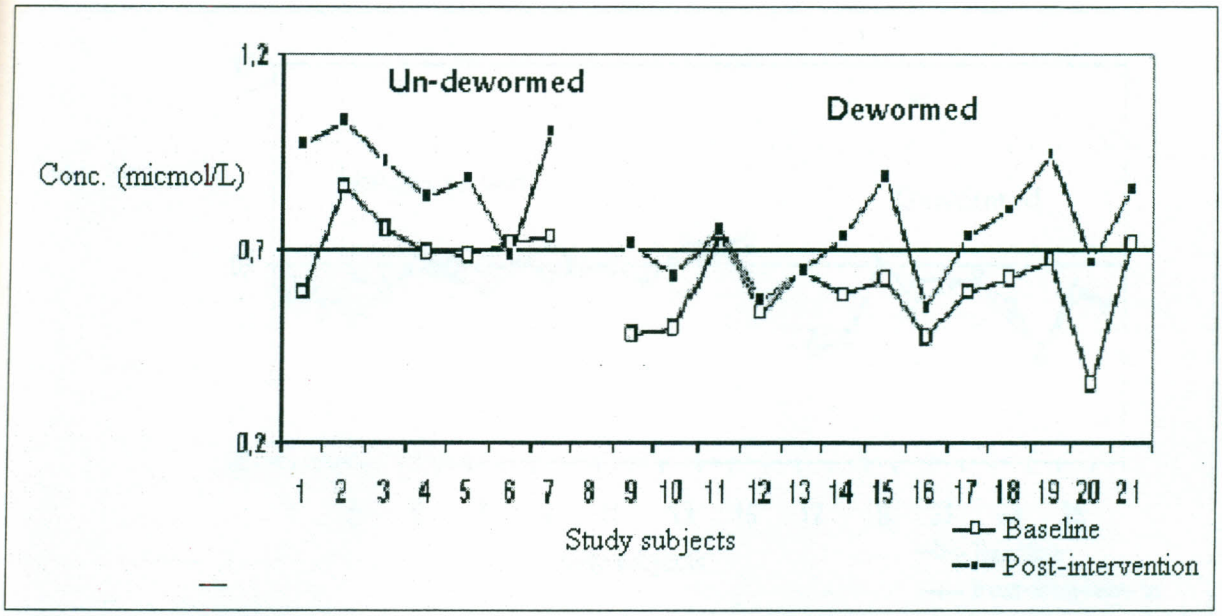


Figure 4.9 c: Serum retinol profile at baseline and post-intervention for individual subjects in the sun-dried vegetable study group

There were inter-individual variations as observed in the profile. McNemars chi-square tests showed that at baseline, 70% of study subjects had S-R concentrations that were <0.70 μmol/L this reducing significantly to 30%. While some subjects who had marginal serum retinol (S-R) concentration at baseline did not show any noticeable increase post-intervention (subjects 11, 12 and 13), others showed improvements (80%) while subject 6 had S-R decrease post-intervention. The latter was however, infected post-intervention thus this may explain the negative change in this subject. The profile indicates that intervention with the dehydrated vegetables improved the S-R of the study subjects. The subjects in the control group had their profile as shown in Figure 4.9 d.

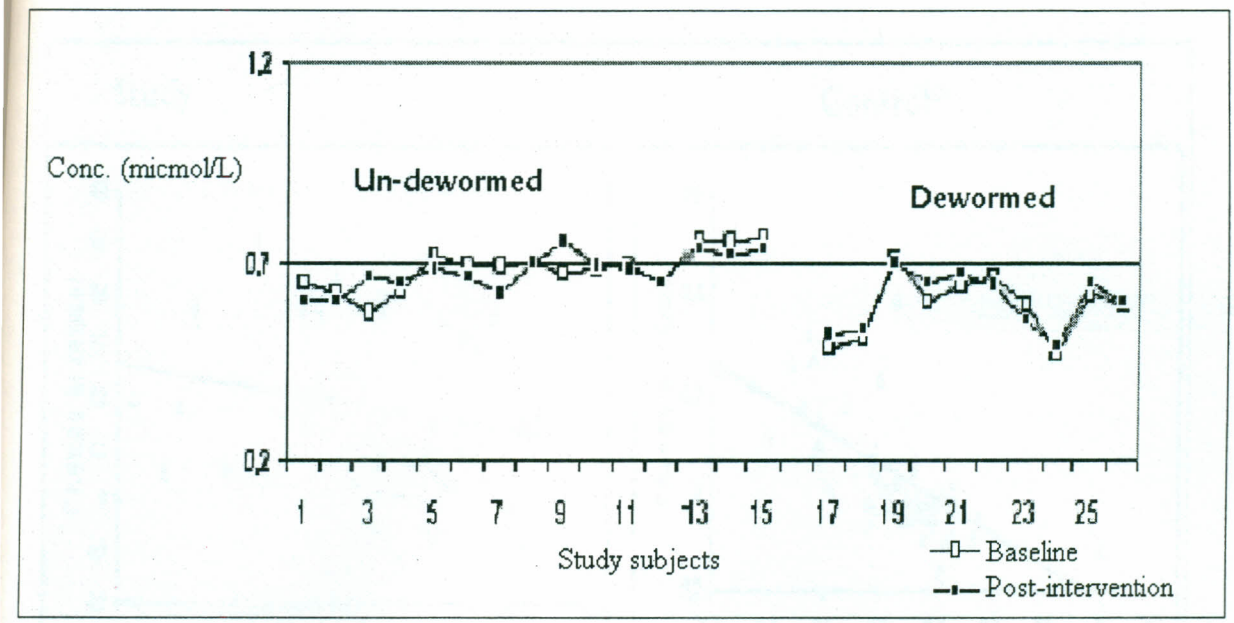


Figure 4.9 d: Serum retinol profile at baseline and post-intervention for individual study subjects in the sun-dried vegetable control group

In the individual profile, changes are described as being a one to one mapping for the baseline and post interventional levels of S-R. This implied the changes were minimal or not noticeable as also noted by McNemars chi-square tests whish showed that 76% of subjects had S-R being less than 0.70 μmol/L at both baseline and post intervention. This is explained by the low-carotene containing recipe for this group.

The correlation analysis was done between baseline S-R values and changes in S-R and the results are presented in Figures 4.10 a and 4.10 b.

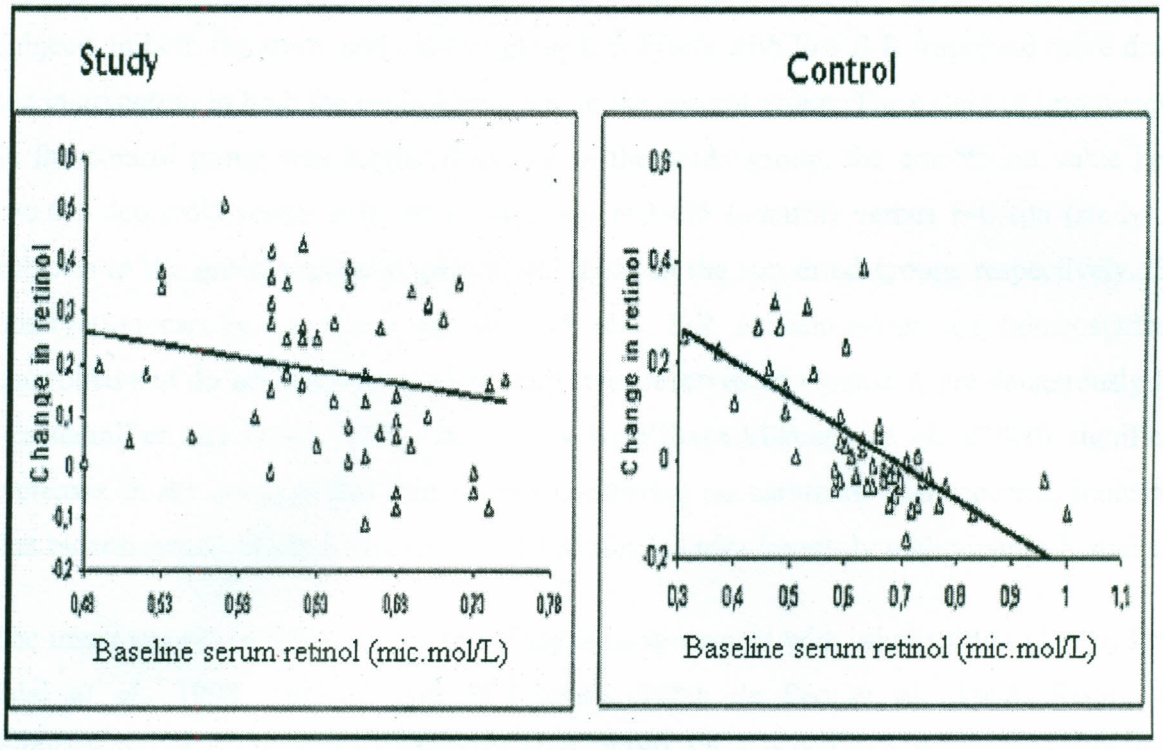


Figure 4.10 a: Correlation of baseline serum retinol and change in retinol post-intervention for subjects in the fresh vegetable group

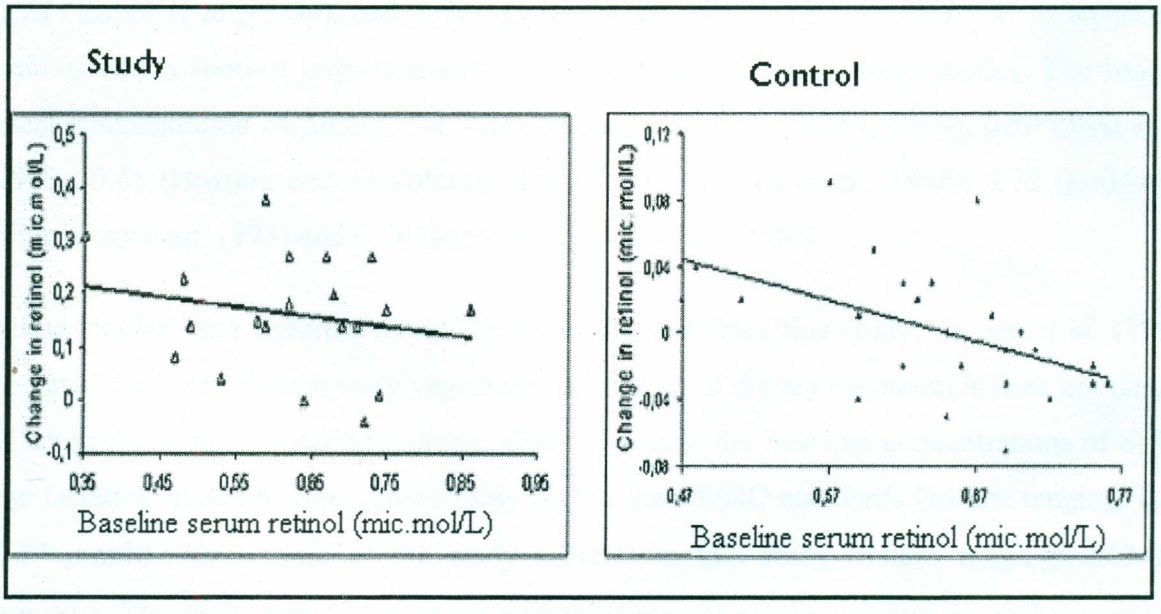


Figure 4.10 b: Correlation of baseline serum retinol and change in retinol post-intervention for subjects in the sun-dried vegetable group

The results indicated a negative correlation between the baseline S-R and change in S-R for subjects in both the study and control groups. Subjects with low S-R improved more during the intervention in both the study group and in the control group. The extent of improvement in the control group was higher than that in the study group, the correlation value being r=0.695 (control) versus r=0.211 (study) and r=0.495 (control) versus r=0.206 (study) for subjects in the green vegetable groups and those in the sun dried groups respectively. This observation can be explained by the fact that, S-R concentrations are homeostatically controlled and do not begin to decline until liver reserves of vitamin A are dangerously low (Castemniller and West, 1998). In a study by Ribaya-Mercado *et al.*, (2000) significant increases in the subjects that had recipes containing no carotenes was reported, indicating that bioconversion of plant carotenoids to vitamin A varies inversely with vitamin A status.

The improvement in S-R noted in this study is in agreement with other studies (Takyi, 1999; Jalal *et al.*, 1998; Hussein and El-Tohamy, 1989; de Pee *et al.*, 1998; Reddy and Vijayaraghavan, 1995; Ribaya-Mercado *et al.* 2000). Studies that showed no improvement in S-R with increased fruit and vegetable intakes, had the baseline S-R of subjects being higher than those in studies where increments were noted. The baseline mean concentrations in µmol/l were: 2.31 (Micozzi *et al.*, 1992), 1.79 (Yeum *et al.*, 1996), 1.45 (Bowen *et al.*, 1993), 1.26 (Bulux *et al.*, 1994), and 0.89 (de Pee *et al.*, 1995). The baseline S-R of subjects in studies which showed improvements were lower than those in these studies. The baseline mean concentration in µmol/L for some studies was: 0.58 (Takyi, 1999), 0.59 (Jalal *et al.*, 1998), 0.65 (Hussein and El-Tohamy, 1989), 0.71 (de Pee *et al.*, 1998), 0.73 (Reddy and Vijayaraghavan, 1995) and 0.68 (Ribaya-Mercado *et al.*, 2000).

Some studies have reported results that are different from this study. de pee *et al.* (1995), reported that use of green leafy vegetables as sources of dietary carotenoids does not raise S-R concentrations in lactating women. This is because the baseline concentrations of S-R in the lactating mothers were considerably higher than WHO standards (means ranging; 0.81-0.89 µmol/L) than those of the study subjects in this study (means ranging; 0.63-0.64 µmol/L). The study population had a high incidence of parasitic infection, and was not de-wormed. It is known that infections, may severely affect the absorption and utilization of carotene (Kücükbay *et al.*, 1997). However, Vuong *et al.*, (2002) found higher baseline S-R

of 0.805 μmol/L to increase to 0.933 μmol/L. The inconsistent results in these studies may be because of differences in study design and the length of study, the amount of beta-carotene supplements used, the source of beta-carotene, and the age and the physiologic status of the target groups among other factors which affect the bio-conversion of S-BC (West and Castenmiller, 1998; Kostic *et al.*, 1995; Castenmiller and West, 1998; Castenmiller *et al.*, 1999; van het Hof *et al.,* 1999; Boileau *et al.*, 1999; Erdman *et al.,* 1993; Roodenburg *et al.*, 1994; Roodenburg *et al.*, 2000; Olmedilla *et al.,* 1994). The laboratory analysis procedures in some works such as that of de pee *et al.* (1998), which involved an overnight saponification step, could also have had implications of the different findings.

The effect of parasitic infection and de-worming on S-R concentrations at baseline and post-intervention was analysed and the results are shown in Table 4.19.

Table 4.19: Serum retinol concentration (μmol/L) and percent change in study groups that had positive parasitic infection and negative stool at baseline and post-intervention

Group	Retinol (μmol/L)[1]		% Change
	Baseline	Post-intervention	
Positive			
-Green	0.598 ± 0.083 (0.35-0.74)	0.764 ± 0.156 (0.49-1.08)	27.76
-White	0.587± 0.111 (0.31-0.83)	0.639 ± 0.107 (0.49-1.02)	8.86
Negative			
-Green	0.672± 0.061 (0.53-0.86)	0.864 ± 0.127 (0.66-1.08)	28.57
-White	0.686 ± 0.097 (0.46-1.00)	0.684 ± 0.068 (0.59-0.92)	(-) 0.29

[1]ranges in parenthesis

The post-intervention mean value of S-R shows an improvement that is significant from that of the baseline in the positive green subjects ($p< 0.001$, $df= 76$). Similarly, the post-interventional change was noted for the negative green subjects ($p< 0.001$, $df= 76$) that was

significant. The control group study subjects (positive white) had significant increase (p=0.020 and df=76). The increases noted here imply that pro-vitamin A carotenoids from DGLV`s would improve the S-R levels when subjects are free of infection. This is supported further by the trend in the negative subjects who were fed on low-carotene containing recipes, whose concentrations decreased by 0.29% although the drop was not significant. Jalal *et al.* (1998) reported an increase in S-R (by 0.17 µmol/L) after 3 weeks intervention in Indonesian children who were treated with an anthelminthic drug and provided with supplemental fat but no carotenes in a basic meal. The improvements noted can also be explained as being due to decreased intestinal parasitic load as a result of de-worming, which enabled liver stores of beta-carotene to be converted to retinol. The use of fat in foods enhance the dioxygenase activity in terms of efficient conversion of beta-carotene to vitamin A after the subjects were de-wormed because S-R concentrations fall during times of infection (Tanumihardjo *et al.*, 1996; Stephensen *et al.*, 1994).

These favourable effects on vitamin A status (serum retinol) after consumption of DGLVs agree with those reported by Jalal *et al.,* (1998), who supplemented basic diets to vitamin A–malnourished Indonesian children aged 3 to 6 years old with carotene-rich foods, mainly from red sweet potatoes in 6 days per week for 3 weeks. They found that in subjects with mild infection, mean S-R increased by 0.54 µmol/L, whereas in subjects with high infection, the increase in mean S-R was less than 0.23 µmol/L. The lower baseline serum retinol concentrations observed in positive subjects would be due to an impaired uptake of carotene by the gut mucosa, reduced bioconversion of pro-vitamin A carotenoids in the intestinal wall, or a reduction in serum retinol in response to the infection (Jalal *et al.,* 1998; Failla and Chitchumroonchokchai, 2005).

Infection is one of the host-related factors that affect bioavailability of beta-carotene and conversion of the same to retinol during intervention periods (West, and Castenmiller, 1998, Olmedilla *et al.,* 1994). However, not all studies have shown an impairment of vitamin A absorption in children with parasitic infection. Tanumihardjo *et al.* (1996) reported that de-worming infected children while treating them with a capsule containing vitamin A did not affect the improvement in vitamin A status. This difference may be explained by the initial S-R concentrations which were substantially higher than the baseline concentrations in the

present study. Further, supplemental preformed vitamin A precursor is quite different from intake of dietary vitamin A precursors, as used in this study. Both the absorption and the metabolism of preformed vitamin A in the gut wall are also different from that of beta-carotene (Harrison and Hussain, 2001; Breithaupt *et al.*, 2002; Borel *et al.*, 1996; Failla and Chitchumroonchokchai, 2005; Linder, 1991; Borel *et al.*, 2001; Lakshman and Okoh, 1993).

Correlation analysis between baseline S-R and change in S-R post-intervention showed that there was negative correlation between baseline S-R and change in S-R post-intervention for both study groups and the control groups. The subjects with lower S-R levels at baseline improved or decreased more (based on the overall group effect) than those with higher levels. However, this correlation was stronger for negative subjects compared to the positive subjects. The correlation was also observed to be stronger for the subjects in the control group (white) than those in the intervention group. The implication here is that the subjects who were free of infection had a better bioconversion of beta-carotene to retinol and hence the improvement in S-R (Jalal *et al.*, 1998). Infection is known to be associated with reduced fat absorption in both animals and humans (West and Castenmiller, 1998). De-worming may have resulted in improved fat absorption in the study subjects, which resulted in improved utilization of different sources of vitamin A and its precursors consumed as part of their diets during the intervention and home diets. De-worming when combined with added fat and beta-carotene either separately or together raises serum retinol concentrations (Jalal *et al.*, 1998).

CHAPTER FIVE

CONCLUSIONS, RECOMMENDATIONS AND AREAS FOR FURTHER STUDIES

5.1 CONCLUSIONS

This study was carried out to investigate the bioavailability of beta-carotene and its bio-conversion to retinol in serum of preschool children after consumption of selected dehydrated dark green leafy vegetables. The results have shown that:

i) Most parents in the study area were aware of vitamin A deficiency (VAD) while a number of indigenous vegetables in the study area are available in the wet seasons but scarce during the dry seasons. The preschool children were preferably fed on fresh vegetables mainly *Cucurbita pepo, Amaranthus sp* and *V. unguiculata* leaves.

ii) The preschool children in both the fresh and dehydrated vegetable study groups had mean values of hemoglobin being below the 12.0 g/L WHO value at baseline but both improved post-intervention.

iii) Fresh *V. unguiculata* leaves contained mean value of 806 µg/g, DM with losses of less than 35% of the all-*trans*-β-carotene due to dehydration by sun drying and solar drying. *Amaranthus* leaves had the mean concentration of 599 µg/g DM when fresh reducing to have retentions of over 64% with solar and sun drying. Dehydration retained levels of beta-carotene that had a potential to provide adequate beta-carotene.

iv) It was hypothesized that the bioavailability of beta-carotene in blood serum increases with consumption of dehydrated dark green leafy vegetables. The mean serum beta-carotene levels were marginally low for study subjects in the dehydrated vegetable group. There was a post-intervention change from 0.163 ± 0.105 to 0.380 ± 0.219 µmol/L (133%) which was highly significant ($p<0.000$, $df = 38$). The study concludes that intervention with sun-dried *V. unguiculata* and *amaranthus* significantly increased the beta-carotene bioavailability levels in serum among the preschool

children. Correlations between baseline levels and change in serum beta-carotene post-intervention were found to be negative, supporting the effectiveness of the intervention.

v) Beta-carotene is assumed to be readily bio-converted to retinol to improve the bioavailability of retinol with consumption of dehydrated DGLVs. The serum retinol concentrations for subjects in the dehydrated vegetable group were marginally lower than the expected mean concentrations of 0.7 μmol/L for children aged below six years. At post-intervention, mean S-R increased in this group from 0.626 ± 0.120 μmol/L to 0.788 ± 0.144 μmol/L reflecting a 25.88% increase that was statistically significant ($p<0.000$, $df = 38$). The results show that sun-dried vegetables positively contribute to the bioconversion of beta-carotene to retinol. This is supported by the comparable levels of retinol of subjects in the dehydrated vegetable group against those in the fresh vegetable group. Correlations between baseline levels and change in serum retinol post-intervention were found to be negative, supporting bioconversion of beta-carotene to retinol due to intervention.

vi) The findings supports that food based interventions are part of an effective strategy for reducing VAD.

5.2 RECOMMENDATIONS

1) Drying of vegetables should be encouraged to ensure their availability during seasons of scarcity to combat VAD.
2) The consumption of indigenous DGLVs by children as sources of pro-vitamin A be enhanced.
3) More VAD awareness programs to mothers and caretakers and regular VAD medical check ups of children be encouraged

5.3 AREAS FOR FURTHER STUDIES

i. The bio-availability of beta-carotene from dehydrated fruits should be investigated.

ii. Although *in-vivo* studies are static, they can be compared with *in-vitro* studies.

iii. Bio-availability studies using simulated systems will remove all other intervening factors to only investigate bioconversion.

iv. The quantification of other pro-vitamin A carotenoids from dietary sources need to be investigated to give a complete picture of bio-availability and bioconversion.

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Appendix 1

Questionnaire

Location_____ Sub-location_____ Village_____

PART 1:- Demographic information

1. How old are you?_____
2. Did you have any basic education? If yes what level? Yes( ) No ( )
3. How many children do you have? _____
4. How many sons are in preschool?_____
5. How many daughters are in preschool? _____
6. Are you a member of any women group? Yes ( ) No ( )
7. What is your monthly income? Below 5000( ) Over 5000( )

PART 2:-Vegetables/preservation

Fill in the table

Local name	Common name	Availability (dry/wet/all round)	Preservation method (sun/solar/other)	Length of preservation

1. Who preserves the vegetables? Mother( ) father( ) son( ) daughter( ) other(specify)_____
2. How do you store the preserved vegetables?_____
3. For how long are the vegetables stored after preservation?_____
4. Do preschool children feed on fresh vegetables? Yes( ) No( )
5. If yes which ones?_____
6. If no why?_____
7. Do preschool children feed on preserved vegetables? Yes ( ) No ( )

8. If yes which ones?_____
9. If no why? _____
10. How do you prepare (cook) the vegetables for the preschool children?_____
11. List other common foods fed to preschool children during the wet season _____
12. List other common foods fed to preschool children during the dry season _____
13. How long is the longest dry period in the year?
14. Where is your source of vegetables during the dry season?_____
15. Fill the table: Name a vegetable, which family member feeds on it, how frequently (daily, weekly, monthly) and in what quantity (small, medium, large).

Vegetable	Fresh/preserved	Family member	Frequency	Quantity

PART 3:- Vitamin A awareness

1. Do you know that children suffer from vitamin A deficiency? Yes ( ) No ( )
2. If yes which signs and symptoms do you know of? _____
3. Do you seek medical attention incase of vitamin A deficiency? Yes ( ) No ( )
4. Which foods do you give to curb the problem? _____
5. Where do you obtain these foods from? _____
6. Do you do a regular check up for your child's vitamin A status? Yes ( ) No ( )
7. Do you give your child vitamin A supplements? Yes ( ) No ( )

If yes what kind? _____

How often do you give the supplements? Daily ( ) weekly ( ) monthly ( )

Appendix 2

Total energy, fat, retinol equivalents and carotenoids in foods reported in the subjects food diary

Food	KJ	Fat (g)	RE (μg)	Car (μg)
White bread	1146	3.0	0	0
Maizemeal, white, dry	1515	3.6	0	0
White rice	544	0.2	0	0
Avocado	674	15.3	4	53
Banana	385	0.5	4	48
Orange	197	0.1	7	82
Papaya,ripe,raw	163	0.1	68	814
Raw/boiled carrots	159	0.3	430	4,877
Onion	150	0.2	0	5
Spinach/sukuma	84	0.4	192	4,615
Tomatoes	88	0.3	22	115
Potato boiled	389	0.1	0	0
Milk	277	3.9	32	13
Vegetable oil	3699	100.0	0	0
Sugar	1619	0.0	0	0
Beef	455	1.9	20	0