BIOAVAILABILITY OF ANTIOXIDANT VITAMINS IN SELECTED INDIGENEOUS VEGETABLES AND THEIR POTENTIAL USE IN MANAGEMENT OF HIV/AIDS IN BUTULA, BUSIA KENYA

BY

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REG NO: I56/CE/12515/04

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

October, 2011

DECLARATION

This thesis is my original work and has not been presented for the award of degree in any other university.

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

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DEDICATION

To loving memory of my late dad Noah Nambafu and mum Mary Wangui from whom I learnt hard work and optimism.

ACKNOWLEDGEMENTS

Writing a thesis like this, apart from the mental exhaustion, the side issues that arise are a testimony that any academic pursuit is a team effort. I would therefore wish to sincerely thank my parents Noah Nambafu and Mary Wangui, whose faith in education and hard work I have immensely benefited from, over the years. Thanks a lot.

Many thanks go to my supervisors Prof. Hudson Nyambaka and Dr Sauda Swaleh for their dedication and insight in the development of once a hazy idea that matured into this thesis. I am also heavily indebted to my lecturers at the department of Chemistry, for their rigorous, firm and fair approach to their teaching assignment. I am also grateful to the technicians in KEMRI for their initial assistance in using the HPLC instrument. I cannot forget the technicians of Kenyatta University for their co-operation and assistance during this study.

I also want to salute my siblings for always being there for me. My heartfelt gratitude also goes to Mr. Mutuku for kindly accepting to type this thesis and for patience during the typing period.

Finally to all those who knowingly or unknowingly helped in the compilation of this work and who I have not mentioned, bear with me but you will always remain appreciated for as long as this work comes to conclusion.

Above all, thanks to God Almighty for grace, strength and sustenance throughout the course of study.

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ABBREVIATIONS AND ACRONYMS

AIDS:	Acquired Immunodeficiency Syndrome
ANOVA:	Analysis of Variance
ARV:	Antiretroviral Drugs
BMI:	Body Mass Index
CDC:	Centre for Disease Control
DCPIP:	2, 6-dichlorophenolindophenol
DW:	Dry weight
ELISA:	Enzyme linked Immunosorbent Assay
FAO:	Food and Agriculture Organization
FSH:	Follicle Stimulating Hormone
Hb:	Hemoglobin
HIV:	Human Immunodeficiency Virus
HPLC:	High Performance Liquid Chromatography
IEC:	Information Education and Communication
IU:	International Units
KDHS:	Kenya Demographic Health Survey
KEMRI:	Kenya Medical Research Institute
LH:	Luteinizing Hormone
MOH:	Ministry of Health
NACC:	National Aids Control Council
NASCOP:	National Aids and STD'S Control Programme
PLWHA:	People Living with HIV and AIDS

PUFA:	Polyunsaturated Fatty Acids
RDA:	Recommended Dietary Allowance
RE:	Retinol Equivalent
REDOX:	Reduction Oxidation
REEP:	Rural Education and Economic Enhancement Programme
RNA:	Ribonucleic Acid
STH:	Somatotrophin Growth Hormone
SPSS:	Statistical Package for Social Science
UNAIDS:	United Nations Programme on HIV and AIDS
UVS:	Ultraviolet Spectrophotometer
VCT:	Voluntary Counselling and Testing
WHO:	World Health Organization

ABSTRACT

HIV infection is characterized by a high preference of micronutrients deficiencies and wasting that may vary considerably among different HIV infected persons. High intake of micronutrients have been associated with reduced progression of HIV to AIDS and improved survival. Butula division in Busia district in Kenya is a region where indigenous vegetables do well during the rainy season but become scarce in the dry season. Vitamin A, C and E deficiency is a problem affecting people living with HIV and AIDS (PLWHA). Deficiency of these antioxidant vitamins contribute to oxidative stress condition that accelerate death of immune cell and increase the rate of HIV replication. The aim of this study was to identify vegetables rich in vitamin A, C and E with a view of assessing the bioavailability of these vitamins to PLWHA. The study investigated the feeding pattern of PLWHA in Butula division, determined vitamin A, C and E content in selected indigenous vegetables and the bioavailability of these vitamins for use in the management of HIV and AIDS. The determination of β-carotene and α-tocopherol content in selected fresh and dry vegetables (amaranthus, cowpea leaves, nightshade, slender leaf, pumpkin leaves and frying spider), and in sera were done using high performance liquid chromatography (HPLC) procedure. Starch, protein, animal and plants food sources accounted for 45.17 %, 21.9 %, 17.48 % and 73 % respectively of total foods consumed by PLWHA in Butula Division. Fresh blanched vegetables, contained high levels of β-carotene; 4000-9700 µg/100g and α-tocopherol levels; 3000-7350 µg/100g. The solar dried vegetables contained β-carotene levels ranging from 572 to 854 µg/g dry weight (DW) and α-tocopherol levels ranging from 281 to 673 µg/g DW. Solar dried vegetables contained significantly lower ($P<0.05$) amounts of β-carotene and α-tocopherol as compared with fresh vegetables. L-ascorbic acid content in selected fresh vegetables and fruit juices was determined by redox titration and contained levels ranging between 6 and 65 mg/100g. Pineapple juice was found to contain high level of vitamin C; with one glass of blended pineapple juice meeting the recommended dietary allowance (RDA) values. The mean serum retinol, α-tocopherol and β-carotene levels were 0.937, 0.144 and 17.787µmol/l respectively. The CD4+ cell counts of the PLWHA at baseline, determined using flow cytometer were within the normal range of 500-1500 cells/µl in blood while the CD8+ cell count was in the range of 300-3300 cell/µl of blood. Haemoglobin was less than 12 g/dL and red blood cell width was less than 15.2 % indicating anaemia. 88.35% of the subjects had a CD4+/CD8+ ratio of less than 1, 9.71% had a CD4+/CD8+ ratio range of 2-3 and 1.94% had a ratio greater than 4. Normal CD4+/CD8+ ratio is in the range of 1-4. In the course of HIV disease CD4 is often decreased and CD8 increased so that the ratio is inverted.77.95% of the subjects had a normal weight, 11.81% were underweight, 8.66% were overweight and 1.57% were obese. The bioavailability in the foods estimated using algorithm indicated a +2.17 change in serum β-carotene while there was +7.776 change in serum α-tocopherol levels indicating that the consumption of the food supplement would result to improving the bioavailability of these nutrients in PLWHA in Butula. Indigenous foods in Butula contain high levels of β-carotene and α-tocopherol which are moderately bioavailable if mixed in good proportion can meet RDA of the vitamins A, C and E which are 750µg retinol equivalent/day, 60mg/day, 8mg/day respectively (Combs, 1998) and boost the immunity of PLWHA. The results will provide nutritional information on the indigenous vegetables grown in Butula division and their potential use in the management of HIV/AIDS.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The global picture on HIV statistics is overwhelming. The UNAIDS and WHO has estimated that 25 million people have died of AIDS and 39.5 million people are currently infected with HIV (WHO, 2006). Approximately 70% of those infected live in Sub-Saharan Africa (UNAIDS, 2010). Adult HIV prevalence in East Africa exceeds 5% in Uganda, Kenya and Tanzania (UNAIDS 2010). UNAIDS estimates indicates that 1.3 million Kenyans are living with the virus of whom 380,000 are children, while over 650,000 children have been orphaned by the disease (UNAIDS, 2010).

In Kenya, despite the fact that there has been a drop in the national prevalence from 10% in 1990s to 7.4% in 2007 and adult HIV prevalence has now stabilized around 6.3 percent, according to the most recent Kenya Demographic and Health Survey (KDHS) 2008-2009 (UNAIDS, 2008) . HIV and AIDS remains a major health concern (NACC, 2007). HIV infection is concentrated in the socially and economically productive groups aged 15-45 years (NASCOP, 2009). According to National Aids Control Council (NACC) 2000-2005 work plan the disease increasingly affects the poor and people with low levels of formal education, thus the share of new infection is rising among low-income earners and less educated people (NACC, 2005).

Gender is also a factor in HIV/AIDS infection. HIV/AIDS prevalence among women aged 15-49 in Kenya is nearly 9% compared to less than 5% for men in the 15-54 age group (NACC, 2005). Busia district in western Kenya has a high prevalence of 7.4 with male prevalence of 5.9% and female prevalence of 6.4% in 2009 (NACC, 2005). Some of the factors cited as contributing to the high HIV/AIDS prevalence in Busia include; poverty, adoption of western lifestyles, socio-cultural

practices including wife inheritance, cross border movements, stigma and discrimination (NACC, 2005).

The management of HIV and AIDS is being done through activities such as awareness creation, condom distribution and provision of ARV's. Access to ARV's is increasingly recognized as a key component to comprehensive AIDS management strategies. However ARV's pose real challenges with respect to compliance, resistance and sometimes have adverse side effects (Saitoih *et al.*, 2005; Dybul, 2002). In addition one of the disappointing aspects of the use of ARVS is that although they reduce the rate of viral replication there is only a small improvement in the immune function (Barter *et al.*, 1993). The role of nutrition in the management of HIV/AIDS has assumed increasing importance over the past decade (FANTA, 2004). Research has shown that nutritional status may affect the progression of HIV to AIDS and survival of HIV individuals (Piwoz and Preble, 2000).

HIV infection is characterized by a high preference of micronutrients deficiencies (vitamin C, α-tocopherol and β-carotene) and wasting that may vary considerably among different HIV infected population (Baum *et al.*, 1995). Foods have the nutrients necessary for providing energy, building and repairing tissues and helping in strengthening immunity (Stine, 2005). High intakes of vitamin C, α-tocopherol and β-carotene have been associated with reduced progression of HIV to AIDS and improved survival (Semba *et al.*, 1995). Studies suggest that micronutrients deficiencies still occur due to malabsorption or altered metabolism and habitual consumption of poor diets (Miller, 1988; Semba, 1995; Baum *et al.*, 1995). Many parts of Butula division in Busia district have indigenous foods such as cowpeas, green grams, soya beans, sweet potatoes and green vegetables rich in immune boosting micronutrients that can be used in the management of HIV and AIDS. Since there is no known cure for AIDS, people living with HIV and AIDS (PLWHA) require care in order to maintain

their health so as to remain productive longer. It is therefore important that locally available foods rich in immune boosting vitamins are identified and used to prepare food products that will enhance the bioavailability of micronutrients in PLWHA and hence improve their health status and quality of life.

1.2 Problem statement and justification

HIV weakens the immune system thus compromising the body's ability to fight infections (Baum *et al.*, 1994). As a result of prolonged illness there is reduced appetite and interference with the body's absorption of nutrients (Piwoz and Preble, 2000). Infection also increases the body's need for essential nutrients. Clinical outcomes of HIV are poorer for individuals with compromised nutrition (Piwoz and Preble, 2000). Many HIV infected individuals are unable to meet these additional nutritional requirements and thus become weak and malnourished. Nutrients most commonly deficient include zinc, selenium and vitamin A, E, B_{12} and B_6 (Liang *et al.*, 1996). Deficiency of these antioxidant vitamins and minerals contribute to oxidative stress condition that may accelerate immune cell death and increase the rate of HIV replication (Romero-Alvira and Roche, 1998). HIV/AIDS and nutrition are intimately linked. Studies have shown that daily micronutrient (vitamin C, α-tocopherol and β-carotene) supplementation improves body weight, body cell mass, reduces HIV RNA levels, improves CD4+ cell counts and reduces incidences of opportunistic infections (Allard *et al.*, 1998; Tang *et al.*, 1993; Semba, 1995). Indigenous vegetables such as pumpkins leaves, cowpea leaves, amaranthus, nightshade and frying spider contain these vitamins, which boost immunity (Ihekoronye, 1992). Butula division (the study area) in Busia district has a high HIV prevalence of 7.4% (NACC, 2005) and produces enough of these vegetables during the rainy season but they become scarce during the dry season. The aim of the study was to determine the vitamin A, C and E content in indigenous vegetables with a view of utilizing them to prepare locally acceptable

food products that will improve the bioavailability of these vitamins in PLWHA and the general population of Busia district.

1.3 Hypothesis

Indigenous vegetables contain high levels of vitamins A, C and E, which are bioavailable to PLWHA.

1.4 Objectives

1.4.1 General objective

To assess the bioavailability of immunity boosting vitamins (A, C and E) in selected indigenous vegetables and their potential use in the management of HIV and AIDS in Butula division.

1.4.2 Specific objectives

i. To determine demographic and food consumption pattern of PLWHA in Butula division.

ii.To determine vitamin A, C and E content and moisture content in cowpea leaves, amaranthus, pumpkin leaves, slender leaf, frying spider and nightshade in Butula division.

iii. To determine the CD4+,CD8+ cell counts, CD4+/ CD8+ ratio, hematological status, clinical status and BMI of PLWHA in Butula division.

iv.To determine the serum vitamin A and E levels in HIV/AIDS patients living in Butula division.

v.To determine the bioavailable vitamin A and E in selected indigenous vegetables by use of algorithms.

1.5 Significance and anticipated output

The findings of the study are useful because they will provide nutritional information on the indigenous vegetables grown in the Butula division of Busia district that form the source of nutrients

for the community. It also provides useful information on the levels of β- carotene and α-tocopherol in the vegetables grown in the Butula division. This information is of great benefit to the ministry of health, ministry of agriculture and non-governmental organizations interested in the management of HIV by use of nutrition intervention and promoting of growth of indigenous vegetables as a way of improving food security.

The use of indigenous food formulation will boost immunity of PLWHA hence improve their health and also delay the use of ARV's. In addition growth of certain indigenous vegetables will be enhanced thus improving food security and socio- economic status of PLWHA and the general population of Busia district.

1.6 Scope and limitations of the study

1.6.1 Scope

The study investigated the β-carotene, α-tocopherol and L-ascorbic acid content in six varieties of indigenous vegetables, their availability and moisture content. Specific variety within each vegetable was not emphasized due to lack of finances. Serum β-carotene, α-tocopherol and retinol levels CD4+cell count, CD8+cell count, CD4+/C8+ ratio, hematological index and BMI in HIV/AIDS patients were also determined. A mathematical model was developed to determine the bioavailability of β-carotene and α-tocopherol in some selected indigenous vegetables.

1.6.2 Limitations

This study looked at the vegetables identified to contain a substantial amount of vitamin A, C and E due to limitation of time and finances. The study focused on indigenous vegetables grown in Butula division, Busia district therefore any generalization of the study findings to other areas should be

done with caution since vitamin content in vegetables differ in different areas; seasons and in different vegetable type. The variation of the vitamin levels in foods especially vegetables and fruits are caused by factors such as variety, maturity of the tissues, growing conditions such as the weather, growing season and soil state. The findings of this study may not reflect feeding habits and vitamin status of PLWHA which vary with season because baseline survey was done once. Bioavailability of vitamins was assessed using algorithms. Accurate algorithms are difficult to develop because of the chemical complexity of the foods and the numerous interactions that can affect the bioavailability of the vitamins.

1.7 Operational definition of terms

AIDS: A combination of illnesses caused by the Human Immunodeficiency Virus (HIV) that weaken the immune system.

Anthropometry: A tool used to identify malnutrition and monitor body measurements.

Antioxidant: Compounds that scavenge free radicals (oxygen molecules) in the body.

Antiretroviral: Drugs that specifically deal with treatment of viruses including the HIV virus.

Asymptomatic: A person infected with a disease but without clinical signs and symptoms.

Beta-carotene: It is a provitamin A carotenoid (precursor of vitamin A) found in plant foods.

Bioavailability: The degree and rate at which a substance is absorbed into the body at the site of physiological activity and absorption.

CD4 cell: A subset of specialized lymphocytes that are key in fighting/attacking infections that are used as marker for HIV progress.

CD8 cell: Suppressor white blood cell with type 8 protein embedded in the cell surface.

Cellular immunity: A collection of cell types that provide protection against various antigens.

Cell-mediated immunity: The reaction to antigenic material by specific defensive cells (macrophages) rather than antibodies.

Dehydration: This is the application of heat under controlled conditions to remove water normally present in food by evaporation.

ELISA test: A blood test that indicates the presence of antibodies to a given antigen. Various ELISA are used to detect a variety of infections. The HIV ELISA test does not detect AIDS but only indicates if viral infection has occurred.

Formulation: The composition of a dosage form, including the characteristics of its raw materials and the operations required to process it.

HIV: The Human Immunodeficiency Virus that causes AIDS.

Humoral immunity: The production of antibodies for defense against infection or disease.

Immunity: Resistance to a disease because of a functioning immune system.

Immunosupression: A weakened body defense system creating vulnerability to infection and other disorders.

Indigenous foods: Local native foods grown in a community.

Infection: The presence of disease causing micro-organism.

Interleukins: Chemical messengers that travel from leukocytes to other white blood cells. Some promote cell development, other promote rapid cell division.

Lean body mass: Weight of the body without fat composed of muscle, bones and other tissues.

Malabsorption: Failure by the digestive tract to absorb nutrients leading to deficiencies.

Malnutrition: A condition in the body brought about by inadequate or excess intake of required nutrients, or malabsorption.

Morbidity: The proportion of people with a disease in a community.

Mortality: The number of people who die as a result of specific cause.

Natural killer cells (also called NK cells): Immune cells that kill infected cells directly within four hours of contact. NK cells differ from other killer cells, such as cytotoxic T lymphocyte, in that they do not require contact with antigen before they are activated.

Nutrient: A substance or component of food, including carbohydrates, proteins, fats, vitamins mineral and water.

Nutrition status: A measurement of the extent in which an individual's physiological needs for nutrients are being met.

Nutrition: Process of food ingested, digested and absorbed to provide the body with required nutrients.

Pandemic: Occurring over a wide geographic area and affecting a high proportion of the population.

P24 antigen: A protein fragment of HIV. The P24 antigen test measures this fragment. A positive test result suggests active HIV replication and may mean the individual has a chance of developing AIDS in the near future.

Polymerase chain reaction: Method to detect and amplify very small amounts of DNA in a sample.

Provitamins: Substances which occur in food and are not themselves vitamins but are capable of conversion into vitamins in the body.

Recommended daily allowance: Average requirement of various nutrients required to maintain nutritional status of a healthy person according to international standards.

Snack: Food or drink readily available and usually taken between main meals.

Suppressor T cells: A subset of T cells that carry the T8 marker and turn of antibody production and other immune responses.

Solar drying: A dehydration process in which the principal source of energy is derived from the enhancement of the suns radiation. It provides higher air temperatures and improved drying rates of vegetables.

Symptomatic: Infection with signs and symptoms.

T helper cells (also called T4 or CD4 cells): A subset of T cells that carry the CD4 marker and are essential for turning on antibody production, activating cytotoxic on antibody initiating many other immune responses.

T 8 cells: A subset of T cells that may kill virus-infected cells and suppress immune function when the infection is over.

Under nutrition: Inadequate nutrients or food intake in the body.

Viral load: Amount of human immunodeficiency virus in blood used as a marker for progress of HIV to AIDS.

Virulence: The quality of expression or the expression of the disease.

Virus: Smallest of all disease causing micro-organisms.

Vitamin: Nutrients whose main function is to protect the body against infection.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 HIV/AIDS infection: Effects and detection

Human Immunodeficiency Virus (HIV) is a retrovirus that targets cells of the body with CD4+ (T helper cells), langerhans cells and microphages being the most affected (Stine, 2005). The depletion of these cells is co-related with clinical progression to AIDS (Acquired immunodeficiency Syndrome). AIDS is a disease caused by the HIV virus, which attacks and impairs the body's natural defense system against diseases and infection. AIDS is the terminal stage of HIV infection, characterized by symptoms that weaken the immune system such as severe weight loss, chronic diarrhea, fever and persistent cough (WHO, 2002). The virus is transmitted through body fluids such as blood, semen, vaginal secretion and breast milk (Burcham and Joyce, 1991).

HIV infection can be detected in three ways: First, by HIV antibody or antigen testing prior to the signs and symptoms of AIDS, second by detecting the presence of HIV nucleic acid and third by physical examination after symptoms occur (Stine, 2005). The most commonly used detection test is Enzyme Linked Immunosorbent Assay (ELISA). Other tests include: Western Blot, Indirect Immunofluorescent Antibody Assay (IFA), Polymerase Chain Reaction (PCR), Rapid HIV testing kit and HIV gene probes (Anderson and Robert, 1991). Prognostic biological markers related to AIDS progression include P24 antigen levels, P24 antibody levels, and T4 and T8 lymphocyte levels (Stine, 2005). T4 and T8 lymphocyte levels are the most extensively used biological markers for AIDS progression (Anderson and Robert, 1991; Burcham and Joyce, 1991; Philips and Andrew, 1991). Viral load assays are good surrogate markers for virus replication and cell killing (CDC, 1993). There are three commercially available viral load assays; Ampricor ultra-sensitive HIV monitoring test, branched DNA (b DNA) technology and nucleic acid sequence based amplification (Stine, 2005).

2.2 HIV infection and immune system

HIV attacks impair the body immune system. HIV infection progresses slowly and may take years before the infected person shows persistent signs of illness (Table 2.1 and Figure 2.1). During this progression period, the virus attacks and destroys defense cells known as CD4's. CD4 cells are critical to immune system functions of the body (Anderson and Robert, 1991). Unlike other infections, it is practically impossible for the body to naturally eliminate HIV. The length of time it takes for untreated and asymptomatic HIV infection to become symptomatic disease depends on several factors, including the general health and nutritional status of a person before and during the infection period (Stine, 2005). For individuals with adequate food and health resources, the average time for an HIV-infected adult to develop to full-blown AIDS is approximately ten years (Rofles *et al.*, 1991) (Figure 2.1).

Table 2.1: Stages for HIV Infection progressing to full blown AIDS for untreated HIV infection

Early Stage	Intermediate stage	Late stage
Asymptomatic • Weight loss of less than 5%. • Increased energy requirement (10% more) • Largely no related symptoms (except in the first few weeks) • Generalized lymph glands enlarged. • Immune system weakening and recurrent upper respiratory tract infections. • Normal activity	Early symptomatic • Increased energy requirement (20% more). • Weight loss greater than 10% failure to thrive. • Persistent fever and diarrhea. • Early opportunistic infections. • Mucous membrane and skin infections Recurring respiratory tract infections. • Normal or partial activity (bed ridden for less than 50% of the time.	Full blown AIDS • Increased energy requirement (30% more) • Weight loss greater than 10% and wasting. • Multiple signs and symptoms. • AIDS defining opportunistic infections: • Chronic diarrhea • Pneumonia • Candidiasis • Tuberculosis (TB) • Kaposi's sarcoma • Weight loss/wasting • Weak and low activity (bed ridden for more than 50% of the time).

Source: Modified from Rofles *et al.*, 1991

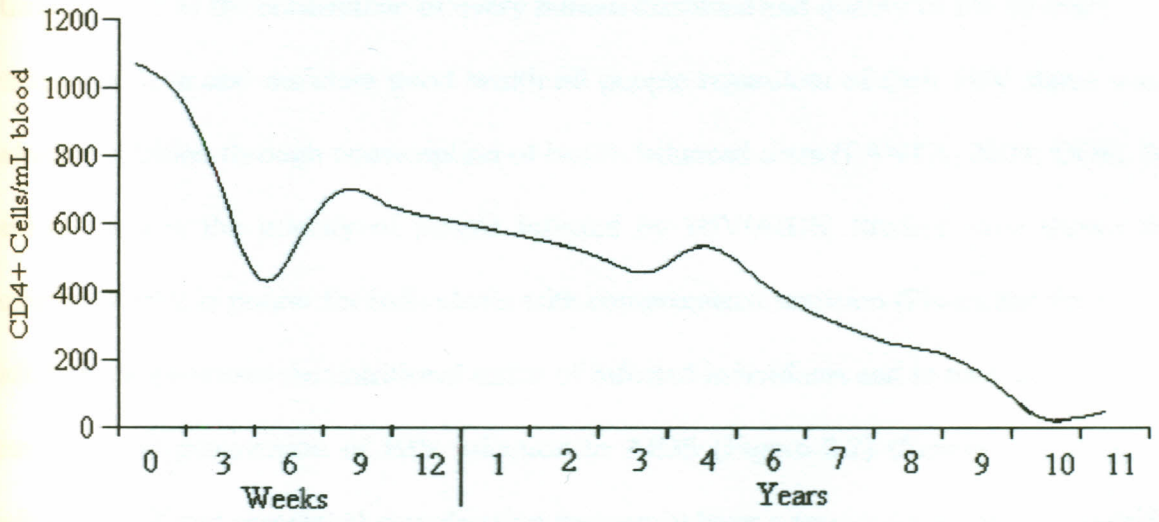


Figure 2.1: CD4 counts for average disease progression of untreated HIV-1 infection

Source: Modified from Rofles *et al.*, 1991

In resource poor settings such as in Kenya, HIV's progression to full-blown AIDS may take a shorter time because majority of people have various nutritional defects and health problems (NASCOP, 2005). The emergence of opportunistic infections (OIs) marks entry into full-blown AIDS (MOH, 2006). At this point, the body's ability to fight against infection from viruses, bacteria and parasites and against non infectious diseases such as cancer and blood disorders is significantly weakened (Stine, 2005). When the immune system is functioning optimally, it helps slow the progression of HIV into AIDS, and increases survival (Semba and Tang, 1999). The development and full function of the immune system requires an array of essential micronutrients and adequate micronutrients achieved through good nutrition. Generally, immune suppression responds rapidly to nutrition intervention and maintainace of good nutrition among PLWHA improves survival and quality of life (Piwoz and Preble, 2000).

2.3 HIV/AIDS and nutrition

Good nutrition is the cornerstone of every human existence and quality of life of every individual. In order to achieve and maintain good health all people regardless of their HIV status need to obtain adequate nutrition through consumption of health balanced diets (FANTA, 2004; GOK, 2006). Food and nutrition is the priority of people infected by HIV/AIDS. Studies have shown that clinical outcome of HIV is poorer for individuals with compromised nutrition (Piwoz and Preble, 2000). HIV infection compromises the nutritional status of infected individuals and in turn, poor nutritional status can affect the progression of HIV infection to AIDS (Figure 2.2) (Semba and Tang, 1999). The infection itself and associated complication frequently have a tremendous effect on nutritional status of individual (Baum, 2000). For example HIV infection has an impact on proteins, fats and carbohydrates metabolism. It also results in increased energy and protein requirements as well as

utilization and loss of nutrients (Macallan, 1999). HIV/AIDS affects nutrition in three sometimes overlapping ways

(i) It's associated with symptoms that cause reduction in the amount of food consumed.

(ii) It interferes with the digestion and absorption of nutrients consumed.

(iii) It changes metabolism, or the way the body transports, uses, stores and secretes many of the nutrients (Piwoz and Preble, 2000).

2.3.1 Dietary intake

Economic factors affect food availability and the nutritional quality of the diet. Reduction in food intake is believed to be the most important cause of slow and progressive weight loss experienced by PLWHA (Macallan, 1999). Reduction of food intake may be due to painful sores in the mouth, pharynx and or esophagus (Semba and Tang, 1999). Fatigue, depression, changes in mental state play a role by affecting appetite and interest in food (Semba, 1994). Side effects from medications like nausea, vomiting, metallic taste, diarrhea, abdominal cramps and anorexia also result in lower dietary intake that causes weight loss (Babemento and Kotler, 1997).

2.3.2 Nutrition malabsorption

Nutrition malabsorption occurs due to frequent bouts of diarrhea and other pathogens that affect persons with compromised immune systems (Stine, 2005). Intestinal infections cause diarrhea, with loss and waste of nutrients (Keating *et al.*, 1995). It is possible that HIV infection itself particularly of intestinal cells may cause epithelial damage and nutrients malabsorption (Babemento and Kotler, 1997). Malabsorption of fats and carbohydrates is common at all stages of HIV infection in adults and children (Semba and Tang, 1999). This further affects absorption and utilization of fat-soluble vitamins thus further compromising nutrition and immune status. Some HIV infected individuals

have increased intestinal permeability and other intestinal defects even when asymptomatic (Semba, 1994).

2.3.3 Metabolic alterations

HIV infection has an impact on protein, fat and carbohydrates metabolism (Semba and Tang, 1999). Infection results in increased energy and protein requirements as well as inefficient utilization and loss of nutrients (Macallan, 1999). These changes occur during infection from severe reduction in food intake as well as from immune response to infection. When food is restricted the body responds by altering insulin and glucagon production which regulate the flow of sugar and other nutrients in the intestines, blood, liver and body tissue (Keating *et al*., 1995). Over time the body uses up its carbohydrates store from muscle and liver tissue and begins to breakdown body protein to glucose (Semba, 1994). Thus causing weight loss and muscle wasting (Babemento and Kotler, 1997). Chronic infections and illness in HIV/AIDS is usually accompanied by fevers and repeated infections. Fevers and infections lead to higher nutrients requirements and poor use of nutrients by the body. Common illnesses caused by HIV infection such as diarrhea, vomiting, constipation, mouth ulcers, thrush, anorexia, pneumonia and fever have serious nutritional consequences that manifest as wasting and micronutrients deficiencies (Baum *et al* ., 1994).

The link between HIV infection and nutrition and can be summarized as follows (MOH, 2001).

(i) HIV infection decreases nutrient intake, absorption and increases the risk of malnutrition through altered food intake and or its nutrient absorption and utilization

(ii) Poor nutrition increases risk of opportunistic infection (OIs) and accelerates the progression of HIV to AIDS.

(iii)Malnutrition and HIV/AIDS are synergistic and weakens the immune system. The emergence of IOs further increases energy and nutrient needs (Semba and Tang, 1999). Moreover,

psychological stress affects nutrient intake and can contribute to the risk of malnutrition. In actual sense, the relationship between nutrition and HIV/AIDS is a vicious cycle (Figure 2.2).

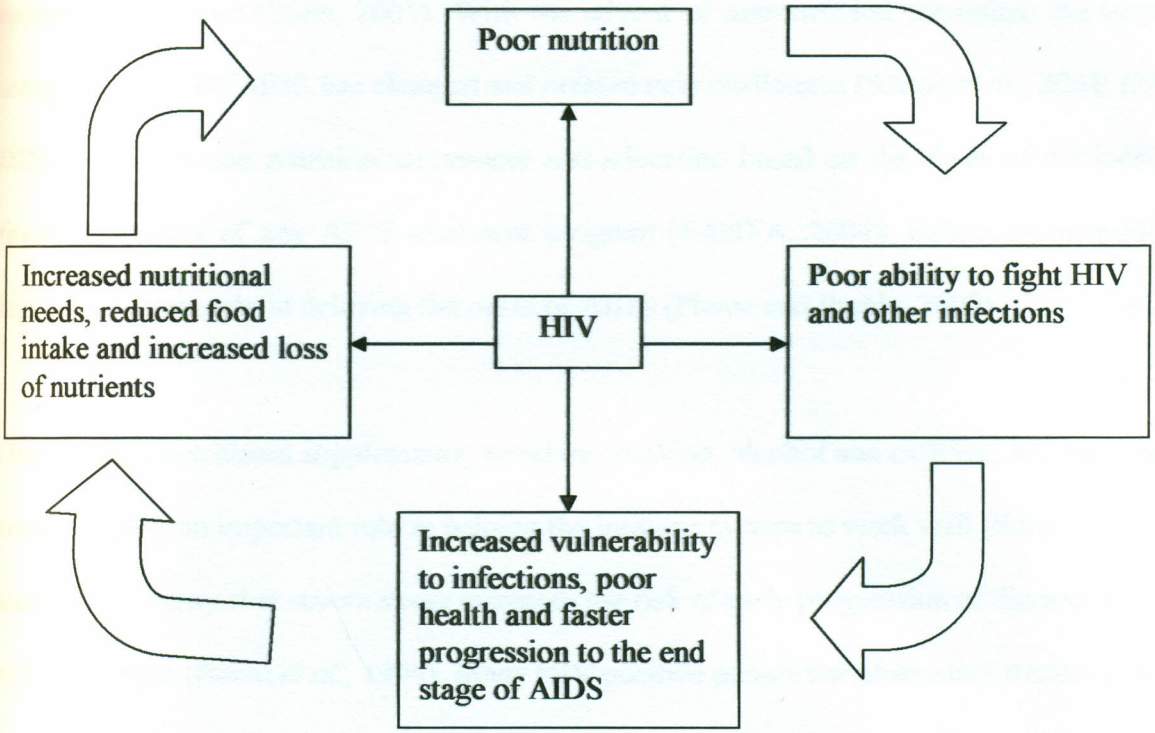


Figure 2.2: The cycle of malnutrition and HIV infection

Source: Semba and Tang, 1999

2.4 Management of HIV/AIDS

HIV management for PLWHA is done through activities such as treating the infection with ARVs, nutrition management, treating sexually transmitted diseases, preventing and treating opportunistic infections (NACC, 2007). Since there is no known cure for full-blown AIDS, HIV management is being done through prevention measures such as awareness campaign in the media, voluntary testing and counseling at VCT centers (Stine, 2005). Other approaches used include participatory education, entertainment through drama and puppetry among youth and women group and free distribution of

condoms (NACC, 2005). Other strategies used include home based care, prevention of transmission in medical settings including safe blood transfusion and proper infection control and work based programmes (Stine, 2005). The aim of therapy is to affect the action of HIV and reduce the immune suppression it causes (Stine, 2005). With the advent of antiretroviral therapies, the treatment and management of HIV/AIDS has changed and created new challenges (Saitoh *et al.*, 2005; Dybul *et al.*, 2002). For this reason nutrition assessment and education based on the needs of the individual is a critical component of any AIDS treatment program (FANTA, 2004). Enhancing immune function plays an important role in delaying the onset of AIDS (Piwoz and Preble, 2000).

A healthy diet, nutritional supplements, avoiding smoking, alcohol and caffeine, adequate sleep and exercise all play an important role in helping the immune system to work well (Stine, 2005). Research has shown that severe stress increases the risk of early progression of disease in those who are HIV positive (Baum *et al.*, 1994). Many HIV positive people use alternative treatment such as relaxation, spiritual and self help therapies (Saitoh *et al.*, 2005). Herbal medicine, acupuncture, homeopathy and dietary therapies are also popular (FANTA, 2004).

2.4.1 Nutritional management of HIV/AIDS

Nutrition support plays a vital role throughout HIV disease in two basic areas. First it is a vital component of care for the involuntary weight loss and body tissue wasting caused by the disease effects on metabolism (Babemento and Kotler, 1997). Secondly and fundamental, it is an intimate and integral component of care through the specific roles of key nutrients in maintaining the body's immunocompetence (Stine, 2005). Individual nutritional status influences the impact of morbidity and mortality irrespective of the disease process (MOH, 2006).

Good nutrition has the following impacts; which are summarized in (Figure 2.2)

(i) Prevents malnutrition and wasting

(ii) Achieves and maintains optimal body weight and strength

(iii)Enhances the body ability to fight opportunistic infections

(iv)May help delay the progression of HIV

(v) Improves effectives of drug treatment (MOH, 2006).

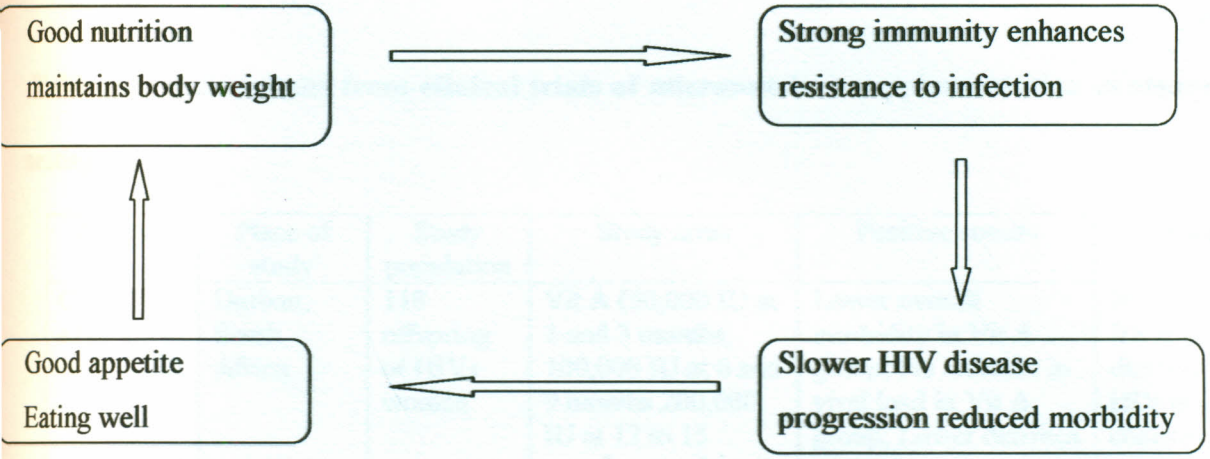


Figure 2.3: Benefits of good nutrition

Source: MOH, 2006

The current focus on increased access to ARV's in low and middle income earners should not obscure the fact that for much of the world's population living with HIV need for food remains an overwhelming priority (UNAIDS, 2002). Clinical outcome has been shown to be poor and risk of death higher for HIV positive adults with micronutrient deficiencies (Baum *et al.*, 1995; Tang *et al.*, 1996). A preliminary study on the use of nutritional preparation with enhanced antioxidant properties in Kenya has shown that HIV/AIDS patients experience reduced clinical signs and symptoms, viral load and improved immune status (Mbakaya *et al.*, 2003). Similar studies have shown that daily

micronutrient (antioxidant vitamins, vitamin C, tocopherol, β-carotene) supplementation improves body weight, body cell mass, reduces HIV RNA levels, improves CD4+ cell counts and reduces incidences of opportunistic infections (Allard *et al.*, 1998). Daily multivitamin supplements were found to reduce HIV disease progression among men, women and children in several longitudinal observational studies and randomized trials. They were also found to provide an important low cost intervention that could be provided to adults in early stages of HIV disease to prolong the time before antiretroviral therapy is recommended (Table 2.2).

Table 2.2: Main results from clinical trials of micronutrient supplementation in resource-poor settings.

Authors	Place of study	Study population	Study arms	Positive results	Negative results
Coutsoudis *e t al.*, 1995	Durban, South Africa	118 offspring of HIV+ women	Vit A (50,000 IU at 1 and 3 months, 100,000 IU at 6 and 9 months 200,000 IU at 12 to 15 months placebo	Lower overall morbidity in Vit A group; No increase in viral load in Vit A group; Lower diarrhea	No effect of Vit A on diarrhea in HIV negative children
Coutsoudis *et al.*, 1997	Durban, South Africa	24 HIV+ pregnant women	Vit A (5000 IU retinyl palmate (RP) and 30mg beta–carotene (BC) daily during pregnancy. At delivery: 200,000 IU RT placebo	No increase in viral load in Vit A group	
Fawzi *et al.*, 1998	Dare salaam Tanzania	1075 HIV + pregnant women, 12–27 weeks gestation	Vit A (30mg BC and 5000 IU preformed Vit A) Multivitamin (MV) (B_1 B_2 B_6 niacin, B_{12} folate, C and E MV + Vit A placebo At delivery, women in groups 1 and 3 received 200000 IU Vit A; groups 2 and 4 received placebo	MV associated with lower risk of : (a) fetal deaths, (b) low birth weight, (c) severe preterm birth, and (d) small size at birth MV associated with increase in CD4+, CD8+ and CD3+ cell counts	No effect of Vit A on birth outcomes or T-cell subsets
Fawzi *et al.*, 2000	Daresalaam	1083 HIV + pregnant	Same as above; additionally all	MVs associated with higher birth weight	No effect of Vit A or MVs

	Tanzania	women, 12–27 weeks gestation; infants born followed for 6 weeks post-partum	infants received 100,000 IU Vit A at 6 months and 200,000 IU Vit A every 6 months thereafter	among babies who were HIV-negative at birth	on risk of vertical transmission up to 6 weeks post partum
Fawzi *et al.*, 2002	Daresalaam Tanzania	1078 HIV + pregnant women, 12–27 weeks gestation; infants born followed for 24 months	Same as above	MVs reduced mortality among children who were HIV negative at birth: MVs reduced vertical transmission among immunologically compromised mothers	No effect of MVs on overall risk of vertical transmission; Vit A associated with significantly increased risk of vertical transmission; no effect of Vit A on child mortality
Villamor *et al.*, 2002	Daresalaam Tanzania	1075 HIV + pregnant women, 12–27 weeks gestation	Same as above	MVs increased weight gain in third trimester; MVs reduced risk of low total weight gain, weight loss, and low rate of weight gain in third trimester, MVs + Vit A resulted in lower risk of low total weight gain than MV alone	No effect of Vit A on overall weight gain or third trimester weight gain.
Fawzi *et al.*, 2003	Daresalaam Tanzania	Same as above, 788 infants born followed from 6 weeks to 24 months	Same as above	MVs decreased risk of infant diarrhea MVs increased CD4+ cell counts among infants Vit A decreased risk of cough, with rapid respiratory rate	No effect of Vit A on infant diarrhea or CD4+ cell counts
Fawzi *et al.*, 2004	Daresalaam Tanzania	1078 HIV + pregnant women; subgroup of 297 women for	Same as above	MVs decreased risk of progression to late stage disease or death MVs reduce risk of oral G1 and other symptoms MVs	No effect of Vit A on disease progression or death

		viral load and end point		associated with higher CD4+ cell counts and lower viral load	
Kelly *et al.*, 1999	Zambia	135 HIV + patients with persistent diarrhea	Albendazole + MV (Vitamins A, C and E, selenium and zinc) Albendazole + placebo	Low serum levels of vit A and E at baseline associated with increased mortality	No effect of MVs on mortality time with diarrhea, CD4+ cell counts, or serum levels of Vit A and E after 1 month
Jiamton *et al.*, 2003	Bangkok, Thailand	481 HIV + patients with CD4+ cell counts between 50 and 550x106 cells/L	MV (vitamins A, B_1 B_2 B_6 B_{12} C, D_3, E and K, beta carotene, folacin, pantothenic acid, iron, magnesium, manganese, zinc, iodine copper, selenium, chromium and cysteine Placebo	MVs reduced death rates among participants with CD4+ cell counts < 200 x 106 cells/L	MVS had no effect on CD4+ cells counts or viral load

Source: Tang *et al.*, 2005

Studies have shown that nutritional supplements especially micronutrient consumption, can be increased through eating specific foods or through special supplements (FANTA, 2004). Establishing home and community gardens to grow local foods and vegetables is important to improve intake of mineral rich foods (Ihekoronye, 1992). Raising of small animals may help improve protein, fat and micronutrients consumption such as iron (FAO, 2002). Nutrition education at an early stage of HIV infection gives a person a chance to build up healthy eating habits (FAO, 2002). Inclusion of nutrition as a core part in any HIV-care package is essential. Among the nutrients required are vitamins.

2.5 Vitamins

Vitamins have been defined as a group of organic substances which in minute amounts are essential for normal metabolism of organism (Combs, 1998). Like other essential nutrients vitamins have to be supplied by the diet in amounts covering the individual requirements of the organism in question which by definition is unable to synthesize these compounds (Dunne, 1990). In certain cases supplementation with synthetic vitamins might be necessary to avoid vitamin deficiency (Piwoz and Preble, 2000).

Vitamins are a chemically and functionally inhomogeneous group of biomolecules; almost all is a family of chemically related substances that is vitamers sharing qualitatively (but not necessarily quantitively biological activities (Osborne and Voogt, 1978). Thus the vitamers composing of a vitamin family may vary in biopotency and the common vitamin is actually a generic descriptor for all the relevant vitamers (Table 2.3). Vitamins are classified into two group's water soluble and fat soluble vitamins (Combs, 1998).

The water-soluble vitamins tend to have one or more polar or ionizable groups (carboxyl, keto, hydroxyl, amino or phosphate), whereas the fat soluble vitamins have predominantly aromatic and aliphatic characters (Dunne, 1990). Fat soluble vitamins include vitamin A, D, E and K. Water soluble vitamins consists of vitamin C and eight vitamins of group B (B_1- thiamin,B_2-riboflavin, B_3-nicotinic acid, B_5- pantothenic acid, B_6- pyridoxine acid, biotin, folic acid, B_{12}- Cyanocobalamin) (Alan, 1995).

Table 2.3: Physical properties of vitamins

Vitamin	Vitamers	MW	Solubility Org[a]	Solubility H_2O	Absorption (nm)	Mp°C	colour/form
Vitamin A	Retinol	286.4	+	–	325	62-64	yellow/crystal
	Retinal	284.4	+	–	373	61-64	orange/crystal
	Retinoic acid	300.4	+	[b]sl	351	180-182	yellow/crystal
Vitamin E	α-tocopherol	430.7	+	–	294	2.5	yellow/oil
	γ-tocopherol	416.7	+	–	298	-2.4	yellow/oil
Vitamin C	free acid	176.1	–	323	245	190-192	white/crystal
	Sodium salt	198.1	–	620	245	218[c]	white/crystal

Org[a] – Organic solubility in organic solvents, fats and oils

[b]sl – Slightly soluble; Mp°C – Melting point

Source: Alan, 1995

2.6 Vitamin A

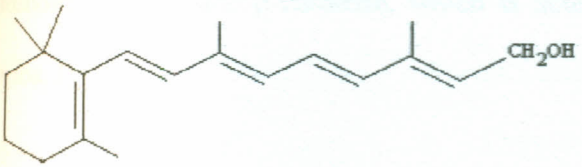
2.6.1 Sources of vitamin A

Vitamin A or all trans retinol is found as preformed vitamin A in foods such as liver and daily products or provitamin A carotenoids in foods such as dark green leafy vegetables and carrots (Combs, 1998). Fruit provitamin carotenoids include apricot, yellow melons, peaches, and prunes. Vegetables provitamin carotenoids include beet green, broccoli greens, carrots, endive, kale, lettuce, mint, mustard, parsley, pumpkins, spinach, sweet potatoes, turnip greens, cress, nuts- provitamin carotenoids in small quantity in most nuts (Ihekoronye, 1992).

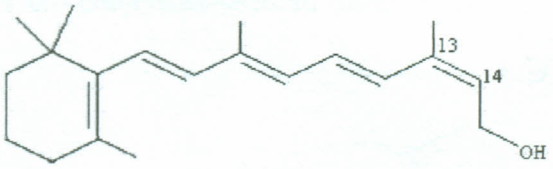
2.6.2 Vitamin A nomenclature and chemistry

Vitamin A is a generic descriptor for compounds with the qualitative biological activity of retinol (Combs, 1998). These compounds are formally derived from a monocyclic parent compound containing five carbon-carbon double bonds and a functional group at the terminus of the acyclic portion. These compounds are called retinoids owing to their close structural similarities to retinol (Kutsky, 1981). The vitamin A active retinoids occur in nature in three forms; the alcohol-retinol, the aldehyde-retinal and the acid-retinoic acid.

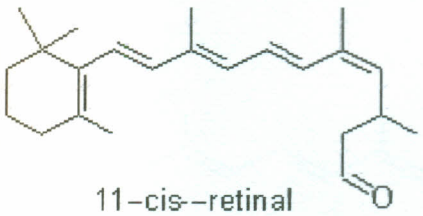
All three basic forms are found in two variants with the β-ionone nucleus (vitamin A_1) or the dehydrogenated β-ionone nucleus (vitamin A_2). However because the former is both quantitatively and qualitatively more important as a source of vitamin A activity, the term vitamin A is usually taken to mean vitamin A_1 (Figure 2.4) (Ball, 1997).



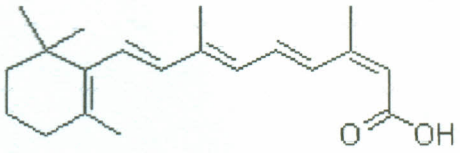
All-trans-retinol (vitamin A_1)



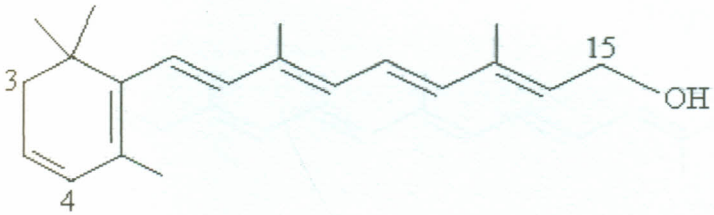
13-cis-retinol



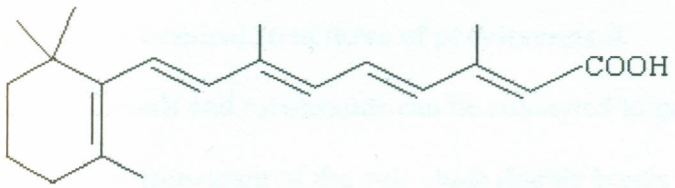
11-cis--retinal



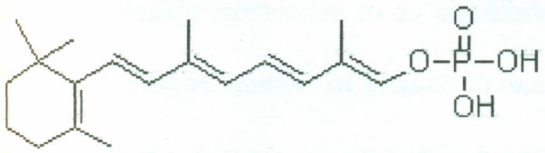
13-cis--retinoic acid



All-trans-3-dehydroretinol
(Sometimes called vitamin A_2)



All-trans-retinoic acid



all-trans--retinylphosphate

Figure 2.4: Chemical structures of the vitamin A group

Some compounds of the class of plant pigments carotenoids, owing to their relation to the carotenes, yield retinoids on metabolism and thus, also have vitamin A activity. They are called provitamin A

carotenoids and include β-carotene, which is actually a tail-conjoined retinoid dimer (Figure 2.5) (Combs, 1998).

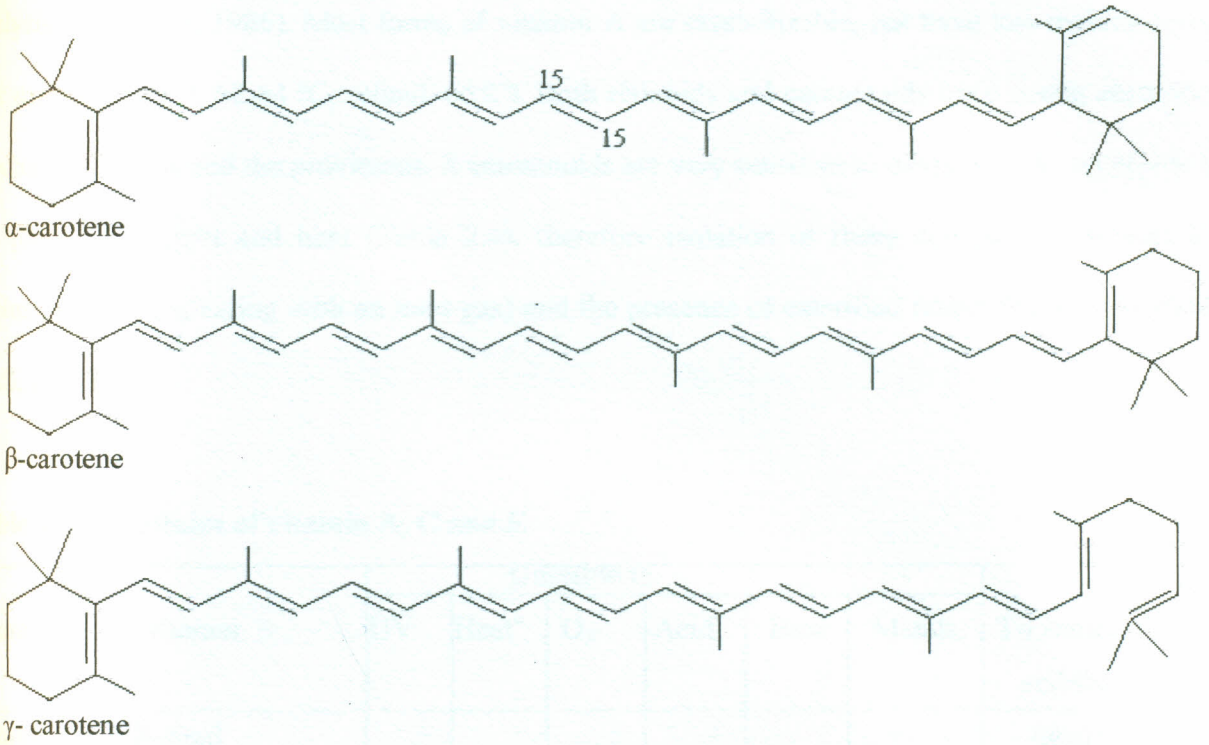


Figure 2.5: Chemical structures of provitamins A

In solution, retinoids and carotenoids can be converted to geometric isomers by light, heat and iodine through cis-trans isomerism of the side chain double bonds for instance in aqueous solution all-trans-retinol spontaneously isomerizes to an equilibrium mixture containing one third cis forms (Combs, 1998). Of the 16 stereoisomers of vitamin A made possible by the four side chain double bonds most of the potential cis isomers are sterically hindered, thus a few isomers are known (Dunne, 1990). Carotenoids in both plants and animals occur almost exclusively in the all-trans form. These conjugated polyene systems absorb light and in the case of carotenoids, appear to quench free radical weakly. For the retinoids, the functional group at position 15 determines specific chemical reactivity (Combs, 1998). Thus retinol can be oxidized to retinoic acid or reduced to retinal and retinoic acid can be esterified with organic alcohols.

Retinol and Retinal each undergo colour reactions with such reagents as antimony trichloride, trifluoroacetic acid and trichloroacetic acids which have been used as the basis of their chemical analysis (Brubacher, 1986). Most forms of vitamin A are crystallizable, but have low melting points (for example retinol, 62-64 °C, retinal, 65°C). Both retinoids and carotenoids have strong absorption spectra. Vitamin A and the provitamin A carotenoids are very sensitive to oxygen in air, especially in the presence of light and heat (Table 2.4), therefore isolation of these compounds requires the exclusion of air (sparging with an inert gas) and the presence of esterified retinoids are fairly stable (Ball, 1997).

Table 2.4: Stabilities of vitamin A, C and E

Vitamin	vitamer	Unstable to						To enhance stability
		UV	Heat[a]	O_2	Acid	Base	Metals	
Vitamin A	Retinol	+		+	+		+	keep in the dark sealed
	Retinal			+	+		+	keep sealed
	Retinoic acid							good stability
	Dehydroretinol			+				keep sealed
	Retinyl esters							good stability
	β-carotene	+	+	+	+			keep in the dark sealed
Vitamin E	Tocopherols	+	+	+	+		+	keep cool, at neutral PH
	Tocopheryl ester							good stability
Vitamin C	Ascorbic acid			+[b]		+	+	keep sealed at neutral PH

a that is, 100°C

b in solution with Fe^{2+} and Cu^{2+}

Source: Ihekoronye, 1992

2.6.3 Vitamin A biopotency

Of the estimated 500 carotenoids in nature, only about 60 have been found to have provitamin A activity that is those that can be cleaved to yield at least one molecule of retinol (Table 2.5). Five or six of these are common in foods (Combs, 1998). While the chemical properties of each determine its biopotency, dietary and physiological factors can affect the physiological utilization of each, referred to as its bioavailability (Ball, 1997). To accommodate differences in biopotency, the reporting of vitamin A activity from its various forms in foods requires some means of standardization. Two systems are used for this purpose. International units (IU) and retinol equivalents (RE)

Reporting Foods vitamin A activity

1 International unit (IU) = 0.3 μg all-trans-retinol

= 0.344 μg retinyl acetate

= 0.6 μg β-carotene

= 1.2 μg other provitamin A carotenoids

1 retinol equivalent (RE) = 1 μg all-trans-retinol

= 0.6 μg β-carotene

= 12 μg other provitamin A carotenoids

In the calculation of RE values, corrections are made for the conversion efficiency of carotenoids to retinol and it is assumed that retinol intermediate is completely absorbed (i.e. absorption efficiency is 100%) (Combs, 1998).

Table 2.5: Relative biopotencies of vitamin A and related compounds

Compounds	Relative biopotency %
All-trans-retinol	100
All-trans-retinal	100
Cis-retinol isomers	23-75
Retinyl esters	10-100
3-dehydro vitamin A	30
β-carotene	50
α-carotene	26
γ-carotene	21
Cryptoxanthin	28
Zeaxanthin	0

Most relative biopotencies were determined by liver storage bioassays with chick and or rats

Source: Combs, 1998

2.6.4 Absorption and storage of vitamin A

The upper intestinal tract is the primary area of absorption of vitamin A .It is here that the fat-splitting enzymes and bile salts convert carotene into a usable nutrient (Stevenson, 1987). This conversion is stimulated by thyroxine, a hormone obtained from the thyroid gland (Ball, 1997). Once converted to vitamin A, carotene is absorbed in the same way as the preformed vitamin. Vitamin A is carried through the bloodstream, readily accessible to tissues throughout the body (Dunne, 1990). Preformed vitamin A is found in fish liver oil or other animal products it is absorbed by the body 3 to 5 hours

after ingestion, whereas the conversion and absorption of carotene takes 6 to 7 hours (Stevenson, 1987).

The conversion of carotene into vitamin A is not 100 percent complete; approximately one third of the carotene in food is converted into vitamin A (Dunne, 1990). Less than one fourth of the carotene in carrots and root vegetables undergoes conversion, and about one half of the carotene in leafy vegetables undergoes conversion (Kutsky, 1981). Some unchanged carotene is absorbed into the circulatory system and stored in the fat tissue rather than in the liver. Unabsorbed carotene is excreted in the feaces. The ability of the body to utilize carotene varies with the food and the form in which the food is ingested. Cooking, pureeing or mashing of vegetable ruptures the cell membranes and therefore makes carotene more available for absorption (Dunne, 1990).

Factors interfering with absorption of vitamin A and carotene include strenuous physical activity performed within 4 hours of consumption, intake of mineral oil, excessive consumption of alcohol, excessive consumption of iron and use of cortisone and other drugs (Combs, 1998). The intake of polyunsaturated fatty acids with carotene results in rapid destruction of carotene unless antioxidants also are present (Stevenson, 1987).

Approximately 90 percent of the body's vitamin A is stored in the liver, with small amounts deposited in the fat tissues, lungs, kidneys and retinas of the eyes (Dunne, 1990). Under stressful conditions the body uses this reserve supply if it is not receiving enough vitamin A from the diet (Combs, 1998). An adequate supply of zinc is needed so the liver can mobilize vitamin A out of storage depots. Gastro intestinal and liver disorders, infection of any kind or any condition in which the bile duct is obstructed may limit the body's capacity to retain and use vitamin A (Dunne, 1990).

Factors affecting absorption of vitamin A include the quantity given, influence of other substance present in the intestines and the amount of vitamin stored in the body. A diet low in fat, resulting in little bile reaching the intestine, can cause carotene and vitamin A to be lost in the feaces (Ball, 1997).

2.6.5 Role of vitamin A in immunity

Vitamin A is essential for immunity, cell differentiation, growth, reproduction and vision (Combs, 1998). All-trans and 9-cis retinoic acid active metabolites of vitamin A, modulate gene transcription via specific nuclear receptors (Passmore and Eastwood, 1986).Vitamin A is important in supporting immunocompetence. Vitamin A plays a central role in growth and function of T and B cell, antibody responses and maintenance of mucosal epithelia, including that of respiratory gastrointestinal and genitourinary tracts (Semba *et al.*, 1995).

Retinol may also play a positive role in immune function. Evidence suggests that retinol may be a specific growth factor for B lymphocytes (Combs, 1998).That lymphocyte proliferation can be impaired by vitamin A deprivation suggests that cell- mediated immunity may be comprised by vitamin A deficiency. Decreased natural killer (NK) cell cytotoxic activity has been reported in vitamin A deficient rats. Vitamin A has been shown to enhance the immune responses of children vaccinated against measles (Semba, 1994).

2.6.6 Vitamin A deficiency and HIV

Vitamin A deficiency can occur either because of a lack of both provitamin A and preformed vitamin A in diets (primary vitamin A deficiency) or because of failures in their physiologic utilization (secondary vitamin A deficiency). Primary vitamin A deficiency can occur among children and adults who consume diet composed of few servings of yellow and green vegetables, fruits and liver (Combs,

1998). The appreciable storage of vitamin A in the body tends to mitigate against the effects of low dietary intakes of the vitamin, as tissues stores are mobilized in response to low vitamin A conditions (IVAGG, 1981). However, it should be noted that, while hepatic stores are great enough to provide retinol, the plasma retinol level is only minimally affected by vitamin A deprivation (Robert and Gooditart, 1974).

Serum or plasma vitamin A levels are commonly used to measure vitamin A status (Liang *et al*, 1996). Abnormally low levels of circulating provitamin A carotenoids have been reported in about 30-80% of HIV-infected individuals (Bogden *et al.*, 1990). Inadequate dietary intake of vitamin A is common and contributes to the development of vitamin A deficiency (Smit *et al.*, 1996). Vitamin A deficiency is associated with accelerated HIV disease progression, and increased mother to child transmission of HIV ((Baum, 1995; Semba, 1995). Higher HIV load has been found in breast milk from mothers with vitamin A deficiency (Nduati *et al.*, 1995). The presence of HIV DNA in vagina has been linked with severe vitamin A deficiency in HIV infected women suggesting that vitamin A deficiency may possibly contribute to heterosexual transmission of HIV (John et *al.*, 1997).

Whether vitamin A supplementation has therapeutic benefit in HIV infection has not been well established. Although β-carotene supplementation has been shown to increase CD4+ lymphocyte counts and natural killer cells in HIV infected patients. A large series of clinical trials suggest that high dose vitamin A supplementation reduces diarrhea, morbidity and enhances immunity in HIV infected children (Coodley *et al.*, 1993; Hussey *et al.*, 1996) (Table 2.2).

2.6.7 Antioxidant activities of vitamin A and carotenoids

Retinol and retinal cannot quench singlet oxygen and have only weak capacities to scavenge free radicals. It however affects tissue levels of other antioxidants several carotenoids on the other hand have been shown to have direct antioxidant activities (Combs, 1998). These include β-carotene, lycopene and some oxycarotenoids (zeanthin, lutein) which quench singlet oxygen ($^1O_2$) or free radicals in the lipid membranes in which they partition (Ihekoronye, 1992). These anti-oxidant activities are due to their extended systems of conjugated double bonds, which are thought to delocalize the unpaired electron of a free-radical reactant (Dunne, 1990). At low (physiologic) partial pressures of oxygen carotenoids can also participate in the reduction of free radicals xanthophylls carotenoids (lutein, lycopene and β-crypoxanthin) are more effective than β-carotene and more efficient than α-tocopherol in vitro (Combs, 1998).

2.6.8 Vitamin A dosage and toxicity

The recommended dietary allowance of vitamin A is 4000-5000 IU for adults and 1500-4000 IU for children (Institute of Medicine, 2002). These amounts increase during disease, trauma, pregnancy and lactation (Dietary Reference Intakes, 2001). Requirements vary for people who smoke, those who live in highly polluted areas, people who easily absorb vitamin A, and those who have had their stored supply of vitamin A depleted by pneumonia or nephritic (Diplock *et al.*, 2000).

The potential for vitamin A intoxication is greater than those for other hyper vitaminoses, as its range of safe intake is relatively small. For humans intakes as low as 25 times the RDA are thought to be potentially intoxicating (Dietary Reference Intakes, 2001). The toxicities of carotenoids are considered low, and circumstantial evidence suggests that for β-carotene intake of as much as 30mg/day are without side effects other than carotenodermia, the accumulation of the carotenoid in

the skin. Most signs of hyper vitaminosis A can be reversed by discontinuing excess exposure to the vitamin (Dunne, 1990).

2.7 Vitamin C

2.7.1 Sources of vitamin C

Vitamin C or ascorbic acid is found in foods such as citrus fruits, green vegetables, peppers, berries and potatoes.

2.7.2 Vitamin C nomenclature and chemistry

Vitamin C is generic descriptor for all compounds exhibiting the qualitative biological activity of ascorbic acid (Passmore and Eastwood, 1986). The terms L-ascorbic acid and ascorbic acid are both trivial designators for the compound 2, 3-didehydro-L-threo-hexuronic-1, 4-lactone, which was formerly known as hexuronic acid (Friedrich, 1988). The oxidized form of this compound is called L-dehydro ascorbic acid or dehydroascorbic acid (Figure 2.10) (Combs, 1998).

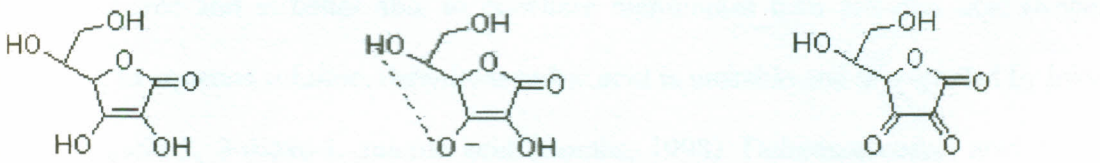


Figure 2.6: Chemical structures of vitamin C

Ascorbic acid is a dibasic acid (Pka values of 4.1 and 11.8), because both enolic hydroxyl groups can dissociate it forms salts, the most important of which are sodium and calcium salts the aqueous solution of which are strongly acidic (Gayla and Kirschmann, 1996). A strong reducing agent,

ascorbic acid is oxidized under mild conditions to dehydroascorbic ascorbic acid (sometimes called monodehydro ascorbic acid) (Friedrich, 1988).

The semiquinoid ascorbic acid radical is a strong acid (Pka = – 0.45); after the loss of a proton, it becomes a radical anion that, owing to resonance stabilization, is relatively inert but disproportionates to ascorbic acid and dehydroascorbic acid, a reversible redox system (Combs, 1998). It is therefore an effective quencher of free radical such as singlet oxygen ($^1O_2$) (Weber *et al.*, 1996). It reduces ferric (Fe^{3+}) to ferrous (Fe^{2+}) iron and other metals analogously and the super oxide radical ($O_2^{\bullet}$) to H_2O_2 and is oxidized to monodehydroascorbic acid in the process (Kutsky, 1981). Ascorbic acid complexes with disulfides e.g. oxidized glutathione, cystine, but does not reduce those disulfide bond (Combs, 1998).

Dehydroascorbic acid is not ionized in environments of weakly acidic or neutral ; therefore, it is relatively hydrophobic and is better able to penetrate membranes than ascorbic acid (Robert and Gooditart, 1974). In aqueous solution, dehydroascorbic acid is unstable and is degraded by hydrolytic ring opening to yield 2, 3-dioxo-L-gulonic acid (Combs, 1998). Dehydroascorbic acid reacts with several amino acids to form brown-coloured products, a reaction contributing to food spoilage (Friedrich, 1988).

2.7.3 Vitamin C biopotency

Several synthetic analogs of ascorbic acid have been made (Gayla and Kirchmann, 1996). Some have biological activity for instance 6-deoxy-L-ascorbic acid, whereas others have little or no activity for

example d-isoascorbic acid and L-glucoascorbic acid (Naidu, 2003). Several esters of ascorbic acid are converted to the vitamin in vivo and thus, have good biological activity (for instance ascorbyl-5, 6-diacetate, ascorbyl-6-palmitate and 6-deoxy-6-chloro-L-ascorbic acids). Esters of the C-2 position show variable vitamin C activity among different species (Combs, 1988). (Table 2.6)

Table 2.6: Relative biopotencies of vitamin C active substances

Compound	Relative biopotency %
Ascorbic acid	100
Ascorbyl-5,6-diacetate	100
Ascorbyl - 6- palmitate	100
6-deoxy- 6- chloro-L- ascorbic acid	70 – 98
Dehydroascorbic acid	80
6- deoxyascorbic acid	33
Ascorbic acid- 2-sulfate	$\pm$ [a]
Isoascorbic acid	5
L-glucoascorbic acid	3

[a]This form is active in fishes, which have an intestinal sulfohydrase that liberates ascorbic acid; it is inactive in guinea pigs, rhesus monkeys and humans, which lack the enzyme.

Source: Combs, 1998

2.7.4 Absorption and storage of vitamin C

The level of ascorbic acid in the blood reaches a maximum in 2 or 3 hours after ingestion of a moderate quantity, and then decreases as it is eliminated in the urine and through perspiration (Ball, 1997). Most vitamin C is out of the body in 3 to 4 hours (Ausman, 1999). Increased urinary output of vitamin C resulting from larger intake of the vitamin does not mean body tissues are saturated (Combs, 1998). The blood levels of vitamin C will return to its average levels in 12 to 13 hours

regardless of the amount ingested. To maintain adequate serum level, the vitamin should be taken throughout the day (Ausman, 1999). Excess vitamin C carried to the bladder may prevent bladder cancer (Naidu, 2003).

Taken orally, most of the vitamin is absorbed through the mucous membranes of the mouth, stomach and upper part of the small intestines (Weber *et al.*, 1996). The larger the dose the less is absorbed (Naidu, 2003). Therefore it is best to take vitamin C in small doses several times a day. The human body when fully saturated contains about 500mg of vitamin C, of which 30mgs are found in the adrenal glands, 200mgs in the extracellular fluids, and the rest distributed in varying concentration throughout the cells of the body (Stevenson, 1987).

The body's ability to absorb vitamin C is reduced by smoking, stress, high fever, prolonged administration of antibiotics or cortisone, inhalation of dimethyldiphenyltrichloroethane fumes of petroleum and ingestion of aspirin or any other pain killers, sulphur drugs increase urinary excretion of vitamin C by two to three times the normal amount (Gayla and Kirchmann, 1996).

Baking soda creates an alkaline medium that destroys vitamin C in addition; drinking excessive amounts of water will deplete the body's vitamin C (Ball, 1997).

2.7.5 Role of vitamin C in immunity

Ascorbic acid has been found to affect immune function in several different ways (Dunne, 1990). It can stimulate the production of interferons, the protein that protect cell against viral attack (Semba and Tang, 1999). It can stimulate the positive chemotactic and proliferative responses of neutrophils associated with the oxidative burst of neutrophils (Tang *et al.*, 1993). Ascorbic acid can stimulate the synthesis of humoral thymus factor and antibodies (Gayla and Kirschmann, 1996).

Phagocytic cells of the immune system produce oxidants during infections that ascorbic acid which is present in high concentrations in phagocytes and lymphocytes will provide some antioxidant protection (Naidu, 2003). Indeed studies have found that vitamin C increases the proliferative response of lymphocytes, is associated with enhanced natural killer cell activity, increases the production of interferon and decreases viral replication in cell culture systems (Combs, 1998).

2.7.6 Vitamin C deficiency and HIV

Acute vitamin C deficiency results in scurvy in individuals unable to synthesize the vitamin C. Signs of the disease occur primarily in mesenchymal tissue. Defects in collagen formation are manifested as impaired wound healing; edema, hemorrhage (due to deficient formation of intracellular substance) in skin, mucous membranes, internal organs and muscles and weakening of collageneous structures in bone, cartilage, teeth and connective tissues (Semba and Tang, 1999).

Mild vitamin C deficiency results in several nonspecific prescorbutic signs and symptoms including lassitude, fatigue, anorexia, muscular weakness, increased susceptibility can be caused by low dietary intakes, as well as by a variety of factors than increase ascorbate turnover in the body e.g. smoking, stress, chronic disease, diabetes (Gayla and Kirchmann, 1996). In addition, elderly people typically show total body vitamin C pools of reduced size; perharps owing to reduce enteric absorption and increased turnover (Alan, 1995). There is limited data to suggest that vitamin C play a significant role in immune function (Weber *et al.*, 1996). During HIV, low vitamin C levels consistent with deficiency occur in 7-27% of homosexual men and 20% of heterosexual adults (Coodley *et al.*, 1993; Skurnick *et al.*, 1996). In homosexual men, high dietary intake of vitamin C is associated with decreased progression to AIDS (Tang *et al.*, 1993) (Table 2.2).

2.7.7 Antioxidant and proxidant functions of vitamin C

Ascorbic acid can act either as an antioxidant or as a proxidant. Its antioxidant activity is based on its ability to react with free radicals, being first converted to ascorbyl radical which quickly disproportionate to ascorbate and dehydroascorbate (Combs, 1998). This ascorbic acid can react with the toxic forms of oxygen, the superoxide anion ($O_2^{\bullet}$) and the hydroxyl radical ($OH^{\bullet}$). This reaction is likely to be of fundamental importance in all aerobic cells which must defend against the toxicity of the very element depended upon as the terminal electron acceptor for energy production via the respiratory chain enzymes. It is this type of reaction that appears to be the basic of most if not all of the essential biological functions of ascorbic acid (Dunne, 1990). One of these is important in extending the antioxidant protection to hydrophobic regions of cell.

Ascorbic acid appears to be able to reduce the semi stable chromanoxyl radical thus regenerating the metabolically active form of the lipid antioxidant Vitamin E. The antioxidant efficiency of vitamin C is the greatest at low concentrations of the vitamin (Combs, 1998). Under these conditions, the predominant reaction is a radical chain-terminating one of ascorbate (AH^{-}) with a peroxyl radical to yield a hydroperoxide and the ascorbyl radical and yield the vitamin in its oxidized form dehydroascorbic (A). At low concentrations of the vitamin two moles of peroxyl radical are reduced for every mole of ascorbate consumed (Friedrich, 1988).

$AH^{-} + ROO^{\bullet} \rightarrow A\bullet + ROO^{-}$ $(H+ \rightarrow ROOH)$

$A^{\bullet -} + R'OO^{\bullet} \rightarrow A + R'OO^{-}$

At higher vitamin C concentrations, however a slower radical chain-propagation reaction of ascorbyl radical and molecular oxygen appears to become significant. It yields dehydroascorbic acid and superoxide radical which in turn can oxidize ascorbate to return ascorbyl radical (Friedrich, 1988).

$A\bullet^{-} + O_2 \rightarrow A + O_2^{\bullet -}$

$O_2^{\bullet -} + AH^- \rightarrow HOO^- + A^{\bullet -}$

It is thought that at high vitamin C concentrations, this reaction sequence can develop into a radical chain auto-oxidation process that consumes ascorbate thus "wasting" the vitamin. Hence, in aerobic systems, the efficiency of radical quenching of ascorbate is inversely related to the concentration of the vitamin. In the presence of metal ions (Fe^{3+}, Cu^{2+}), high concentration of vitamin C can also function as a pro-oxidant (Combs, 1998). It does so by donating a single electron to reduce such ions to forms that in turn can react with oxygen to form oxygen radicals (the metal ions being re-oxidised in the process). Thus ascorbate can react with copper or iron and lead salts and lead to the formation of superoxide radical anion and hydroxyl radical. Ascorbic acid increases the bio-availability of iron in foods (Combs, 1998). This effect is associated with increased enteric absorption (which is normally low) of the mineral. Its effect involves its reducing the ferric form of the element (Fe^{3+}, Fe^{2+}) and then forming a soluble stable chelate that stays in solution in the alkaline environment of the small intestine and is thus rather well absorbed. The effect depends on the presence of both ascorbic acid and iron in the gut at the same time (Dunne, 1990).

2.7.8 Vitamin C dosage and toxicity

The National Research Council recommends 60 milligrams of vitamin C for adult (Dietary Reference Intakes, 2001). The requirement may vary due to differences in weight, amount of activity, rate of metabolism, ailments and age, periods of stress, such as anxiety, infection, injury, surgery, burns or fatigue, and increase the body's need for this vitamin (Institute of medicine, 2002).

The only adverse effects of large doses of vitamin C that have been consistently observed in humans are gastro intestinal disturbances and diarrhea occurring at levels of intake nearly 20-80 times the RDAS. Dietary vitamin C concentrations of 100-1000 times the allowance levels appear safe for most species (Ausman, 1999).

2.8 Vitamin E

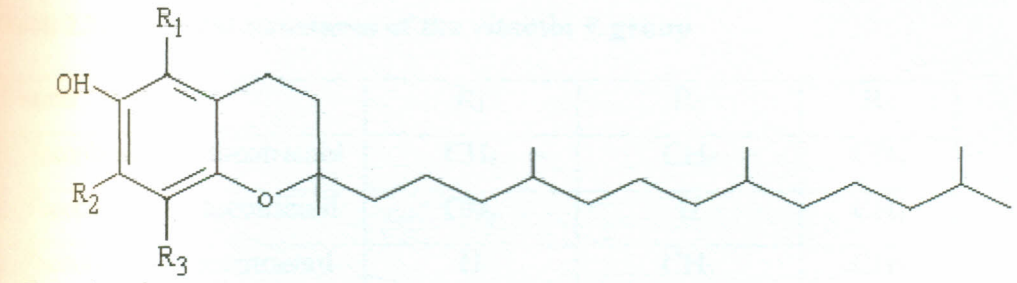
2.8.1 Sources of vitamin E

Vitamin E is synthesized only by plants, the richest source being plant oil. All higher plant appears to contain α-tocopherol in their leaves and other green parts (Kaur and Kapoor, 2001). Because α-tocopherol is contained mainly in the chloroplasts of plant cell (whereas the β-, γ-, δ-vitamers are usually found outside of these particles), green plants tend to contain more vitamin E than yellow plants (Combs, 1998). The tocotrienols are not found in green leaves but, rather in the bran and germ fractions of certain plants (Ihekoronye, 1992). They can occur naturally in esterified form, unlike the tocopherols, which exist only as free alcohols (Dunne, 1990).

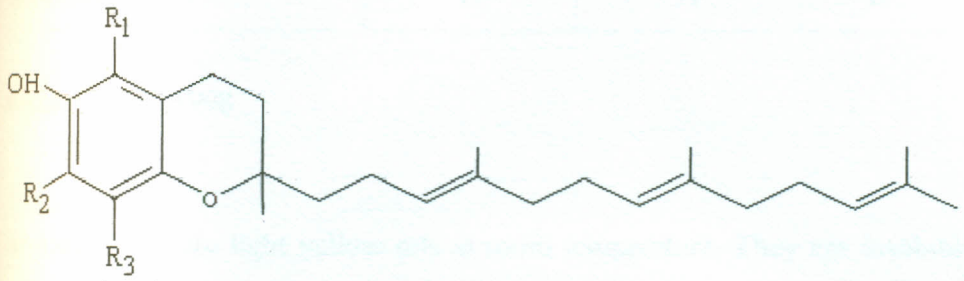
Animal tissues tend to contain low amounts of vitamin E, the highest levels occurring in fatty tissue. These levels vary according to the dietary intake of the vitamin. Because vitamin E occurs naturally in fat oils, reductions in fat intake can be expected also to reduce vitamin E intake. The vitamer of greatest interest in nutrition is the most biopotent one is α-tocopherol, which occurs naturally as the *R,R,R*- stereoisomer (*R,R,R*-α-tocopherol) (Combs, 1998).

2.8.2 Vitamin E nomenclature and chemistry

Vitamin E is a generic descriptor for all tocol and tocotrienol derivatives that exhibit qualitatively the biological activity of α-tocopherol. These compounds are isoprenoid side-chain derivatives of 6-chromanol (Combs, 1998).The term tocol is the trivial designation for the derivative with a side chain consisting of three fully saturated isopentyl units; tocopherol denotes generically the mono-, di-and trimethyltocols irrespective of biological activity (Figure 2.7).



Tocopherols



Tocotrienol

Figure 2.7: Chemical structures of the vitamin E group

Tocotrienol is trival designation of the 6-chromanol derivative with a similar side chain containing three double bonds. **Individual tocopherol and tocotrienol are named according** to the position and number of methyl groups on their chromanol nuclei (Table 2.7) (Dunne, 1990). Because the tocopherol side chain **contain two anomeric carbons (C-4, C-8) in addition** to the one at the point of its attachment to the nucleus (C-2), eight stereoisomers are possible. However only one stereoisomer occurs naturally the *R,R,R*-forms. **The chemical synthesis of vitamin E** produces mixtures of other stereoisomers, depending on the starting materials (Combs, 1998).

Table 2.7: Chemical structures of the vitamin E group

Vitamer	R_1	R_2	R_3
α-Tocopherol/ α – tocotrienol	CH_3	CH_3	CH_3
β-Tocopherol / β- tocotrienol	CH_3	H	CH_3
γ-Tocopherol / γ- tocotrienol	H	CH_3	CH_3
δ-Tocopherol / δ- tocotrienol	H	H	CH_3
Tocol / tocotrienol	H	H	H

Source: Combs, 1998

The tocopherols are light yellow oils at room temperature. They are insoluble in water, but readily soluble in non-polar solvents. Being monoethers of hydroquinone with phenolic hydrogen in hydroxyl group at position C-6 in the chromanol nucleus, with the ability to accommodate an unshared electron within the resonance structure of the ring they are good quenchers of free radicals and thus, serve as antioxidants (Fredrick, 1988). They are, however, easily oxidized and can be destroyed by peroxides, ozone and permanganate in a process catalyzed by light and accelerated by polyunsaturated fatty acids and metal salts. They are very resistant to acids and to bases.

Tocopheryl esters, by virtue of the blocking of the C-6 hydroxyl group, are very stable in air and are, therefore, the forms of choice as food/ feed supplements (Alan, 1995). Because tocopherol is liberated by the saponification of its esters, extraction and isolation of vitamin E call for the use of protective antioxidants (e.g. propyl gallate, ascorbic acid, metal chelators, inert gas environments and subdued light (Brubacher, 1986). The UV absorption spectra of tocopherols and their acetates in ethanol have maxima of 280-300nm (α-tocopherol, 292nm) (Alan, 1995); however their extinction coefficients are not great. Because their fluorescence is significant (excitation, 294nm; emission, 330nm), this property has analytical utility (Diplock *et al.*, 2000).

2.8.3 Vitamin E biopotency

The vitamers E vary in biopotency, mainly according to the positions and numbers of their nucleus, methyl groups, and the most biopotent being the trimethylated (α) form (Table 2.8) (Combs, 1998).

Table 2.8: Relative biopotencies of vitamin E active compound

Trivial designation	systematic name	biopotency (IU/mg) [a]
R,R,R-α-tocopherol[b]	2*R*, 4'*R*,8'*R*-5, 7, 8-trimethyltocol	1.49
R,R,R-α-tocopheryl acetate	2*R*, 4'*R*,8'*R*-5, 7, 8-trimethyltocyl acetate	1.36
all-rac-α-tocopherol[c]	2*RS*, 4'*RS*,8'*RS*-5, 7, 8-trimethyltocol	1.1
All-rac-α-tocopherol acetate	2*RS*, 4'*RS*,8'*RS*-5, 7,8-trimethyltocyl acetate	1.0
R,R,R-β-tocopherol	2*R*, 4'*R*,8'*R*-5, 7, 8-dimethyltocol	0.12
R,R,R-γ-tocopherol	2*R*, 4'*R*,8'*R*-5,7-dimethyltocol	0.05
R-α-tocotrienol	*trans*-2*R*-5, 7, 8- trimethyltocotrienol	0.32
R-β-tocotrienol	*trans*-2*R*-5,8- dimethyltocotrienol	0.05
R-γ-tocotrienol	*trans*-2*R*-5, 7- dimethyltocotrienol	–

a International unit per milligram of material, based chiefly on rat gestation-resorption bio assay

b formally called d- α-tocopherol

c formally called dl-α-tocopherol

Source: Combs, 1998

2.8.4 Absorption and storage of vitamin E

Vitamin E as other fat soluble vitamins, is absorbed in the presence of bile salts and fats from the intestines, it is absorbed in the lymph and is transported in the blood stream as tocopherol to the liver, where high concentration are stored (Dunne, 1990). It is also stored in the fatty tissues, heart,

muscles, testes, uterus, blood, adrenal and pituitary glands. Vitamin E in ointment form can be absorbed through the skin and mucous membranes (Combs, 1998). Excessive amounts of vitamin E are excreted in the urine and all effects of vitamin E disappear within three days (Kutsky, 1981). Chlorine in drinking water, ferric chloride, rancid oil or fat and inorganic iron compounds destroys vitamin E in the body. Mineral oil used as a laxative depletes vitamin E. Vegetable oils dissolve α-tocopherol and readily release it in the body, whereas mineral oil dissolves it but does not readily release it (Dunne, 1990).

Large amounts of polyunsaturated fats or oils in diet increase the rate of oxidation of vitamin E; thus the more unsaturated fat or oil consumed the more vitamin E is necessary (Combs ,1998). The female hormone estrogen is a vitamin E antagonist. Intake of this hormone makes it very difficult to estimate the amount of α- tocopherol the individual is lacking (Kutsky, 1981). Improper absorption may be partly responsible for muscular problems, such as muscular dystrophy and poor performance in athletes and digestive problems, such as peptic ulcers and cancer of the colon (Stevenson, 1987). Poor absorption can impair the survival of red blood cells (Piwoz and Preble, 2000).

Factors that decrease the availability of vitamin E includes; presence of antagonist, mineral oil ingestion, presence of vitamin E oxidation product. Occurrence with other less active analogues, excretion in feaces, impaired fat absorption, chemical binding in foods, increased destruction (stress), cooking losses-heat and oxygen, labile losses in frozen storage, steatorrhea, variability of natural sources. Factors that increase the availability of vitamin E include; storage in (adipose and muscle) tissue, esterification increases stability, use of unprocessed fresh food sources and bile salts aid absorption (Ball, 1997).

2.8.5 Role of vitamin E in immunity

Vitamin E acts as anti-oxidant that protects against cellular damage by inhibiting peroxidation of polyunsaturated fatty acids in cell membranes, normal growth maintenances, aids intestinal absorption of unsaturated fatty acids, maintains normal muscle metabolism. It also maintains integrity of vascular system, central nervous system, kidney tubules, lungs, genital structures, liver and red blood cell membranes (Combs, 1998).

Vitamin E is essential for normal immune function, which is also stimulated by supranutritional levels of intake (Combs, 1998). Its role appears to be that of a biological antioxidant protecting immune cells from reactive oxygen species produced during the inflammatory process (Stevenson, 1987). These species are produced mostly by phagocytic cells attracted to the site of tissue injury. On encountering or ingesting a bacterium or other foreign particles, activated neutrophils, and macrophages produce large amount of super oxide radical (O_2^-) and hydrogen peroxide in a process called respiratory burst (Dunne, 1990).

These reactions are important in killing pathogens, but they can also be deleterious to immune cells themselves. If not controlled, they can contribute to the pathogenesis of disease (Semba, 1994). Therefore, it appears that adequate antioxidant status is required to maintain appropriate peroxide tone (Combs, 1998). Supranutritional intakes of vitamin E have been found to stimulate many immune functions including antibody production (Liang *et al*, 1996).

2.8.6 Vitamin E deficiency and HIV

Vitamin E deficiency can result from insufficient dietary intake or impaired absorption of the vitamin. Several other dietary factors affect the need for vitamin E (FANTA, 2004). Two are most important

in the regard; selenium and polyunsaturated fatty acids (PUFAs) (Dunne, 1990). Selenium spares the need for vitamin E (Semba, 1994). Animals fed low-selenium diet generally require more vitamin E than animals fed the same diets supplemented with available source of selenium (Combs, 1998). In contrast, the dietary intake of PUFAs directly affects the need for vitamin E; animals fed high PUFA diets require more vitamin than those fed low PUFA diets (Combs, 1998). Other factors that can be expected to increase vitamin E needs are deficiencies of sulfur- containing amino acids, deficiencies of copper, zinc, manganese and riboflavin (Dunne, 1990). Conditions involving the malabsorption of lipids can also lead to vitamin E deficiency such conditions include those resulting in loss of pancreatic tumor, nutritional pancreatic atrophy in severe selenium deficiency, those involving luminal deficiency of bile (biliary stasis due to mycotoxicosis, biliary atrosia), and those due to defect in lipoprotein metabolism (abetaliproteinemia) (Combs, 1998).

The various signs of vitamin E deficiency are believed to be manifestation of membrane dysfunction resulting from oxidative degradation of polyunsaturated membrane phospholipids and or the disruption of other critical cellular processes (Semba, 1994). A clear cut syndrome of human vitamin E deficiency has not been defined, although muscle weakness, neuronal degeneration and haemolytic anaemia have been described in individuals with chronic malabsorption (Macallan, 1999).

Vitamin E deficiency is thought to compromise immunity because of immune effector cell by free-radicals. Vitamin E supplementation has been shown to enhance delayed type hypersensitivity and interleukin 2-production (Meydani *et al.*, 1990). Total serum or plasma tocopherol levels are used to measure vitamin E status (normal range 11.6-30.8 μmol/L, deficient <11.6μ mol / L (Meydani *et al.*, 1990). During HIV infection, vitamin E deficiency occurs in 4% of heterosexual adults and in 10-20% of homosexual men and malabsorption may contribute to vitamin E deficiency (Baum *et al.*,

1995). Elevated immunoglobulin E levels, a marker of immune dysregulation are associated with vitamin E deficiency in early HIV infection (Miquez-Burbano *et al.*, 1995).

A longitudinal study of over 300 HIV infected heterosexual men showed that individuals with serum vitamin > 3.2μ mol/L had a 30% decrease in risk of progression to AIDS (Tang *et al.*, 1996). Daily vitamin E supplementation has been shown to reduce oxidative stress and enhance T-cell function in healthy adults (Meydani, 1995). Whether there is a role for vitamin E supplementation during HIV infection is unclear, but available data, suggest that intakes of vitamin E above the recommended dietary allowances (RDA) may be beneficial to health (Meydani *et al.*, 1997) (Table 2.2).

2.8.7 Vitamin E as a scavenger of free radical

Because of the reactivity of the phenolic hydrogen on its C-6 hydroxyl group and the ability of the chromanol ring system to stabilize an unpaired electron, vitamin E has anti-oxidant activity capable of terminating chain reactions among PUFAS in the membranes where in it resides (Combs,1998). This action termed as free-radical scavenging involves the donation of phenolic hydrogen to a fatty acyl free radical (or $O_2^{\bullet}$) to prevent the attack of that species on other PUFAS. The anti-oxidant activities of the vitamins E, thus relate to the leaving ability of their phenolic hydrogen (Dunne, 1990).

The tocopherols have greatest reactivities towards peroxyl and phenoxyl radicals but can also quench such mutagenic electrophiles as reactive nitrogen oxide species (NO_x) when assessed in vitro in chemically defined systems, activities are greatest for α-tocopherol with the β- and γ-vitamers roughly comparable and greater than the δ-vitamer (Kaur and kapoor, 2001). An exception to this rank order appears to be the case for NO_x, which is trapped more effectively by γ-tocopherol than by

the α-vitamer. In serving its antioxidant function, vitamin E is converted from its alcohol form to a semi stable radical intermediate, the tocopheroxyl (or chromanoxyl) radical unlike the free radicals formed from PUFAS, the tocopheroxyl radical is relatively unreactive thus stopping the destructive propagative cycle of lipid peroxidation (Combs, 1998). In fact tocopheroxyl is sufficiently stable to react with a second peroxyl radical to form inactive, non-radical products including tocopheryl quinine. Because α-tocopherol can compete for peroxyl radicals much faster than PUFAS small amounts of the vitamin are able to affect the antioxidant protection of relatively large amounts of the latter (Dunne, 1990). Although α-tocopherol is clearly the most abundant lipid soluble chain breaking antioxidant the fact that it is typically present in such low amounts relative to the membrane PUFAS it protects suggests that it is both recycled and highly mobile within the membrane (Combs, 1998).

2.8.8 Vitamin E dosage and toxicity

The daily intake of vitamin E recommended by the National Research Council is based upon the metabolic body size and the level of polyunsaturated fatty acids in the diet rather than upon weight or calorie intake (Dietary Reference Intakes, 2001). The requirements increase with gains in polyunsaturated fatty acids in the diet. Air pollution also increases the need for vitamin E. The RDA for infants is 4 to 5 IU daily; for children and adolescents the range is 7 to 12 IU; for adult males, 15 IU for adult females, 12 IU, in pregnancy and lactation, needs increase to 15 IU daily (Combs, 1998).

The toxic potential of vitamin E is low; intake of 100 (or more) times the typical allowance levels are tolerated without adverse reaction by all species tested (Institute of Medicine, 2002). Studies with animals indicate that excessive dosages of tocopherols exert most, if not all of their adverse effects by antagonizing the utilization of the other fat-soluble vitamins, probably at the level of their common micelle-dependent absorption (Combs, 1998).

2.9 Interaction among vitamin C, vitamin E and β-carotene

The effects of vitamin c (ascorbic acid) and vitamin E (α-tocopherol) and β-carotene as antioxidants and their cooperative action against the oxidation of lipid in solution, membranes and lipoprotein have been studied and reviewed.(Combs, 1998).Ascorbic acid and α-tocopherol act as potent and probably the most important hydrophobic and lipophilic antioxidants respectively (Dunne,1990). They function at their own site individually and furthermore act synergistically. β-carotene has lower reactivity towards radicals than does α-tocopherol and acts as a weak antioxidants in solution. It is more lipophilic than α-tocopherol and is assumed to be present at the interior of membranes or lipoproteins which enables it to scavenge radical within the lipophilic compartment more efficiently than does α-tocopherol. The co-operative interaction between vitamin C and vitamin E may be quite probable, that of vitamin C and β-carotene is improbable, whereas that between vitamin E and β-carotene may be possible (Combs, 1998).

2.10 Indigenous vegetables

Vegetables form an important part of the diet in just about every household in Africa. Various types of vegetables are cultivated, mostly in small back or front yard gardens, but also increasingly in medium to large-scale commercial enterprises. The types of vegetables grown vary with agro-ecology and consumption preferences. Consumer preferences are influenced to an extent by culture, traditions and income available to the household (Ihekoronye, 1992). In almost all countries the vegetables grown can be divided into two categories:

(a) Introduced vegetables

(b) Indigenous vegetables

In East Africa, i.e. Kenya, Tanzania and Uganda, introduced vegetables may include kale , white and red cabbage, tomatoes, French beans, carrots, spinach, some onions, green peas, some eggplants, and

green pepper (Sarah and Maina,2008). These vegetables are more popular in urban areas where many households cultivate their own small gardens to meet some of the household requirements. For many urban households however, a good proportion of vegetables consumed are purchased from markets. Some common indigenous vegetables include: spider weed, amaranthus, pigweed, black nightshade, pumpkin leaves, cowpeas and black jack, as well as the less common sun hemp, jute plant, stinging nettle, African eggplant and okra (Ihekoronye, 1992).

Indigenous Green Leafy Vegetables (GLVs) occupy an important place among the food crops as these provide adequate amounts of vitamins and minerals for humans. The nutritive value of greens remains underutilized due to lack of awareness and promotion of appropriate technologies for their effective utilization. Green leafy vegetables are a very good source of minerals and vitamins and when consumed regularly they can substantially improve micronutrient status of the Kenyan population. Several of these are used for medicinal purposes (Ihekoronye, 1992). These health promoting properties along with their rich nutrient profile make these GLVs an important nominee for their use in the food based approach to combat several public health problems of Kenya (Sarah and Maina, 2008).

However, GLV's are highly perishable due to their high water activity, therefore methods involving removal of this moisture helps in their preservation (Macrae *et al.*, 1993). The percent retention of essential nutrients on dehydration using several techniques such as direct solar drying, shade solar drying, drying using electric cabinet and pretreated shade drying have been studied for different foods and they vary from 25.7-90% (Nderitu,2006; Manuche,2003; Arya,1979).

Vitamins (β-Carotene, α-tocopherol and L-ascorbic acid) are found primarily in fruits and vegetables. In GLVs the leaf contains Vitamin A, C, folic acid, riboflavin, thiamine, β-carotene, minerals like iron and calcium, a lot of fiber and antioxidants. The carotene in GLVs is converted in the body into Vitamin A that prevents blindness. The nutritive value of vegetables varies with the season, type of chemical used and the variety used for cultivation. Vitamin C, B, sugar and antioxidants are comparatively less in chemically grown vegetables.

Some indigenous green leafy vegetables consumed in Kenya and their β-carotene and α-tocopherol levels are listed in Table 2.9.

Table: 2.9 Average content of β-carotene and α-tocopherol (µg/100g) in some vegetables

Common name	Botanical name	Local name	β-carotene	α-tocopherol
Cowpea leaves	*Vigna unguiculata*	Likhubi	7500	6420
Pumpkin leaves	*Cucurbita maxima*	Lisebebe	8500	7530
Amaranthus LL	*Amaranthus hybridus*	Lidodo	7600	7040
Miro	*Crotalaria brevidens*	Miro	6250	-
Frying spider	*Gynandropsis gynandra*	Chisaka	7600	3020
Bean leaf	*Phaseolus vulgaris*	Sukuma wiki	9500	3250
Night shade	*Solanun nigrum*	Lisutsa	6760	7500

Source: Nyambaka and Nyaga, 1991

.2.11 Vitamin bioavailability

Bioavailability relates to the proportion of an ingested nutrient that is absorbed, retained and metabolized through normal pathways to exert normal physiological function (Combs, 1998). The chemical analysis of such vitamins will yield measures of the total vitamin contents which will be overestimates of the biologically relevant amounts. In the cases of niacin, biotin, pyridoxine, vitamin B_{12} and choline which in certain foods and feedstuffs can be poorly utilized, only the biologically available amounts have nutritional relevance (Alan, 1995). Vitamin bioavailability can be affected by several intrinsic and extrinsic factors (Table 2.10).

Table 2.10: **Factors determining vitamin bioavailability**

Factors	Description
Extrinsic factors	
Differing biopotencies	Different vitamers can have inherent differences in biopotencies
Losses	Some vitamins in food show significant losses during storage, processing and/ or cooking e.g. the vitamin C content of potatoes can drop by one-third within 1 month of storage
Dietary effects	The compositions of meals and diets can affect the absorption of some vitamins by affecting intestinal transit time or/ the enteric formation of mixed micelles, e.g. vitamin A and provitamin A carotenoids are absorbed very poorly from very low fat diets (Mclaren, 1992)
Intrinsic factors	
Physiological effects	Age-related in differences in gastro intestinal function can affect the absorption and post absorptive utilization of certain vitamins e.g. the absorption of vitamin B_{12} is reduced in many older persons who experience loss of gastric parietal cell function.
Health status	Some illness can affect the absorption and post absorptive utilization of certain vitamins e.g. folate absorption is impaired in patients with sprue.

Source: Combs, 1998

2.12 Assessment of nutritional status

General assessment in any application has three general purposes: Detection of deficiency states, evaluation of nutrition qualities of diets, food habits and or food supplies and prediction of health effects (Dietary reference intake, 2001).

2.12.1 System of nutritional assessment

Three types of nutritional assessment systems have been employed both in population based studies and in the care of hospitalized patients (Combs, 1998).

Nutrition survey: Cross-sectional evaluations of selected population groups; conducted to generate baseline nutrition status, and to identify subgroups at nutritional risk.

Nutrition surveillance: Continuous monitoring of the nutritional status of selected population groups (risk groups) for an extended period of time, conducted to identify possible causes of malnutrition.

Nutritional screening: Comparison of individuals parameters of nutritional status with predetermined standards, conducted to identify malnourished individuals requiring nutritional intervention (Dunne, 1990).

2.12.2 Methods of nutritional assessment

Systems of nutritional assessment employ a wide variety of specific methods. These methods fall into five categories; Dietary evaluation, anthropometric evaluation, clinical evaluation, biochemical evaluation and sociological evaluation (Stevenson, 1987).

Dietary evaluation: Estimation of nutritient intakes from evaluation of diets, food availability and food habits (using instruments such as food frequency questionnaires, food recall procedures, diet histories, food records). Diets and food habits are likely to provide insufficient amounts of available vitamins identified by dietary evaluation. However these methods are almost imprecise with respect to vitamin and the parameters they measure usually have greater inherent variability than those for

other nutrients. The detection of early-stage vitamin deficiencies is therefore best achieved using the various biochemical methods that are available (Passmore and Eastwood, 1986).

Anthropometric evaluation: Estimation of nutritional status on the basis of measurements of the physical dimension and gross composition of an individual's body. Anthropometric evaluation can be informative regarding energy and protein status, but yield no information relevant to vitamin status.

Clinical evaluation: Estimation of nutritional status on the basis of recording a medical history and conducting a physical examination to detect signs (observations made by a qualified observer) and symptoms (manifestation reported by the patient) associated with malnutrition. Clinical evaluation can be effective in the diagnosis of late stage vitamin deficiencies that is those involving physiologic dysfunction and morphological changes.

Biochemical evaluation: Estimation of nutritional status on the basis of measurements of nutrient stores, functional forms, excreted forms and or metabolic functions.

Sociologic evaluation. Collection of information on non-nutrient related variable known to effect or be related to nutritional status (e.g. socio-economic status, food habit and beliefs food prices and availability, food storage and cooking practices and age cause- specific mortality rates, birth order, family structure.

Nutritional assessment systems employ many or all of these types of methods for the complete evaluation of nutritional status. Some of these approaches however are more informative than others with respect to specific nutrients and particularly to early stages of vitamin deficiencies (Combs, 1998).

2.12.3 Biochemical methods for assessing vitamin status

The ideal parameter of vitamin status is a measure of actual metabolic function of the vitamin (Combs, 1998). In some cases this is possible, however, in most cases; direct measurement of vitamin metabolic function is not possible owing to the absence of a discrete functional parameter. The existence of more than one metabolic function with different sensitivities to vitamin supply, the function of the vitamin in a loosely bound form which is unstable to the methods of tissue preparation may also hinder measurement of actual metabolic function (Augustine *et al.*, 1985). Therefore, other parameters are useful for assessing vitamin status. These include measurements of the vitamin in accessible tissues or urine certain metabolites or other enzyme related to the metabolic function of the vitamin (Diplock *et al.*, 2000) (Table 2.11).

Table 2.11: Tissue accessible for biochemical assessment of vitamin status

Tissue or cell type	Relevance
Blood plasma/serum	Contains newly absorbed nutrients as well as vitamin being transported to other tissues and therefore tends to reflect nutrient intake; this effect can be reduced by collecting blood after a fast.
Erythrocytes	With a half- life of about 120 days, they tend to reflect chronic nutrient status analyses can be technically difficult.
Leukocytes	Have relatively short half-lives and therefore, can be used to monitor short-term changes in nutrient status isolation of these cells can present technical difficulties.
Liver, adipose, bone marrow, muscle	Sampling is invasive, requiring research in clinical settings.
Hair, nails	Easily collected and stored specimens offer advantages, particularly for population studies of trace element status.

Source: Augustine *et al.*, 1985

2.12.4 Interpretation of analytical data in relation to bioavailability

The nutritional value of foods is not synonymous with their content of nutrients as determined by chemical analysis. Obviously, it is not enough to know how much of nutrient, for example a vitamin is present in the food, but there is need to know how much of the biological potential inherent in the analyzed substance can be realized when the food is eaten. Thus 'bioavailability' has to be considered, which has been defined as the portion of a nutrient capable of being absorbed and available for use or storage (Ball, 1997). Sensitive components of food, like vitamins, are prone to much possible interaction in the food itself and or in the body. Such interaction have a major influence on the final bioavailability, some beneficial while others are harmful (Table 2.12 and Table 2.13).

Table 2.12 Synergisms (+) and antagonisms (-) between vitamins and hormones

Hormones[a]	Vitamin												
	A	B_1	B_2	B_6	B_{12}	C	D	E	K	Biotin	F.A.[b]	Niacin	P.A.[c]
ACTH	+									+		+	+
Aldos													
Cort						±	-						
Epi				+		+							
Est	-		-				+	+			+		
FSH													
Gluc				+									
GH (STH)	+	+	+	+	+	+	+	+	+	+	+	+	+
	All related to growth												
HRH													
Ln		+	+			-							
LH								+					
H	+												
Norepi				+		+							
Oxy													
PTH							±						
Prol[e]	+	+	+	+	+	+	+	+	+	+	+	+	+
Relax													
Test	+	+	+			+	+	+		+	+		
TCT							-						
T4	±[d]	+	+	-		+		-				-	
TSH	-[d]		+										
aso.													

[b]Folic acid. [c]Pantothenic acid. [d]Large Doses. [e]All synergistic when acting as a growth hormone

Table 2.13: Synergisms (+) and antagonisms (-) among the vitamins

Vitamins	A	B_1	B_2	B_6	B_{12}	C	D	E	K	Biotin	F.A[a]	Niacin	P.A[b]
A			+		+	+		±					
B_1			+	+	+							+	+
B_2	+	+		+	+					+	+	+	+
B_6		+	+			+	+		+	+	+	+	
B_{12}	+	+	+			+	+		+	+	+	+	+
C	+			+	+		+	+		+			+
D												+	
E				+	+	+		+			+		
K						+		+					
Biotin			+	+	+						+		+
F.A[b]			+	+	+	+		+		+		+	+
Niacin		+	+	+	+		+				+		+
P.A[c]		+	+		+	+				+	+	+	

Source: Kutsky, 1981

As a first level, problems related to nutrient availability might be located in storage, preparation and processing of food stuffs (Ihekoronye, 1992). Bioavailability, in the widest sense of the term is also implicated. A few examples may elucidate this .Heat is the most commonly used agent in the preparation and processing of food stuffs in order to achieve preservation, favourable texture and flavour changes, increased palatability (Augustine *et al.*,1985). Some vitamins are very sensitive to high temperature and can be entirely inactivated under different time/ temperature conditions (Alan, 1995). But heating may also have advantageous effects releasing some vitamins from binding proteins, and thus converting them into absorbable form, or by destroying compounds like thiaminase, ascorbic acid oxidase or avidin which decompose or in activate vitamins by binding (Ball, 1997).

Losses of vitamins also occur as a result of various operations like washing, peeling, and crushing in house hold preparation as well as in industrial preparation, oxygen mostly being the deleterious agents (Ihekoronye, 1992). Interaction of some vitamins with heavy metal ions, especially copper, may also cause destruction (Kutsky, 1981). These few examples also illustrate that data obtained by analysis of raw food materials have to be weighed critically with regard to their significance concerning real supply of certain vitamins. The next process which has to be taken into account is intestinal digestion and absorption. Some interaction with food and non food components can be presumed on this level too.

Dietary fibre on the other hand are candidates for such interaction, the significance of which has to be closely examined (Alan, 1995). Even when the intestinal absorption of vitamin is normal, further problems of bioavailability might arise when the vitamin is transported in the blood stream in a bound form. For example retinol deficiency occurs despite a plentiful supply of retinol when protein

deficiency limits the retinol-binding protein (Dunne, 1990). Research on bioavailability of vitamins is a modern field of biochemistry with a strong need for non biological and biological analytical techniques to promote the understanding of the complicated subject of bioavailability.

2.13 Methods commonly used in analysis of vitamins

The methods available in the literature for determination of vitamins can be classified into biological, microbiological and physicochemical methods (Kirc-Othmer, 1984).

2.13.1 Biological or bioassay methods

Biological assays measure the effect on instance growth, reproduction, storage in tissues by adding a certain vitamin to a diet lacking only in this nutrient (Alan, 1995). The measurements rely on the response in animals in terms of growth reaction time or the degree of variation with the vitamin dosage. Nowadays other criteria based on specific roles of vitamins in metabolic processes especially influence on enzyme activity are used. Biological assays are inevitably expensive and imprecise and are directly relevant only to the species under investigation. Never the less the bioassay is still used as a check against physiochemical methods to provide evaluation of vitamin biopotency and bioavailability (Osborn and Voogt, 1978).

2.13.2 Microbiological methods

Microbiological methods of vitamin analysis are based on the requirement of microorganism for certain vitamin. For the assay, a culture medium is formulated that provides all the nutrients essential for growth of the micro organism except for the vitamin to be determined. When a sample containing the vitamin to be assayed is added to the initially translucent medium, followed by inoculation with the test organism the micro organism reproduces in proportion to the vitamin content, at least over a

defined concentration range. Growth rate can be monitored for instance by turbidimetry or by determination of metabolites produced by the micro organism (Augustine et *al.*, 1985).

Compared with animal experiments microbiological techniques have some advantages, especially minimal requirements of space, material and time. But to be appropriate for the assay the test microorganism must have the following characteristics: specific need for the vitamin forms which are biologically active in higher animals, genetic stability during prolonged response, rapid growth cycle, stability against chemicals in the sample to be measured. Non pathogenic lactobacilli have been the most widely employed microorganism but also yeasts, mould and protozoa have been used. Microbiological methods of vitamin analysis are common for clinical purposes even at the present time (Alan, 1995).

2.13.3 Enzyme assays

Enzyme assays for determination of vitamins are of special interest regarding estimation of the body status in man. In principle, all the vitamins which have coenzyme function could be quantified by measuring enzyme activity which depends on the saturation rate of the apoenzyme with the vitamin functioning as the coenzyme (Alan, 1995). For tests in humans the material used for enzyme assays is almost exclusively blood that is blood serum and plasma as well as corpuscular blood components, most importantly erythrocytes (Augustine *et al.*, 1985).

The use of human biopsy material is confined to rare special cases, while organ tissues of experimental animals are often employed to examine tissue saturation with a vitamin by means of enzyme assays. Some vitamins have coenzyme function in more than one enzymatic reaction. To

select the most appropriate enzyme it is necessary to know how sensitive the relationship is between the vitamin supply and the activity of different enzymes having the same coenzyme (Ball, 1997).

2.13.4 Radioisotope methods

Competitive protein–binding methods and radioimmunoassay have been successfully applied to the determination of vitamin B_1, folic acid and vitamin B_{12}. The principle of these methods may be explained using vitamin B_{12} as an example. A standard amount of vitamin B_{12} binding agent, intrinsic factor or transcobalamin I, is added to a mixture of a standard amount of labeled vitamin B_{12} and the serum extract containing an unknown amount of unlabelled endogenous vitamin B_{12} which is to be quantified when binding has taken place the bound and free vitamin B_{12} can be separated by several methods. The bound radioactive vitamin B_{12} varies inversely with the amount of unlabelled vitamin B_{12} (Brown, 1990).

The concentration of vitamin B_{12} in serum extract can be determined by comparing the percentage of bound radioactivity with a standard curve which can be obtained by replacing the serum extract by various vitamin B_{12} solution of known concentration. Generally the applicability of this type of method presupposes the existence of a binding agent, usually a protein, which binds the vitamin to be quantified in a specific manner (Alan, 1995).

2.13.5 Colorimetric methods

The colorimetric methods are based on the formation of colour when a reagent has been added to a sample extract. The production of colour, which demonstrates the presence of a vitamin, has been exploited in many procedures of vitamins analysis, including the AOAC methods (Kirc-Othmer, 1984). In the analysis of vitamin E the colorimetric method is known as Emmeric-Engel reaction. It is

based on the fact that when α-tocopherol reduces ferric to ferrous iron in the presence of α,α-dipyridyl; a red complex is formed between the ferrous ions and α,α-dipyridyl. The red complex then may be readily measured in a colorimeter or spectrophotometer (Brown, 1990).

Other reagents used in the formation of colour include 4-amino-N, N-dimethylaniline, diphenyl (batho) phenanthroline, terphyridyl and 2, 4, 6-triphyridyl-5-triazine. The fact that other reducing agents such as carotenoid, sterols and phenolic compounds in plants also react with the reagents especially α, α-dipyridyl makes the method lack specificity (Alan, 1995). Most of the procedures given for the analysis of the vitamin by this method include a purification step that removes interfering compounds. However these procedures are characterized by loss of the vitamin by degradation since it is exposed to air and ultraviolet light. This method also involves long sample preparation and detection time (Brubacher, 1986).

2.13.6 Fluorimetric methods

Fluorimetric methods utilize the fact that some vitamins fluoresce strongly when exposed to light of a particular wave length. The intensity of the fluorescence is proportional to the amount of vitamin in the sample being examined (Dicesare *et al.*, 1981). The major drawback in the fluorimetric procedures is that fluorescence is affected by visible light, various pigments and anions such as halides, cyanides, thiocyanides, sulphites and nitrites. In addition a variety of other interfering fluorescent materials are often present in extracts of natural foods (Brown, 1990).

2.13.7 Spectrophotometric methods

The spectrophotometric procedures are based on the principle that some vitamins contain conjugated double bonds which are associated with absorption of ultraviolet and visible light. The spectra of such vitamins display maximum absorbance at certain wave lengths.

The UV absorbance has been used in quantitative analysis of vitamins for over 50 years. Various spectrophotometric procedures for determination of provitamin A and vitamin E have been reported. However since some vitamins have low UV absorption and there are many interfering substances in foods this methods has found limited use (Synder *et al.*, 1983).

2.13.8 Other methods

There are other analytical methods which are used for the determination of vitamins, especially vitamin E as α-tocopherol. Some of the methods for determination of α-tocopherol include titrimetry, polarimetry and Gas liquid chromatography (Nyambaka, 1988). The labour-intensive manual wet chemical methods have been replaced by mechanized procedures based especially on different types of chromatography. Chromatography as used today is the skill of separation and quantification of closely related compounds, as vitamers. Both Gas-Liquid Chromatography (GLS) and Liquid Chromatography (LC) have the advantage of speed, selectivity and sensitivity. Vitamins analysis not only involves quantification of the analyte of interest but frequently also the separation of the analyte from a complex biological matrix (Alan, 1995). Chromatography is an excellent tool in this context too. Appropriate choice of apparatus, stationary phase and mobile phase, specific detectors and data recorders allows the quantification of almost all vitamins and the vitamers (Diop *et al.*, 1988).

2.13.9 High performance liquid chromatography (HPLC)

High performance liquid chromatography has two chromatographic phases (mobile phase and stationary phase) that enable various compounds to be resolved. Separation is due to differences in the partition coefficients of solutes between the stationary liquid and the liquid mobile phase.

After passing through the column the separated solutes are sensed by an in line detector. The output of detector is an electrical signal the variation of which is displayed on a computing integrators or a visual display unit (V D U) screen (Meyer, 1984).

2.13.9.1 Liquid chromatography modes

Liquid-Liquid partition Chromatography (LLC)

LLC involves a liquid stationary phase, which is either dispersed on to a finely divided inert support or chemically bonded to the support material, and a liquid mobile phase. The sample to be analyzed is dispersed in the mobile phase and is partitioned between the mobile phase and the stationary phase according to its partition coefficient K, thus partitioning leads to a differential rate of migration and separation occurs (Simpson, 1987).

In normal LLC the stationary phase would be a polar liquid e.g. water while the mobile phase is relatively non-polar e.g. hexane, benzene or chloroform. This mode of separation is used to separate polar compounds which would be distributed preferentially in the polar stationary phase (Meyer, 1984).If the stationary phase is non- polar e.g. a hydrocarbon and a polar mobile phase e.g. water is used, the technique is referred to as reversed phase LLC (Diop *et al.*, 1988).

Paired Ion Partition Chromatography (PIC) is a special form of LLC used for the separation of ionic or ionizable compounds e.g. quaternary ammonium salts, sulphonates, amino-acids and aminophenol

(Brown, 1990). The ionic or ionazable sample molecule forms an ion pair by association with a suitable large organic counter-ion in aqueous media. The ion pair is then partitioned into an aqueous-organic phase of moderate solvating ability (e.g. water-methanol) to give a high degree of selectivity (Meyer, 1984). PIC is an alternative to ion exchange chromatography but offers the advantages of longer column life and greater reproducibility. Furthermore, PIC allows the simultaneous determination of acids, bases, and neutral compounds (Kirc -Othmer, 1984).

Liquid-Solid (Adsorption) Chromatography (LSC)

The separation is carried out with a liquid mobile phase and a solid stationary phase which reversibly adsorbs the solute molecules. The stationary phase may be either polar (e.g. silica, gel, porous glass beads or alumina). When the mobile phase would be relatively non-polar (e.g. hexane or chloroform), or non-polar (e.g. polymer beads) when a polar mobile phase (e.g. water or ethanol) would be used. This latter mode is known as reverse phase adsorption (Alan, 1995).

Ion Exchange Chromatography (IEC)

Ion exchange chromatography is a form of adsorption chromatography. Ion exchange involves the substitution of one ionic species for another. The stationary phase consists of a rigid matrix, the surface of which carries a net positive charge to give an ion exchange site (R^+). If a mobile phase containing anions is used, the exchange site will attract and hold a negative counter ion (Y^-). Sample anions (X^-) may then exchange with the counter ions (Y^-) (Brown, 1990).

The process can be represented in terms of the equilibrium.

$$R^+Y^- + X^- \rightleftharpoons R^+X^- + Y^-$$

Since the process involves the exchange of anions it is known as anion exchange. The complementary process of cation exchange occurs when the surface carries a net negative charge to give an exchange site (R^-). The counter ions (Y^+) and the sample ions (X^+) are then both cations and their exchange may be represented by:

$$R^- Y^+ + X^+ \rightleftharpoons R^- X^+ + Y^+$$

The separation is thus based on the strength of the interactions between the sample ions and the exchange site (Meyer, 1984).

Gel Permeation Chromatography (GPC)

Gel permeation (GPC) also referred to as gel filtration, exclusion chromatography, or gel chromatography.

GPC separates substance according to their molecular size and shape. Small molecules that can enter freely into the pores of the stationary place are said to have a distribution coefficient K=1, and large molecules which are completely excluded from all pores have a distribution coefficient K=0, whilst molecules of an intermediate size will have distribution coefficients between 0 and 1. Thus large molecules will move more rapidly through the column than will the smaller molecules and they are eluted first (Simpson, 1987).

2.13.9.2 HPLC instrumental principles

In HPLC, eluent from the solvent reservoir is filtered, pressurized and pumped through the chromatographic column. A mixture of solutes injected at the top of the column is separated into components on traveling down the column and individual solutes are monitored by the detector and recorded as peaks on a chart recorder. The main components of a high performance liquid chromatograph are a high pressure pump, a column, injector system and a detector. In addition,

components such as solvent reservoirs, in- line filters, pressure gauges, recorders, integrators and other minor components may be required (Synder *et al.*, 1983) (Figure 2.8).

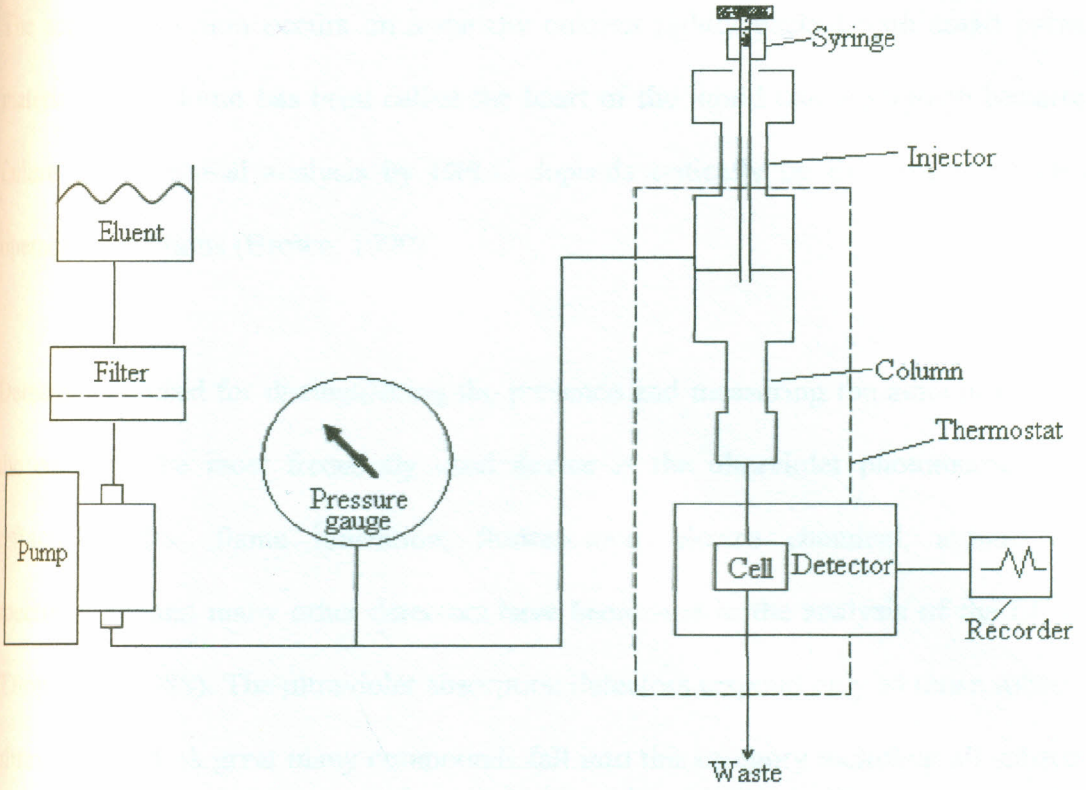


Figure 2.8: Components of high performance liquid chromatograph

Source: Meyer, 1984

The primary function of the solvent reservoir is to hold the composition of the mobile phase constant during the operation of the instrument. The flow stream from the solvent reservoir usually travels through a filter or series of filters that remove particles that could damage the pump or column. A central component of a modern L C instrument is the pump.

Three principal types of pumps-pneumatic, syringe type and reciprocating piston have been used in HPLC (Meyer, 1984). These pumps are designed to maintain a constant, pulse- free flow rate at very

high pressure. An injection device is also very important component in a LC system. It is used to introduce the sample at the head of the column with minimum disturbance of the column packing. The actual separation occurs on a narrow column tightly packed with small particles of packing material. The column has been called the heart of the liquid chromatograph because the success or failure of a chemical analysis by HPLC depends critically on the proper choice of column and operating conditions (Brown, 1990).

Detectors are used for distinguishing the presence and measuring the amount of solute eluting from the column. The most frequently used device is the ultraviolet photometric detector. Infra-red, refractive index, flame ionization, fluorescence, electro chemical, atomic absorption, mass spectrometry, and many other detectors have been used in the analysis of the LC column effluents (Diop *et al.*, 1988). The ultraviolet absorption detectors respond only to those substances that absorb ultraviolet light. A great many compounds fall into this category including all substances having one or more double bond (Π electrons) and substances having unshared (non bonded) electrons e.g. all olefins, all aromatics and compounds containing C=O, C=S, –N=O, –N=N–. The detector cell usually consists of a short length of tube, which is made to carry the eluent from the column and through which a beam of UV light is focused on to a photoelectric cell. When solutes are present in the mobile phase, light is adsorbed and thus the intensity of light falling on the photo cell is reduced producing an electrical output which can be amplified and fed to a recorder (Meyer, 1984).

2.13.10 Flow cytometry

Measurement of peripheral blood CD4 lymphocytes is probably the most important laboratory assay for evaluation and monitoring of patients with HIV. The CD4 count is critical for determining the clinical stage of HIV infection, for deciding when to start ART, for evaluating the efficacy of

treatment and for changing the medications when necessary. Most HIV treatment decisions are therefore based upon the CD4 count (Mellors *et al.*, 1997). The most common technique for measuring CD4 counts is flow cytometry. Flow cytometer use lasers to excite fluorescent antibody probes specific for various cell surface markers such as CD3, CD4 andCD8, which distinguish one type of lymphocyte from another (Fahey *et al.*, 1998).

Flow cytometry is a technology that simultaneously measures and then analyzes multiple physical characteristics of single particles, usually cells, as they flow in a fluid stream through a beam of light. The properties measured include a particle's relative size, relative granularity or internal complexity, and relative fluorescence intensity. These characteristics are determined using an optical-to-electronic coupling system that records how the cell or particle scatters incident laser light and emits fluorescence. A flow cytometer is made up of three main systems: fluidics, optics, and electronics (Margolik *et al.*, 1991).

- The fluidics system transports particles in a stream to the laser beam for interrogation.
- The optics system consists of lasers to illuminate the particles in the sample stream and optical filters to direct the resulting light signals to the appropriate detectors.
- The electronics system converts the detected light signals into electronic signals that can be processed by the computer. For some instruments equipped with a sorting feature, the electronics system is also capable of initiating sorting decisions to charge and deflect particles.

In the flow cytometer, particles are carried to the laser intercept in a fluid stream. Any suspended particle or cell from 0.2-150 micrometers in size is suitable for analysis. Cells from solid tissue must be disaggregated before analysis. The portion of the fluid stream where particles are located is called the sample core. When particles pass through the laser intercept, they scatter laser light. Any

fluorescent molecules present on the particle fluoresce. The scattered and fluorescent light is collected by appropriately positioned lenses (Margolik *et al.*, 1991). A combination of beam splitters and filters steers the scattered and fluorescent light to the appropriate detectors. The detectors produce electronic signals proportional to the optical signals striking them. List mode data are collected on each particle or event. The characteristics or parameters of each event are based on its light scattering and fluorescent properties. The data are collected and stored in the computer. This data can be analyzed to provide information about subpopulations within the sample (Fahey *et al.*, 1998).

2.13.11 Determining vitamin C level in fruits by Reduction oxidation (Redox) titration

2, 6-dichlorophenolindophenol (DCPIP) is a blue chemical compound used as a redox dye. Oxidized DCPIP is blue, reduced DCPIP is colorless. DCPIP is a redox dye commonly used as a monitor of the light reactions in photosynthesis because it is an electron acceptor that is blue when oxidized and colorless when reduced. DCPIP can also be used as an indicator for Vitamin C (Vanderjagt *et al.*, 1986). If vitamin C, which is a good reducing agent, is present, the blue dye, which turns pink in acid conditions, is reduced to a colorless compound by ascorbic acid.

$$\text{DCPIP (blue)} + H^+ \longrightarrow \text{DCPIPH (pink)}$$

$$\text{DCPIPH (pink)} + \text{VitC} \longrightarrow \text{DCPIPH2 (colorless)}$$

$$C_6H_8O_6 + C_{12}H_7NCl_2O_2 \longrightarrow C_6H_6O_6 + C_{12}H_9NCl_2O_2$$

In a titration, when all the ascorbic acid in the solution has been used up, there will not be any electrons available to reduce the DCPIPH and the solution will remain pink due to the DCPIPH. The end point is a pink color that persists for 10 seconds or more. The oxidation of vitamin C is used to determine the mass of vitamin C in a known mass of food. The amount or concentration of vitamin C

is reported as milligrams of vitamin C per 100 grams of food. 2, 6-dichlorophenolindolphenol, reacts with vitamin C in a one-to-one ratio as shown by the redox reaction above. A solution of DCPIP added to a solution of vitamin C will be colorless until the equivalence point is reached. At the equivalence point any addition of dye will cause the solution to be pink. The amount of juices used to turn the DCPIP colourless is recorded and the vitamin C content determined by measuring the amount of drops of a known vitamin C concentration solution, used to turn the DCPIP colorless.

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study area

The study area was Busia district which is geographically located between 34 ° 54' 32'' E and 34 ° 25' 24'' longitude and 0 ° 1' 36'' s and 0 ° 1' 33'' N latitude. The district covers an area of 1,261.3 km^2. The district has a varied topography with low altitude of 1,130 m above sea level and the highest altitude of 1,375 m above sea level. The area experiences two main rainfall seasons. Long rains fall between March to May whereas the short rainfalls between August to October. The average temperature of the area is 26 ° C (GOK, 2002).

Busia district has a population density of 321 people/ km^2 (GOK, 2002). Crops grown in Busia district include; maize, sorghum, sweet potatoes, soya beans, cowpeas, green gram, kales, simsim, sunflowers, avocadoes, oranges, bananas, sugarcane and indigenous vegetables (GOK, 2002).

3.2 Research design

The study was a pre-intervention nutritional status study of PLWHA assessing the potential use of indigenous foods in the management of HIV and AIDS. Study subjects were selected randomly from members of Rural Education and Economic Enhancement Programme (REEP), a local NGO operating in Butula Division especially those that were available and could easily access the REEP VCT centre. The baseline information involved collection of demographic and food consumption pattern using a questionnaire, collection of blood and vegetable samples. Levels of vitamin A, C and E in selected indigenous foods known to be rich in the vitamins and serum β-carotene and α-tocopherols levels and were determined by HPLC technique. Indigenous foods found to contain high

levels of vitamins were used to prepare a food supplement. The bioavailability of the vitamins in the food supplement was determined using algorithm estimates.

3.3 Sample size

Sample size for the study was determined according to Equation 3.1

$$n = \frac{2\delta^2 (Z\alpha/_2 + Z_\beta)^2}{d^2} \qquad [3.1]$$

Where δ is the variance

$Z\alpha/_2$ = Normal standard deviate relating to the chances of making type 1 error

When $\alpha = 0.05$ $Z\alpha/_2 = 1.96$

Z_β = Normal standard deviate relating to chances of making a type II error = 1.282

n = Number of observations

d = Difference to be detected = $\mu_1 - \mu_2$ = 10µg/dl serum and $\delta^2 = 18^2$

Therefore

$$n = \frac{2 \times 18^2 (10.51)}{10^2}$$

$$= \frac{2 \times 18^2 \times 10.51}{100}$$

$$= 68.1048$$

The next larger integer is 69. Hence 69 subjects were recruited per Arm; giving a total sample of 138 subjects.

3.4 Inclusion and exclusion criteria

Subjects who were included in the study were HIV infected adults living in Busia district with a positive HIV test result from VCT's. They were in clinical stages one and two as per the centre for disease control (CDC) HIV/ AIDS staging criteria with CD4+ counts of 350 cells / mm^3 and above. Those who were excluded were HIV patients using or qualifying to use ARV's and those using other nutritional supplements. HIV patients who refused to give a written informed consent and those who were terminally ill and bed ridden for 50% of time were also excluded from the study.

3.5 Recruitment and ethical consideration

Permission was sought from the ethical review committee of Kenya Medical Research Institute (KEMRI); a research permit was also obtained from the Ministry of Education. The Provincial administration and medical officers of health in the study area were informed of the objectives of the study and their support sought. The recruitment of PLWHA was carried out at REEP VCT centre. The subjects were sampled from those that were HIV positive attending the REEP VCT centre and willing to participate. This was done through the involvement of medical officer of health, nutrition officer and other hospital staff. The selected subjects were briefed of the objectives and procedures of the study. Consenting subjects were requested to sign consent forms.

3.6 Field work

Field work involved determination of availability of indigenous vegetables, feeding habits, nutritional and clinical status of PLWHA by use of a questionnaire. Purchase of food samples and collection of blood sample for laboratory analysis. Pre-testing of working of the questionnaire was done by giving to potential subjects who were not included in the study.

3.7 Administration of questionnaire

A physician in the REEP centre with the help of a nurse administered the questionnaire on clinical evaluation. The subjects were examined and details of HIV associated opportunistic infections recorded and treatment of any conditions requiring management was taken accordingly. A dietician with the help of trained research assistant administered the questionnaire on feeding habits, socio-economic, nutritional status and determination of BMI.

3.8 Anthropometric measurements and immunological status

Height and weight of HIV/AIDS patients were measured to be used for calculating the body mass index (BMI). The height measurements were taken using a stadiometer, placed against a hard flat surface. The patients stood on the foot plate with no shoes, with minimum clothing and head gear, with feet parallel and with heels, shoulders, buttocks and back of the head touching the vertical board. The head was held comfortably erect and measurements taken to the nearest 0.1 cm. A bathroom scale was used to take weight. The subject with light clothing stood on a calibrated bathroom scale with body weight evenly distributed between both feet and weight was read to the nearest 0.1 kg. This was repeated a second time and values averaged.

Flow cytometer was used to determine CD4+ cell count, CD8+cell count, CD4+/CD8+ ratio and haematological index. All immunological and haematological status analyses were carried out in KEMRI.

3.9 Apparatus and instruments

3.9.1 Glassware

All the glassware used was cleaned with chromic acid followed by a washing detergent. They were then rinsed with distilled water. Before the glassware were used in the analysis of fat- soluble vitamins (α- tocopherol and β-carotene) they were rinsed with methanol.

3.9.2 HPLC instrument and operating system

The HPLC chromatograph used was model L-6000 with dual plunger reciprocating pump (Hitachi instrument inc model L-6000). The sample was introduced into the column through a 50µl sample injector of Rheodyne (Model B/N 655-0890). Separation was achieved by a reversed phase column consisting of a stainless steel (15cm long x 4mm internal diameter) packed with µ Bondapak C_{18} material (10µm particle size). The column was obtained from water associates. The column effluents were monitored by ultraviolet (UV) absorption using a variable wavelength (UV-VIS) detector (Hitachi, model L-4000H/L-4200H).The chromatographic peaks were recorded by model D-2500 chromato- integrator.

Twenty microlitres of both the standard solutions and the sample extracts were injected into HPLC system. The sensitivity used was mainly 0.02 absorbance units' full scale (AUF'S). The detection wavelength was 297nm for β-carotene and α–tocopherol in the vegetables. The mobile phase for β-carotene and α-tocopherol was methanol-acetonitrile-chloroform-water in the ratios 46:30:18:6 (v/v/v/v). The wavelength for retinol, β-carotene and α-tocopherol in the serum sample was set at 325 nm, 450 nm and 292 nm respectively. The mobile phase for retinol was acetonitrile: water in the ratios 85:15(v/v), while that for β-carotene and α-tocopherol was methanol: DCM: water in the ratios

83:15:2 (v/v/v).The flow rate was 1.0ml/ min while the chart speed was 10mm/ min for the two sets of vitamins.

3.9.3 Flow cytometer

Beckman coulter cytometer interfaced with a detector module (BD Fascount manufactured by San Jose ca 95131 USA B9320) was used for determining immunological and hematological index in the blood.

3.10 Chemical reagents and solvents

All the analytical standards were purchased from Sigma Aldrich (United States). All other chemical reagents used were purchased from Kobian (Kenya) chemical stores. The chemicals used included anhydrous sodium sulphate, sodium sulphite, potassium hydroxide, phenolphthalein indicator, L-ascorbic acid (purity 99%), α-tocopherol (purity 95%) and β-carotene (purity : 97%) and all-trans retinol (purity : 99%), carotene (purity : 97%) and all-trans retinol (purity : 99%),Methanol, Acetonitrile, Ethanol, Dichloromethane (DCM), 2,4-Dichlorophenolindophenol (DCPIP), Hexane, Ethyl acetate, Butylated Hydroxytoluene (BHT). All the solvents were of HPLC grade. All the reagents were used without further purification. Deionized water, purified by milli Q system (Millipore, milford, MA, USA) was used throughout the study.

3.11 Sampling and pre-treatment of blood and food samples

3.11.1 Blood and food sampling

Ten ml blood samples were obtained from HIV patients by venipucture of the anticubital vein of the left arm by a registered nurse. The blood was then transferred in polycarbonate tubes containing lithium heparin as an anticoagulant and stored at -80°C in a freezer till transported to the analytical

laboratory in KEMRI where they were stored in the same conditions before analysis. All samples were wrapped with black paper and transported on dry ice.

The food samples were obtained from Butula market and household gardens. The samples were washed and trimmed to remove the fibrous materials in the laboratory. The trimmed samples were blanched by dipping into boiling water for 1-3 minutes. The blanched samples were cut with a knife into small pieces then solar dried. The solar dried samples were packed into clean polythene bags and sealed awaiting analysis. Samples which were to be analysed while fresh were washed, trimmed, blanched and stored in clean polythene bags in a refrigerator awaiting analysis.

3.11.2 Food and blood sample pre-treatment

Fresh raw vegetables samples (cowpea leaves, frying spider, pumpkin leaves, slender leaf and nightshade) collected from Butula market and household gardens were thoroughly washed under tap water and then destalked and all inedible parts removed before shredding according to common household practice. The samples were then blanched for 1-3 minutes in boiling water and divided into two portions, one for solar drying and another for analysis when fresh. Fresh samples that were not analyzed on the same day were kept frozen until use. The vegetables were solar dried using an indirect solar dryer. The model is shown in plate 3.1.

Plate 3.1: Solar drier used in this study

Samples were spread in a wire mesh tray before inserting into dryer. The dryer was made of wood and covered on top with a black polythene bag. The inside was painted black to concentrate the heat and ensure that air inside was heated appropriately. The dryer measured 1.5m in length, 1.2m in width, and the front height was 0.9m and a back height of 0.6m making an angle of 30° towards the incident light. It was raised 1.5m from the ground. A small opening of 1inch was left beneath the tray and chimney was inserted in the front of the tray to allow free circulation of air into and out of the dryer. The vegetable samples were spread onto the tray forming a uniform layer. After inserting the tray containing the samples into the dryer, the dryer was kept in the open and positioned in such a way that the sunrays fell directly on top of the dryer. The dryer was rotated appropriately with the change in light direction throughout the day. It took 6 to 8 hours when the temperature ranged from

25°C to 27°C for the vegetables to dry. The solar dried samples were stored in polythene bags, nitrogen flashed and then sealed tightly to prevent any oxygen getting in.

Serum was obtained by centrifuging whole blood at 800 rpm for 10 minutes at room temperature. The serum was separated transferred in cryo tubes and kept at -20 °C in ice packs before being transferred to KEMRI where they were kept at -80 °C until analysis. The serum was used for analysis of both α-tocopherol, β-carotene and all-trans retinol. Whole blood was used for analysis of immunological and haematological status.

3.12 Procedures for analysis

3.12.1 Preparation of standards

(a) Preparation of β-carotene and α-tocopherol standards

β-Carotene and α-tocopherol containing 100µg/ml were prepared by weighing 10mg standard reagents and dissolving each in n- hexane and making up the solution to 100ml. The stock solutions were kept under refrigeration conditions to be used for 2 weeks. The working solutions of different concentrations were prepared daily by serial dilution of the standard.

(b) Standardization of DCPIP with standard L-ascorbic acid

An amount, 0.038g of DCPIP was measured and dissolved in 500 cm^3 of distilled water and filtered. Twenty five cubic centimetre of this solution required 25cm^3 of standard L-ascorbic acid made by dissolving 4.9910mg and made up to one litre with distilled water. The standardized DCPIP (0.038g/500ml) was then used to find the concentrations of acid in sample extracts.

(c) Preparation of standards for serum analysis

The standards stock solutions (10mg/ml) of α- tocopherol and β-carotene were dissolved in 20 ml of ethanol and topped up to 250ml with methanol. All stock solutions were protected from light and stored at -20^{0}C. Standard stock solution (5mg/ml) of retinol was prepared in a few drops of ethanol and then topped up to 250ml with methanol. The stock solutions were further diluted with methanol to give a series of working standard. The working solution for spiking blank human serum sample were prepared fresh daily.

(d) Preparation of 5 % metaphosphoric acid (m-HPO_4)

1g of M-HPO_4 was dissolved in distilled water and the solution was made up to 100ml without heating. The solution was filtered and stored in refrigerator for two weeks.

3.12.2 Method Validation

The reliability of the method was validated through its linearity, reproducibility and recovery. Samples were quantified using peak areas of α-tocopherol, β-carotene and retinol standards. Limit of quantification was based on the lower concentration validated by the methods.

(a) Linearity

Calibration curves were used to test for linearity. Calibration curves for the peak areas against concentrations of standards were used. The blood samples calibration curves were constructed using spiked serum with a known amount of retinol, α-tocopherol, and β–carotene.

(b) Reproducibility studies

Some food samples were pretreated as above and five sub sample, removed, extracted and analyzed as per procedures in 3.12.3 and 3.12.4. The coefficients of variation were used to determine reproducibility of the method.

(c) Recovery

Recovery data was obtained by adding known amounts of β- carotene, α- tocopherol and L- ascorbic acid standards to the homogenized food samples that were ready for extraction and comparing the increased calculated vitamins content to the amounts added. Serum standards spiked with a known amount of retinol, α-tocopherol and β-carotene were used to determine the extraction recovery, within day and between day precision (n=5) of the method. The recoveries of retinol, α-tocopherol and β-carotene after liquid- phase extraction were calculated by comparing observed retinol, α-tocopherol and β-carotene peak areas in extracted serum, to those of non- processed standard solutions.

3.12.3 Food sample preparation, extraction and analysis

All extraction steps were performed in glass apparatus covered 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.

i) Extraction of β–carotene and α–tocopherol in vegetables

Twenty five grams of vegetable and fruit samples were blended for 5 minutes with 0.3 g ascorbic acid to form a puree. Five grams of the pretreated sample was weighed and transferred into 150 ml round bottomed flask. 30 ml of hexane: dichloromethane mixture in the ratio of 3:2 was added to the flask and the mixture shaken for 2 minutes and allowed to separate. The hexane layer was then decanted

into a 250 ml separating funnel which was then corked. The residue was similarly re-extracted with 50 ml of n-hexane three times each time decanting the hexane layer into the separating funnel. The combined hexane layer was then washed with 50 ml of saturated potassium hydroxide in methanol followed by portions of 50ml distilled water until there was no colouration on phenolphthalein indicator. The hexane layer was then dried by filtering over anhydrous sodium sulphate and evaporated to dryness under a stream of nitrogen. The residue was immediately dissolved in 10ml of methanol. An aliquot of the solution was filtered with 0.45 µm millipore filter and injected into HPLC system for analysis.

ii) Extraction of L-ascorbic acid in fruits and vegetables

Five grams of the blended fruits and vegetables samples was mixed with 50ml of 1% HPO_4 acid and shaken with a mechanical shaker for 15 minutes. The mixture was then centrifuged at 10,000 rpm for 5 minutes. The supernant was then a vacuum filtered and put in to a 50ml volumetric flask and made to the mark with 1% phosphoric acid. The burette was cleaned thoroughly using hydrochloric acid and rinsed with distilled water. The burette was filled with the fresh fruit and vegetable juice using a funnel. The juice was then drained until it came exactly to the zero line. The juice was slowly dripped into the conical flasks with DCPIP until a clear observation in colour change was observed. The conical flask was swirled gently to ensure mixing. The amount of juice taken for a full colour change to take place in the conical flask containing DCPIP was recorded. The burette was then cleaned by pouring distilled water through it twice. The process was repeated with each fresh fruit and vegetable juice.

3.12.4 Blood sample preparation, extraction and analysis

(a) β-carotene and α- tocopherol extraction from serum

Frozen serum samples were let to thaw for 20 minutes before 200 µl aliquots were pipette into serum vials using a micropipette and diluted with 200 µl double distilled de-ionised water. Samples were then de-proteinised by vortex mixing for 30 seconds with 400µl ethanol containing BHT (0.0599 g / ml). The sample was extracted with 3ml hexane, vortex mixed and centrifuged at 800 rpm at 5°C for 15 minutes. The extraction was repeated twice and the resultant supernatant was combined and evaporated under nitrogen at 30 °C. The residue was re-dissolved in 150 µl DCM: methanol (4:1), vortex mixed and ultrasonically sonicated for for10 seconds before injection into HPLC column. The mobile phase for the isocratic elution of serum extracts of β- carotene and α-tocopherol consisted of a mixture of methanol: DCM: water in ratios of 83:15:2 (v: v: v) containing 0.1% BHT.

(b) Retinol extraction from serum

Frozen serum samples were let to thaw for 20 minutes before 300 µl aliquots of serum were pipette into serum vials using a micropipette and diluted with 300 µl double de-ionised water. Samples were then de-proteinised by vortex mixing for 30 seconds with 600 µl ethanol containing BHT (0.0599 g/ml) as an antioxidant. Extraction was repeated twice with 2 ml hexane and the combined supernant was evaporated under a stream of nitrogen at 30°C.The residue was dissolved in 70 µl ethyl acetate and vortex mixed for 10seconds.The sample was diluted with 200 µl of the mobile phase and ultrasonically agitated for 10 seconds before injection into the column. The mobile phase for the isocratic elution of serum extracts for retinol analysis consisted of an aqueous mixture of acetonitrile: water in ratios of 85:15 (v: v).

3.12.5 Determination of moisture content

1 gram of dried/fresh vegetable material was weighed and placed in a dry pre-weighed crucible. This sample was placed in an oven at 105°C for at least 3hours. The dry weight of the vegetables was then taken. The moisture content in the vegetables was calculated using the initial weight before drying and final weight after drying. As shown below in Equation 3.2.

$$100\left(\frac{initial\ weight - final\ weight}{initial\ weight}\right) \qquad [3.2]$$

3.13 Preparation of food supplement

Locally available vegetables confirmed to be rich in vitamin A, C and E were used to prepare locally acceptable food products. The samples constituted raw foods, which include indigenous vegetables and fruits. This study used frying spider, cowpea leaves, and pineapple fruit in the preparation of the food supplement. Dried samples were ground into powder, weighed, mixed and packed in ratios which would meet the RDA. The pineapple was washed peeled and chopped into small pieces which were put into a juicer. The juice was filtered and put into 200 ml plastic containers.

3.14 Bioavailability studies

Two mathematical models were developed using β-carotene and α-tocopherol nutrition intervention data obtained from similar studies in the internet (Appendix vi). Data collected was subjected to multiple linear regression analysis. β-Carotene and α-tocopherol absorption were each separately regressed against dietary, pre-study serum and duration of intake of the diet.

3.15 Data analysis

Data was analyzed using the SPSS, version 12 program. The quantitative data was analyzed using descriptive statistical methods. Tables and graphs were used to illustrate the data collected from the field and experimental analysis. The detection and identification of β-carotene, α-tocopherol and retinol in the standards and samples were recorded as HPLC chromatograms showing the detector response in the form of peak areas against retention times. Pearson correlation coefficient was calculated to determine within day and between day reproducibility of the method for analysis of vitamin content in serum samples. Regression analysis was used to test the relationship between absorbance and concentration of β-carotene and α-tocopherol standard solutions. The amount of juice taken for a full colour change to take place in the conical flask containing DCPIP was used to quantify the amount of L-ascorbic acid in the fruits. Multiple regression analysis was used to develop the algorithms for determining the bioavailability of β-carotene and α-tocopherol in indigenous foods.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 Introduction

The study investigated the indigenous vegetables grown in Butula division of Busia district, their annual availability and how they are preserved as a measure of improving food security and particularly as a potential means of nutritional management of HIV and AIDS. The results of the study are presented and discussed under the following subheadings:

i. Demographic information and food consumption pattern of PLWHA in Butula.

ii. β-carotene, α-tocopherol and L-ascorbic acid content of indigenous vegetables and their moisture content

iii. Immunological, haematological, clinical status and anthropometric characteristics of PLWHA in Butula.

iv. Vitamin status of PLWHA in Butula.

v. Bioavailability using algorithms

4.2 Demographic information

The demographic information was obtained using a questionnaire (Appendix I).The information obtained included; personal information, clinical status and feeding habits. Table 4.8 gives a summary of personal information which included mean, frequency and percentages in age, smokers, alcohol consumption and marital status.

4.2.1 Personal information

Table 4.1 Personal characteristics of PLWHA in Butula division

		Male (N = 17)			(Female N = 111)		
		Mean	Frequency	%	Mean	Frequency	%
Age (years)		43.6	-	15.5	37.8	-	84.5
Smokers		-	1	5.8	-	2	1.8
Use of alcohol		-	2	13.3	-	5	4.4
Marital status	Single	-	3	60.0	-	0	0.0
	Monogamous married	-	1	20.0	-	6	26.1
	Polygamous Marriage	-	0	0.0	-	2	4.3
	Widowed	-	1	20	-	10	43.5
	Other	-	0	0.0	-	6	26.1

As can be seen in Table 4.1 the number of men who smoke were 5.8 % of men while 1.8 % of women also smoke. Smoking in HIV patients is discouraged since smoking weakens the immune system and makes it harder to fight off HIV-related infections. Smoking increases the risk of some long term side effects of HIV disease and treatment, including osteoporosis and lung related infections (Hasley *et al.*, 1992). For women, smoking can increase the risk and severity of infection with human papilloma virus (Hasley *et al.*, 1992).

Smoking tends to lower concentrations of carotenoids compared to non smokers; and also affects vitamin C metabolism (Romero-alvaro and Roche, 1998). Some researchers have hypothesized that smoking in early HIV disease may increase risk of progression to AIDS by activating T4 cells thus facilitating HIV replication (Hasley *et al.*, 1992).

About 13.3 % men and 4.4 % women do consume alcohol. Alcohol intake is inversely associated with serum carotenoid concentrations (Comb, 1998). Those who chronically consume large quantities of alcohol are often deficient in many nutrients, but it is unknown whether the deficiency is the result of poor diet or the metabolic consequences of chronic alcoholism (Romero-alvaro and Roche, 1998). Alcohol consumption may adversely affect immunologic function in HIV infected persons by various mechanisms, including HIV replication in lymphocytes (Stine, 2005).

Marital status of the subjects was as follows; 60% of the male were single, 20% were in monogamous marriage and 20% were widowed; the female subjects marital status was as follows 26.1% were in monogamous marriages, 4.3 % were in polygamous marriages, 43.5 were widowed while 26.1% were in the category of others (separated, divorced and separated and remarried).

4.2.2 Gender and age of subjects

Figure 4.1 shows the number of subjects by gender and age who participated in the study. The number of females who participated was higher than that of male in all age groups. Gender is a factor in HIV/AIDS infection. HIV/AIDS prevalence among women aged 15- 49 in Kenya is nearly 9% compared to less than 5% for men in the 15-54 age group (NASCOP, 2009). In Busia district the male HIV prevalence was 4.2% and female HIV prevalence was 6.4% in 2004 (NAAC, 2005).This

could be the reason for the lower participation of the male subjects as compared to the female. The female who participated were 86.72% while the male were 13.27 % as shown in Figure 4.1.

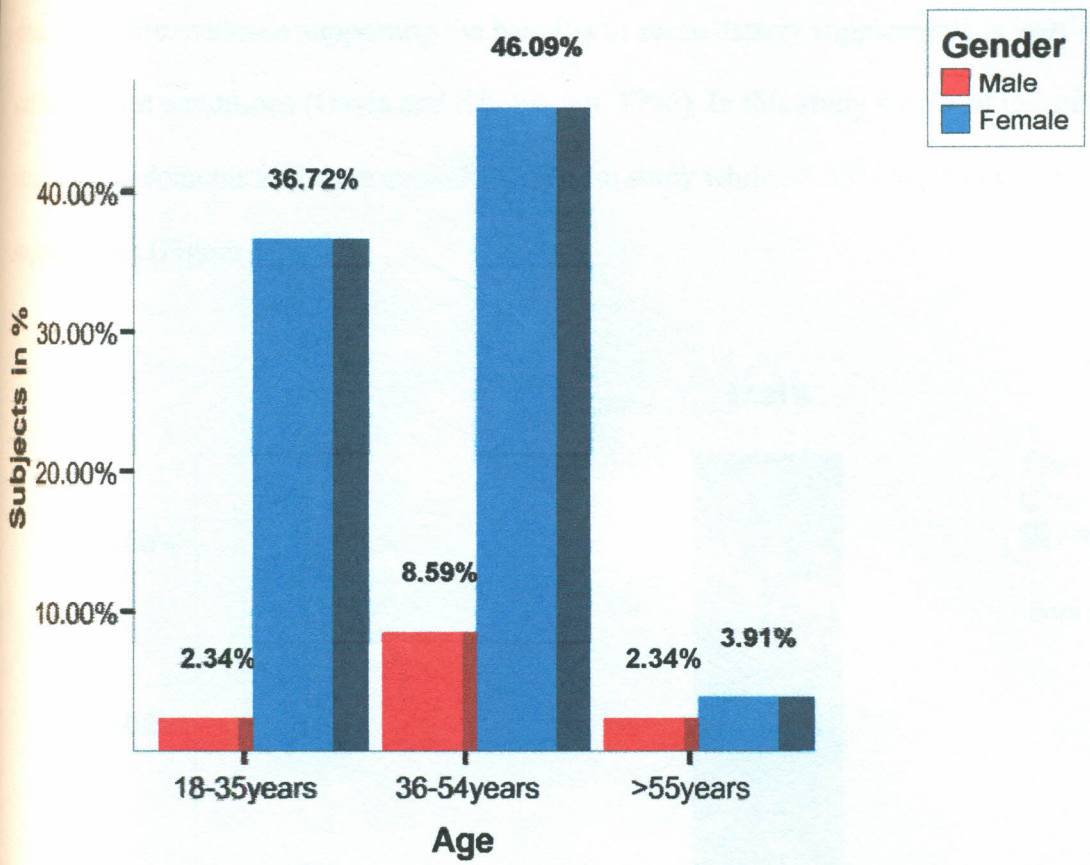


Figure 4.1: Gender and age of subjects

HIV prevalence in young women is reportedly high at the age of 18-29; however women at the age of 36-54 were the highest number (46.09%) of participants in this study. HIV prevalence is lower among people aged 55 years and above (NACC, 2005).This group was poorly represented in this study.

4.2.3 Nutrient supplements

Dietary supplements, often containing vitamins are used to ensure that adequate amounts of nutrients are obtained on daily basis, if optimal amounts of the nutrients cannot be obtained through a varied diet. Scientific evidence supporting the benefits of some dietary supplements is well established for certain health conditions (Gayla and Kirchmann, 1996). In this study 4.65% of the subjects were on nutrient supplements and were excluded from the study while 95.35% were not using nutrient supplements (Figure 4.2).

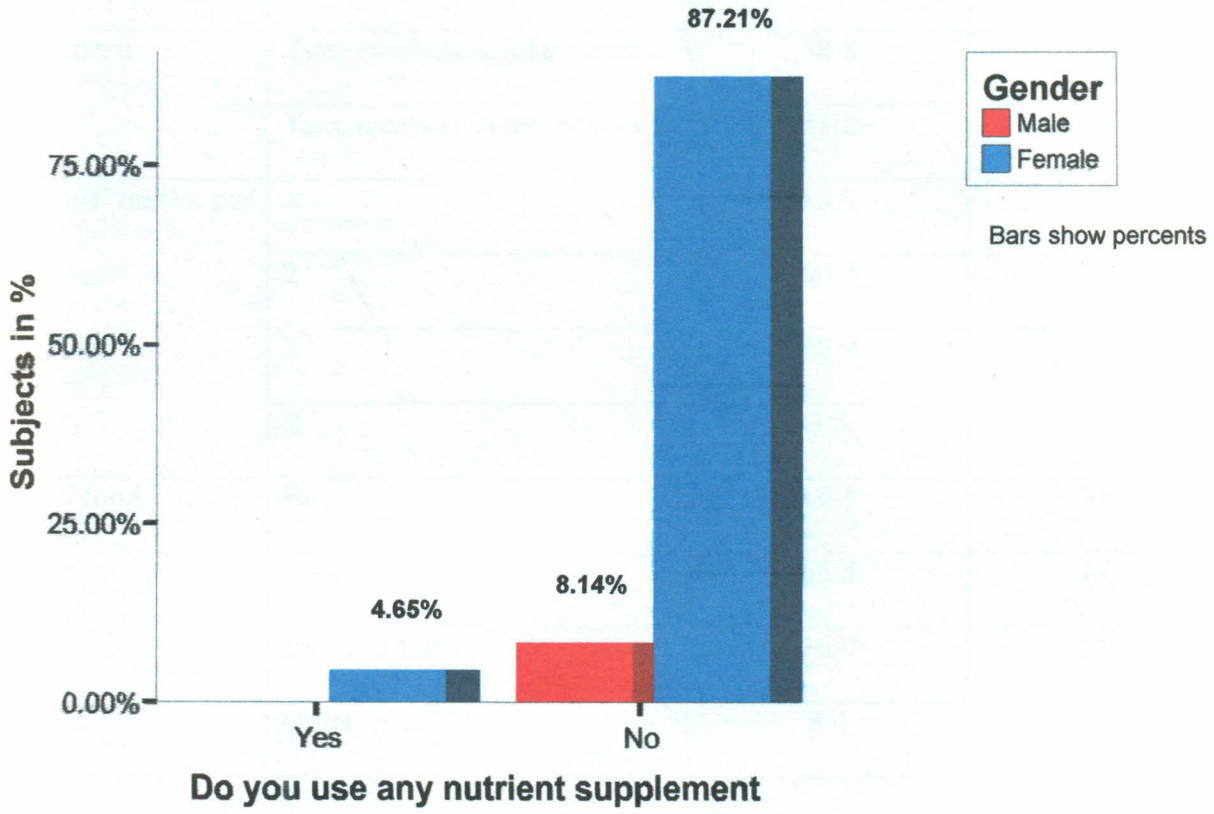


Figure 4.2: Number of subjects (%) taking nutrient supplements

4.2.4 Feeding habits

Table 4.2 gives the feeding pattern of PLWHA in Butula division. As can be seen in Table 4.2, 58.8% of the subjects take their meals at regular times while 41.2 % take their meals at irregular times. 3.8 % of the subjects eat four times in a day, while 22.9% took three meals in a day. 60.3% took two meals in a day while 13.0% ate once a day.

Table 4.2: Feeding pattern and sources for food of PLWHA in Butula division

		Percentage %	Frequency
Eating pattern	Take meals at regular times	58.8	77
	Take meals at irregular times	41.2	51
Number of meals per day	1	13.0	17
	2	60.3	79
	3	22.9	30
	4	3.8	3
Source of food	Buy	19.1	25
	Farm	56.5	14
	Buy and farm	16.0	21
	Other	8.4	11

The sources of food included buying; farming and others. 19.1% of the subjects buy food from the local markets, 56.5% obtain their foods from their farms, 16.0% buy and farm while 8.4% obtain their foods from other sources. Good feeding habits are essential to alter the course of HIV progression. Good nutrition can play apart in preventing weight loss and other AIDS complications (Stine, 2005).

In order to maintain health it is necessary to eat a variety of foods since different foods protect the body in different ways. Each meal should include all three food types; that is energy giving foods, body building foods and protective foods (Passmore and East wood, 1986).

The foods commonly taken by the PLWHA living in Butula division include; vegetables, animal foods sources, pulses, tubers nuts, beverages and others (Table 4.3). Twenty two percent of the subjects take vegetables, 18% take animal foods, 4% take pulses, 11% take tubers, 35% take cereals and 8% take other types of foods.

Table 4.3: Percentage of major types of foods taken by PLWHA in Butula division

Food type	Percentage %
Vegetables	22
Animal foods sources	18
Pulses	4
Tubers	11
Cereals	35
Beverages	2
Others	8

The reasons for not consuming some foods by PLWHA in Butula included; some of the foods are expensive, rare, lack of money to buy some of the foods, lack of seeds to grow some of the foods and their source of income was so little to afford to buy some of the foods (Table 4.4). The subjects who could not consume some of the foods because they were expensive were 3.4%, 68.5% lacked money, and 0.4% lacked seeds to grow some of the foods, 0.4% of the subjects income was little to afford to

buy some of the foods. 0.4% of the subjects said some of the foods were rare. Those subjects who didn't eat some of the foods because they were not their favourite foods were 0.4%. Kitchen garden and keeping of small animal should be encouraged since it provides affordable foods and provides a variety of foods. The foods taken by PLWHA in the study include; bananas, beans, cassava, "chapati", eggs, "githeri", fish, milk, jute mallow, potatoes, sweet potatoes, rice, "ugali" and porridge (Appendix IV).

Table 4.4: Reasons for not consuming some foods by PLWHA in the study

Reasons	Percentage %
Expensive	3.40
Not favourite	0.40
Lack of money	68.5
Lack of seeds	0.40
Rare	0.40
Source of income	0.40
Others	30.0

Figure 4.3 shows the percentage number of subjects consuming different nutrients as accompaniment to the main dish. Those who consume proteins are 12.09% which are obtained from animal and plant sources. 80.64% of the subjects consume vitamins which are obtained from indigenous vegetables and fruits which are locally available. The subjects who consume carbohydrates as accompaniments to the main dish were 7.26%.

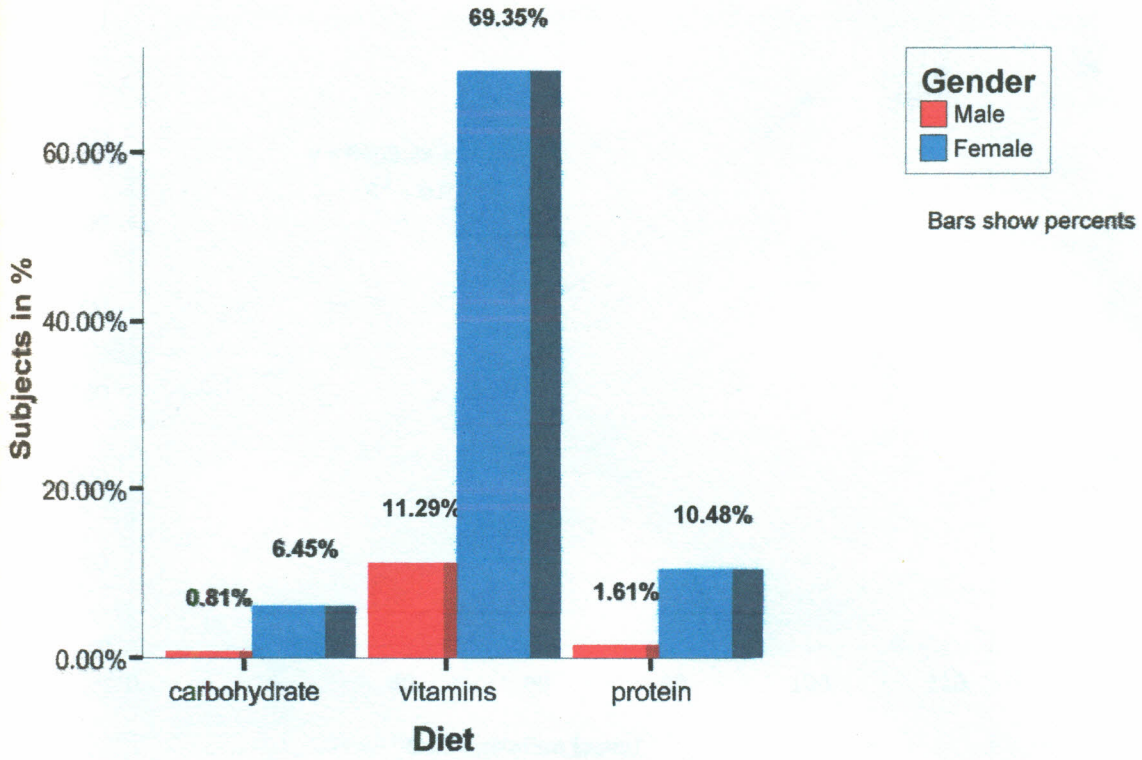


Figure 4.3: Number of subject's (%) consuming different nutrients as accompaniment to the main dish

4.3 Vitamins content of selected indigenous vegetables and their moisture content

4.3.1 Method validation

4.3.1.1 Calibration curve

The β-Carotene and α-tocopherol standards were prepared as in Section 3.12.1. The solutions were injected into the HPLC column and eluted using a mobile phase consisting of methanol -acetonitrile-chloroform-water (46:30:18:6). The β-carotene and α-tocopherol were eluted at relatively sharp peaks at a retention time of 5 minutes for α-tocopherol and 10 minutes for β-carotene. Calibration curves of peak areas against the concentrations of β-carotene and α-tocopherol standard solutions were plotted (Figure 4.4 and 4.5).

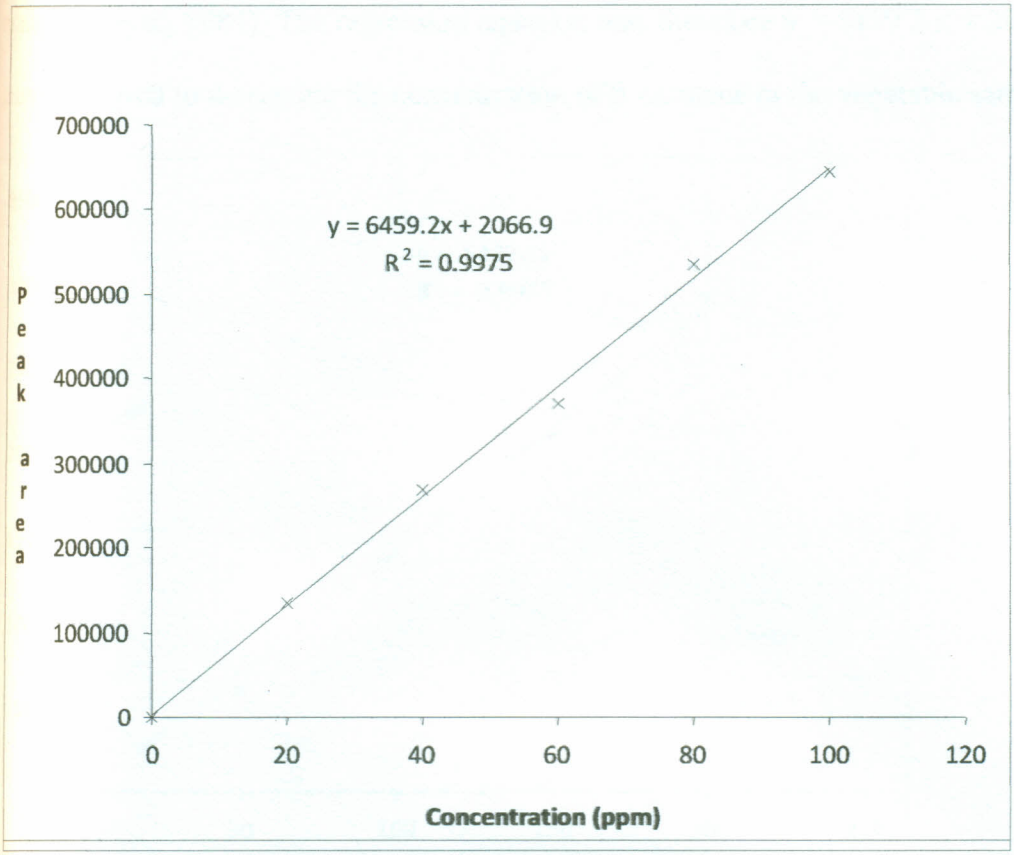


Figure 4.4: The calibration curve for β-carotene standard solution

The β-carotene curve was linear within the concentration range determined (0 to 100mg/ml). This calibration line gave a correlation coefficient with $r^2 = 0.9975$. The correlation coefficient obtained in this study was comparable to those obtained in other studies. Nyambaka and Nyaga (1991) obtained $r^2 = 0.9970$ using a HPLC system consisting of μ Bondak C_{18} reversed phase column and a mobile phase of methanol-acetonitrile-chloroform-water in ratio of 46:30:18:6 and detection limit of 297nm for both β-carotene and α-tocopherol. Nderitu (2006) reported $r^2 = 0.9987$ using a HPLC system and a mobile phase of methanol-dichloromethane-water (79:18:3) and detection limit of 450nm. Nawiri (2008) reported $r^2 = 0.9981$ using a HPLC system and a mobile phase methanol: DCM: water (83:15:2) and a detection limit of 450 nm. This value indicates that there was a linear relationship between the chromatographic peak area and the β-carotene concentration. The linearity also indicates that the detectors of the HPLC equipments were responding positively to different concentrations of the

analyte (Meyer, 1984). The regression equation was therefore y = 6459.2 x + 2066.9. The calibration curve was used to determine the concentration of β-carotene in the vegetable samples (Table 4.7).

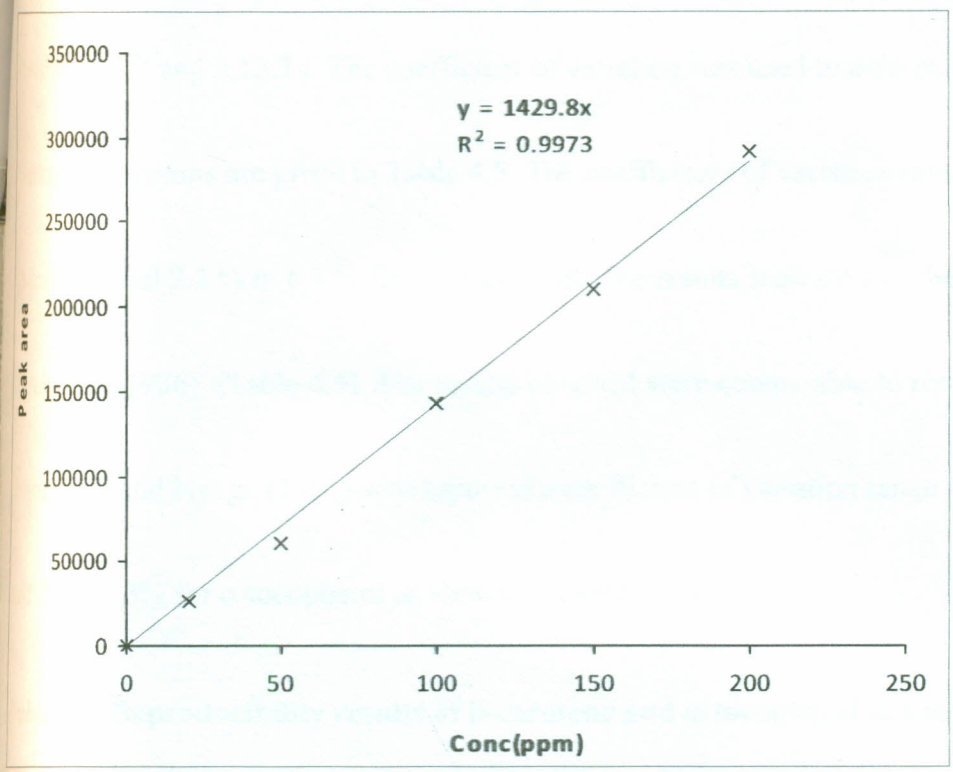


Figure 4.5: The calibration curve for α-tocopherol standard solution

The α-tocopherol curve was linear within the concentration range determined (0 to 200 mg/ml). This calibration line gave a correlation coefficient with $r^2 = 0.9973$. The correlation coefficient obtained in this study was comparable to that obtained in the study by Nyambaka and Nyaga (1991) who obtained $r^2 = 0.9970$ using a HPLC system consisting of μ Bondak C_{18} reversed phase column and a mobile phase of methanol- acetonitrile - chloroform -water in ratio of 46:30:18:6 and detection limit of 297nm for both β-carotene and α-tocopherol. This value indicates that there was a linear relationship between the chromatographic peak area and the α-tocopherol concentration. The regression equation was therefore y = 1429.8 x. The calibration curve was used to determine the concentration of α-tocopherol in the vegetable samples (Table 4.7).

4.3.1.2 Reproducibility studies

Some food samples (0.1-1.0kg each) were pre-treated, extracted and analyzed as per procedure outlined in section 3.11.2 and 3.12.3 i. The coefficient of variation was used to determine the reproducibility of the method. The results are given in Table 4.5. The coefficients of variation ranged from 2.0 % to 3.1 % for β-carotene and 2.3 % to 6.3 % for α-tocopherol. The results indicate that the method is reproducible (Brubacher, 1986) (Table 4.5). The results obtained were comparable to results obtained in the study by Nyambaka and Nyaga (1991) who reported a coefficient of variation range of 2.0 to 7.3% for β – carotene and 2.1 to 6.3% for α-tocopherol as shown in Table 4.5.

Table 4.5: Reproducibility results of β-carotene and α-tocopherol in some vegetables

Vegetables	β-carotene		α-tocopherol	
	Mean µg/100g±SD	Variation %	Mean µg/100g±SD	Variation %
Flying spider	7585 ± 149.9	2.0	3035±112.4	6.3
Amaranthus	7623 ±216.2	3.1	7200 ± 222.1	3.1
Pumpkin leaves	8564 ±269.6	3.1	7450 ± 175.0	2.3
Cowpea leaves	7550 ± 222.0	2.9	6545 ±207.4	3.1

4.3.1.3 Recovery studies

Since both β-carotene and α-tocopherol are affected by heat and presence of oxygen and other Oxidizing agents, their stability during sample extraction was determined by measuring their recovery when added to some food samples .The samples were analyzed in duplicate for β-carotene and α-tocopherol

with addition of the standard. The results gave mean recoveries of 95.5% for β-carotene and 93.6% α-tocopherol (Table 4.6).this shows that were significantly affected by oxidation (Brubacher, 1986).

Table 4.6: Recovery of β-carotene and α-tocopherol added to some vegetable leaves

Vegetable	β-carotene			α-tocopherol		
	Added	Retained	Recovered	Added	Retained	Recovered
	µg	µg	%	µg	µg	%
Pumpkin leaves	50.0	47.4	94.8	80.0	76.2	95.3
Nightshade	50.0	49.2	98.4	80.0	78.0	97.6
Amaranthus	50.0	48.8	97.6	60.0	54.3	90.5
Cow peas L	60.0	54.7	91.2	60.0	54.3	90.8

The results were comparable to those of other studies. Nyambaka (1988) reported a mean recovery 94.3% for β-carotene and 93.5% for α-tocopherol while Nderitu (2006) reported a mean recovery of 94.3 % for β-carotene. The results indicate that the extraction process was satisfactory and no significant losses occurred during the extraction and analysis process. Therefore the results presented in this work are valid.

4.3.2 Vitamins levels, availability and moisture content of selected vegetables and fruits

4.3.2.1 Levels of β-carotene and α-tocopherol

Table 4.7 shows the β-carotene and α-tocopherol levels in dry and fresh vegetables. The β-carotene content in the dry vegetable samples ranged from 548.00 µg/g to 854.00 µg/g dry matter while in the fresh vegetables the β-carotene content ranged from 7000 µg/100g to 9700 µg/100g. The α-tocopherol content in the dry vegetable samples ranged from 281.60 µg/g to 693.55 µg/g dry matter while in the fresh vegetables

the α-tocopherol content ranged from 2800 µg/100g to 7500 µg/100g.The concentration β-carotene and α-tocopherol varied with the type of vegetables. Frying spider had the highest β-carotene level while slender leaf had the lowest while night shade had the highest α-tocopherol level while slender leaf had the lowest (Table 4.7).

Table 4.7: β-carotene and α-tocopherol Mean content in µg/g (± Standard deviation) of some selected vegetables (dry matter) and µg/100g wet weight

Vegetable	β-carotene Dry matter	α-tocopherol Dry matter	β-carotene wet weight	α-tocopherol wet weight
Cow pea leaves	680.00 ± 4.35	513.60 ± 13.95	7437 ± 391.38	6400 ± 582.54
Pumpkin leaves	548.00 ± 54.68	693.55 ± 66.53	8000 ± 604.18	7350 ± 941.60
Amaranthus LL	650.00 ± 9.065	653.63 ± 48.67	7400 ± 337.40	6750 ± 714.83
Slender leaf	572.60 ± 43.68	281.60 ± 117.70	7000 ± 226.21	2800 ± 778.13
Frying spider	854.00 ± 82.17	445.75 ± 44.29	9700 ± 1246.72	3000 ± 702.54
Night shade	717.00 ± 20.90	680.60 ± 60.74	7625 ± 462.44	7500 ± 998.30

The values obtained in this study are comparable to those from other studies (Nyambaka and Ryley, 1995; Mulokozi *et al.*, 2000; Manuche, 2003; Nderitu, 2006; Nawiri, 2008). The concentration of β-carotene reported for cowpeas were 7416 µg/100g wet weight (Gomez, 1981), 526 µg/g, dry weight (DM) (Mulokozi *et al.*, 2000), 691 µg/g DM (Nyambaka and Ryley, 1995). The concentration of β-carotene in amaranthus leaves have been reported as 677 µg/g DM (Mulokozi *et al.*, 2000). The

concentration of β-carotene in amaranthus, nightshade and cowpea leaves was reported as 712 µg/g, 693 µg/g, 653 µg/g DM respectively by Nderitu (2006). Manuche (2003) reported the following concentrations of β-carotene for various vegetables: Pumpkin leaves 548 µg/g, spider herb 1048 µg/g, black night shade 717 µg/g, cowpea leaves 507 µg/g, amaranthus 650 µg/g and slender leafo 668 µg/g DM. The concentration of β-carotene reported by Nawiri (2008) were : cowpea leaves fresh 806 µg/g, solar dried 579 µg/g and sun dried 553 µg/g while those for amaranthus leaves were fresh 599 µg/g, sundried 402 µg/g and solar dried 412 µg/g. Nyambaka and Nyaga (1991) reported the following concentration of β-carotene for various vegetables: kale 4920 µg/g, cowpea leaves 7500 µg/g, pumpkin leaves 8500 µg/g, frying spider 7600 µg/g, nightshade 6760 µg/g, amaranthus leaves 7600 µg/g, DM. The concentration of α-tocopherol for various vegetables was also reported by Nyambaka and Nyaga (1991) to be as follows: cowpea leaves 6420 µg/g, pumpkin leaves 7530 µg/g, frying spider 3020 µg/g, nightshade 7500 µg/g, amaranthus 7040 µg/g wet weight.

The values for β-carotene and α-tocopherol obtained in this study are generally within the range of values reported although some values obtained cannot be directly compared. The variation could be due to the fact that β-carotene and α-tocopherol content is dependent on sample varieties, stage of maturity, soil fertility, climate or geographical site of production, harvesting and post harvesting handling, processing and storage conditions (Ihekoronye, 1992). Another reason why values obtained cannot be directly compared with those published could be due to use of different methods of analysis. Also the samples used in this study had stayed for more than a day from the time they were harvested and transported from busia to Nairobi. Since they were kept under normal conditions enzymatic destruction of carotene was rapid (Gomez, 1981).

4.3.2.2 L-ascorbic acid content

The standard for L-Ascorbic acid was prepared as in Section 3.12.1 b. L-ascorbic acid content in vegetables was determined by a titration procedure using DCPIP. When testing for vitamin C, a colour change took place from blue to clear or from blue to pink to clear. Chemically, vitamin C is both a reducing agent and a weak acid and the vitamin concentration in the vegetables and citrus juices was determined using an oxidation and reduction titration. The titration reaction is as follows

$$H_2C_6H_6O_6 \text{ (Vitamin C)} + HC_{12}H_6Cl_2O_2N \text{ (DCPIP)} \longrightarrow C_6H_6O_6 + H C_{12} H_8 Cl_2 O_2 N$$

In this reaction the reactants and products are colorless or pale yellow with the exception of DCPIP which is either blue at PH>4 or purple at a PH< 4 hence vitamin C bleaches DCPIP and the colour of the reaction mixture is used to detect the end point of the titration. Vitamin C standard prepared as per Section 3.12.1b was used to standardize DCPIP. The standardized DCPIP (0.0038 g/500ml) was used to find the concentrations of ascorbic acid in sample extracts as per Equation 4.1 and 4.2 and the results obtained are shown in Table 4.8.

$$\text{Ascorbic acid concentration in 100g of wet sample} = 176\left(\frac{100}{M_s} x \frac{50}{500} x \frac{V_d}{V_s} x \frac{0.038}{268}\right) \quad [4.1]$$

$$\text{Ascorbic acid concentration in 100g of dry sample} = \left(\frac{V_d}{M_s} x \frac{0.038}{268} x \frac{176}{V_s} x \frac{100}{P_s}\right) \quad [4.2]$$

Where:

P_S – Percentage of matter sample without water

M_S – Mass of the sample taken

V_S – Volume of the sample used in the titrations against DCPIP

V_d- Volume of DCPIP

268 – Mass of DCPIP

176 – Mass of L -ascorbic acid

Table 4.8: Mean vitamin C content (mg/100g) in selected fruits and vegetables

Vegetable/Fruit	Mean vitamin C (mg/100g) ± SD
Banana (raw)	8.20 ± 5.36
Amaranthus	62.50 ± 10.32
Pineapple	24.50 ± 0.65
Water melon	6.22 ± 5.93
Cow pea leaves	0.20 ± 7.67
Pawpaw	52.00 ± 7.29
Pumpkin leaves	4.99 ± 6.28
Avocado	17.85 ± 2.57
Slender leaf	0.41 ± 7.61
Orange	50.00 ± 6.71
Banana (Ripe)	51.00 ± 7.14

The vitamin C levels in fruits ranged from 8.20 mg/100g to 52.00 mg/100g. Raw banana had the lowest concentration (8.20mg/100g) while pawpaw had the highest concentration (52.00 mg/100g). The vitamin C levels in vegetables ranged from 0.20 mg/100g to 62.50 mg/100g. Cow pea leaves had the lowest concentration (0.20 mg/100g) while amaranthus had the highest (62.50 mg/100g). The concentration values in this study are comparable to those cited in the literature (Ihekoronye, 1992).

Vitamin C values published by Ihekoronye (1992) for the various vegetables and fruits are as follows: pawpaw (112 mg/100g), pineapple (34 mg/100g), orange(46 mg/100g), amaranthus (68 mg/100g), ripe banana (51 mg/100g), raw banana (9 mg/100g), avocado (18 mg/100g), slender leaf (4-7 mg/100g),pumpkin leaves (5 mg/100g), water melon(13 mg/100g) and cow pea leaves(0.10 mg/100g).

4.3.2.3 Moisture content in some selected vegetables and fruits

The percent moisture content of some fresh and dried vegetables and fresh fruits was determined and the results are given in Table 4.9.

Table 4.9: Moisture content in some selected vegetables and fruits

Vegetable/Fruit	Moisture content %	Moisture content %
	Fresh	Dry
Banana	73.50 ± 6.67	_
Amaranthus	96.00 ± 0.17	6.05 ± 0.85
Pineapple	85.00 ± 3.35	_
Water melon	93.00 ± 1.04	_
Paw paw	92.00 ± 1.33	_
Pumpkin leaves	89.00 ± 2.19	5.98 ± 0.83
Avocado	80.00 ± 4.79	_
Slender leaf	94.00 ± 0.75	5.20 ± 0.61
Cabbage	92.00 ± 1.33	6.35 ± 0.94
Orange	86.00 ± 3.06	_
Night shade	96.14 ± 0.31	6.40 ± 0.96
Cow pea leaves	94.14 ± 0.71	5.18 ± 0.60

The moisture content ranged from 73.50 to 96.14 % for the fresh vegetables and fruits. The moisture content of most fresh tissue foods like vegetables and fruits 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 moisture content for the dry vegetable sampled ranged from 5.18% to 6.4%. The total moisture content for 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 unbound water is lost during the dehydration process. The bound water constitutes the moisture content of the dry samples while free unbound water constitute the moisture content of the fresh sample (Arya *et al* ., 1979) to ensure safe storage the final moisture content of the food should be less than 20% for fruits and less than 10% for vegetables (Ihekoronye, 1992). Since dried fruits are generally eaten without being rehydrated, they should not be dehydrated to the point of brittleness (Macrae *et al.*, 1993).

Most foods are preserved through canning, sun drying, freezing and refrigeration, use of chemical additives and packaging (Ihekoronye, 1992). Some of these techniques such as canning, freezing and refrigeration require sophisticated equipment; their cost is high and need electricity to provide the energy for running them. In most rural areas in Kenya, electricity is unavailable therefore less sophisticated methods such as solar drying; smoking; curing and fermentation are commonly used. In this study, solar drying was advocated for to be used in Butula division. A number of studies carried out to examine the loss of β-carotene on drying have shown that there are lower loss of β-carotene and α-tocopherol while using the solar drier. Nderitu (2006) reported β-carotene losses of 16.41% in cowpea leaves, 31.93% for nightshade and 31.93% for amaranthus for solar dried vegetables. Manuche (2003) reported retentions of β-carotene of between 55% and 90% for solar dried green leafy vegetables as compared to the sun dried vegetables whose retention was between 20% and 70%. The vegetables which were preserved in the study area included the cowpeas, spider herb, black night

shade and pumpkin leaves. Preservation of these vegetables ensures that they are available during the dry season when they are scarce.

4.3.2.4 Vegetable availability

Information obtained from field work, Busia district development plan 2002 and government of Kenya nutritional micronutrients survey 1988 indicates that production of food crops in Butula location is mainly done on a small scale basis (GOK, 2002). The crops grown include; maize, sorghum, sweet potatoes, soya beans, cowpeas, green grams, kales, "simsim", sunflower, cassava, avocadoes, oranges, watermelons, bananas, sugarcane, slender leaf, amaranthus, pumpkins, frying spider, night shade, and other local vegetables. The foods act as a source of food and income for the inhabitants of this region. However in many households, maize, millet, sorghum and indigenous vegetables are dried and stored for use in times of scarcity. Most of the indigenous vegetables are available throughout the year such as pumpkin leaves and sun hemp (Table 4.9).

Table 4.10: Vegetables availability calendar

Month	High	Medium	Low
January		A,H	C,F,G, D, B, I
February		A,H	C,F,G, D, B, I
March	A	B,F,G,H	C,D,E,I
April	A,B,E,F,G,H	C,D,I	
May	A,B,E,F,G,H	C,D,I	
June	B,C,D,F,G,H,I	A,E	
July	C,D,F,G,H,I	A,B,E	E
August	C,D,F,G,I	A,B,E,H	
September	B	A,C,D,F,G,H,I	F,G,I
October	C,H	A	F,G,H,B,C,D,E
November		A,B,C,D,E,H	I
December		A	

Key

A	Pumpkin Leaves	F	Pigweed
B	Sun Hemp	G	Amaranthus
C	Spider Flower	I	Kale (sukuma wiki)
D	Black Night Shade	H	Cowpea leaves
E	Jute Plant		

4.4 Health status of HIV/AIDS subjects

4.4.1 Cluster designation numbers (CD4 counts, CD8 counts and CD4/CD8 ratios)

CD4 cells also known as T4 or helper T cells are the primary target to HIV (Stine, 2005). The cells perform critical functions such as signaling other parts of the immune system to respond to an infection. Treatment decisions are often based on viral load and CD4 counts. Normal counts range

from 500 to 1500 cells per µl of blood (Augustine *et al.*, 1985). CD4 counts in a man without HIV infection is approximately 400-1200 cells per µl of blood and 500 to1600 cells per µl of blood in women (American Association for Clinical Chemistry, 2009).

The study subjects were classified into groups according to their CD4 cells counts. The CD4 levels of the subjects were in the range of 6 -1400 cells per µl in blood. 65.05% of the subjects were in the CD4 counts range of 6-670 cells per µl of blood, while 30.09% of subjects were in the range of 671-1335 cells per µl of blood and those with CD4 count greater than 1336 cells per µl of blood were 4.85% from Figure 4.6. The female subjects in all the groups had a higher CD4 cell counts than their male counterparts. CD4 cell counts are often higher in HIV positive women compared to HIV positive men (American Association for Clinical Chemistry, 2009). The CD4 count is used as a basis for initiating antiretroviral therapy. The WHO recommends intake of ARVs for HIV patients with a CD4 count of 200- 250 or below (WHO, 2002).The number of subjects on ARVs was 15 (6%) and were eliminated from the study since they did not fulfill the inclusion criteria. The mean CD4 count for the subjects considered in this study was 838.24 cells per µl of blood.

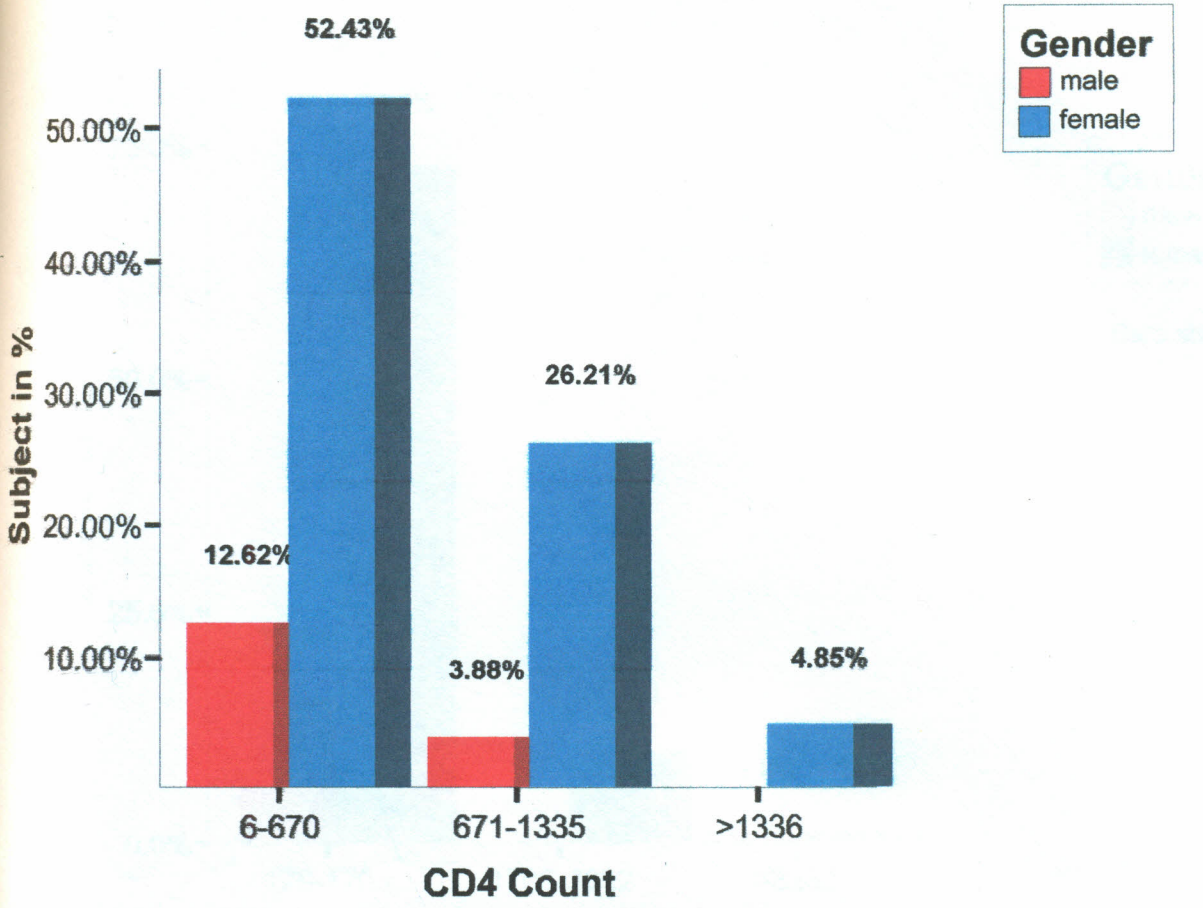


Figure 4.6: Number of subjects (%) in the study in different CD4 counts groups

A CD8+ cell refers to T cell with CD8 receptor that recognizes antigens on the surface of a virus infected cell and binds to the infected cell and kills it. A normal CD8 cell count is about 300 -1000 cells. CD8 cells counts usually rise overtime in HIV positive people (Augustine *et al.*, 1985). The CD8 cell count of the subjects was in the range of 179 - 3300 cells per µl of blood. The mean CD8 count was 888.70 cells per µl of blood. 88.3% of the subjects had a CD8 count in the range of 179-1705, 9.7% were in the range of 1706- 3232 and 2% had a CD8 greater than 3233 cells per µl of blood (Figure 4.7).

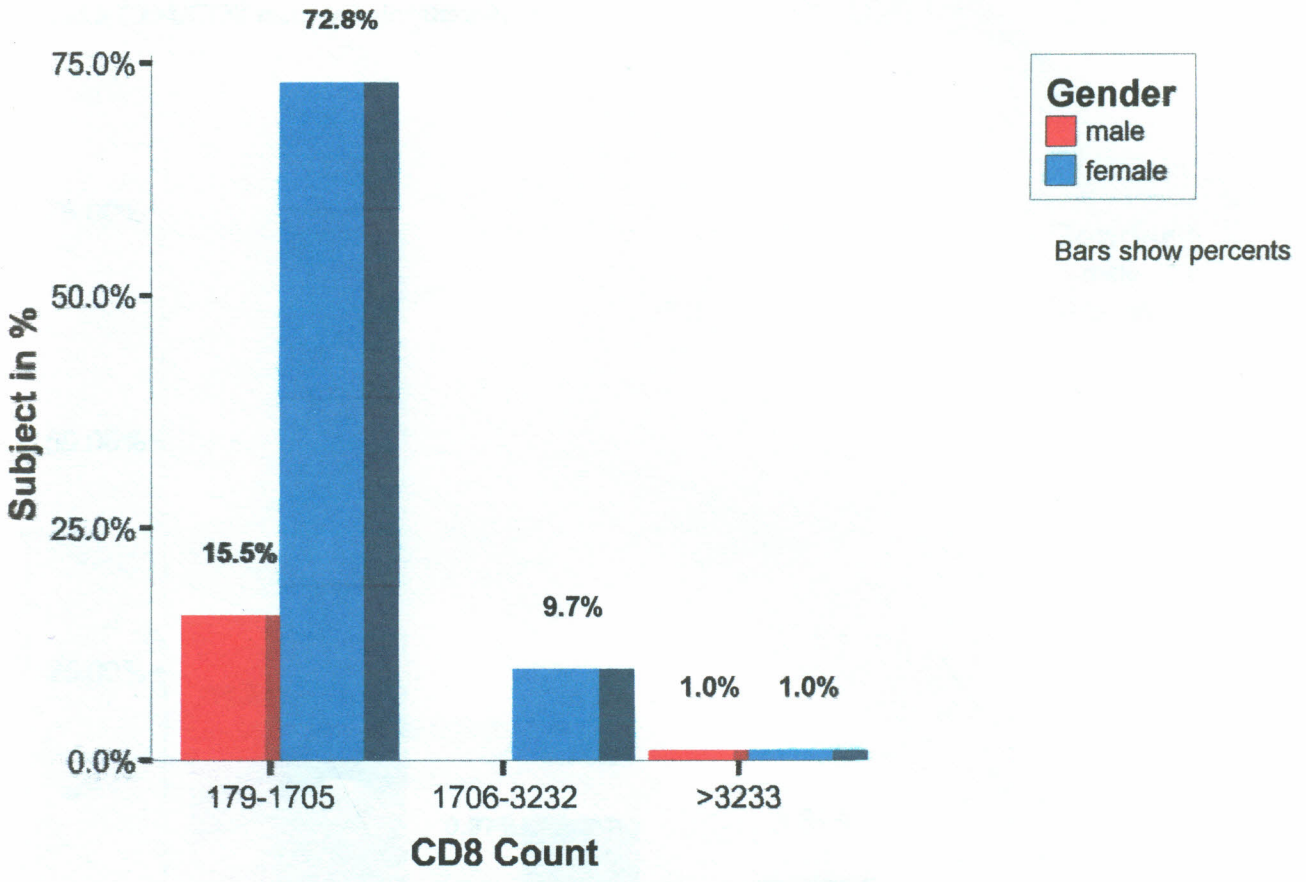


Figure 4.7: Number of subjects (%) in the study in different CD8 cell counts groups

During the course of HIV infection CD4+ cell counts decrease, while CD8+ cell counts are elevated and CD3+ counts remain constant (Giorgi, 1993).

CD4/CD8 ratio of the subjects was determined. It is a reflection of the health of the immune system and the normal ratio is between 1 and 4 (Augustine *et al.*, 1985). In HIV infection the CD4 count is often decreased and CD8 count increased so the ratio might be inverted, that is it becomes less than1 (Stine, 2005). Figure 4.8 shows the CD4/CD8 ratios obtained where 88.35% of the subjects had a

CD4/CD8 ratio of less than 1, 9.71% had CD4/CD8 ratio in the range of 2 - 3 while 1.94% of the subjects had a CD4/CD8 ratio greater than 4.

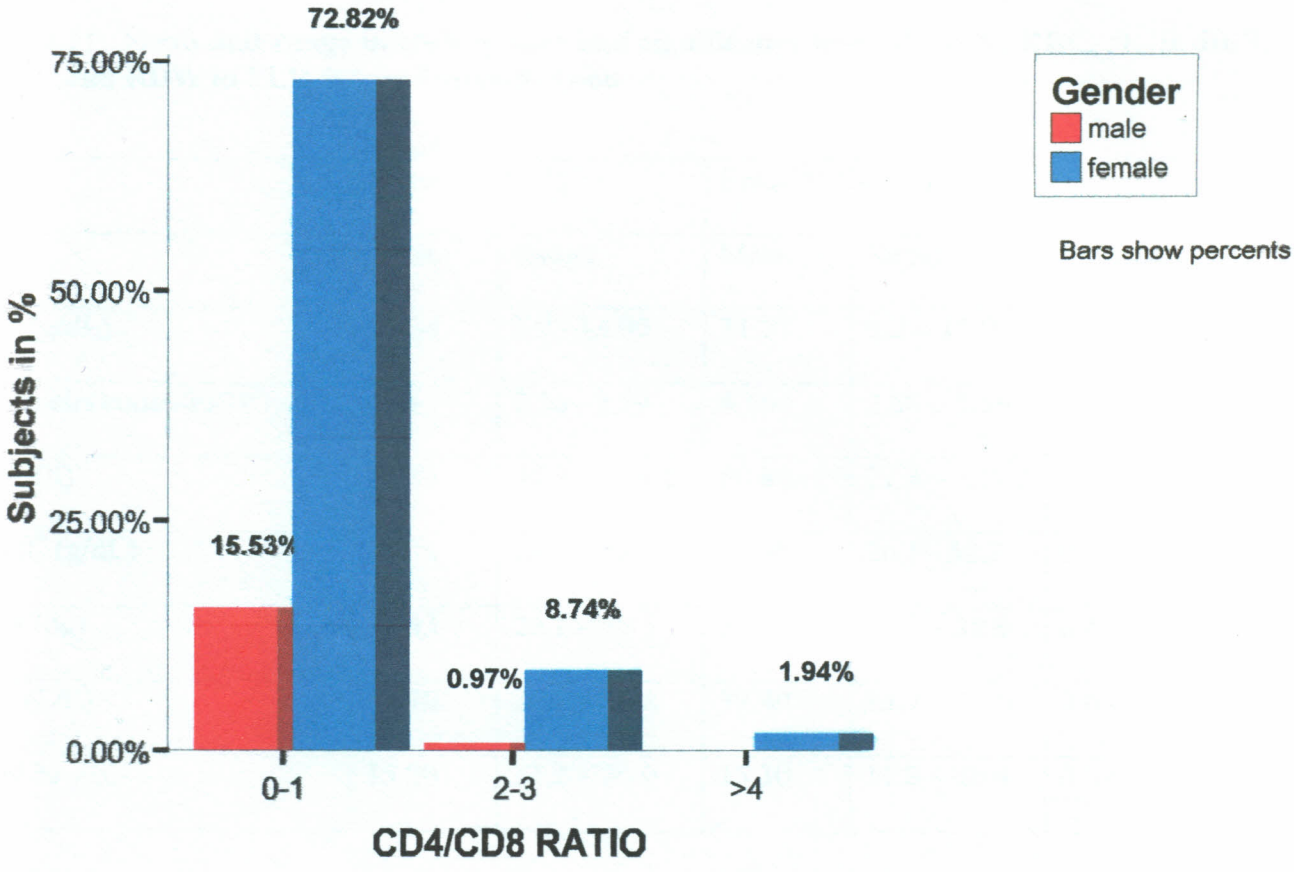


Figure 4.8: Number of subjects (%) study group in different CD4/CD8 ratios

4.4.2 Haematological index

Table 4.11 shows the mean, range and significance level of MCV, RBC, HGB, HCT, MCHC and RDW in PLWHA in Butula division.

Table 4.11: Mean and range in each gender and significance level of MCV, RBC, HGB, HCT, MCHC and RDW in PLWHA in Butula division

Gender	Males (N = 17)		Females (N = 111)		
	Mean	Range	Mean	Range	P = value
HGB (g/dL)	11.34	7.7 –13.90	11.23	6.2 – 15.9	0.809
RBC (cells counts) $x10^6/\mu L$	4.31	2.56 –5.39	4.16	2.86 – 5.59	0.338
MCV (fl)	88.84	68.7 – 103.4	89.80	27.3 – 123.2	0.741
MCHC (g/dL)	29.74	28.4 – 31.1	29.98	26.1– 32.7	0.325
MCH (pg)	26.43	20.1 – 30.5	27.13	17.9 – 38.9	0.435
HCT (L/L)	38.10	26.4 – 46.8	37.40	23.7 – 51.6	0.631
RDW %	15.20	12.2 – 20.0	15.30	11.8 – 30.4	0.333

The range of hemoglobin (HGB) was 7.7 - 13.9 g/ dL for male; and 6.2-15.9 g/ dL for female. The normal range for HGB is different between sexes and is approximately 13 to18 g/ dL for men and 12 to 16 g/ dL for women (Zucker, 2007).The range of red blood cell count(RBC) for women is 2.8 - 5.59 million cells/µl of blood and 2.5 - 5.39 million cells/µl for men. The normal range for RBC is 4.2 -5.4 million cells/µl of blood for women and 4.6 - 6.1 million cells/µl of blood (Zucker, 2007). The mean corpuscular volume (MCV) range for men was between 68.7 and 103.4 fl while that for women was 27.3 - 123.2 fl. The normal range of MCV is 80 – 100 fl for men and 96-108 fl for women (Stine, 2005). The mean corpuscular hemoglobin concentration (MCHC) range for the

subjects was as follows 28.4 - 31.1 g/dL in males and 26.1 - 32.7 g/dL for females. Normal MCHC range is 32 to 36 g/dL. The hematocrit (HCT) range for female subjects was 23.7 -51.6 L/L while that for male was 26.4 - 46.8 L/L. The normal range is 45% -52% in men and 37 - 48% in women (Augustine *et al.*, 1985). Red cell distribution width (RDW) range in males 12.2 - 20.0% while that in female is 11.8 -30.4%, normal RDW range is 1I - 15 % (Margolik *et al.*, 1991).

Levels of MCV (89.8 and 88.8 fL) and RBC (4.3 and $4.16\times10^6/\mu L$) observed were above the cut off values of MCV (89 and 81.0 fL) and RBC (4.3 and $3.8\times10^6/\mu L$) for anaemia in males and females respectively (UNCF/UNU/WHO, 2001). However, low levels of MCHC (29.7 and 29.98 g/dL) and high levels of RDW (15.2 and 15.3 %) observed are indicative of anaemia, which are set at below 32 g/dL (MCHC) and above 15.0 % (RDW) for adult person (UNCF/UNU/WHO, 2001; CDC, 1998).

Low levels of HGB in the males (11.3 g/dL) and females (11.2 g/dL) also showed anaemia in males and mild iron deficiency in females, based on the cut-off of below 12 g/dL as recommended by UNCF/UNU/WHO (2001). Presence of anaemia in the male subjects was further confirmed by levels of haemocrit (38.1%) that are lower than the cut off levels for anaemia (39.9 %) (CDC, 1998).

4.4.3 Clinical status

The clinical status was assessed by looking at the opportunistic infections affecting PLWHA living in Butula division. Those who suffered from opportunistic infection in this study were 77.7%. Most of the PLWHA in Butula division were suffering from common opportunistic infection as shown in Table 4.12. Common HIV-related opportunistic infections include; tuberculosis, malaria, pneumonia, skin rash, diarrhea, fever, oral thrush, fatigue, Kaposi sarcoma, anorexia, head ache, vomiting, constipation and pneumocystis carinii pneumonia (PCP) (Stine, 2005).

These diseases do also affect people with normal immune systems, but with HIV they occur at a much higher rate. It also takes longer for a person with HIV to recover than it takes for someone with a healthy immune system. When the immune system is very weak due to advanced HIV disease or AIDS opportunistic infections such as PCP, toxoplasmosis and cyptococcosis develop (Dybul *et al.*, 2002). Zero percent of the subjects were suffering these opportunistic infections commonly found in people with AIDS (Appendix II). Some infections can spread to a number of different organs; which is known as systematic disease. Many of the opportunistic infections that occur at this late stage can be fatal (CDC, 1993).

Table 4.12: Percentage and frequency of clinical conditions of PLWHA in the study area

Clinical conditions	Male (N= 17)		Female (N=111)		All subjects (N=128)	
	Frequency	Percentage %	Frequency	Percentage %	Frequency	Percentage %
Opportunistic infections	13	81.3	85	77.3	98	77.7
Fatigue	7	43.8	56	51.0	63	50.4
Lack of appetite	2	12.5	23	21.3	25	20.0
Fever	3	18.8	22	20.6	25	20.3
Weight loss	10	62.5	43	39.1	53	42.1

In this study 50.4% of the patients were suffering from fatigue. Several studies suggest that most people with HIV experience fatigue at some point during illness, with estimates ranging from less than 50% to more than 80%.The rate of fatigue increases as HIV disease progresses (WHO, 2002).

During the initial acute period of HIV infection many people experience a flue like illness that includes fatigue. After this initial period, the incidence of fatigue decreases, but then rises again later in the course of illness; people with advanced AIDS are more likely to report fatigue than people at earlier stages of HIV infection (Stine, 2005). Studies suggest, fatigue in HIV disease is associated with a variety of factors. Among these are anaemia, hormonal imbalance, depression, anxiety, poor nutrition, insufficient or poor quality sleep, lack of physical activity and medication side effects, active infections, including AIDS related opportunistic infections also play a role.

The number of subjects who suffered fever was 20.3%. Nearly all patients infected with HIV experience fever and skin rash at some point after diagnosis. A fever and rash are symptoms of an underlying medical condition (Fahey *et al.*, 1998). An estimated 80-90% of HIV patients develop fever and rash when they first become infected with HIV. This is because the virus is multiplying rapidly in the body and infecting immune cells. This phase generally lasts several weeks. Other common causes of fever and rash in HIV patients include allergic reactions to medications, infections and a cancer called Kaposi's sarcoma (Stine, 2005).

About 20% of the subjects suffered from lack of appetite and 42.1% weight loss. Weight loss in HIV infection could be caused by loss of appetite, medications that patients take to control the disease, opportunistic infections which increase the calorie needs, mouth and tooth infections which make it difficult to eat. Diarrhea is a common problem for HIV positive people may be directly related to weight loss. With or without diarrhea, malfunction of the intestine may lead to an in ability to absorb nutrients and may also contribute to weight loss (Macallan, 1999). Metabolism, including the number of calories patients expend at rest or physical activity also affect weight loss. Weight loss late in the

course of HIV disease is common and increased energy expenditure in response to opportunistic infections, as well as infection itself (WHO, 2005).

4.4.4 Mean body mass index

Body mass index is obtained from weight in kilograms divided by the square of height in metres. The WHO regard a BMI of less than 18.5 kg/m^2 as underweight and may indicate malnutrition, eating disorder or other health problems, while a BMI greater than 25 kg/m^2 is considered overweight and above 30 kg/m^2 is considered obese (Rofles, 1991). The subjects were classified into four groups; less than18.5, 18.6-25.0, 25.1-30.0, 31.0-39.0 in kg/m^2 indicating underweight, normal weight, overweight and obese respectively (Rofles, 1991). Figure 4.9 shows the number of subjects in each group. Where 77.95% of the subjects were normal weight, 11.81% were underweight, 8.66% were overweight and 1.57% were obese. The majority of the subjects were normal weight.This is because they were asymptomatic whereby there is weight loss of less than 5% (Stine, 2005). HIV patients who are overweight and obese usually have low antioxidants such as β-carotene, α-tocopherol and vitamin C which are free radical scavengers and are therefore, at a risk of high oxidative stress (Macallan, 1999). High oxidative stress is associated with a high risk of progression of HIV to AIDS (Semba and Tang, 1999), hence the need to change their feeding habits. The subjects who were underweight need to obtain adequate nutrition through consumption of a balanced diet. HIV positive individual who are underweight have been found to have low CD4+ cells which puts them at a higher risk of HIV progressing to AIDS (Macallan, 1999).

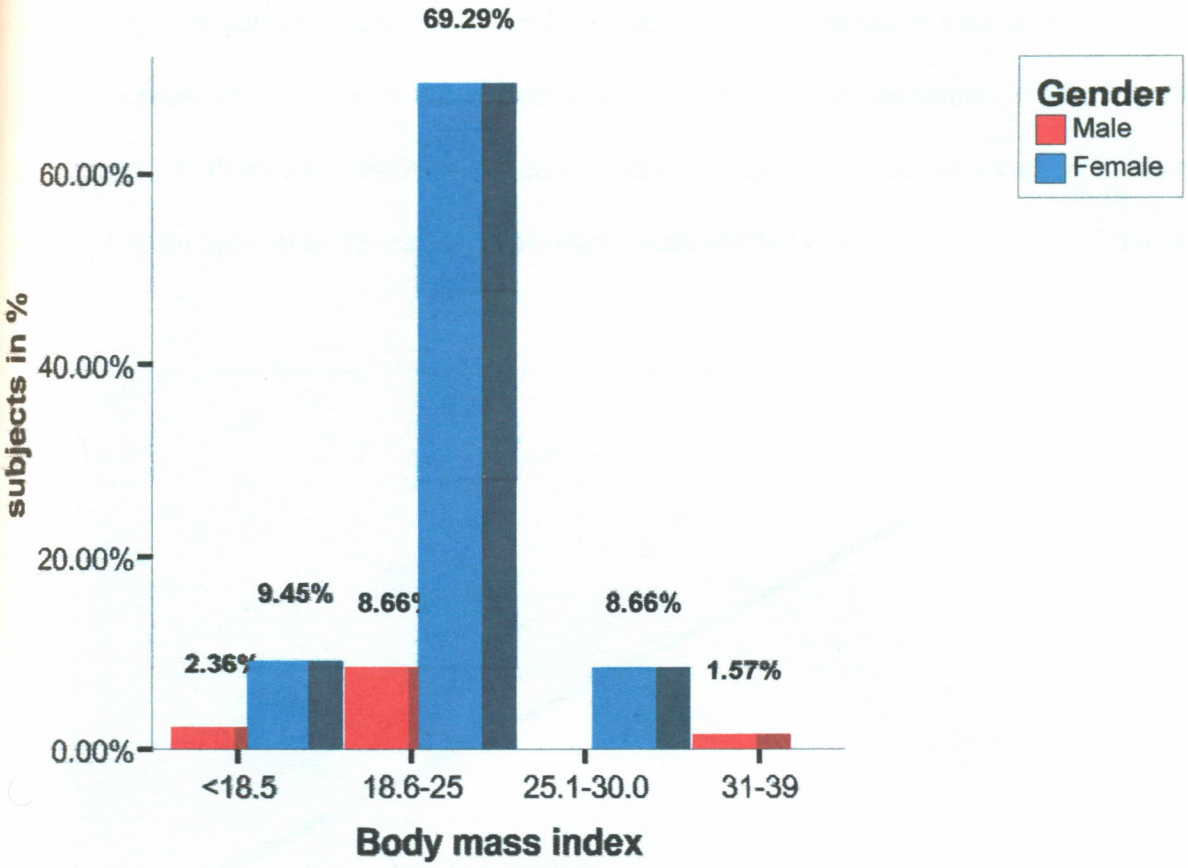


Figure 4.9: Mean body mass index (kg/m^2) of the subjects in the study group

4.5 Serum vitamins level

In this study retinol, α-tocopherol and β-carotene serum levels were determined using HPLC procedures, with UV detection at 325 nm, 292 nm and 450 nm respectively. The purpose of this study was to assess the vitamin levels in sera of subjects with aim of determining the bioavailability of these vitamins from a food formulation based on indigenous vegetables.

4. 5.1 Method validation

4.5.1.1 Calibration curve

The retinol, α-tocopherol and β-carotene standards solutions were prepared as described in Section 3.12.1 c. The solutions of α-tocopherol and β-carotene were injected and eluted using a mobile phase consisting of methanol: DCM: water (83:15:2) containing 0.1% BHT, while retinol was injected and

eluted using acetonitrile: water (85:15) mobile phase. The β- carotene, α-tocopherol and retinol were eluted at a relatively sharp peak at a retention time of 3.97 minutes for retinol, 4.19 minutes for β-carotene and 8.39 minutes for α-tocopherol .Calibration curves of peak areas against concentration of retinol, α-tocopherol and β-carotene standards were plotted (Figures 4.10, 4.11 and 4.12).

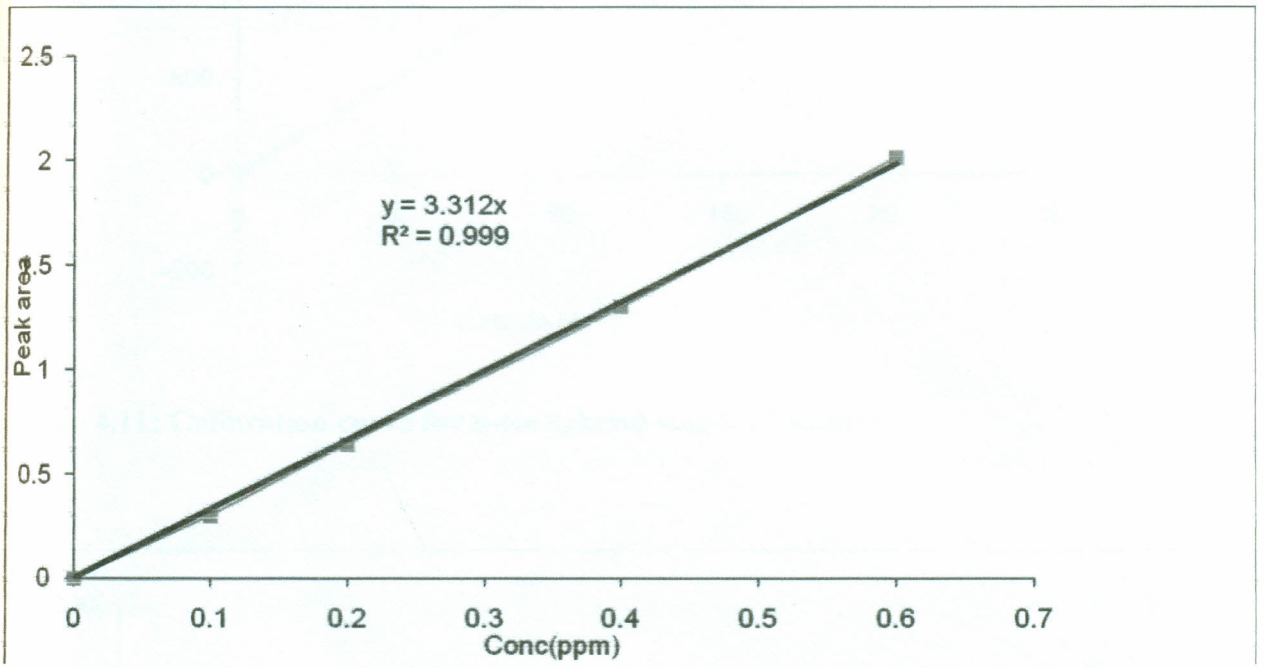


Figure 4.10: Calibration curve for retinol standard solution

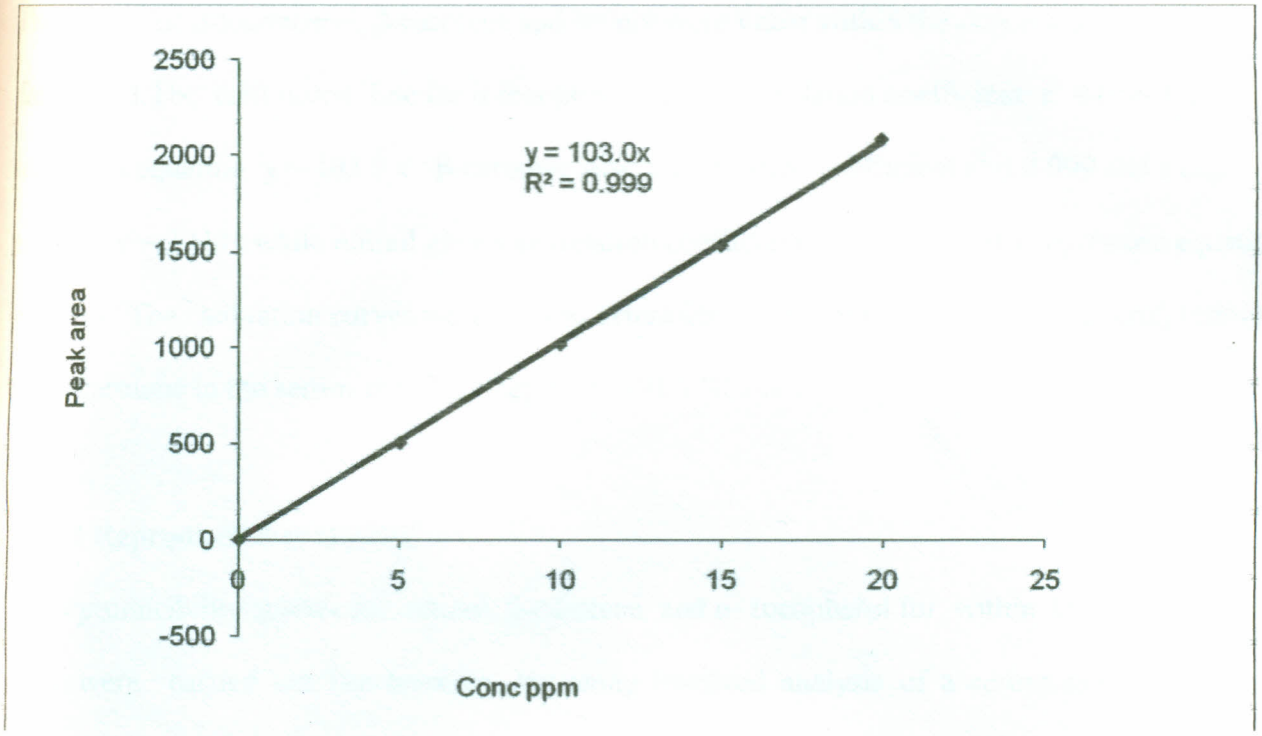


Figure 4.11: Calibration curve for α-tocopherol standard solution

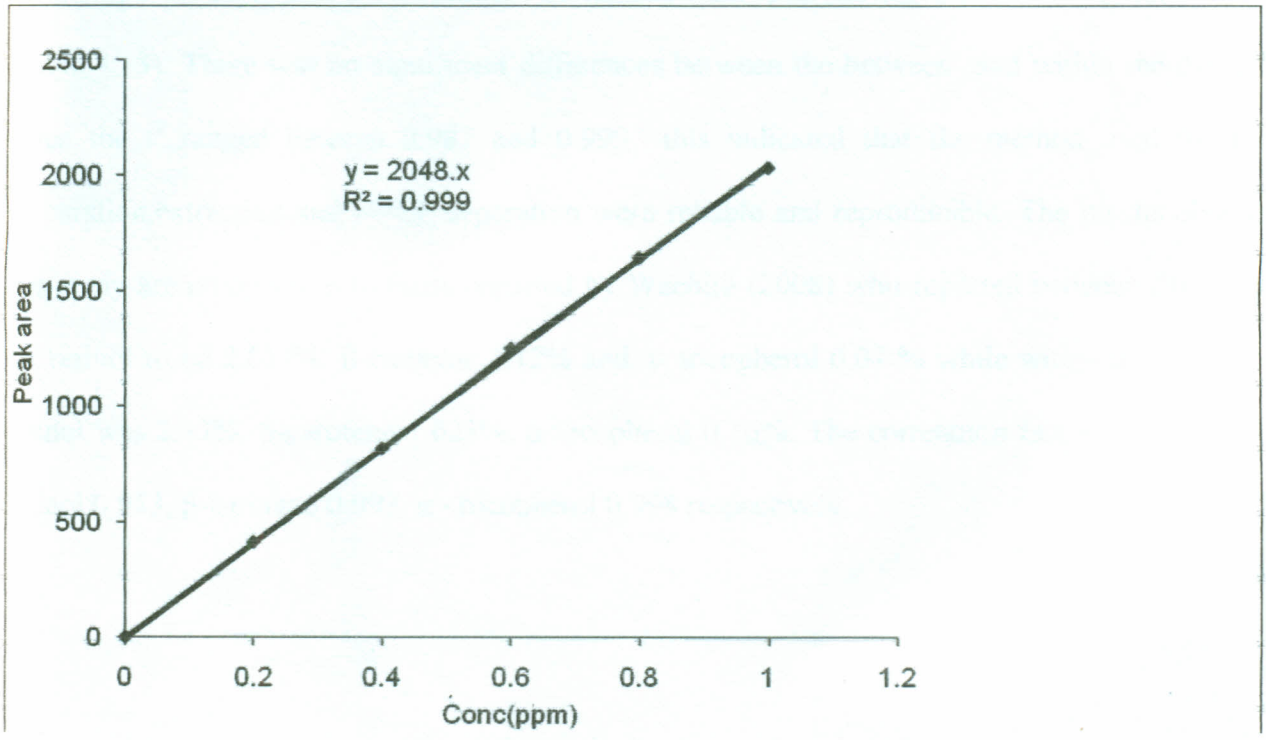


Figure 4.12: Calibration curve for β-carotene standard solution

The curves for α-tocopherol, β-carotene and retinol were linear within the concentration range determined.The calibration line for α-tocopherol gave a correlation coefficient $r^2 = 0.999$ and regression equation $y = 103.0 x$. β-carotene gave a correlation coefficient $r^2 = 0.999$ and a regression equation $y = 2048x$ while retinol gave a correlation coefficient $r^2 = 0.999$ and a regression equation $y = 3.312x$ .The calibration curves were used to determine the concentration of α-tocopherol, retinol and β-carotene in the serum samples (Appendix VII,VIII and IX).

4.5.1.2 Reproducibility studies

The reproducibility studies for retinol, β-carotene and α- tocopherol for within day and between day assay were carried out.The between day assay involved analysis of a serum sample three times within a day for five continuous days.The within day assay involved analysis of three different serum samples three times everyday for five days in order to ascertain the extent of reproducibility of the method. In each case the coefficent of variation and pearson correlation factor were computed (Table 4.13). There was no significant differences between the between and within the day assays. Since the r^2 ranged beween 0.987 and 0.999 this indicated that the method used for sample preparation,extraction and HPLC separation were reliable and reproducible. The results obtained in this study are comparable to those reported by Wachira (2008) who reported between day variation for retinol to be 2.014%, β-carotene 1.12% and α- tocopherol 0.07 % while within day variation of retinol was 2.17%, β-carotene 1.623%, α-tocopherol 0.16%. The correlation factor were as follows: retinol 0.953, β-carotene 0.997, α - tocopherol 0.998 respectively.

Table 4.13: Reproducibility study results for β -carotene and α -tocopherol in serum samples

Vitamins	Between day % variation	Within day % variation	Correlation factor (r^2)
Retinol	2.03	2.19	0.987
β – carotene	0.41	0.53	0.998
α – tocopherol	0.04	0.07	0.999

4.5.1.3 Recovery studies

Recovery studies were assesed by adding progressively increasing concentrations of retinol β-carotene and α-tocopherol (0.5-2.5 µmol/l) standards to serum samples. The extraction recoveries were calculated. The mean recoveries of retinol, β-carotene and α-tocopherol within the concentration range of the assay were 94%, 91% and 92% respectively (Table 4.14).

Table 4.14: Recovery study results for β -carotene and α -tocopherol in serum samples

Retinol			β –carotene			α – tocopherol		
Added µg/ml	Retained µg/ml	Recovered %	Added µg/ml	Retained µg/ml	Recovered %	Added µg/ml	Retained µg/ml	Recovered %
0.25	0.24	96	0.25	0.235	94	0.25	0.225	90
0.50	0.48	96	0.50	0.46	92	0.50	0.465	93
2.5	2.25	90	2.5	2.4	96	2.5	2.35	93
3.25	2.97	94%	3.25	3.095	91%	3.25	3.040	92%

4.5.2 Vitamin levels

The concentration range for α-tocopherol, retinol and β-carotene in the serum samples were as follows 1.602 to 28.051 µg/ml, 0.022 to 0.835µg/ml, 0.08 to 4.029 µg/ml respectively (Table 4.15). The mean β-carotene, α-tocopherol and retinol was 0.144 µmol/l, 17.787 µmol/l and 0.937 µmol/l respectively. The number of male subjects whose serum β-carotene, α-tocopherol and retinol were within the normal range were:10, 11 and 11 while the female were 21, 24 and 23 respectively. Those whose serum was deficient were as follows:male, β-carotene(6), α-tocopherol(5) and retinol(5)while the female were β-carotene (20), α-tocopherol (17) and retinol (18) as shown in Table 4.15.

Table 4.15:Vitamins status of PLWHA in Butula division

Vitamin	Mean serum level (µmol/l)	Serum level range (µmol/l)	Number of male PLWHA		No of female PLWHA	
			deficient	normal	deficient	Normal
β-carotene	0.144	0.080-4.029	6	10	20	21
α-tocopherol	17.787	1.602-28.051	5	11	17	24
Retinol	0.937	0.022-0.835	5	11	18	23

The values obtained in this study are comparable to those obtained in the study byWachira (2008) who reported a retinol concentration range of 14.7 to 63.8 µg/100 ml, β-carotene concentration range of 15.4 to 288.9 µg/100ml and a concentration range of 199.8 to 1010.8 µg/100ml for α - tocopherol. Total serum or plasma α-tocopherol levels are often used to measure vitamin E status.The normal range is 11.6 - 30.8 µmol/l, deficiency occurs at levels below 11.6 µmol/l (Robert and Gooditart, 1974). Retinol deficiency occurs at retinol serum $\leq$ 0.7 µmol/l, while a healthy person should have $\geq$ 1.745 µmol/l (Semba *et al.*, 1995). The recommended range of β-carotene in blood is

0.09 -0.466 μmol/l (Semba and Tang, 1999). In this study the mean α-tocopherol, retinol and β-carotene levels suggest that most of the subjects were within the normal range and would therefore be encouranged to continue taking foods rich in β-carotene and α-tocopherol. The subjects who were deficient would also be encouranged to take foods rich in β-carotene and α-tocopherol since this vitamins have been shown to have antioxidant properties which help delay the progression of HIV to AIDS.

4.6 Bioavailability of vitamins in indigenous vegetables by use of algorithms

Bioavailability algorithms, or mathematical models to estimate nutrient bioavailability from different diets have great appeal because of the potential to apply general principles to a complex dietary matrix, the increase in predictability without direct measurement of absorption and retention and ability to facilitate dietary assessements and recommendations (Ball,1997). Application of bioavailabilty algorithms may be useful for populations with low nutrient status (Combs, 1998)).

The bioavailabilty of vitamins in indigenous foods was estimated by use of algorithms derived from results of regression analysis of data obtained from information in Appendix VI and which were used to come up with Equations 4.3 and 4.4.The results are shown in Table 4.18. The data on absorption (Appendix VI) was obtained fom the internet using search words such as β-carotene and α-tocopherol intervention, absorption and retention.

4.6.1 Regression analysis of β-carotene

The data on β-carotene used are: absorption ranged from 0.01 to 7.8435 μmol/l, the duration of intake ranged from 30hours to 4 years, the number of subjects ranged from 2 to 29,133 ,while the amount of β-carotene administered ranged from 1.32 to 25 mg (David *et al.*, 1997; Sonia *et al.*, 2003; Cheryl *et*

al., 1992; Chingjang *et al.*, 2000; Ribaya *et al.*, 2007; Matti *et al.*, 1997; Francois *et al.*,1999; Sabrina *et al.*, 2002). β-Carotene absorbed was regressed against the duration of intake of supplement (days) the dietary level of β-carotene and the pre-study serum level of β-carotene (μmol/l) and results presented in Table 4.16.

Table 4.16: Regression analysis for β - carotene

Predictor variables	Unstandardized coefficients (B)	P=value
Constant	-3.010	0.000
Pre- study serum level(μmol/L)	12.806	0.000
β-carotene levels in food (mg)	0.657	0.000
Duration of study(days)	0.009	0.002

Dependent variable: change in serum vitamin μmol/l

Adjusted $R^2 = 0.877$

The dietary level of β-carotene had (B=0.657; p<0.000), pre-study serum level (B=12.806; P<0.000) while duration of study (B=0.009; P<0.002). Pre-study serum level was the most influential positive significant predictor. The three predictors accounted for over half of the variance in absorption (r^2=0.877) which was highly significant. Therefore the regression equation 4.3 is given as follows.

$$Y= -3.010 +12.806X+0.657Z+0.009W \qquad [4.3]$$

The B's (–3.010, 12.806, 0.657 and 0.009) are the regression coefficients representing the amount the dependent variable Y changes when the corresponding independent changes by one unit. In the

equation pre-study serum β -carotene level is represented by X, dietary β-carotene levels is Z, duration of study (days) by W and Y is change in serum level β-carotene. The equation shows that the change in serum β -carotene level is partly dependent on dietary β -carotene levels, pre study serum and duration of intervention. –3.010 is the constant, where the regression line intercepts the y axis, representing the amount the dependent (Y) will be when all the independents variables are zero. The negative constant (–3.010) may be due to influence of absorption efficiency by other food related factors such as food matrix effect, cooking technique, dietary fat, dietary interactions and host related factors such as clinical status, age, metabolic rate and physiological changes (Combs, 1998).

The model validity, goodness of fit, satisfaction of regression assumptions and quality of parameter estimates were evaluated using standard statistical criteria. The goodness of fit as reflected by r^2=0.877 is supportive of the model indicating that 87.7% of the variance is explained by the model. Plots of residual squares (Figure 4.13 and 4.14) obtained from regression analysis of β -carotene absorption show that, assumptions about linearity, normal distribution and equal variances were met.

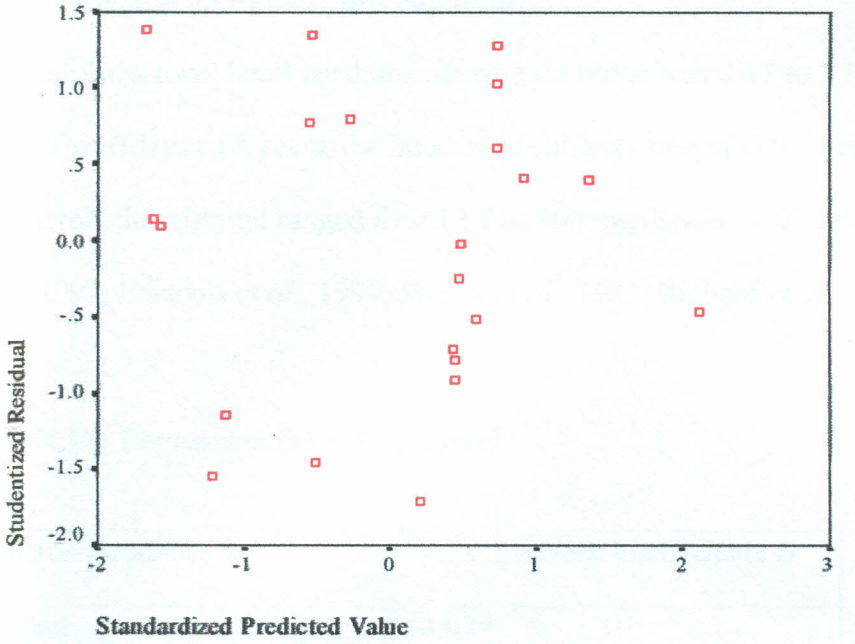


Figure 4.13: Regression residual plot for β- carotene

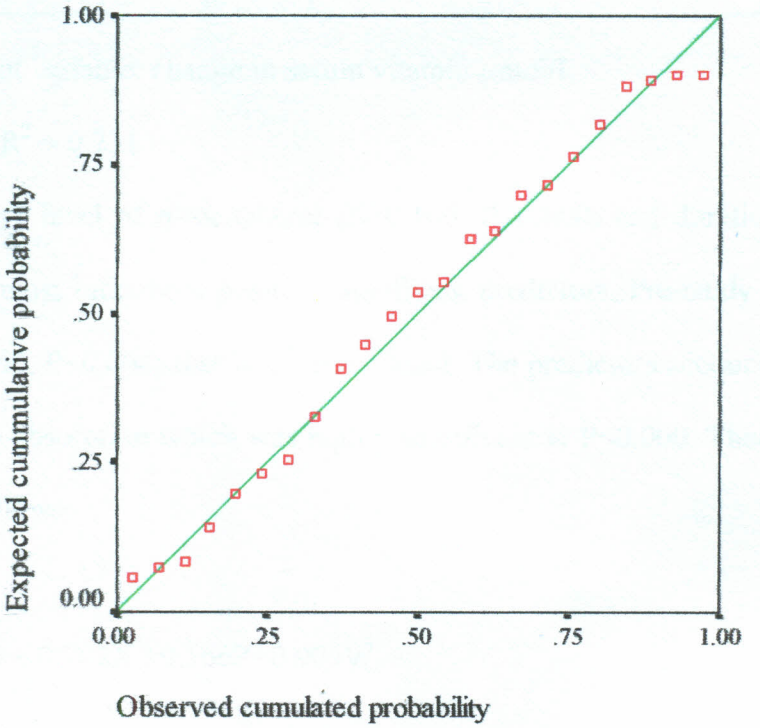


Figure 4.14: Normal probabilty plot of regression residuals β-carotene

4.6.2 Regression analysis of α-tocopherol

The data for α-tocopherol used are: absorption range was 0.17 to 27.2 µmol/l, the duration of intake ranged from7days to 3 years, the number of subjects ranged from 5 to 29,133, while the amount of α-tocopherol administered ranged from12.7 to 800 mg (Sonia *et al.*, 2003; Chingjang *et al.*, 2000; Matti *et al.*, 1997; Francois *et al.*, 1999; Walter *et al.*, 1983; Richard *et al.*, 2006; Meydani *et al.*, 1997).

Table 4.17: Regression for α-tocopherol

Predictor variables	Unstandardized coefficients B	P=value
Constant	16.824	0.006
Pre- study serum level (µmol/L)	- 0.702	0.008
Vitamin levels in food (mg)	0.166	0.000
Duration of study (days)	0.005	0.000

Dependent variable: change in serum vitamin µmol/L

Adjusted $R^2 = 0.731$

The dietary level of α-tocopherol (B=0.166; P<0.000) and duration of study (B=0.005; P< 0.000) were the most influential positive significant predictors. Pre-study serum had a negative coefficient (B= - 0.702; P<0.008) that was insignificant. The predictors accounted for over a half of the variance (0.731) in absorption which was highly significant at P<0.000. Therefore the regression equation 4.4 was as follows.

$$Y=16.824 - 0.702X + 0.166Z + 0.005W \qquad [4.4]$$

The B's (16.824, –0.702, 0.166 and 0.005) are the regression coefficients representing the amount the dependent variable Y changes when the corresponding independent changes one unit. Pre-study serum α-tocopherol level is represented by X, dietary α-tocopherol level is Z and duration of study (days) is W, where Y is change in serum level α-tocopherol. The equation shows that the change in serum α-tocopherol level in serum is partly dependent on dietary α-tocopherol levels, duration of intervention and partly independent on pre study serum. The positive constant (+16.824) may be due to influence of absorption efficiency by several foods related factors such as dietary fat and host related factors (Combs, 1998).

The model validity, goodness of fit, satisfaction of regression assumptions and quality of parameter estimates were evaluated using standard statistical criteria. The goodness of fit as reflected by r^2=0.731 is supportive of the model indicating that 73.1 % of the variance is explained by the model. Plots of residual squares (Figures 4.15 and 4.16) obtained from regression analysis of α-tocopherol absorption show that, assumptions about linearity, normal distribution and equal variances were met.

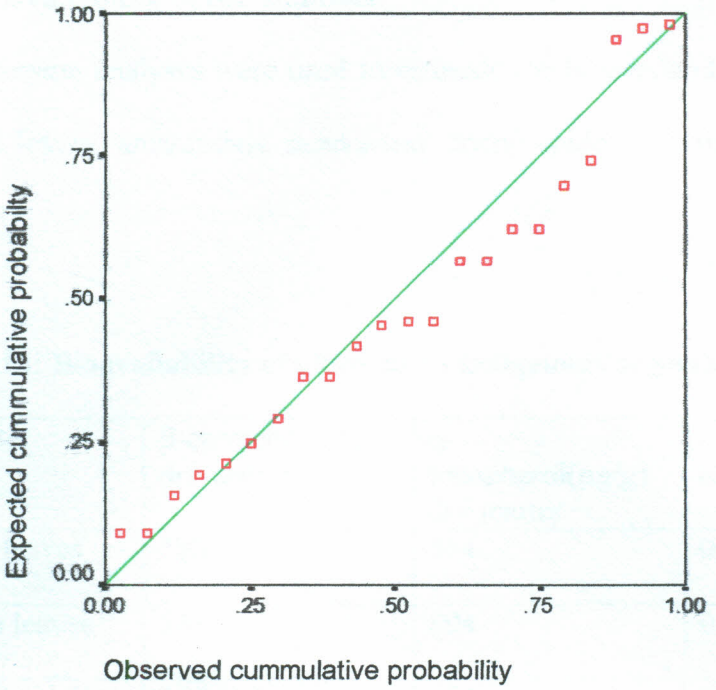


Figure 4.15: Normal probability plot for α-tocopherol

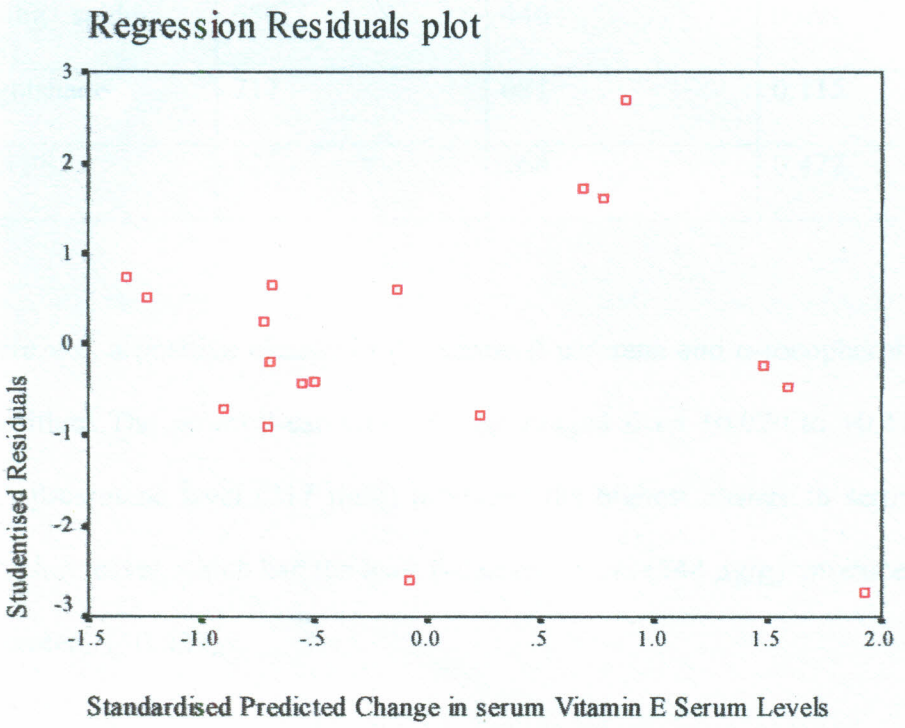


Figure 4.16: Regression residuals plot for α-tocopherol

4.6.3 Bioavailability of the vitamins

The regression analyses were used to estimate the bioavailability of the vitamins in cowpea leaves, pumpkin leaves, amaranthus, slender leaf, frying spider and nightshade and results are presented in Table 4.18.

Table 4.18: Bioavailability of vitamins in indigenous vegetables obtained using algorithms

Vegetable	β-carotene(µg/g) dry matter	α-tocopherol(µg/g) dry matter	Serum β-carotene change(µmol/l)	Serum α-tocopherol change(µmol/l)
Cowpea leaves (CL)	580	514	0.025	4.873
Pumpkin leaves	548	694	0.004	4.903
Amaranthus	650	654	0.019	4.897
Slender leaf	572	281	0.020	4.840
Frying spider (FS)	680	446	0.091	4.862
Nightshade	717	681	0.115	4.901
CL+FS	1260	960	0.472	4.947

There was a positive change in the serum β-carotene and α-tocopherol levels determined by use of algorithms.The serum β-carotene change ranged from +0.020 to +0.115. Nightshade which had a high β-carotene level (717 µg/g) produced the highest change in serum β-carotene (+0.115) while pumpkin leaves which had the least β-carotene level (548 µg/g) produced the lowest change in serum β-carotene (+0.020).

The serum α-tocopherol change ranged from +4.840 to +4.903. Pumpkin leaves which had a high α-tocopherol level (694 µg/g) produced the highest change in serum β-carotene (+4.903) while slender leaf which had the least α-tocopherol level (281 µg/g) produced the lowest change in serum β-carotene (+4.840).

The results are comparable to a study carried out by Wachira (2008) who reported an increase in β-carotene and α-tocopherol levels after a 3 months intervention study with PLWHA using a food supplement consisting of cowpea leaves,pumpkins and carrots. At the onset of the three months intervention study the mean β-carotene level was 77.6 ± 58.93 µg/dL while at the end term the mean β-carotene level was 82.1 µg/dL ± 61.82 µg/dL. The mean α-tocopherol at the on-set of intervention period was 648.44 ± 186.83 µg/dL while at end term the α-tocopherol mean level was 711.0 ± 216.15 µg/dL. It was therefore hypothesized that the bioavailabilty of β -carotene and α-tocopherol in blood serum increases with consumption of green leafy vegetables.

The low relative bioavailabilty of β-carotene from vegetables is in line with reports from other studies. De pee *et al* ., 1995 reported only 7% relative bioavailabilty of β-carotene from green leafy vegetables. Later De Pee *et al* ., 1998 reported a relative bioavailabilty of 23% for β-carotene from green leafy vegetables and carrots. The presence of carrots in the diet may have resulted to he higher bioavailability.

Cowpeas and frying spider were used to prepare food supplement because they contained high levels of β-carotene and α-tocopherol and are grown in large quantities. Table 4.19 shows nutrients composition used to prepare the food supplement. The vitamin A value is calculated by dividing β-

carotene by 6 as approved by WHO/FAO, 1988. The contribution to the RDA is based on the assumption of 100% bioconversion that is set at 750 µg β-carotene and 8 mg α-tocopherol for adults.

Table 4.19: Preparation of food supplement

Vegetables	β-carotene µg/g dry weight	α-tocopherol µg/g dry weight
Cowpea leaves (CL)	580	514
Frying spider (FS)	680	446
Total	1260	960
RDA	750 µg RE/day	8 mg/day
Fruit (Pineapple)	L-ascorbic acid	
RDA	60 mg/day	
pineapple	24.5 mg/100g	
pineapple required to meet RDA	244.90g	
Serum β-carotene change	+2.170	
Serum α-tocopherol change	+7.776	

The food supplement consists of cowpea leaves and frying spider in the ratio of 1:1. One gram of cowpea leaves produces 580 µg while one gram of frying spider produces 680 µg making up a total of 1260 µg in 2 grams. Therefore 2 g of CL+FS mixture provide 1260 µg β-carotene which is equivalent to 210 µg RE vitamin value obtained by dividing by 1260 µg by 6 .The RDA is based on the assumption of 100% bioconversion set at 750 µg RE/day for adults. Required mixture to meet RDA is 7.14g of CL and FS mixture in the ratio of 1:1.α- tocopherol RDA is set at 8 mg/day for adults. Two grams of CL+FS mixture provide 960 µg/g with cowpea leaves contributing 514 µg/g

while frying spider contributing 446 µg/g to the mixture. Required mixture to meet the RDA is 16.67 g CL+FS mixture in the ratio of 1:1. If 18 g of the CL+FS mixture was to be taken by each of the PLWHA on daily basis the mixture would provide 2.5xRDA β- carotene and 1xRDA α-tocopherol.

L-ascorbic acid RDA is set at 60 mg/day. 100g pineapple provides 24.5 mg; therefore 244.90 g of pineapple is required to meet the RDA. The food supplement should be taken together with other foods in order to provide all the nutrients required by the body. Algorithms for β-carotene and α-tocopherol were used to determine the effect the food supplement would have on the serum levels. There was a positive change in serum β-carotene and α-tocopherol levels by +2.17 and +7.776 respectively thus indicating an improvement in the bioavailabilty of these nutrients in the blood.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The questionaire results indicated that there was a lower participation of male than female. The female who participated were 86.72% while the male were 13.27%. The subjects who smoke are 6.7% men while 2.2% were women. Those who consumed alcohol were 11.7% men and 4.8% women. Most of the subjects (58.8%) take their meals at regular time while 39.7% take their meals at irregular times. The sources of food for the subjects included buying, farming and others. 19.1% of the subjects buy food from the local market, 56.5% get the food from their farms while 8.4% obtain their food from other sources.

The study indicated that the fresh vegetables have high β-carotene (4000 to 9700 µg/100g) and α-tocopherol (2800 to7500 µg/100g) wet weight; 548 to 854 µg/g and 281 to693 µg/g dry weight repectively. The vitamin C levels for fruits and vegetables ranged between 0.019 to 62.5 mg/100g.

Information from the questionnaire indicated that most of the indigenous vegetables are available throughout the year. The moisture content of the fresh vegetables and fruits ranged from 73.5% to 96.4% while the dry vegetable samples were in the range of 5.18% to 6.4%. Solar drying was the method used to dry vegetables and advocated for as a method of preservation in this study since it has been shown in other studies to result to lower loses of β-carotene .

More than half of the subjects (65.05%) had a CD4+ cell count in the range of 6-640 cells per µl of blood while 30.09% were in the range between 641- 1335 cells per µl of blood and 4.85% had a cell count greater than 1336 cells per µl of blood.CD4+ cell count is used as a basis of intiating ARV

therapy. Low levels of CD4+ cells was associated with low levels of MCH ($r=0.262, p>0.01$) and MCHC ($r=0.243, p>0.05$). 77.7% of the subjects suffered from opportunistic infections.The subjects had BMI which was within the normal range (77.95%), 11.81% were under weight while 1.57% were obese.

The mean serum levels for the subjects were as follows: β-carotene (0.144 µmol/l), α-tocopherol (17.787 µmol/l) and retinol (0.937 µmol/l). They were within the recommnded range. Bioavailability studies using algorithms show that all the selected indigenous vegetables produce a positive change in serum levels with β-carotene showing a positive change of range between +0.019 to +0.472 while α-tocopherol shows a a positive change of range +4.862 to +4.947. Consumption of the food supplement also showed a positive change in both serum β-carotene (+2.170) and α-tocopherol (+7.776). The positive change in serum level would indicate an improvement in bioavailability of the three nutrients in the blood, hence strengthening the immune system of PLWHA and delay in the progression of HIV and early use of ARV's.

5.2 Recommendations

5.2.1 Recommendations from this study

Based on the above conclusions the following are recommended:

(i) PLWHA in Butula division need to be encouraged to grow and feed on green leafy indigenous vegetables since they have high levels of α-tocopherol and β-carotene both during the wet and dry seasons. The vegetables can be consumed in the fresh or dry forms. This may help preserve immune function and alter the rate of disease progression.

(ii) PLWHA should be advised to avoid unhealthy lifestyles that include alcohol consumption and tobacco use which may affect nutritional processes, all infections that affect appetite, ability to eat and nutrient retention should be treated immediately.

(iii) α-Tocopherol and β-carotene play a critical role in the maintenance of immune function and overall metabolism (Stine, 2005). Indigenous vegetables are rich in α-tocopherol and β-carotene. These vegetables can be used to come up with food formulations which can be used to enhance the bioavailability of the immune boosting vitamins and thus prolong the time before which ARVs therapy is intiated in PLWHA.

(iv) Green leafy vegetables are a very good source of minerals and vitamins and should be consumed regularly.

5.2.2 Recommendations for further research

(i) More indigenous foods should be analysed in other parts of the country to determine geographical and interspecies variation of vitamins levels.

(ii) In vitro and in vivo studies are required to come up with algorithms to estimate bioavailability of vitamins

(iii) An intervention study should be carried out on PLWHA using the food supplement prepared in this study to determine the actual uptake of the vitamins.

(iv) Determine shelf life of the food supplement.

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APPENDICES

APPENDIX I

CONSENT FORM

ASSESSMENT OF BIOAVAILABILITY OF ANTIOXIDANT VITAMINS IN SELECTED INDIGENOUS VEGETABLES AND THEIR POTENTIAL USE IN THE MANAGEMENT OF HIV/AIDS IN BUTULA, BUSIA KENYA

An MSC Research project by Rachel Wanjiru Nambafu of Chemistry department, Kenyatta University

Name of patient..... I.D No.....

Date of Birth..... Age..... sex.....

There exists a synergistic relationship between infection, nutritional status and immune function. The immune system protects an individual against infections. Malnutrition worsens disease, which in turn worsens malnutrition. The cycle begins when impaired immunity opens the way for disease, which distorts food assimilation and nutrition status. Disease and poor nutrition together form a downward spiral that must be broken for recovery to occur. This can be achieved by treatment of the disease and provision of adequate nutrition, which is a key player in maintaining a healthy immune system to defend against infectious disease.

The study aims at assessing the bioavailabity of vitamin A, E and C in selected indigenous foods and their use in the management of HIV/AIDS. The study shall aid in improving the quality of nutrition care and diet therapy for PLWHA. In this assessment you will be interviewed and observed, your answers will be put together with a lot of other people's so you will not be identified in any way in the overall result. In addition your CD4+/CD8+/CD3+cell counts, serum vitamin A, E and C levels will be assessed and BMI measurements will be carried out as a basis of normal hospital routine.

Absolute confidentiality will be observed in the information you give. You will not be identified in any public report or publication or to any other parties your cooperation with this study will help the researcher to come up with recommendations that will be useful to improve food service for PLWHA in Busia district.

I have read the information above and had the opportunity to ask questions and all were answered satisfactorily. I hereby give consent for my participation as explained to me.

Patient sign or thumbprint: .....

Date: .....

APPENDIX II

QUESTIONNAIRE

Section a

Social demographic and economic characteristic

Name of Interviewer: .....

Date of interview: .....

1. Respondent's age.....
2. Sex: 1 = Male 2 = Female
3. Marital status: 1 = Single 2 = Married 3 = Monogamous
   4 = Polygamous 5 = Widowed 6 = others
4. Where do you get your food? 1 = Own farm 2 = Buy
   3=Buy and farm 4 = others

Section b: Medical details (To be filled by a physician)

5. Have you had a recent illness or infection lasting 10 or more days?
   1 = Yes 2 = No
   If yes specify.....
6. Diagnosed illness.....
7. HIV/AIDS stage according to CDC criteria
   (1) (2) (3) (4)
8. How do you manage HIV/AIDS?
   1= Use of vitamin supplements 2= Use of ARVs 3=Use prophylaxis 4=others, specify

Section c: Dietary pattern

9. How many meals do you have in a day?
   1 = 1 2 = 2 3 = 3

10. Do you eat at regular times each day?

1 = Yes 2 = No

11. Are there foods you don't eat?

1= Yes 2 = No

12. If yes which ones?

1 = Dairy 2 = Meat 3 = Green vegetables

4 = Fats 5 = Legumes 6 = Fruits 7 = Cereals

13. For what reason don't you eat the food?

1 = Religion 2 = Allergic 3 = Taboo

4 = Medical 5 = Other

14. Which food do you particularly like?

1 = Dairy 2 = Meat 3 = Vegetables

4 = Legumes 5 = Fruits 6 = Cereals 7 = Fat

15. Are you on any special diet?

1 = Yes 2 = No

If yes specify.....

16. Do you drink alcohol? 1 = Yes 2 = No

17. Do you smoke? 1 = Yes 2 = No

18. **Simplified Food frequency check list**

Meal: Ingredients/ serving size	**Breakfast**	**Lunch**	**Supper**	Others / Specify
Monday				
Tuesday				
Wednesday				
Thursday				
Friday				
Saturday				
Sunday				

Section d

19. **Anthropometry**

	1st reading	2nd reading	3rd reading	Average
Weight (kg)				
Height (m)				
BMI				

Section e:

20. **Vegetables grown, their availability and method of preservation**

21. Which types of indigenous vegetables do you grow? .....

22. Do you get these vegetables throughout the year?

1=Yes 2= No

23. Which months of the year are the vegetables grown available? .....

24. Do you preserve vegetables?

1=Yes 2=No

25. Which preservation method do you use?

1=Sun drying 2=Solar drying 3=Fermentation 4=other

APPENDIX III

OPPORTUNISTIC INFECTIONS AFFECTING PLWHA IN BUTULA DIVISION

Opportunistic infections	Male		Female		Total	
	Frequency	Percentage %	Frequency	Percentage%	Frequency	Percentage %
Pallor	2	14.3	17	15.6		15.4
Lymp	5	31.3	6	5.5		8.8
Oedema	0	0	1	0.9		0.8
Urti	4	25.0	37	33.9		32.8
Headache	4	23.5	40	36.4		34.6
Skin rash	1	5.9	27	25.0		22.4
Diarrhea	1	5.9	6	5.5		5.6
Fever	3	18.8	22	20.6		20.3
Oral thrush	1	6.3	5	4.7		4.9
Herpes	1	5.9	4	3.7		4.0
Kaposi Sarcoma	1	5.9	0	0		0.8
PCP	0	0	0	0	0	0
Cryptococci	0	0	0	0	0	0
Fatigue	7	43.8	56	51.4		50.4
Pneumonia	2	11.8	17	15.6		15.1
Boils	1	5.9	2	1.8		2.4
TB	2	11.8	2	1.8		3.2
Piles	1	5.9	3	2.8		3.2
Genital	0	0	16	14.8		12.8
Cough	7	43.8	38	35.5		36.6

APPENDIX IV

MAIN SOURCES OF DISHES CONSUMED BY PLWHA IN THE STUDY

Type of foods	Foods	Percentage of food accompaniment of main dish in household 1	Percentage of food accompaniment of main dish in household 2	Average of percentages of food accompaniments of main dish in household 1and 2
Cereals	Ugali	0.0	0.4	0.2
	Porridge	0.0	1.9	0.95
	Maize	1.5	0.2	0.85
	Rice	0.4	0.4	0.4
	Chapati	0.0	0.0	0.0
	Total	01.9	2.9	2.4
Tubers	Potatoes	1.5	0.8	1.15
	Sweet potatoes	0.4	0.4	0.4
	Yams	0.0	0.0	0.2
	Cassava	0.0	0.4	0.0
	Nduma	0.0	0.0	0.0
	Total	**1.9**	**1.6**	**1.75**
Fruits	Bananas	0.0	0.8	0.4
	Avocado	0.4	0.0	0.2
	Matoke	0.0	0.0	0.0
	Total	0.4	0.8	0.6
Plant proteins	Beans	1.2	1.9	1.55
	Peas	0.0	0.4	0.2
	Total	**1.2**	**2.3**	**1.75**

Nuts	Groundnut	0.4	0.0	0.2
	Total	**0.4**	**0.0**	**0.2**
Animal proteins	Meat	0.8	0.4	0.6
	Fish	6.4	32.3	19.35
	Pork	0.4	0.0	0.2
	Milk	0.4	0.0	0.2
	Eggs	0.0	0.8	0.4
	Total	**8**	**33.5**	**20.75**
Vegetables	Cabbages	0.8	1.6	1.2
	Sukuma wiki	2.7	2.7	2.7
	Cowpea leaves	35.0	16.4	25.7
	Managu	0.8	1.5	1.15
	Miro	1.5	6.8	4.15
	Mrenda	0.8	3.8	2.3
	Chisaka	0.8	1.5	1.15
	Vegetables	40.0	10.7	25.35
	Pumpkin	0.0	1.5	0.75
	Tomatoes	0.0	0.4	0.2
	Total	**82.4**	**46.9**	**20.75**

Cereals	53.05	2.1	47.6	34.25
Tubers	29.3	1.75	1.7	10.92
Pulses	2.1	2.15	9.0	4.42
Animal sources	0.0	20.75	31.7	17.48
Vegetables	0.0	63.2	0.4	21.2
Nuts	0.0	0.2	1.2	0.05
Beverage	0.0	2.1	2.3	2.20
Others	14.1	6.1	2.3	7.50
Total	**99.5**	**98.95**	**99.3**	**99.59**

APPENDIX V

FOODS LIKED BY PLWHA IN THE STUDY

Foods	Percentage %
Bananas	0.4
Beans	4.6
Cassava	2.3
Chapati	1.9
Eggs	0.4
Fish	1.5
Githeri	3.4
Milk	0.4
Mrenda	0.4
Nduma	0.4
Potatoes	3.1
Sweet potatoes	1.5
Rice	5.3
Tea	0.4
Ugali	69.6
Uji	0.4

APPENDIX VI

β-CAROTENE AND α-tOCOPHEROL REGRESSION ANALYSIS DATA

(i) β-carotene internet data

Author	Intervention study	Sample	β-carotene				
			Dietary supplement (mg)	Baseline serum (µmol/l)	serum after intervention µmol/l	Absorbed µmol/l	Duration of study hrs/days/wks/yrs/months
David *et al.*,1997	Effects of 4 years oral supplementation with β-carotene on serum concentration of retinol, tocopherol and five carotenoids	54	25	0.405	0.735	0.330	4yrs
Sonia *et al.*,2003	Changes in retional,α-tocopherol,vitamin c,carotenoids, Zinc and selenium after supplementation during alcohol rehabilitation	53	6	0.11	0.76	0.650	3wks
Cheryl *et al.*,1992	Plasma β-carotene response in humans after meals supplemented with dietary pectin	7	25	1.638	2.577	0.939	30 hrs
		7	25	1.36	2.021	0.661	48hrs
		7	25	0.99	1.29	0.300	192hrs
Ching-jang *et al.*,2000	The bioavailability of β-carotene and α-						

	tocopherol in stir- or deep-fried vegetables in men determined by measuring the serum response to a single ingestion	10	12.7	2.47	3.03	0.560	26wks
Ribaya *et al.*,2007	Carotene-rich plant foods ingested with minimal dietary fat enhance the total body vitamin A pool size in Filipino school as assessed by stable isotope dilution methodology	39	4.2	0.24	1.11	0.87	9wk
		39	4.2	0.19	1.12	0.93	9wks
		39	4.2	0.20	1.06	0.86	9wks
Matti *et al.*,1997	The effect of α-tocopherol and β-carotene supplementation on the incidence of lung cancer and other cancers in male smokers	29133	20	0.3167	5.589	5.272	3yrs
		29133	20	0.1863	2.9808	2.7945	3yrs
		29133	20	0.5403	8.3838	7.8435	3 yrs
Francois *et al.*,1999	Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients	725	6	0.8649	2.43288	1.56798	6months
		725	6	0.8649	2.43474	1.56984	12 months

Sabrina *et al.*,2002	Variability in conversion of β-carotene to vitamin A in men as measured by using a double tracer study design	3	3.44	0.34	0.36	0.02	22days
		4	1.52	0.17	0.23	0.06	22days
		2	3.09	0.12	0.13	0.01	22days
		11	10.38	0.84	0.9	0.06	22 days
		12	6.2	0.36	0.39	0.03	22days
		10	1.32	0.34	0.63	0.29	22days
		37	1.69	0.25	0.31	0.06	22days

(ii) α-tocopherol internet data

Author	Intervention	Sample size	α-tocopherol				
			Dietary supplement (mg)	Baseline serum (μmol/l)	Serum after intervention (μmol/l)	Absorbed (μmol/l)	Duration of study hrs/wks/months/days/yrs
Annette *et al.*,2000	Amount of fat in the diet affects bioavailability of lutein esters but does not ofα-carotene, β-carotene and vitamin E in humans	15	50	25.1	30.1	5.0	7days
		15	50	24.4	29.9	5.5	7days

Sonia *et al.*,2003	Changes in retional,α-tocopherol,vitamin c,carotenoids,Zinc and selenium after supplementation during alcohol rehabilitation	53	30	25.6	25.77	0.17	3wks
Ching-jang *et al.*,2000	The bioavailability of β-carotene andα-tocopherol in stir- or deep-fried vegetables in men determined by measuring the serum response to a single ingestion	10	12.7	11.22	13.35	2.23	26wks
Walter *et al.*,1983	Vitamin A,E and carotene: effects of supplementation on their plasma levels	15	35	24.61	25.58	0.97	16wks
Matti *et al.*,1997	The effect of α-tocopherol	29133	50	26.703	40.1706	13.4676	3yrs

	and β-carotene supplementation on the incidence of lung cancer and other cancers in male smokers	29133	50	21.5946	33.2046	11.61	3yrs
		29133	50	32.9724	48.9922	16.0198	3yrs
Francois *et al.*,1999	Impact of trace elements and vitamin supplementation on immunity and infections in institutionalized elderly patients	72	15	29.0018	32.1368	3.135	6months
		72	15	29.0178	32.1992	3.18144	12months
Richard *et al.*,2006	Human vitamin E requirements assessed with the use of apples fortified with deuterium-labeled α-tocopheryl acetate	5	22	20.8	25.6	4.8	72 hrs
		5	22	21.1	27.9	6.8	72hrs
		5	22	20.7	29.9	9.2	72hrs
Meydani *et al.*,1997	Vitamin E supplementation and in vivo immune response in healthy elderly subjects	18	60	9.4	36.6	27.2	1month
		19	200	23.4	49	25.6	1month
		18	800	31.1	56.9	25.8	1months
		18	60	11.2	38.4	27.2	4months
		19	200	25.4	51	25.6	4months
		18	800	45.2	71	25.8	4months

APPENDIX VII

SERUM α-TOCOPHEROL LEVELS

SAMPLE ID	µg/ml	µg/dl	µmol/l
2	4.0571	405.7071	9.420519
3	5.6974	569.737	13.22929
4	6.6097	660.9725	15.34778
5	5.6974	569.737	13.22929
6	10.5503	1055.033	24.49785
7	5.6974	569.737	13.22929
8	3.4553	345.5304	8.023216
10	10.395	1039.503	24.13726
11	2.0091	200.9124	4.665186
12	10.7347	1073.474	24.92606
13	3.0477	304.7656	7.076657
14	17.9948	1799.476	41.78383
15	11.7733	1177.327	27.33753
16	2.1159	211.5889	4.913094
17	8.5315	853.1496	19.81013
18	6.0030	50.0311	13.00392
19	12.608	1260.798	29.27572
20	7.2891	728.9139	16.92538
21	9.2303	923.0321	21.43281
22	1.1259	112.5886	2.614307
23	12.5497	1254.974	29.1405
25	6.5127	651.2666	15.12241
27	2.2518	225.1771	5.228612
28	7.6871	768.7081	17.8494
29	15.4809	1548.093	35.94671
31	15.9565	1595.652	37.05103
32	20.0524	2005.241	46.5617
33	2.3877	238.7654	5.544133
34	8.3083	830.826	19.29178
35	8.0171	801.7082	18.61566
36	6.1341	613.4136	14.24346
37	5.4450	544.5016	12.64333
39	3.7853	378.5305	8.789478
41	12.2294	1222.945	28.39678
42	5.0762	507.6191	11.78692
43	7.5124	751.2375	17.44373

44	2.3003	230.0301	5.341299
45	11.0162	1101.621	25.57964
46	14.7821	1478.21	34.32404
47	8.2888	828.8848	19.24671
52	8.2694	826.9436	19.20163
55	20.0524	2005.241	46.5617
57	7.0368	703.6785	16.33941
58	9.3759	937.591	21.77086
62	13.4136	1341.357	31.14631
66	6.0468	604.6782	14.04063
68	7.3280	732.7963	17.01553
71	5.9400	594.0017	13.79272
73	6.6583	665.8255	15.46047
76	10.298	1029.797	23.91189
81	2.4459	244.589	5.679357
82	14.5686	1456.857	33.82822
85	8.4830	848.2966	19.69745
86	5.0568	505.678	11.74184
87	4.3871	438.7072	10.18678
88	3.4553	345.5304	8.023216
89	2.0577	205.7653	4.77787
91	4.4259	442.5895	10.27693
92	4.2997	429.9719	9.983948
95	5.2994	529.9427	12.30527
96	4.7074	470.7367	10.93051
97	5.2606	526.0604	12.21512
98	1.8441	184.4123	4.282054
99	1.6015	160.1475	3.718625
100	2.5721	257.2066	5.972337
102	7.3959	739.5904	17.17329
103	4.4259	442.5895	10.27693
104	5.6683	566.8252	13.16168
106	2.4653	246.5301	5.724429
107	3.1641	316.4127	7.347103
110	5.7168	571.6782	13.27437
117	3.6882	368.8246	8.564107
118	3.3291	332.9127	7.730233
123	5.9983	599.8253	13.92794
128	5.2412	524.1192	12.17005
137	5.3091	530.9133	12.32781
138	2.1353	213.53	4.958167
140	6.3283	632.8254	14.69421

143	4.4065	440.6484	10.23186
145	7.3668	736.6786	17.10568
146	5.5130	551.2957	12.80109
148	28.0501	2805.008	65.13229
150	12.6565	1265.651	29.38841
152	9.5797	957.9734	22.24414
154	12.1906	1219.062	28.30663
155	16.3448	1634.475	37.95252
162	9.9486	994.8559	23.10055
163	10.9289	1092.886	25.3768
164	11.5403	1154.033	26.79664
165	25.7595	2575.949	59.81353
166	16.0633	1606.328	37.29894
170	6.2603	626.0313	14.53645
171	8.9391	893.9144	20.75669
173	11.0259	1102.592	25.60217
180	3.9309	393.0894	9.127536
182	15.0053	1500.534	34.84239
184	8.2888	828.8848	19.24671
191	3.3777	337.7657	7.84292
195	5.7750	577.5017	13.40959
201	8.3665	836.6495	19.427
203	11.1618	1116.18	25.91769
211	13.5106	1351.063	31.37168
217	5.5227	552.2663	12.82362
218	5.2994	529.9427	12.30527
219	14.1318	1413.181	32.81405
220	12.8603	1286.033	29.86169
221	9.9486	994.8559	23.10055
222	5.5130	551.2957	12.80109
223	7.3377	733.7669	17.03807
224	2.5041	250.4125	5.814578
228	4.5521	455.2072	10.56991
229	10.9191	1091.915	25.35427
230	10.0553	1005.532	23.34846
231	9.5700	957.0028	22.22161
233	7.7162	771.6199	17.91701
235	1.1938	119.3827	2.772066
238	4.7074	470.7367	10.93051
241	3.1544	315.4421	7.324566
242	3.1156	311.5597	7.234416
245	8.3083	830.826	19.29178

248	2.4847	248.4713	5.769504
253	6.2603	626.0313	14.53645
254	2.2809	228.0889	5.296224
255	8.3083	830.826	19.29178
259	6.0468	604.6782	14.04063
260	3.9115	391.1482	9.082461
261	5.0568	505.678	11.74184
262	3.5718	357.1775	8.293662
263	8.2888	828.8848	19.24671
Total			2294.49
Mean			17.787

APPENDIX VIII

SERUM RETINOL LEVELS

SAMPLE ID	µg/100ml	µg/dl	µmol/l
2	0.0216	2.1586	0.0753
3	0.2809	28.0859	0.9802
4	0.3108	31.0765	1.0846
5	0.3526	35.2621	1.2306
6	0.1065	10.6497	0.3717
7	0.2221	22.2064	0.7750
8	0.1285	12.8490	0.4484
10	0.1710	17.1037	0.5969
11	0.2257	22.5714	0.7877
12	0.2179	21.7940	0.7606
13	0.1935	19.3500	0.6753
14	0.2279	22.7866	0.7953
15	0.1144	11.4364	0.3991
16	0.3206	32.0592	1.1189
17	0.2632	26.3167	0.9185
18	0.3496	34.9602	1.2201
19	0.2613	26.1262	0.9118
20	0.2021	20.2066	0.7052
21	0.1667	16.6683	0.5817
22	0.2849	28.4934	0.9944
23	0.2427	24.2699	0.8470
25	0.2619	26.1940	0.9142
27	0.1375	13.7495	0.4799
28	0.4995	49.9465	1.7431
29	0.2280	22.7998	0.7957
31	0.3294	32.9448	1.1498
32	0.1766	17.6575	0.6162
33	0.3880	38.8028	1.3542
34	0.3739	37.3947	1.3051
35	0.2012	20.1167	0.7021
36	0.2195	21.9525	0.7661
37	0.1667	16.6745	0.5819
39	0.2699	26.9893	0.9419
41	0.1501	15.0066	0.5237
42	0.2327	23.2682	0.8121
43	0.1360	13.5995	0.4746
44	0.3591	35.9070	1.2532

45	0.4039	40.3861	1.4095
46	0.1170	11.7009	0.4084
47	0.4978	49.7762	1.7372
52	0.1012	10.1159	0.3530
55	0.2310	23.0962	0.8061
57	0.1652	16.5187	0.5765
58	0.2572	25.7178	0.8976
62	0.1886	18.8586	0.6582
66	0.2685	26.8528	0.9372
68	0.1855	18.5484	0.6473
71	0.5062	50.6221	1.7667
73	0.1503	15.0281	0.5245
76	0.0883	8.8253	0.3080
81	0.3225	32.2504	1.1255
82	0.2052	20.5163	0.7160
85	0.0878	8.7816	0.3065
86	0.3170	31.6958	1.1062
87	0.2529	25.2925	0.8827
88	0.1692	16.9222	0.5906
89	0.3034	30.3447	1.0590
91	0.5605	56.0487	1.9561
92	0.1884	18.8410	0.6576
95	0.1866	18.6570	0.6511
96	0.3326	33.2621	1.1608
97	0.2421	24.2132	0.8450
98	0.1454	14.5392	0.5074
99	0.1919	19.1908	0.6698
100	0.2778	27.7760	0.9694
102	0.1187	11.8723	0.4143
103	0.2878	28.7787	1.0044
104	0.2532	25.3232	0.8838
106	0.3068	30.6754	1.0706
107	0.1612	16.1206	0.5626
110	0.2655	26.5500	0.9266
117	0.3417	34.1688	1.1925
118	0.1962	19.6207	0.6848
123	0.1367	13.6660	0.4769
128	0.1901	19.0144	0.6636
137	0.1902	19.0160	0.6637
138	0.2945	29.4491	1.0278
140	0.2706	27.0563	0.9443
143	0.0437	4.3669	0.1524

145	0.2413	24.1275	0.8420
146	0.3126	31.2598	1.0910
148	0.1412	14.1215	0.4928
150	0.1991	19.9091	0.6948
152	0.1721	17.2109	0.6007
154	0.2396	23.9583	0.8361
155	0.0474	4.7366	0.1653
162	0.3248	32.4751	1.1334
163	0.3202	32.0203	1.1175
164	0.3442	34.4183	1.2012
165	0.2147	21.4650	0.7491
166	0.5894	58.9412	2.0570
170	0.2210	22.1018	0.7714
171	0.2498	24.9836	0.8719
173	0.8353	83.5267	2.9151
180	0.2412	24.1210	0.8418
182	0.2954	29.5357	1.0308
184	0.3289	32.8875	1.1478
191	0.2110	21.0977	0.7363
195	0.2065	20.6513	0.7207
201	0.2221	22.2110	0.7752
203	0.1564	15.6385	0.5458
211	0.2364	23.6358	0.8249
217	0.4900	48.9997	1.7101
218	0.4368	43.6755	1.5243
219	0.3633	36.3283	1.2679
220	0.2019	20.1926	0.7047
221	0.3179	31.7886	1.1094
222	0.2480	24.7998	0.8655
223	0.1629	16.2883	0.5685
224	0.5642	56.4215	1.9691
228	0.3033	30.3333	1.0586
229	0.3395	33.9466	1.1847
230	0.2922	29.2152	1.0196
231	0.3423	34.2341	1.1948
233	0.2022	20.2246	0.7058
235	0.1312	13.1151	0.4577
238	0.3041	30.4149	1.0615
241	0.3473	34.7310	1.2121
242	0.3324	33.2403	1.1601
245	0.2812	28.1220	0.9815
248	0.2324	23.2416	0.8111

253	0.3376	33.7620	1.1783
254	0.3423	34.2270	1.1945
255	0.3505	35.0469	1.2231
259	1.6195	161.9527	5.6521
260	0.2150	21.5024	0.7504
261	0.2237	22.3684	0.7807
262	0.2626	26.2586	0.9164
263	0.3396	33.9608	1.1852
Total			120.85
Mean			0.937

APPENDIX IX

SERUM β-CAROTENE LEVELS

Sample ID	µg/ml	µg/dl	µmol/l
2	0.532969	53.29689	0.09929211
3	0.222558	22.25584	0.04146264
4	0.695007	69.50071	0.12947982
5	0.587632	58.76324	0.10947591
6	0.783835	78.38352	0.1460285
7	0.700376	70.03758	0.13048001
8	0.883889	88.38889	0.16466851
10	0.758456	75.84558	0.14130031
11	0.082971	8.297135	0.01545756
12	0.801406	80.14056	0.14930187
13	0.398262	39.82625	0.0741963
14	4.029479	402.9479	0.75069198
15	1.002977	100.2977	0.18685465
16	0.354336	35.43365	0.06601288
17	0.364586	36.45859	0.06792235
18	0.782371	78.2371	0.14575572
19	1.084972	108.4972	0.20213036
20	0.306018	30.60179	0.05701113
21	0.936600	93.66001	0.1744886
22	0.244033	24.40334	0.04546342
23	1.707746	170.7746	0.31815301
25	0.737957	73.7957	0.13748138
27	0.896579	89.65787	0.1670326
28	1.331446	133.1446	0.24804842
29	2.008883	200.8883	0.37425487
31	0.158134	15.81336	0.0294603
32	2.483284	248.3284	0.46263576
33	0.289912	28.99117	0.05401054
34	0.781395	78.13949	0.14557387
35	0.570550	57.05501	0.10629347
36	0.657914	65.7914	0.12256938
37	0.748206	74.82064	0.13939084
39	0.234760	23.47601	0.04373581
41	1.153302	115.3302	0.21486012
42	0.600322	60.03221	0.11184001
43	1.142564	114.2564	0.21285973
44	0.288936	28.89355	0.05382869
45	1.309971	130.9971	0.24404764

46	0.632535	63.25345	0.11784118
47	0.486603	48.66026	0.09065406
52	0.767241	76.7241	0.14293699
55	2.132852	213.2852	0.39735029
57	0.824345	82.43448	0.15357543
58	0.282103	28.21026	0.05255571
62	0.398262	39.82625	0.0741963
66	0.151301	15.13007	0.02818732
68	0.431451	43.1451	0.08037933
71	0.310899	31.08985	0.0579204
73	0.850700	85.07004	0.15848548
76	0.759432	75.94319	0.14148216
81	0.170823	17.08234	0.03182439
82	1.559861	155.9861	0.29060218
85	0.338718	33.87183	0.06310323
86	0.568598	56.85978	0.10592977
87	0.55298	55.29796	0.10302011
88	0.080043	8.004295	0.014912
89	0.311387	31.13866	0.05801132
91	0.938064	93.80643	0.17476138
92	0.258187	25.81873	0.0481003
95	0.385085	38.50847	0.07174128
96	0.472449	47.24486	0.08801718
97	0.886329	88.63292	0.16512314
98	0.327493	32.7493	0.06101195
99	0.308946	30.89463	0.05755669
100	0.423154	42.31539	0.07883357
102	0.785299	78.52994	0.14630128
103	0.453414	45.3414	0.08447103
104	0.718922	71.89223	0.13393523
106	0.294304	29.43043	0.05482888
107	0.092733	9.273269	0.0172761
110	0.531993	53.19928	0.09911025
117	0.091757	9.175655	0.01709425
118	0.545171	54.51706	0.10156528
123	0.784323	78.43233	0.14611943
128	0.578847	57.88472	0.10783923
137	0.780907	78.09068	0.14548294
138	0.165455	16.54546	0.0308242
140	1.267997	126.7997	0.23622793
143	0.643760	64.37601	0.1199325
145	0.526136	52.6136	0.09801913

146	0.744790	74.47899	0.13875436
148	2.116258	211.6258	0.39425877
150	1.253355	125.3355	0.23350012
152	0.287471	28.74713	0.05355591
154	0.348480	34.84797	0.06492176
155	1.074235	107.4235	0.20012997
162	0.843867	84.38674	0.1572125
163	0.516375	51.63746	0.0962006
164	0.670604	67.06037	0.12493348
165	0.585192	58.51921	0.10902128
166	0.298209	29.82088	0.0555563
170	0.859486	85.94856	0.16012216
171	0.952706	95.27063	0.17748919
173	0.772610	77.26097	0.14393719
180	1.080580	108.058	0.20131202
182	0.594953	59.49534	0.11083982
184	0.76236	76.23603	0.14202772
191	0.750647	75.06467	0.13984548
195	0.997608	99.76085	0.18585446
201	0.850212	85.02123	0.15839455
203	0.815072	81.50715	0.15184782
211	1.105959	110.5959	0.20604022
217	0.613012	61.30119	0.11420411
218	0.602762	60.27625	0.11229465
219	0.500757	50.07565	0.09329094
220	0.481722	48.17219	0.08974479
221	0.277222	27.72219	0.05164644
222	1.803407	180.3407	0.33597467
223	2.20899	220.899	0.41153487
224	1.046903	104.6903	0.19503807
228	0.368978	36.89785	0.06874069
229	0.537850	53.78496	0.10020138
230	0.275270	27.52697	0.05128274
231	1.253355	125.3355	0.23350012
233	0.277222	27.72219	0.05164644
235	3.884035	388.4035	0.72359578
238	1.783396	178.3396	0.33224667
241	1.392943	139.2943	0.2595052
242	0.549563	54.95632	0.10238362
245	0.368978	36.89785	0.06874069
248	0.205476	20.54761	0.0382802
253	1.178681	117.8681	0.21958832

254	0.426570	42.65704	0.07947006
255	1.263605	126.3605	0.23540959
259	0.335302	33.53019	0.06246674
260	0.275270	27.52697	0.05128274
261	0.256723	25.67231	0.04782752
262	0.436332	43.63317	0.08128859
263	0.422178	42.21778	0.07865172
Total			18.601
Mean			0.144

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THIS IS TO CERTIFY THAT:

Prof./Dr./Mr./Mrs./Miss. RAUHNI KANJIRU

KAMBATU

of (Address) KENYATTA UNIVERSITY

P.O.BOX 43844 KAIREBA

has been permitted to conduct research in.....

Location,

BUSIA District,

WESTERN Province,

on the topic ASSESMENT OF THE BIOAVAILABILITY

OF VITAMIN A,E AND C IN SELECTED

INDIGENOUS FOODS AND THEIR USE IN

MANAGEMENT OF HIV/AIDS

for a period ending 30TH MAY, 20 08

PAGE 3

Research Permit No. MOST 13/001/37c 217

Date of issue 4th May 2007

Fee received SHS 500

Applicant's Signature

P.O.ADERA

Permanent Secretary
Ministry of
Science and Technology