TRADITIONAL VEGETABLE PREPARATION METHODS IN RURAL TANZANIA AND CAROTENOIDS RETENTION AND IN-VITRO IRON BIOAVAILABILITY IN VEGETABLE DISHES

BY

JOYCE BONIFACE NGEGBA

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
HUMAN NUTRITION OF SOKOINE UNIVERSITY OF AGRICULTURE,
MOROGORO, TANZANIA

2007

ABSTRACT

Vitamin A and iron deficiency problems affect a huge proportion of population in Sub-Sahara Africa including Tanzania and vegetables are potential rich and sustainable sources of these nutrients. This study was designed to investigate feasible food preparation methods to increase carotenoids retention and iron bioavailability of leafy vegetable dishes traditionally prepared by Tanzanian households. The first part of the study used structured questionnaire to interview 156 women in Arumeru, Singida, and Kongwa districts to identify the traditional methods of preparing indigenous vegetables. The second phase assessed lutein and β-carotene retention and in-vitro iron bioavailability in sweet potato leaf dishes. The most consumed vegetables identified by the survey were amaranth (81.4%), jute mallow (75%) and sweet potato leaves (64.7%), most commonly cooked by boiling in Singida and Kongwa and by stir-frying in Arumeru districts. In the laboratory, fresh and dried sweet potato leaves were prepared by traditional modified methods. Traditional methods involved cooking with sunflower oil and without oil. Modified methods involved the use of oil, tomato, lemon and soybean. In-vitro iron bioavailability was done by simulating gastrointestinal digestion system. Carotenoids were analysed by High Performance Liquid Chromatography method. Traditional methods retained carotenoids in the range of 17 – 72%, while modified methods retained carotenoids in the range of 60 – 116%. Iron bioavailability was improved by 1.52 – 3.30% in modified dishes. The highest levels of β-carotene and lutein retention and iron bioavailability were obtained in dishes cooked with oil + tomato. The study concludes that modified preparation methods with appropriate ingredients have potential for retaining lutein and β-carotene; and improve in-vitro iron bioavailability. Furthermore, dishes with higher retention of lutein and β-carotene also showed higher iron bioavailability, suggesting a relationship between the two. Hence there is a need for further studies to explain this relationship.

DECLARATION

I, JOYCE BONIFACE NGEGBA, do hereby declare to the Senate of the Sokoine University of Agriculture that this dissertation is my original work and that it has never been submitted for a degree award in any other University.

14/11/2007

Joyce Boniface Ngegba
(MSc. Candidate)

Date

The above signature is confirmed

14/11/2007

Prof. John Msuya
(Supervisor)

Date

COPYRIGHT

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to Prof. J. Msuya, my supervisor in the department of Food Science and Technology, Sokoine University of Agriculture, whose wise guidance and hard work led me to accomplish this task. I am also very much grateful to him for his facilitative role in linking me with AVRDC where I did data collection. I am grateful to Dr. Ray- Yu-Yang, head of Nutrition Unit, AVRDC, Taiwan for her quality guidance during data collection in Taiwan. Her constructive criticism and tireless efforts enabled me to accomplish laboratory analysis and literature review in Taiwan successfully. My special gratitude goes also to Dr. Moses Ngegba, my beloved husband, for his great efforts and contributions in data analysis and editing drafts. I also thank him for his prayers, moral and material support that played a great role in my accomplishment.

This work could not be accomplished without financial support. Hence I acknowledge Belgium Technical Co-operation (BTC) for granting me a scholarship which enabled me to pursue this two year study at Sokoine University of Agriculture (SUA). I am also indebted to AVRDC, Regional Center for Africa (RCA), Arusha, for the financial support through the project "Promotion of neglected indigenous vegetable crop for nutritional health in Eastern and Southern Africa" - Phase II. I am indeed very grateful to Dr. Detlef Virchow, the RCA director, Dr. Mel Oluoch, the training specialist, and Ms. Genya Jacob, the secretary, whose roles facilitated a comfortable and valuable stay at RCA, and my travel to Taiwan. Along with these, my sincere thanks go to Ms. Gudrun Keller, a PhD student at Giessen University in

Germany, for her willingness to travel with me and arranged other logistics during our field visits. I also recognize and appreciate the role of Mr. Swai, who arranged my field visits and Mr. Hassan Mndiga who guided me during my field visits in Arumeru district.

I also acknowledge and appreciate the roles of Ms. Jane and Ms. Lin and the rest of the laboratory technicians for their tireless efforts in helping me to analyze the samples at Nutrition laboratory, AVRDC Taiwan. The staff, trainees and friends who provided support and company during my three month stay at Shanhua deserve my sincere gratitude. My special thanks to Pastor and members of Jiankang Baptist Church, Tainan, for their warm fellowship which made me feel at home in Taiwan. To my church pastors and members of Bethel Revival Temple in Morogoro, I am grateful for their prayers, moral and physical support. I am grateful to my children Nicanor and Ebenezer, whose patience and endurance allowed me to study at their tender age and to the rest of my family members for their prayers and encouragement. Last, but not the least, to the many people who have contributed in diverse ways to the success of this study. Though I can not mention all of them by name, but to them all I extend my gratitude.

DEDICATION

To the Almighty God through His Son Jesus Christ, our Lord and Savior who gives learning and skill in literature and wisdom. I dedicate this work.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND SYMBOLS

ACC/SCN - Administrative Committee on Coordination/ Sub-Committee on Nutrition.
AVRDC - Asian Vegetable Research and Development Centre
FAO - Food and Agriculture Organization
FW - Fresh Weight
HPLC - High Performance Liquid Chromatography
pH - Hydrogen ion Concentration
RCA - Regional Centre for Africa
ROC - Republic of China
SAS - Statistical Analysis System
SUA - Sokoine University of Agriculture
UNICEF - United Nations International Children's Emergency Fund
UNU - United Nations University
URT - United Republic of Tanzania
VAD - Vitamin A Deficiency
WHO - World Health Organization
α - Alpha
β - Beta
γ - Gamma

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information and Problem Statement

Micronutrients deficiency, also known as hidden hunger is a public health problem that is affecting a huge proportion of human population especially in developing countries. Most physiological disorders, poor health, reduced productivity. reduced intellectual potential and increased mortality are associated with it (Maberly *et al.*, 1994; Friis, 2005). Micronutrient deficiencies are affecting the population at all stages in the lifecycle; however, children at rapid stages of growth and development and pregnant women are the most vulnerable (Sanghvi *et al.*, 2007).

Vitamin A and iron are among the three nutrient deficiencies of public interest as they affect over 2 billion people worldwide. It is estimated that 25% of preschool children and 18% of women are vitamin A deficient; whereas 37% of the world's total population is iron deficient (Borwankar *et al.*, 2007). Data for developing countries show that about 40% of the population suffers from iron deficiency and 40% of children are growing up with insufficient vitamin A (UNICEF, 2003). In Tanzania, 65% of children under 5 years of age suffer from iron deficiency while 37% are estimated to have sub-clinical vitamin A deficiencies (UNICEF, 2003). Approximately 35% of these children are growing up with lowered immunity (AVRDC, 2005). The prevailing situation calls for urgent and effective measures to overcome micronutrient deficiency which is precondition for ensuring rapid and appropriate development (Maberly *et al.*, 1994).

Micronutrients are vitamins and minerals that are needed in small amounts in the body. The human body cannot synthesize vitamins and minerals; they must be provided by the diet. Recent studies have also confirmed the importance of micronutrients in HIV infection. HIV infection seems to impair micronutrient status whereas micronutrient status may affect HIV transmission, progression and morbidity (Fawzi, 2003). In a study done in Rwanda, a relationship was found between maternal serum retinol and mother-to-child HIV transmission (Friis, 2005). In another study in Tanzania iron deficiency was found to be associated with all-cause and AIDS-related deaths and a 50% decline in CD4 cell count among pregnant women (O'Brien *et al.*, 2005). Vitamin A has a role in immune functions and in the prevention of diarrhoea and respiratory tract infections (Bobat *et al.*, 2005; Friis, 2005).

1.1.1 Strategies to combat micronutrients deficiencies

Strategies to combat micronutrients deficiencies include fortification, supplementation and food based strategies. Fortification involves addition of nutrients in foods and other consumable products. However, fortification has been associated with increased price of fortified foods and difficulties in enforcement of fortification regulations (FAO. 1997). Supplementation provides nutrients other than those taken in the normal diets (SCN, 1993). However, the routine provision of single micronutrient supplementation has not been effective in combating the coexisting micronutrient deficiencies in developing countries (Gibson. 2004). Among all the strategies, food based strategies seem to be a sustainable solution to micronutrients deficiencies especially in developing countries (WHO. 2001; FAO/WHO, 2003). The strategies involve intake of micronutrients rich foods such as fruits and vegetables and optimizing processing methods (Tontisirin *et al.*, 2002). This is because micronutrient deficiencies rarely occur in isolation. For example, deficiencies of iodine and vitamin A or of iron and vitamin A or zinc are often observed in the same populations (Allen and Gillespie, 2001).

1.1.2 Sweet potato leaves and other indigenous vegetables source of micronutrients

Indigenous vegetables are defined as domesticated or semi-wild vegetable crops that are grown in a particular region as an integral part of a local food (AVRDC. 2005). The plants usually evolve naturally or are introduced and then become adapted in a given area. Sweet potato (*Ipomea batatas*) is a dicotyledonous plant that belongs to *convolvulaceae* family. The plant is indigenous to tropical and subtropical regions; it is thought to originate from South or Central America (Padda, 2006). Sweet potato leaves and other indigenous vegetables are important relish for staple foods in Tanzania (Mosha *et al.*, 1991). In Africa, vegetables provide most requirements of vitamin A and iron in forms of provitamin A carotenoids and non–heme iron respectively (SCN, 1993). This is because of the high costs of foods of animal origin which are not affordable to majority of the population (Tontisirin *et al.*, 2002).

Indigenous vegetables provide high levels of micronutrients and fiber, and are sometimes better nutritional sources than the modern vegetables (Sato *et al.*, 2002; Rensburg *et al.*, 2004). An assessment of nutrients contents in sweet potato leaves found high mineral contents especially iron and vitamins such as provitamin A carotenoids (Ishida *et al.*, 2000). Studies carried out in Zimbabwe confirmed the increasing role of indigenous vegetables in food security and income generation (Dhewa, 2003). They also require less capital investments, are better adapted to local areas and less-labor intensive management. Indigenous vegetables are highly preferred. For example, a study in Tanzania showed that most of the indigenous vegetables were preferred by 50 to 90 percent of the people surveyed (Lyimo *et al.*, 2003). Since the vegetables are affordable they provide a sustainable source of micronutrients to rural and urban families (Sato *et al.*, 2002; Dhewa, 2003). The production and nutritional potential of sweet potato make it to be among the promising crops in improving nutritional and food security in developing countries. It is a relatively hardy crop which can resist erratic rainfall patterns in Sub-Saharan African (Stathers, 2005). The leaves of sweet potato can continuously be harvested throughout the year for a number of months, which makes it a dependable source of micronutrients as compared to other vegetables (Yashimoto *et al.*, 2002; Stathers, 2005).

1.2 Justification

In spite of the proven potential of sweet potato leaves and other indigenous vegetables in alleviating micronutrients deficiency in Sub-Saharan Africa, they are often neglected in research (Rensburg *et al.*, 2004, Madisa and Tshamekang, 2006). A number of studies in Africa (Mwajumwa *et al.*, 1991; Raja *et al.*, 1991; Kinabo *et al.*, 2004) have focused on nutritive value of the uncooked vegetables. This tendency might be associated with high costs and analytical difficulties involved in the assessment of cooked vegetables (Marcela and Rodriguez-Amaya, 2004). For example, although cooking makes the extraction of carotenoids easier, the incorporation of oil and formation of degradation products during cooking cause analytical difficulties. Therefore, information about carotenoid in cooked vegetables is insufficient (Marcela and Rodriguez-Amaya, 2004). Studies on traditional vegetable processing methods in Tanzania found significant nutrient losses (Mosha *et al.*, 1991; Lyimo *et al.*, 2003; Mulokozi *et al.*, 2004). However, none of these assessed the effects of the same on iron bioavailability. Other studies elsewhere reported changes in physical and chemical composition in vegetables due to cooking and other processing methods (Severi, 1998; Reddy and Love, 1999; Agte, 2002; Turkmen *et al.*, 2004).

In Tanzania, vegetables are prepared in different ways and with varying cooking methods. Different ingredients are added at varying proportions and therefore difficult to obtain standard recipes from the communities (Weinberger and Msuya, 2004). Less is known about standard recipes that are of high nutritional quality in terms of minerals and vitamins. Hence, there is a gap in our knowledge regarding carotenoids and iron bioavailability of vegetables under varying cooking methods and the enhancement of these micronutrients. There is perhaps a great deal that we can learn from the local experience of preparations, cooking and preservation methods. The questions addressed in this study were:

(a) How are vegetables traditionally prepared in rural Tanzania households?

(b) How much carotenoids and iron are left in vegetable dishes prepared by traditional methods?

(c) What are the critical steps that are causing most nutrient loss?

(d) What feasible methods could be used to modify the traditional methods to improve nutrient retention and iron bioavailability?

(e) By how much could the carotenoids retention and iron bioavailability be increased by modified methods?

1.3 Objectives

1.3.1 General objective

This study was designed to investigate feasible food preparation methods to increase carotenoids retention and iron bioavailability in leafy vegetable dishes traditionally prepared by rural households in Tanzania.

1.3.2 Specific objectives

The major objective was achieved by the following specific objectives:

(a) To identify commonly consumed leafy vegetables and traditional vegetable preparation methods in Arumeru, Singida and Kongwa districts of north and central Tanzania.

(b) To determine the effects of the traditional preparation methods on carotenoids retention and in-vitro iron bioavailability of selected leafy vegetable (sweet potato leaves) dishes.

(c) To modify the traditional methods and assess the effects of modification on carotenoids retention and in-vitro iron bioavailability in sweet potato leaf dishes.

The findings of this study are expected to:

(a) Guide households in identifying and utilizing indigenous vegetable recipes that are of high nutritional quality, locally available and of low cost, in improving their food security and nutritional status.

(b) Contribute to scientific knowledge on micronutrients status of indigenous vegetables dishes in local communities

(c) Help policy makers to attach the due importance to indigenous vegetables in developing policies that would contribute to the prevention of micronutrients deficiency in communities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview

Food processing has advantages of making the food safer, tasteful and shelf-stable. It plays role in increasing digestibility, destruction of antagonists of enzymes and toxins. Food processing also has some disadvantages on the nutrients quality of foods (Severi *et al.*, 1998; Reddy and Love, 1999). For example, blanching results in leaching losses of vitamins and minerals, while milling and extrusion can cause the physical removal of minerals (Rensburg *et al.*, 2004). Losses in nutrients are high for vegetables which are easily perishable. The losses can also occur through chemical reactions such as Maillard reaction, oxidation, isomerisation and hydrolysis (Severi *et al.*, 1998).

African indigenous vegetables which could provide high amount of carotenoids and iron constitute part of the traditional legume and cereal based diets. The diets are characterized by high levels of nutrient inhibitors such as phytic acid, which reduces the bioavailability of nutrients (Ruel, 2001). The contents and bioavailability of some key micronutrients in foods can be enhanced by right food combinations and appropriate food processing methods (Gibson, 2007; Tontisirin, 2002). This chapter highlights some of the current knowledge on carotenoids chemistry and iron bioavailability in vegetables. The effects of processing on the former are also discussed.

2.2 Micronutrients: An Overview

Micronutrients are vitamins and minerals; they can not be synthesized by the human body; therefore they are supplied through the diet. The body needs very small amounts of these compounds, i.e. micrograms or milligrams a day; hence they are called "micro" nutrients (SCN, 1993).

2.2.1 Vitamins

Vitamins belong to organic compounds; each vitamin differs in chemical composition from the others. The primary role of vitamins is catalytic and therefore they are required in small amounts in the body (FAO/WHO, 2003). On the bases of solubility; vitamins are divided into two main groups; fat soluble vitamins (vitamin A, D, E and K) and water soluble vitamins (vitamin C and B group). Fat soluble vitamins are usually found in fat deposits in the body. The use of fat soluble vitamins is greatly dependent on the amount of their deposits in the fat tissues and not absolutely on day to day intake (Sardesai, 1998). For example, an excess amount of vitamin A is stored in the liver; this storage increases with age. Therefore, deficiency of vitamin A is more common in children who have less storage compared to adults (UNICEF, 2003). On the contrary, water soluble vitamins are not stored in the body; the excess is excreted; especially through urine. The day to day intake of water soluble vitamins is highly required (Mudambi and Rajagopal, 2000).

2.2.2 Minerals

Minerals are inorganic elements by nature. The functions and requirements of minerals in the body are related each to other (Hurrell. 2002). For example, phosphorus and calcium have a relationship in the formation of bones and teeth. Potassium, sodium, magnesium, phosphate and chloride ions have a collective function in the control of body fluids (Hurrell, 2002). Most of the minerals act alone or in combination with others as catalysts for essential enzymic processes. Minerals are divided into two main groups. First are those required in large amounts e.g. sodium, magnesium, potassium, phosphorus, chlorine and sulphur. The second group is comprised of those which are needed in smaller amounts or trace minerals, e.g., iron, iodine, copper, cobalt, manganese, molybdenum and zinc (Sardesai, 1998).

2.3 Micronutrient Deficiencies

The deficiency diseases due to vitamin and minerals are termed as micronutrient deficiencies (Sanghvi *et al.*, 2007). Under normal circumstances the deficiencies of most micronutrients are not common in human because most of them are available in varieties of foods in sufficient quantities for the body requirements. Also, with some micronutrients, the body has a strong homeostatic control mechanism to maintain adequate status and any decrease in absorption can be compensated for by a decrease in excretion (Hurrell, 2002).

Vitamin A, iron deficiency anaemia and iodine deficiency disorders are among the most common forms of micronutrient malnutrition (FAO, 1997). Of recent, zinc has also been identified as a mineral of public health concern worldwide (Solomons, 2001). The intake of most of these elements is heavily dependent on few food items such as meat, particularly liver, shell–fish, nuts and leafy vegetables. Iodine's main food sources are sea and shell fish and vegetables grown in iodine rich soils. This is the reason for occurrence of iodine deficiencies in certain sections of the population (Solomons, 2001).

2.3.1 Vitamin A deficiency

Vitamin A deficiency (VAD), often in association with protein-energy malnutrition, principally affects preschool children (Sanghvi *et al.*, 2007). It is estimated that almost 250 million children in developing countries are at risk, of whom at least 2.8-3 million are clinically deficient. VAD causes night blindness and may lead to xerophthalmia and eventually total blindness (UNICEF, 2003). Every year 250,000-500,000 children lose their sight as a result of VAD; two-thirds of these children are likely to die. An estimated 1 million additional children die each year of infectious diseases because VAD impairs their resistance to infection (FAO, 1997, UNICEF, 2003).

Vitamin A intake is often inadequate because of several reasons such as the seasonality of food sources, early abandonment of breastfeeding, and the practice of not giving vitamin A-rich foods to young children (Maberly *et al.*, 1994). Vitamin A is also destroyed by improper processing and storage because it decomposes in light (Severi *et al.*, 1997). Vitamin A absorption may also be impaired where the diet is very low in fat or where intestinal parasites are prevalent (SCN, 1993).

2.3.2 Iron deficiency

It is estimated that up to half of all anaemia is caused by dietary iron deficiency (WHO. 2001). The groups most affected by anaemia are adolescent girls. women of childbearing age and preschool-age children (Borwankar *et al.*. 2007). In adults anaemia is associated with abnormal lethargy and low work potential. Work output of men working on piece rate jobs has been directly related to the severity of anaemia. For a 10% increase in haemoglobin, there is a 10-20% increase in work output (WHO, 2001).

Maternal iron deficiency during pregnancy is associated with babies being born smaller, having an altered placental fetal ratio and an increased risk of cardiovascular diseases in adulthood (Gambling, 2006). According to SCN (1993), iron deficiency is also associated with poor attention span, inadequate fine motor skills. and reduced memory retention in adolescents. Iron deficiency causes anorexia, fatigue. irritability, susceptibility to infection. stunted growth, delayed cognitive development and impaired school performance in children (Moy, 1999; WHO, 2001).

2.4 Consumption of Indigenous Vegetables in Tanzania

A small and negligible minority of the world's population consumes the generally recommended intake of vegetables. For example, in 1998, only 6 of the 14 WHO regions had an availability of fruits and vegetables equal to or greater than the earlier recommended intake of 400 g per capita per day (FAO/WHO, 2003). It is therefore important to promote indigenous vegetables that are under-utilized but are highly adapted to local areas in growth and taste preferences (Sato *et al.*, 2002).

Indigenous vegetables were widely consumed in Tanzania before exotic species were introduced. The vegetables are currently more available and consumed in rural areas where exotic species are limited due to high cost and other factors (Lyimo *et al.*, 1991). Most indigenous vegetables are available in rainy season. They are cultivated in farms, gathered from the wild or grown in home gardens. In semi-arid areas, where their availability is seasonal, they are dried traditionally and preserved for consumption during dry season (Mosha *et al.*, 1995; Keller, 2004; Mulokozi *et al.*, 2004).

Households consume vegetables on the basis of taste, availability and affordability (Sato *et al.*, 2002). Information on the availability and consumption of foods in an area or population can be obtained through market survey or household surveys (WHO, 2001). The information can also be useful in identifying nutrients enhancers and inhibitors. At the market level, vendors are interviewed to obtain information regarding the type of foods available, rough estimates of the quantities available per season and the unit costs. At the household level, use of focus groups of women and elders is recommended to generate a list of important foods available in households from the market, home production, hunting, gathering, imported foods and the frequency of food intake (WHO, 2001). Detailed information on availability and consumption of indigenous vegetables in north eastern and central Tanzania are available (Keller, 2004; Weinberger and Msuya, 2004).

2.5 Vegetables as Sources of Carotenoids

Vitamin A is supplied from vegetables in the form of pro-vitamin A carotenoids, while animal food sources provide preformed retinol; or retinol derivatives (retinol esters, retinal) (Sardesai, 1998). Vitamin A is a generic name for fat soluble substances called retinoid which is unsaturated monohydric alcohol (Sardesai, 1998). The vitamin A has great roles in the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune functions and reproduction (SCN, 1993). In Africa, it is estimated that more than 80% of dietary intake of vitamin A comes from vegetable sources (Table 1). This is because the vegetables are accessible to majority of low income groups (Codjia, 2001; Sato *et al.*, 2002).

Table 1: The supply of vitamin A by regions

Region	Total µg RE/day	Animal sources µg RE/day	Vegetable sources µg RE/day
Africa	775	122	654 (84)
America	814	295	519 (64)
Southeast Asia	431	53	378 (90)
Europe	738	271	467 (63)
Eastern Mediterranean	936	345	591 (63)
Western Pacific	997	216	781 (78)

Source: SCN, 1993; Numbers in parentheses indicate the percent of total retinol equivalents from carotenoids food sources.

2.5.1 The chemistry of carotenoids

Carotenoids are plant pigments widely distributed with high degree of structural diversity and large variations in biological functions (Tanumihardjo, 2002). There are about 600 naturally occurring carotenoids, with 40 carotenoids regularly consumed in diets (Ruel, 2001). Carotenoids are C_{40} isoprenoid compounds, derived from eight C_5 isoprenoid units. They are divided into two main groups namely oxygenated and hydrocarbon compounds (Kopsell and Kopsell, 2006). The oxygenated carotenoids are called xanthophylls (lutein, zeaxanthin and violaxanthin). Hydrocarbon carotenoids are carotenes (β-carotene, α-carotene and lycopene) (Rodriguez–Amaya and Kimura, 2004). Carotenes are hydrocarbon with no hydroxyl side chain; xanthophylls are similar to carotenes in structure, but have hydroxyl side chains in their terminal rings (Graham and Rosser, 2000).

Structurally, carotenoids may be acyclic (e.g. lycopene), monocyclic (e.g. gamma–carotene), or dicyclic (e.g., α- and β-carotene) (Fig.1) (Graham and Rosser, 2000). In nature carotenoids exist primarily in the more stable all-trans (or all-E) form, but small amounts of cis (or Z) isomers do occur (Mosquera-Minguez *et al.*, 2002). Carotenoids are made up of a basic conjugated double bond skeleton system which gives them a light absorbing molecular group (chromophore) that produces colour. The chromophore acts as a basis of their identification and quantification. The skeleton can be modified by several reactions; cyclization, dehydrogenation and introduction of oxygen functions (Rodriguez–Amaya and Kimura, 2004).

Beta carotene (β-carotene) is the most available form of carotenoids in many plants. Other carotenoids such as lycopene, lutein and zeaxanthin are also found in plants but the body can not convert them to vitamin A. One international unit of vitamin A is equal to 3μg of retinol or 6μg of β-carotene (Tanumihardjo, 2002). The relation of retinol to carotene is shown in figure 1. Cleavage of β-carotene molecule exactly in the middle at the double bond yields two 20-carbon molecules of retinol, while α-carotene is not symmetrical as β-carotene. Therefore, only one molecule is produced when α-carotene cleavages in the middle (Graham and Rosser, 2000). This explains the reason why the conversion of α-carotene to retinol is about half as efficient as that of β-carotene (Tanumihardjo, 2002).

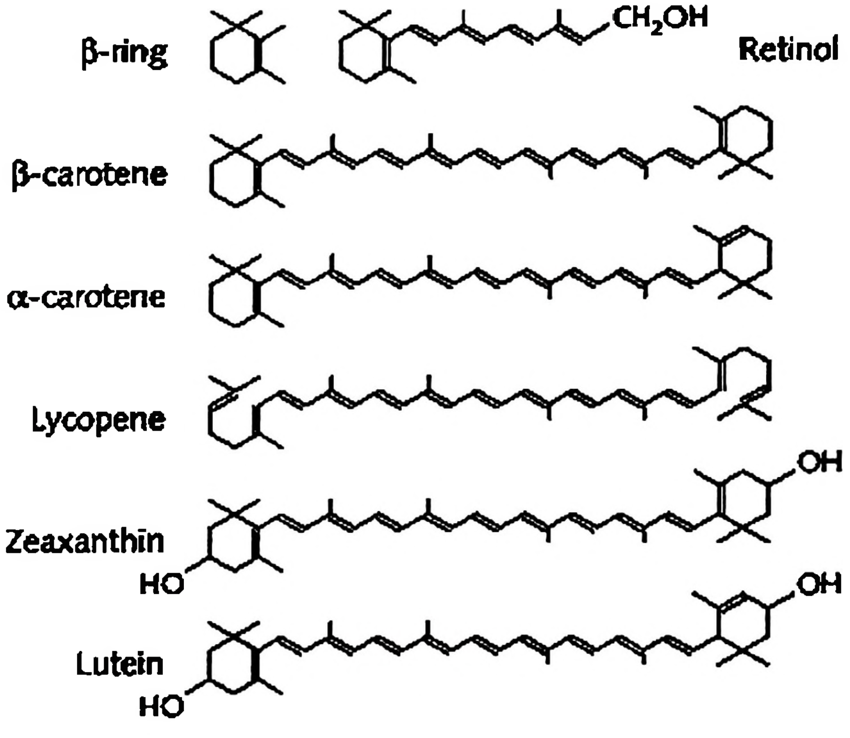

Figure 1: Structures of important carotenoid pigments in plants and of retinol, showing that a β-ring is characteristic of both retinol and α- and β-carotene
Source: Graham and Rosser (2000)

According to FAO/WHO (2003), one molecule of retinol is produced from six molecules of β-carotene (One retinol equivalent (RE) = 6 μg β-carotene). The conversion of β-carotene to retinol takes place in the intestinal mucosa by the enzyme β-carotene–15, 15'-dioxygenase; giving rise to two molecules of retinal, which is reduced into retinol. The retinol is esterified with long-chain fatty acids, transported and stored in the liver (FAO/WHO, 2003).

2.5.2 Biosynthetic pathway of carotenoids

The biosynthesis of carotenoids take place in the plant plastids whereby isopentenyl diphosphate (IPP) is isomerised to dimethylallyl diphosphate (DMAPP) by the enzyme GGPP-synthase. The enzyme also converts DMAPP to C_{20} geranylgeranyl diphosphate (GGPP). Two molecules of GGPP are condensed to form a colourless C_{40} phytoene by the enzyme phytoene synthase. Phytoene desaturase and γ-carotene desaturase enzymes convert phytoene to lycopene (Kopsell and Kopsell, 2006). Lycopene is a coloured caroteinods found much in tomatoes (Howard, 2003). Lycopene undergoes cyclization to produce two groups of carotenoids (Fig. 2); two β-ring (zeaxinthin, β-carotene, violaxanthin, neoxanthin and antheraxanthin) (Graham and Rosser, 2000).

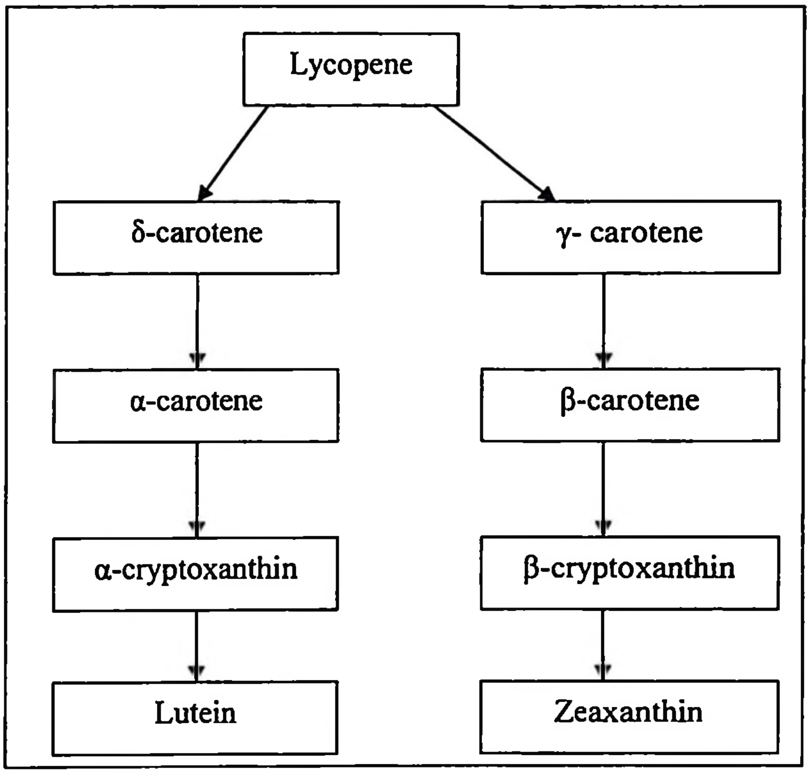

Figure 2: **Biosynthetic pathways of carotenoids**

Source: Modified from Graham and Rosser, (2000)

The second group includes the one β-ring and one ε-ring (α-carotene and lutein). Addition of oxygen converts hydrocarbon carotenoids (β- and α-carotenes) to oxygenated carotenoids (xanthophylls) (Rodriguez–Amaya, 1997). The xanthophylls (zeaxanthin and violaxanthin) undergo a reversible epoxidation reaction, which converts vioalaxanthin back to zeaxanthin via the intermediate antheraxanthin. The reaction called violaxanthin cycle is responsible for energy dissipation from incoming solar radiation (Mosquera-Minguez *et al.*, 2002).

2.5.3 Properties of carotenoids

Chemical and physical properties of carotenoids form the basis of their biological function (Fig. 3). Carotenoids are lipophilic substances and thus are insoluble in aqueous medium. Lipophilic properties are not present in certain cases where highly polar functional groups are present such as in the norbixin, a carotenoid with dicarboxyl acid structure (Krinsky, 1994). Their water insoluble nature makes them to be found in hydrophobic environment in biological systems (Mosquera-Minguez *et al.*, 2002).

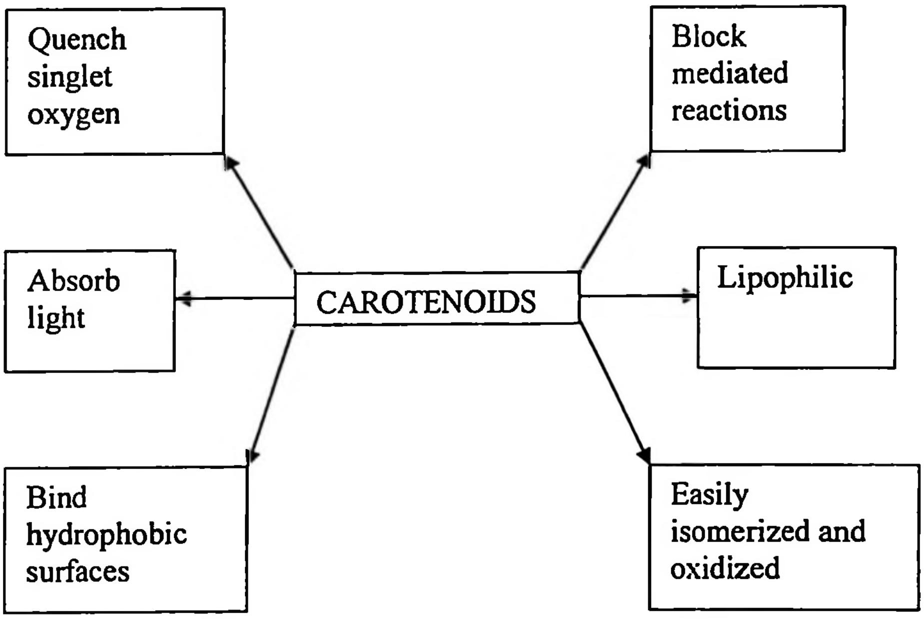

Figure 3: Properties of carotenoids
Source: Rodriguez– Amaya, 1997

The presence of extensive system of double bonds (or polyene), is responsible for their light absorption ability. The polyene chain makes the carotenoid molecule extremely susceptible to oxidizing and isomerising conditions such as light, heat and acids (Krinsky, 1994). Carotenoids can be excited by an energy transfer reaction to form triplet state species. The best known energy transfer reactions are the transfer of energy from a suitable triplet sensitizer to a carotenoid. Another reaction is the transfer of excitation energy from singlet oxygen to a carotenoid; in each case, triplet species are formed (Krinsky, 1994).

2.5.4 Role of carotenoids in human health

Provitamin A carotenoids are precursors of vitamin A, which plays a role in the maintenance of epithelial cell differentiation, normal reproductive performance and visual function. The process of isomerisation is important in visual system (Krinsky, 1994). They are free radical scavengers. The scavenging is done by provitamin A carotenoids (i.e., β-carotene, α-carotene and cryptoxanthins and non-provitamin A carotenoids; i.e., lutein, zeaxanthin and lycopene) (Kopsell and Kopsell, 2007). The antioxidant nature of carotenoid gives it a big role in reducing oxidative stress in the immune system. The oxidative stress is resulting from cumulative damage caused by reactive oxygen species. The immune system is particularly vulnerable to oxidative damage because many immune cells produce those reactive compounds as part of the body's defence mechanisms (Hughes, 2001). Degenerative disorders associated with oxidative damage include cancer, cardiovascular diseases, stroke, cataract, degeneration of retina, and aging (Hughes, 2001). The sufficient intake of carotenoids is reported to delay the onset of these disorders (Britton, 1995; Watkins, 2000; Seddon, 2007).

2.5.5 Carotenoids contents in vegetables

Weinberger and Msuya (2004), reported high contents of β-carotene in amaranth and nightshade of up to 7.54 mg per 100g of the edible portion. Other studies reported varying results on the same type of vegetables. Kidmose *et al.* (2006), reported higher levels of 1780μg/100g, 2310μg/100g and 2530 μg/100g fresh weights of β-carotene, lutein and other xanthophylls respectively in amaranth. Kidmose *et al.* (2006) also reported highest levels of the three carotenoids in drumstick as compared to all the vegetables analysed. According to Benjamin (2007) and in table 2, sweet potatoes leaves have relatively higher levels of β-carotene and other vitamins as compared to kale and cabbage.

Table 2: Comparison of the vitamins contents in sweet potato leaves with other leafy vegetables in East Africa (per 100g raw)

Leaves	β-carotene (μg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Pyridoxine (mg)	Folic acid (mg)
Sweetpotato leaves	2700	0.13	0.35	0.9	0.21	88
Sweetpotato leaves (tender tips)	2290-7050	-	0.29-0.41	0.9	-	-
Amaranthus	6545	0.04	0.22	0.7	-	85
Cassava	8280	0.16	0.32	1.8	-	
Kale	1200	0.04	0.14	0.5	-	
Taro	5535	0.13	0.34	1.5	0.19	163
Cabbage	trace	0.06	0.05	0.6	0.15	26

Source: Modified from Stathers *et al.*, (2005)

2.6 Vegetables Processing Methods

Information on vegetable processing methods and their effects on nutrients are limited in Tanzania. A survey done in Singida region reported on the cooking procedures for vegetables (Mulokozi *et al.*, 2004). The procedure involved sorting to remove dirt; and sand was removed by hitting or rubbing the leaves gently by hand. The cooking of vegetables involved boiling in water for about 20 to 39 minutes in uncovered clay pots. Most of the households surveyed were also shown to cook vegetables without oil due to economic constraints (Mulokozi *et al.*, 2004). A laboratory simulation of cooking practices in Tanzania by Lyimo *et al.* (1991) used cooking time of 90 min for cassava leaves, 50 min for pumpkin leaves and *mwage* leaves.

In semi-arid areas, the availability of leafy vegetables is limited during dry season. Traditional sun drying is widely used to preserve vegetables for use during dry season (Svanberg, 2007). Drying in enclosed (cabinet) solar driers which protect the vegetables from direct sunlight is also used to some extent (Mulokozi and Svanberg, 2003). Among all methods of drying, traditional sun-drying is the cheapest and most common. Other preservation methods include storage in earthenware pots or ventilated containers and blanching (Lyimo *et al.*, 1991; Mosha *et al.*, 1997).

2.6.1 Effects of processing on carotenoids

The changes in carotenoids composition in foods occur through physical removal, enzymatic or non-enzymatic oxidation and geometric isomerization. Isomerization changes trans-carotenoids, the usual configuration in nature, to the cis-forms (Rodriguez–Amaya and Kimura, 2004). Isomerization is prompted by light, acids or heat, while the level of oxidation depends on extent of exposure to light, type of packaging material, and presence of oxygen, storage materials, metals, enzymes, unsaturated lipids, antioxidants and physical state of the carotenoids (Rodgruez–Amaya, 2003).

2.6.1.1 Effects of thermal processing

According to Booth *et al.* (1992), carotenoids are reduced in vegetables and other foods during thermal processing, although nutrient data are inconsistent among studies. The changes differ according to method used; but as a general rule, foods boiled in an open container showed the greatest losses (Booth *et al.*, 1992). Nevertheless, values between studies are difficult to compare, given variation in conditions such as time and temperature. Different foods are processed differently; making comparisons of processing methods difficult (Rodriguez-Amaya, 1997).

According to Rodriguez-Amaya (2003), the losses during cooking generally increase in the following order microwaving < steaming < boiling < sautéing. Other methods such as deep-frying, prolonged cooking, combination of several preparation and cooking methods, baking and pickling all result in substantial losses of carotenoids. Conventional blanching and cooking is reported to cause a significant increase in the concentration of carotenoids in cowpea, peanut and pumpkin leaves (Mosha *et al.*, 1997). While in amaranth and sweet potato leaves, cooking resulted in a significant decrease in the concentration of carotenoids (Mosha *et al.*, 1997).

Table 3: **Beta–carotene contents (mg/100g dry weight) of raw selected leafy vegetables and cooked in different time intervals**

Green leaves	Raw	Cooked		
		15 minutes	30 minutes	60 minutes
Amaranth	19.12	14.23	15.85	16.66
Cowpea	14.72	21.06	29.40	22.89
Pumpkin	11.20	20.38	24.86	25.90
Sweet potato	8.99	5.38	4.73	3.78

Source: Mosha *et al.*, (1997)

2.6.1.2 Effects of drying and other methods of preservation

A number of studies have reported on the effects of traditional drying and storage practices on the retention of provitamins A carotenoids (Speek, 1988; Mosha *et al.*, 1997; Lyimo *et al.*, 2003; Koskei, 2006; Svanberg, 2007). Sun-drying and storage in ventilated containers resulted in a significant ($p<0.05$) decrease in the concentration of total carotenoids, β-carotene and α-carotene for all the vegetables (Mosha *et al.*, 1997).

According to Svanberg (2007), amaranth and cowpea leaves lose more carotenoids when they are open sun-dried than when they are solar dried or blanched. Loses of carotenoids during processing differ within vegetable species. Carotenoids contents from cooked oven, shade and direct sun dried two species of nightshade (*Solanum* *spp*) have been found to be different. Loses in *Solanum scabrum* ranged at 15.4 - 57.8%, while loses in *Solanum villosum* were 13.5- 62.2% (Koskei, 2006). Speek (1988) found carotenoids losses due to cooking. frying, fermenting, sun-drying and sun-drying followed by cooking leafy vegetables to be 14, 24, 29, 44 and 60%, respectively.

The effects of blanching, sun and oven-drying and storage conditions on β-carotene content of fresh leaves have also been reported by Badifu *et al.* (2001), who found that leaves dried by oven had lower loss values compared to sun-dried leaves. Vegetables dried by open sun method were found to have lower contents of β-carotene and α-carotene as compared to those dried by solar dryer method (Table 4).

Table 4: Beta-carotene and alpha-carotene contents of sweet potato leaves and other vegetables dried in different methods.

Vegetable/Processing	β-carotene (µg/g d.m.)	α-carotene (µg/g d.m.)
(i) Sweet potatoes		
Blanched	771	39
Solar dried	522	30
Open Sun-dried	425	12
(ii) Amaranthus		
Blanched	725	36
Solar dried	513	15
Open-sun dried	358	8
(iii) Cowpea		
Blanched	586	16
Solar dried	488	15
Open-sun dried	358	8

Source: Modified from Mulokozi and Svanberg (2003); Svanberg (2007)

2.6.2 Modification of processing methods for retention of carotenoids

2.6.2.1 Improved cooking and preparations methods

Optimizing traditional processing is among the most common ways of reducing loss of nutrients in vegetables (WHO, 2001). Simple measures such as avoiding prolonged cooking, cooking with the lid on, washing before peeling and cutting are important in retention of vitamins and minerals (FAO, 1997). Studies have shown that retention of carotenoids decreases with longer processing time, higher processing temperature, and cutting of the food. Therefore, retention can be significantly improved by reducing the processing time, lowering the temperature, and shortening the time lag between peeling, cutting and cooking (Rodgruez–Amaya, 2003). Blanching may provoke some losses of carotenoids, but the inactivation of oxidative enzymes that occurs in this type of heat treatment prevents further and greater losses during holding before thermal processing, slow processing, and storage. For example blanching cowpea leaves improved β-carotene and vitamin C retention by 15% and 7.5% respectively (Ndawula, 2004). Foods are also recommended to be consumed soon after peeling, slicing, pulping, or juicing to avoid enzymatic oxidation (Azevedo-Meleiro and Rodriguez- Amaya, 2004).

Herman and Muhilal (1995) reported high retention of carotene in vegetables cooked by modified methods as compared to Indonesian traditional methods (Table 5). Modification involved shorter time of cooking, addition of water (i.e., stewing) and the use of lid during cooking (Herman and Muhilal, 1995). Mulokozi *et al.* (2004) found significant lower amounts of 9-cis-β-carotene in vegetables cooked in modified method without oil as compared to the amounts of the same in blanched vegetables. The modification which involved reducing cooking time, covering the pot with lid while cooking and cutting leaves in small pieces did not increase carotenes contents in vegetables (Table 6).

Table 5: Comparison of carotene retention in laboratory simulated Indonesian traditional cooking methods and in modified methods

	Retention (%)	
Vegetable/Cooking method	Traditional method	Modified method
Boiling		
Cassava leaves	45	94
Papaya leaves	61	71
Stewing		
Amaranth	38	73
Chinese cabbage	20	94
Steaming		
Pumpkin leaves	-	69
Swamp cabbage	-	85

Source: Herman and Muhilal (1995)

Table 6: Total content of 9-cis- β-carotene in vegetables cooked in modified methods as compared to the blanched samples

Green leaves	Total amount (µg/g d.m)		
	Blanched	Traditional preparation	Modified preparation
Amaranth	89	96	60
Cowpea	121	93	77
Sweet potato	83	102	73
Pumpkin	83	78	75

Source: Mulokozi *et al.*, (2004)

(a) Effects of addition of oils

The addition of oil has been found to increase the contents and bioavailability of carotenoids (Table 7). Mulokozi *et al.* (2004) found that vegetables cooked in modified method with oil had 2-5 times higher amount of in-vitro accessible all-trans-β-carotene than vegetables cooked without oil. Hedren *et al.* (2002), reported accessibility of 8-29% of the β-carotene content from vegetables cooked without oil and 39-94% from leaves cooked with sunflower oil or red palm after an in-vitro digestion. Studies have reported higher effects of red palm and other types of oil as compared to sunflower oil. For example, adding red palm oil to vegetables instead of sunflower oil resulted in about twice as much accessible β-carotene, due to the high accessibility of its β-carotene content (Hedren *et al.*, 2002). Red palm oil in the maternal diet increased provitamin A carotenoids in breastmilk and serum of the mother (Canfield *et al.*, 2001). Supplementation with red palm oil, increased α-and β-carotene concentrations significantly ($P < 0.001$) in both plasma and breast milk (Lietz *et al.*, 2001). Red palm oil supplementation was also found to significantly improve maternal and neonatal vitamin A status and reduced the prevalence of maternal anaemia (Radhika *et al.*, 2003).

The consumption of tomato products with olive oil but not with sunflower oil is reported to improve lycopene content of the plasma (Lee *et al.*, 2000). Soybean has been reported to have lipids contents of 18 – 20% (Laswai, 2006). Vitamin A when added to soybean oil is well absorbed even after heat treatment (Dutra, 1998).

Table 7: Total Beta-carotene content of vegetables cooked with and without sunflower oil (μg/100g)

Green leaves	Without oil	With sunflower oil
Amaranth	2851	2974
Cowpea	1475	1418
Sweet potato	3659	4008
Pumpkin	1211	1526
Cassava	2233	2728

Source: Hedren *et al.* (2002)

(b) Effects of addition of tomatoes

Tomatoes are rich source of lycopene followed by γ-carotene, phytoene and β- carotene (Howard, 2003, Kristie *et al.*, 2005). Tomatoes and tomato products are the major source of lycopene compounds. Lycopene in fresh tomato fruits occurs essentially in the all-trans configuration (Shi and Magner, 2000). Carotenoids present in tomato fruits and various tomato products ranges from 2.62-6.29 mg/100g lycopene and 0.23-2.83 mg/100 g β-carotene (Bransca *et al.*, 2006). Studies on the effects of processing on carotenoids in tomatoes have given contradictory results. For instance, Tekeoka *et al.* (2001) found lycopene losses during processing of tomatoes into final paste to range from 9 to 28%, while no consistent changes in the other carotenoids were observed due to processing. The main causes of tomato lycopene degradation during processing are isomerization and oxidation. Isomerization converts all-trans isomers to cis-isomers (Shi and Magner, 2000). Kirstie *et al.* (2005) found that processed tomatoes have high levels of lycopene, phytoene and phytofluene but low in β-carotene α-carotene and lutein as compared to raw tomatoes (Table 8). Dewonto *et al.* (2002) found that thermal processing elevates total antioxidant activity and bioaccessible lycopene content in tomatoes, although loss of vitamin C was observed. Howard (2003), found that tomato paste or sauce has high bioavailability of carotenoids, due to change in isomers, decrease in physical size and addition of oil during processing. Tomatoes have also been reported to be rich in vitamin C. For example, a dietary intervention with tomato products increased lycopene concentration in plasma and in lymphocytes. Tomato intake also increased the concentration of vitamin C in plasma by 35% and in lymphocytes by 230% (Riso *et al.*, 2004).

Table 8: Carotenoids content of tomatoes and related tomato products

Carotenoids µ/100g)	Tomato products		
	Raw tomato	Tomato juice	Tomato sauce
β-carotene	499	270	290
α-carotene	101	0	0
Lycopene	2573	9037	15152
Lutein and zeaxanthin	123	60	0
Phytoene	1860	1900	2950
Phytofluene	820	830	1270

Source: Kirstie *et al.*, 2005

2.6.2.2 Improved preservation methods

Modern ways of drying vegetables such as the use of solar and oven driers are reported to improve the nutrient content in dried products and retain higher quantities of carotenoids and other nutrients that are destroyed by traditional methods of drying (FAO. 1997; Rensburg *et al.*, 2004). For example, in Zimbabwe several organizations are currently adopting solar drying technology to produce dried indigenous vegetables (Dhewa, 2003). Ndawula (2004) reported greatest β-carotene and vitamin C losses: 58% and 84% respectively in vegetables dried by traditional method. and the least loss: 34.5% and 71% respectively by solar dryer method. Badifu *et al.* (2001) found high levels of nutrients retention in vegetables dried by oven compared to sun-dried vegetables.

Freezing and blanching vegetables has been found to improve the retention of nutrients. Freezing (especially quick-freezing) and frozen storage generally preserve carotenoids, but slow thawing can be detrimental, particularly when the product had not been properly blanched (Mdziniso *et al.*, 2006). For example, storage of spinach leaves and amaranth in refrigerator, drying in oven, blanching for shorter time and cooking in pressure cooker were found to be better in retention of β-carotene (Yaday and Sehgal, 1995).

2.7 Vegetables Sources of Iron

Iron is transition metal, believed to be the sixth most abundant element in the universe and the fourth most abundant on the earth (Kannah, 2006). Despite its abundance, iron deficiency is a leading nutritional disorder in the world (Moy, 1998; Zlotkin, 2003). Most of the world's population, especially in developing countries get their iron requirements from plant sources, particularly vegetables. Vegetables are good sources of iron although the iron from vegetable sources is less absorbed compared to iron from animal sources (Singh, 2001).

2.7.1 The functions of iron in the body

The human body contains about 2 to 4 grams of iron. Over 65% of body iron is found in erythrocytes as hemoglobin. Up to about 10% is found as myoglobin, about 1% to 5% is found as part of enzymes, and the remaining body iron is found in the blood or in storage (Kannah, 2006). Haemoglobin is a molecule composed of four units, each containing one heme group and one protein chain. The structure of haemoglobin allows it to be fully loaded with oxygen in the lungs and partially unloaded in the tissues (Zlotkin, 2003). The iron-containing oxygen storage protein in the muscles, myoglobin (about 10%), is similar in structure to haemoglobin but has only one heme unit and one globin chain (Moy, 1998). Several iron-containing enzymes, the cytochromes (about 1 to 5%), also have one heme group and one globin protein chain (Zlotkin, 2003). These enzymes act as electron carriers within the cell and their structures do not permit reversible loading and unloading of oxygen. Their role in the oxidative metabolism is to transfer energy within the cell and specifically in the mitochondria (Mudambi and Rajagopal, 2000). Other key functions of the iron-containing enzymes include the synthesis of steroid hormones and bile acids; detoxification of foreign substances in the liver; and signal controlling in some neurotransmitters, such as the dopamine and serotonin systems in the brain (Kannah, 2006).

2.7.2 Composition of iron in vegetables

Studies of African indigenous vegetables have reported high levels of iron and other minerals (Ishida *et al*, 2000; Singh *et al.*, 2001; Lyimo *et al.*, 2003). A study of thirty types of indigenous vegetables commonly used in rural areas of Tanzania recorded values of up to 7.7 mg/100 g of iron in fresh vegetables (Lyimo *et al.*, 2003). Mosha *et al.* (1995 found iron content per 100 g of fresh vegetables in the range of 0.96 - 5.90 mg in amaranth, cowpea, peanut, pumpkin and sweet potato leaves. Fresh Amaranth assessed by Weinberger and Msuya (2004) indicated high values of iron of up to 37.05mg per 100g (table 9). Studies elsewhere reported varying results; for example Ishida *et al.* (2000) found 5.54mg per 100g of fresh sweet potatoes leaves from Japan, while Singh *et al.* (2001) reported higher levels ranging from 7.7mg per 100g for carrots to 66.8mg per 100g for cauliflower vegetables from India.

Table 9: Iron and beta–carotene contents of amaranth, nightshade and African eggplant (mg/100g)

Vegetable/District/Vegetable type	Fe	Beta - carotene
Amaranth		
Kongwa distirict	37.05	3.29
Singida „	22.95	2.70
Arumeru „		
-variety 1 (common type)	13.15	1.71
-variety 3 (indigenous type)	6.50	0.13
Nightshade		
Kongwa district	8.90	3.23
Singida „	14.55	1.09
Arumeru „		
-variety 1 (common type)	15.90	3.97
-variety 2 (broadleaf type)	9.75	1.82
Sweet potatoes leaves		
Kongwa district	8.35	1.93
Singida „	11.10	2.39
Arumeru „	8.05	3.24

Source: Weinberger and Msuya (2004)

2.8 Iron Bioavailability

Bioavailability is defined as the proportion of a dietary nutrient capable of being absorbed and available for use and storage (Hurrell, 2002). In defining bioavailability absorption is the most important criteria, thus avoiding the problem of measuring utilization which is difficult to quantify in human subjects with the exception of iron (Hurrell, 2002). Iron in food is present in two states: ferric (Fe^{3+}) and ferrous (Fe^{2+}) iron. At neutral pH most iron is in the ferric state, which is virtually insoluble. In order to make it more soluble it is reduced to the ferrous state (Allen and Ahluwalia, 1997). Ferrous iron is absorbed easier because it crosses the mucous layer of the small intestine more readily to reach the absorptive cells. In the intestine, Fe^{2+} loses an electron to form Fe^{3+}, before it enters the absorptive cells. Fe^{3+} binds to a receptor protein which finally transfers the iron inside the cell (Lunch, 2005).

Iron absorption is affected by two main groups of factors: physiological and dietary factors (Gibson, 2007). The major factors include iron status of the individual, amount of iron in the meal, the presence of other nutrients in the same meal and the chemical form of iron and nature of food. Other factors are the amount of acid secreted by the stomach during digestion and rate of passage of acid in the digestive tract (Lunch, 2005).

2.8.1 Factors affecting bioavailability of iron

2.8.1.1 Chemical form of iron

The chemical form of iron and the nature of food affect the absorption of iron. There are two kinds of iron, heme iron from animal sources and non-heme iron form plant sources (Gambling, 2006; Moy, 1998). Heme iron is highly bioavailable (15 to 35% is absorbed), whereas non- heme iron is less bioavailable, with absorption rates ranging from 2 to 20% (Allen and Ahluwalia, 1997). Some plant sources of non - heme iron include green leafy vegetables (Table 10), legumes, soya foods, nuts and whole-grain. The main sources of heme iron are the haemoglobin and myoglobin from consumption of meat, poultry and fish. Heme iron can be degraded and converted to non-heme iron if foods are cooked at a high temperature for too long (Gibson, 2007).

Table 10: Total, ionisable and in-vitro iron of green leafy vegetables and carrots (dry weight basis)

Vegetable	Total iron (mg/100g)	Ionizable iron (mg/100g)	Ionizable iron (% of total iron)	In vitro iron (% of total iron)
Coriander	22.3 ± 0.12^{b}	$1.7 \pm 0. 10^{a}$	7.5 ± 0.48^{b}	4.0 ± 0.22^{b}
Spinach	35.8 ± 0.11^{d}	1.7 ± 0.21^{a}	4.8 ± 0.58^{c}	2.7 ± 0.27^{c}
Cauliflower	$66. 8 \pm 0.09^{e}$	2.6 ± 0.07^{c}	3.9 ± 0.10^{a}	2.3 ± 0.05^{a}
Amaranth	26.8 ± 0.09^{f}	2.0 ± 0.11^{d}	7.6 ± 0.42^{b}	4.0 ± 0.19^{b}
Carrot	7.7 ± 0.01^{g}	0.6 ± 0.10^{c}	7.7 ± 1.36^{b}	4.1 ± 0.64^{b}
CD ($p < 0.05$)	0.30	0.34	1.84	0.85

Values are mean ± SEM of three replicates.
Means within the same column bearing different superscripts are significantly different.
Source: Singh *et al.* (2001)

2.8.1.2 Interaction of iron with other compounds in the meals

Bioavailability of non-heme iron is influenced by both dietary inhibitors and enhancers. Inhibitors are phytate, polyphenols, fibre, oxalate and to a certain extent, calcium. Calcium is the only dietary factor that negatively influences the absorption of heme iron and non-heme iron. Enhancers are ascorbic acid, citric acid, sugars, amino acids and alcohol (Sardesai, 1998; Reddy and Love, 1999; Naidu, 2004). Improvement of iron bioavailability can be achieved by degrading inhibitors that prevent the absorption from the food and by formation of promoting factors uptake (Gibson, 2004).

(a) Phytates

Phytate refers to phytic acid (myo–inositol hexaphosphate) as well as salts of calcium, magnesium or potassium phytate. Phytate is a major form of storage of phosphorus in cereals, legumes, seeds, nuts and tubers (Sardesai, 1998). Phytate chelates metal ions such as iron and zinc and therefore inhibit their absorption (Zijp, 2000). Recently, vitamin A and ß-carotene have been shown to enhance non-heme iron absorption by preventing the inhibitory effect of phytates (Manju *et al.*, 2000).

(b) Polyphenols

Tea, coffee, cocoa, red wine and some vegetables can severely inhibit iron absorption when taken at the same meal (SCN, 1993). These foods are known to contain polyphenols which are present in the form of phenolic acids, flavonoids and their polymerization products. The compounds form insoluble complexes with iron and may exist as an "iron-tannin" complex and thus inhibit iron absorption (Walker, 1989). The relative level of polyphenol content per cup of beverage follows the following order: black tea>coffee>cocoa>herbal teas (Kannah, 2006). It has been reported that the presence of sufficient amounts of iron absorption enhancers such as ascorbic acid, fish, poultry and meat in industrialized countries overcome the inhibition of iron absorption from large consumption of tea (Zijp, 2000). This is different as compared to developing countries whereby the consumption of plant foods such as legumes and cereals are higher than animal foods (Ruel, 2001).

(c) Dietary fibre

The inhibitory effects of fibre to iron absorption are greatly dependent on the presence of other factors such as phytate, oxalic acid, minerals and protein in the foods (Zijp, 2000). Fibre may have a greater effect on iron balance in infants and children compared to adults. In adults, up to 32 g per day of dietary fibre and 2 g per day of phytic acid may not cause a significant effect. In children, up to 25 g per day of dietary fibre and 1 g per day of phytic acid is also unlikely to have a significant effect on iron bioavailability (Kannah, 2006).

(d) Ascorbic acid

Ascorbic acid promotes non-heme iron absorption by reducing ferric iron to the ferrous state, which is soluble with high pH in the duodenum and small intestine (SCN, 1993). The acid is more effective when present in high enough quantities (Davidsson, 2003; Hurrell, 2004). Eating acidic foods, especially those rich in ascorbic acid, along with iron containing foods can increase absorption three to seven fold. Food sources of ascorbic acid are inexpensive, culturally acceptable, and widely available. Fresh fruits like orange, lemons, grapefruit, watermelon, papaya and pineapple are rich in ascorbic acid (Naidu, 2003).

According to Teucher (2004), the promotion of iron absorption in the presence of ascorbic acid is determined by the amount of inhibitors in the meals. Meals containing low to medium levels of inhibitors require less amount of ascorbic acid as compared to meals with high levels of phytic acid. The limitation of using ascorbic acid as enhancer to iron bioavailability is associated with its instability during processing and unwanted sensory changes (Davidsson, 2001; Teucher, 2004).

2.8.2 Influence of processing on iron bioavailability

Studies on iron suggest that treatment of food before consumption has a remarkable effect on bioavailability. The treatments involve traditional household food preparation and processing practices as well as commercial processing practices (Gibson, 2007). Available data also suggest the idea that food or food process can be chemically optimized to yield the best possible bioavailability of iron (Lee, 1982). Major food processing methods in which iron bioavailability can be affected includes: removal or addition of energy, micro-organisms, or chemicals, and food rearrangements (Table 11).

Table 11: **Classification of major forms of food processing**

	Type of process	Some examples
1.	Add energy	Cooking, pasteurization, heating
2.	Remove energy	Refrigeration, freezing
3.	Add micro-organisms	Fermentation, curing, aging
4.	Remove micro-organisms	Cleaning, sorting
5.	Add chemicals	Dilution, fortification, additives, pickling
6.	Remove chemicals	Extraction, dehydration, distillation, detoxification
7.	Rearrangements	Homogenization, emulsification, gelation, blending, enzymatic conversions and packaging

Source: Lee (1982)

2.8.2.1 Thermal processing

Heat processing increases the bioavailability of iron when the process involve heating a predominantly aqueous food (wet-heat processing), as well as when ascorbic acid is added (Lee, 1982). It has been reported that cooking and blanching are good ways of improving iron bioavailability (Yaday and Sehgal, 2002). A simple cooking method such as boiling has been reported to improve the bioavailability of iron (Yang *et al.*, 2002).

Similar findings have been reported by Yaday and Sehgal (2002) and Shashi (2003), where blanching and cooking resulted in significant improvement of iron availability by a significant reduction of oxalic acid, phytic acid and polyphenol contents in foods. On the other hand, addition of some ingredients during cooking can cause inhibitory effects. For example, addition of milk which is a common practice used in rural households can inhibit iron absorption (Hurrell, 2004).

2.8.2.2 Food to food fortification

One way of enhancing the bioavailability of certain micronutrients is to combine foods so that when eaten together, they increase the bioavailability of these micronutrients. This strategy is called food-to-food fortification (SCN, 1993). Vijayalakshmi *et al.* (2003) reported increased bioavailability of iron in some vegetables when cooked together compared to when they were cooked separately. For example, cabbage, tomato, moringa (*Moringa oleifera*), kale (*Brassica carinata*), and sweet pepper (*Capsicum annuum*) were added to raw and soaked mungbean and then boiled together. Tomato and moringa leaves were found to be most effective in enhancing the iron bioavailability of mungbean. The bioavailability of iron was observed to be as high as over 20 µg per 20 g of a mixture of mungbean and vegetables after cooking. Similar results were observed when these vegetables were cooked with other legumes such as soybean and lima bean (Vijayalakshmi *et al.*, 2003).

Soybean is cultivated in a number of regions in Tanzania; it is a rich source of proteins 40 – 45%, lipids 18 – 20% and carbohydrates 30% (Laswai, 2006). It also contains minerals such as sodium, potassium, iron, zinc and iodine, selenium, magnesium and copper. It can therefore be used to enrich traditional foods with micronutrients (Rweyemamu, 2007). However, some studies have associated soybean proteins and polyphenols with decreased bioavailability of iron (Sandberg, 2002).

2.8.2.3 Germination

Germination involves the soaking of seeds in water in the dark for up to three days to promote sprouting (Ruel, 2001). Seeds that are commonly germinated before their consumption include lentils, peas, soybeans and mung bean (Sandberg, 2002). Germination increases the activity of phytase enzymes, which cause the breakdown of phytic acid. It also reduces other antinutrients such as tannins and polyphenols. Germination increases the bioavailability of iron, zinc and calcium. Vitamins such as B6, riboflavin and vitamin C can also be improved during germination (Sandberg, 2002).

2.8.2.4 Fermentation

Fermentation reduces the amount of phytic acid in cereals, legumes and vegetables by enzymatic hydrolysis; therefore improves the bioavailability of iron and zinc. Fermentation is useful in improving the nutritional quality and the physical characteristics of these foods (Teucher, 2004).

2.8.2.5 Soaking

Soaking is effective in increasing the amount of soluble iron. Soaking wheat or rye flour for two hours completely decomposes phytic acid (Sandberg and Svanberg, 1991). Soaking flour for 24 hours increased the amount of soluble iron up to 10 fold. A combination of the above three methods is said to be highly effective in reducing phytic acid by activating endogenous phytase enzymes to degrade phytic acid and reduce the amount of polyphenols that inhibit non-heme iron absorption (Svaberg, 1995).

2.8.2.6 Vitamin A – iron interaction

It has been reported that just adding 500IU (International unit) of vitamin A or β-carotene to a cereal meal doubles the iron absorption from the gut of human subjects. These findings suggest in the presence of high levels of phytate and tannins in the diet, vitamin A or β-carotene will enhance the bioavailability of iron in humans (Graham and Rosser, 2000).

2.8.3 In-vitro estimation of iron bioavailability

The absorption and bioavailability of iron is complex. Although absorption can take place along the entire intestine, it occurs primarily in the duodenum and the jejunum (Walker, 1989). Three approaches have been established to estimate iron bioavailability in meals. These include two in-vitro assays: measurement of dialyzable iron and Caco-2 cell uptake, both carried out after in-vitro simulated gastric and pancreatic digestion (Reddy *et al.*, 2000; Lunch, 2005). The in-vitro assays are done by simulating gastrointestinal digestion using a commercially available enzyme and then measurement of the soluble iron released by the digestion to dialysis tubing (Miller and Schricker, 1982). For example, the in-vitro method developed by Miller and Schricker (1982) is simple, rapid, and inexpensive. It permits the simulation of in-vivo digestion conditions, permit pH control, provision of gradual adjustment with a mild base. It also distinguishes between low and high molecular weight soluble iron and it accommodates food mixtures.

The methods have been more useful for identifying and characterizing factors that affect non-heme iron absorption. They are more useful for assessing the effects of isolated determinants than for evaluating the overall iron bioavailability of a complex meal (Reddy *et al.*, 2000). For example, data indicate that dialysis and Caco-2 cell uptake are useful for ranking meals and single food items in terms of predicted iron bioavailability, but may not reflect the magnitudes of the effects of factors that influence absorption accurately (Lynch, 2005).

The third method is the use of algorithms based on the predicted effects of specific meal components on absorption derived from isotopic studies in human volunteers. The precision with which meal iron bioavailability can be predicted in a population, for which a specific algorithm has been developed, is improved by measuring the content of the most important enhancers and inhibitors. However, the accuracy of such predictions appears to be much lower when the algorithm is applied to meals eaten by different populations (Lynch, 2005). The method is having restrictions, since few researches are to administer radioisotopes to human subjects (Miller and Schricker, 1982).

2.9 Other Factors Affecting Nutrients Content of Vegetables

The composition of carotenoids in vegetables also is affected by soil conditions, weather conditions, stage of vegetable maturity and part of the vegetables (leaves, stems and roots) (Rodgruez-Amaya, 2003). For example, maturity of vegetables from stage I (15 days) to stage II (30 days) increased iron and manganese contents; whereas zinc and copper contents decreased (Khader and Rama, 1998). In a study on sweet potatoes leaves Ishida (1999) reported high concentration of protein and minerals particularly iron in leaves as compared to other parts. Carotene, vitamin B2, vitamin C and vitamin E were also high in leaves. All parts of the vegetable were rich in dietary fibre, but leaves had soluble dietary fibre and stems had insoluble dietary fibre. Polyphenol content in leaves was also high compared to other parts (Ishida *et al.*, 2000). According to Speek (1988), the carotenoids of a plant are mainly deposited in the leaves which, in general, have higher relative β-carotene content than the stalks (Speek, 1988).

Variation in nutrient content with species has likewise been reported. Aletor *et al.* (2003) found higher levels of Ca, Mg, Na and K to be higher in four leafy vegetables species compared to P and Cu. Annother study on six traditional vegetables found that *Amaranthus spinosus* and *Adansonia digitata* leaves were highest in iron (38.4 mg/100 g and 30.6 mg/100 g dry weight, respectively) compared to other vegetables (Barminas, 1998). In the same study, Zinc content was reported highest in *Moringa oleifera*, *Adansonia digitata* and *Cassia tora* leaves (25.5 mg/100 g, 22.4 mg/100 g and 20.9 mg/100 g dry weight, respectively), while manganese content was comparatively highest in *Colocasia esculenta*. All the vegetables contained higher level of calcium compared to exotic vegetables (Barminas, 1998). A similar study by Singh *et al.* (2001) reported highest iron contents in carrots, while copper; manganese and zinc contents were higher in spinach (Singh *et al.*, 2001).

2.10 Summary of the Reviewed Literature in Light of the Current Study

Literature for this study has been reviewed in light of the specific objectives. The review establishes the importance and level of consumption of vegetables as major sources of micronutrients. The consumption of indigenous vegetables as cheap and highly available source of nutrients in rural areas of Tanzania is also established. The review highlights the physical and chemical properties and functions of carotenoids and iron in food. The effects of food processing on carotenoids retention and iron bioavailability which are relevant in explaining the effects of various preparation practices identified and simulated in this study are discussed. Lastly the use of in-vitro estimation of iron bioavailability is discussed, as a simple, rapid and inexpensive method.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Approach of the Study

In accomplishing the objectives, this study was designed to be carried out in two approaches, i.e. field survey and laboratory analysis. The field survey was conducted to identify indigenous vegetables and their respective preparation methods in three districts of Tanzania (Singida, Arumeru and Kongwa districts). The laboratory analysis was done to determine the retention of carotenoids and iron bioavailability of a representative vegetable, i.e. sweet potato leaves. The laboratory analysis was carried out in nutrition laboratory at Asian Vegetable Research Centre (AVRDC), Shanhua, Taiwan. The detailed descriptions of the two approaches are presented below.

3.2 Description of the Study Area

Arumeru district is a district in Arusha region. The district belongs to the northern highlands zone which is characterized by volcanic uplands and volcanic soils from lava and ash. The soils are deep fertile loam and clays. The altitude is between 1000-2500m with bimodal rainfall in the range of 1000-2000mm per year. Singida rural and Kongwa districts of Singida and Dodoma regions respectively are in central semi-arid zone. These districts are characterized by undulating plains, with rocky hills and low scarps. The soils are well-drained but with low fertility. The altitude is 1000 - 1500m, while rainfall is unimodal and unreliable between 500 - 800mm per year (URT, 1999).

3.3 Field Survey for Identification of Indigenous Vegetable Preparation Methods

To identify the indigenous vegetables consumed in Tanzania, the field survey was conducted in Singida, Arumeru and Kongwa districts from November to December, 2006. The survey aimed at assessing the vegetable preparation methods such as pre-cooking and cooking practices as well as the extent of use of sun-drying as a preservation method in the districts. A total of 16 villages were surveyed (Appendix 1).

Since the survey required information on cooking of vegetables which is mainly done by women in villages, women respondents were therefore purposively selected. The women were selected on the criteria that they are aged above 18 years and each woman representing a household. The interviews were conducted by using a pre-tested closed ended structured questionnaire. A total of 156 respondents were interviewed i.e. 10 from each village, except in one village where only 6 respondents turned up. Consumption of vegetables as well as factors affecting the quality of vegetable dishes such as method of cooking, method of chopping, time of cooking, type of preservation and ingredients used were assessed (Table 12).

Table 12: Information collected in assessing preservation and cooking methods of vegetables

Information category	Type of information collected
Personal details	➢ Age, marital status, ➢ Level of education, ➢ Size of household and main occupation
Consumption of indigenous vegetables	➢ Local name ➢ Source of the vegetables ➢ Reasons of preferences
Method of preparations and cooking	➢ Preferred method of cooking ➢ Time of cooking ➢ Ways of cutting, washing ➢ Availability and use of ingredients
Preservation of vegetables	➢ The use of sun-drying ➢ Reasons for preferences

The information on traditional preparation methods of vegetable collected in the field survey was used to investigate feasible preparation methods to increase carotenoids retention and in-vitro iron bioavailability of leafy vegetable dishes. In order to have a consistent way of comparing the effects of preparation methods on the nutrients the use of one type of vegetable was necessary (Rodriguez–Amaya, 1997). Three most consumed vegetables in the districts were identified in this current study (Table 13), sweet potato leaf inclusive. Among the three vegetables identified, sweet potato leaf was chosen for analysis, as it was the available vegetable in the analysis site.

3.4 Sample Preparation for Determination of Carotenoids Retention and Iron Bioavailability in Sweet Potato Leaf Dishes

Fresh sweet potato leaves produced from different sites were purchased from a local market at Shanhua, close to AVRDC in April, 2007. Sample preparation was done at AVRDC Nutrition laboratory. All samples were weighed before and after preparations. The preparations were divided into traditional and modified methods each with three stages, i.e. pre-cooking, cooking and reheating (Fig. 4 and 5).

The traditional cooking had four treatments which involved boiling fresh leaves with oil and without oil and boiling sun-dried leaves with oil and without oil. Traditional reheating was done by boiling previously cooked dishes with oil and without oil. Modified cooking on the other hand, involved boiling fresh leaves with oil, with oil + tomato, with oil + lemon and with soybean as well as boiling oven-dried leaves with oil and with oil + tomato. Modified reheating involved boiling previously cooked dishes with oil and with oil + tomato. The summary of the traditional and modified treatments in a schematic form is shown in figures 4 and 5 respectively.

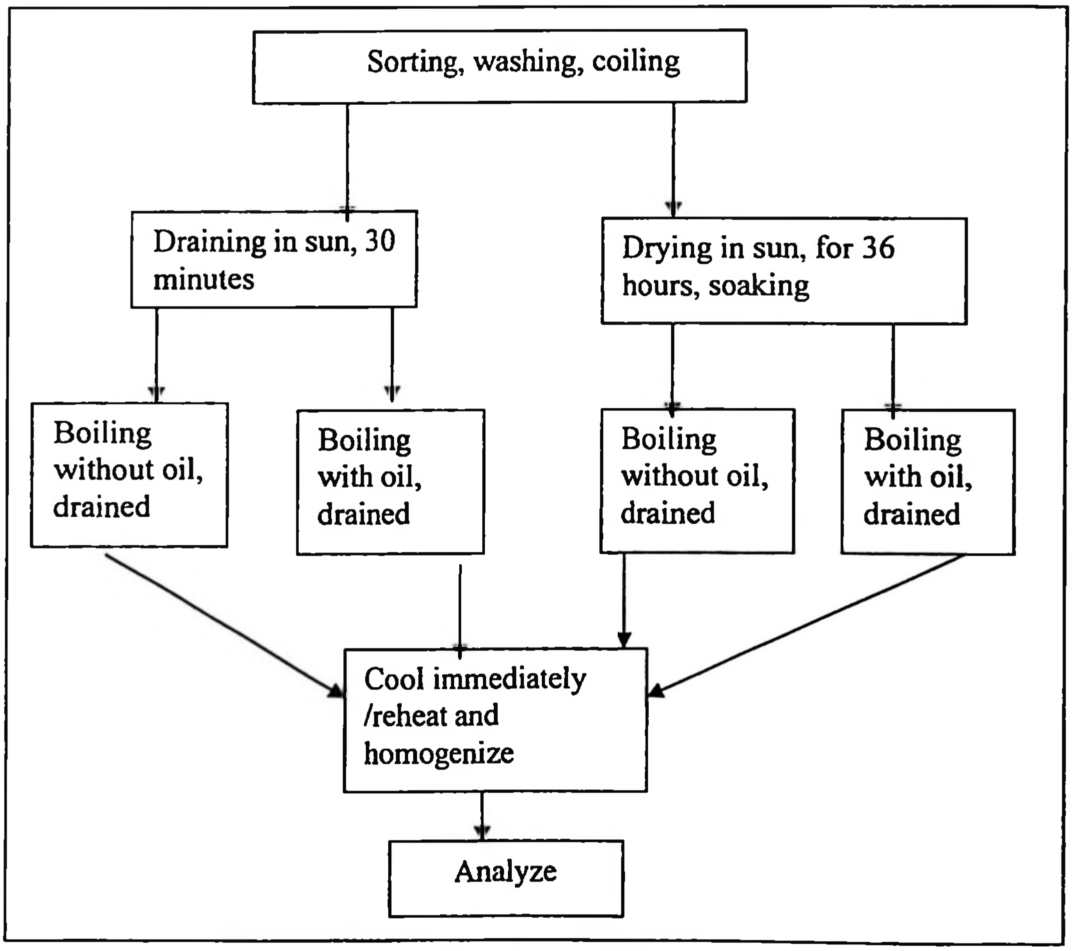

Figure 4: **Schematic diagram of traditional preparation methods of sweet potato leaf dishes**

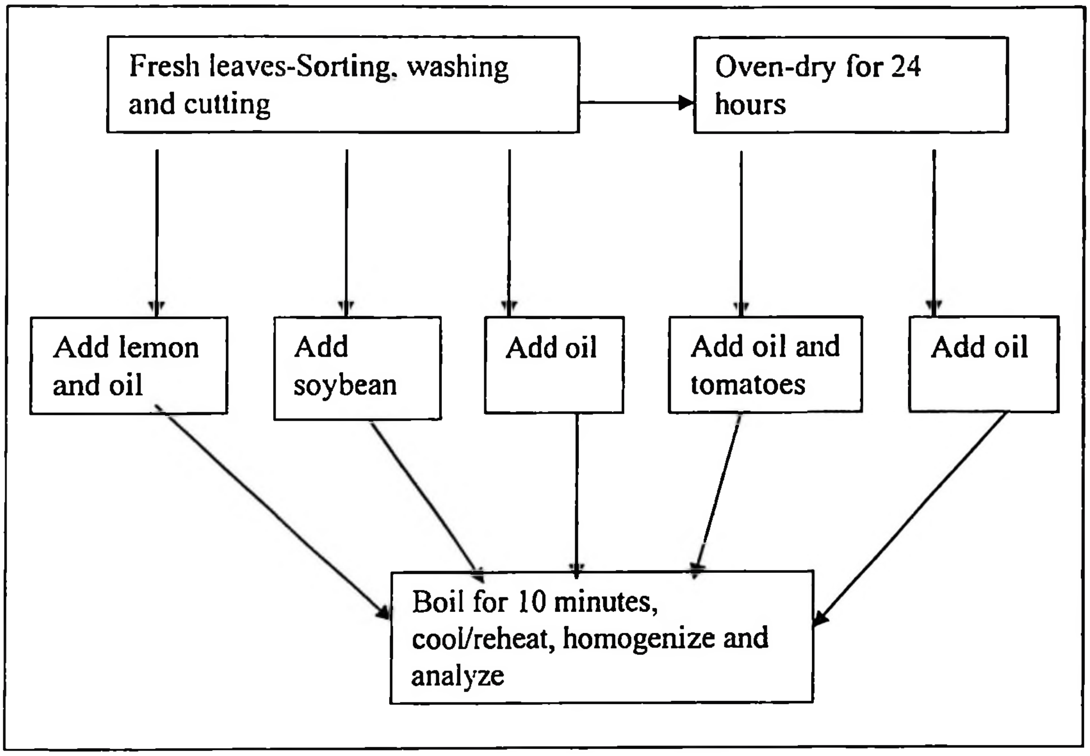

Figure 5: **Schematic diagram of modified preparation methods for sweet potato leaf dishes**

3.4.1 Traditional preparation methods

3.4.1.1 Pre–cooking

The sweet potato leaves were prepared in two portions, i.e. fresh and dried samples. The first portion (fresh leaves) were sorted, stalks removed and washed thoroughly. The leaves were coiled between palms, drained in the open sun for 30 minutes. The second portion of leaves (about 400g) were coiled and dried in the sun for 36 hours at temperature range of 25°C to 26°C. All samples were cooked as explained in section 3.4.1.2.

3.4.1.2 Cooking

(i) Boiling fresh leaves without oil

About 200g of sweet potato leaves were prepared. Two beakers (200ml) were used; in each beaker 100g of sample was measured. Cold water (100g) and salt (2.5g) were added to all the beakers. All samples were placed on electric plate cooker and boiled for 30 minutes with frequent stirring. After boiling, the stock was drained and the remaining samples weighed and recorded. The samples were immediately cooled in tap water, and then homogenized by a warring blender.

(ii) Boiling of fresh leaves with oil

About 200g of prepared leaves were divided in two beakers of 100g in each. Cold water 100g, 5g of sunflower oil and 2.5g of salt were added in each. The samples were all boiled for 30 minutes, and water drained off. The samples were immediately cooled in tap water, and then homogenized.

(iii) Boiling sun-dried leaves with and without oil

The dried leaves were divided in four beakers, each containing 11g, soaked for 10 minutes, the water drained off. The samples were boiled with and without oil as explained above.

3.4.1.3 Reheating

Eight fresh and sun-dried samples were prepared and boiled with oil and without oil (two samples each). They were left to stand at room temperature for 16 hours, then reheated to boiling point, and prepared for analysis as explained above.

3.4.2 Modified preparation methods

3.4.2.1 Pre–cooking

Fresh and dried leaves were prepared. The fresh sweet potato leaves were sorted to remove stalks and dirt; washed and then cut in longer pieces equivalent to one cut per leaf. The leaves were drained on a smooth and clean piece of cloth under room temperature. About 220g of the fresh leaves were dried in oven at 50°C for 16 hours. Samples were cooked as explained below.

3.4.2.2 Cooking

(i) Boiling fresh leaves with oil

About 200g were divided in two beakers, 100g each. Boiled hot water of 100g, common salt 2.5g and sunflower oil 5g were added in all samples. The samples were boiled for 10 minutes, weighed and immediately cooled in tap water, then homogenized for analysis.

(ii) Boiling fresh leaves with oil + tomatoes

Fresh tomatoes (200g) purchased from the market were washed and cut. A warring blender was used to homogenise the tomatoes. The tomatoes were divided into two beakers of 100g each. In each beaker 100g of leaves, 100g boiled hot water, 2.5g salt and 5g sunflower oil were added. All samples were boiled and homogenized as above.

(iii) Boiling fresh leaves with oil + lemon

Leaves 200g were divided in two beakers, each 100g. In each beaker boiled hot water (100g), 2.5g salt, oil (5g), and lemon juice (5g) were added. All samples were boiled and homogenized as above.

(iv) Boiling fresh leaves with soybean

Soybeans collected from AVRDC soybean project were soaked overnight. 50g of soaked soybean were blended in a warring blended. The soybean were mixed in two different beakers containing 100g leaves, 100g boiled hot water and salt (2.5g). All samples were boiled and homogenized as above.

(v) Boiling oven-dried leaves with oil

Oven–dried leaves were divided in two beakers each containing 13g. The samples were soaked in 100g of water for 10 minutes. In all the beakers boiled hot water (50g) was added followed by oil (5g) and salt (2.5g). All samples were boiled and homogenized as above.

(vi) Boiling oven-dried leaves with oil + tomatoes

Tomatoes (200g) were homogenized, and then divided into two beakers (100g) each. In each beaker oven-dried leaves (13g), boiled hot water (50g), oil (5g) and salt (2.5g) were added. All samples were boiled and homogenized as above.

3.4.2.3 Reheating

Four samples were boiled with oil and with oil + tomato (two samples each) as explained above. They were then stored in refrigerator at 4°C for 16 hours, afterward the samples were reheated to boiling point. They were cooled and prepared for analysis as explained above.

3.5 Laboratory Determination of Carotenoids Retention in Sweet Potato Leaf Dishes

Determination of carotenoids retention in sweet potato leaf dishes was done by using the formula by Murphy *et al.* (1975). The contents of carotenoids in each sample dish were determined by high performance liquid chromatography (HPLC).

3.5.1 Determination of carotenoids contents by high performance liquid chromatography (HPLC)

Sample preparation and extractions, identification and quantification of carotenoids (β-carotene, lycopene and lutein) were done as explained by Rodriguez–Amaya and Kimura (2004) and Taungbodhitham *et al.* (1998).

(i) Reagents used

HPLC solvents including hexane, acetone, acetonitrile and methonal and nitrogen gas were used.

(ii) Sample extraction

Sample extraction was done in order to release all carotenoids from the sweet potato leaf dishes and bring them into solution, without altering them. To achieve this, 10g of sample was used. 100ml of hexane: acetone (hexane 60ml and acetone 40ml) mixed with the sample and homogenized for 5 minutes by using a polytron homogenizer. The sample was filtered under vacuum with Whatman filter paper no.1 in a funnel. During filtering the sample was washed with acetone, until the residue was colourless.

(iii) Partitioning

The extract was transferred to a 500ml separatory funnel. Salted water (NaCL) was added gently, by allowing it to flow along the walls of the funnel. The two layers, were allowed to separate, the lower layer was discarded. More salt water was added; the washing repeated three times, the extract layer was collected in volumetric flasks, diluted to 100ml with hexane for saponification.

(iv) Saponification

Saponification was done in order to remove lipids and chlophylls. 20ml of extract was placed in a flask, and evaporated to dryness by nitrogen gas. The residue was dissolved in 2ml diethyl ether, followed by 10ml methanol and 5ml of 15% potassium methanol. Nitrogen gas was employed to remove the ether. The mixture was placed in an incubator at 30°C for 2 hours with shaking, and then stored in a freezer overnight at -20°C. 15ml hexane and 15ml distilled water were added, and shaken for 30 seconds. In a separating funnel, the mixture was extracted three times with hexane. The hexane layers were collected in another separating funnel and extracted with salted water. The washing was repeated three times, the lower layer of potassium methanol discarded each time. The upper layer was then diluted with 100ml hexane. In test tubes, 10 ml of the extract was taken and dried under nitrogen gas. The residue dissolved in 100µl of hexane, filtered by 0.45µl membrane and 20 µl placed in the HPLC.

(v) HPLC instrumentation, conditions and identification

The analysis was done with a water separation module (model 2695) equipped with an autosampler injector and a waters model UV 996 photodiode array detector. Carotenoids were separated by LiChrospher 100, RP – 18e column (5μm: 244 x 4mm). The mobile phase was composed of acetonitrile: methanol (75:25). Flow rate was set at 1.5ml/minute. Isocratic conditions were used, reading done at 436nμ. Identification was done on the basis of retention times and comparison with co–chromatography standards of β–carotene, lutein and lycopene (Taungbodhitham *et al.*, 1998).

3.5.2 Calculation of carotenoids retention

Percent retention of carotenoids was calculated according to Murphy *et al.* (1975), using the following formula:

$$\text{Retention} = \frac{\text{Carotenoids content per g of cooked food x g of cooked food}}{\text{Carotenoids content per g of raw food x g of raw food}} \times 100$$

3.6 Laboratory Determination of Iron Bioavailability in Sweet Potato Leaf Dishes

Iron availability of sweet potato leaf dishes was determined by in-vitro method. Then iron bioavailability was expressed as a percent of total non-heme iron in the original sample.

3.6.1 Determination of iron availability by in-vitro digestion method

The determination of iron availability was done each day of sample preparation. An in-vitro method for estimating iron availability was used by a modified calorimetric method as described in Miller and Schricker (1982) and modified by Kapanidis and Lee (1995).

(i) Reagents used

The chemicals and reagents used were hydrochloric acid (0.01 N HCL, 0.1 N HCL, 6 N HCL) sodium hydroxide (0.5 M NaOH,), 0.1 M sodium bicarbonate ($NaHCO_3$), 0.5 M sodium bicarbonate and Iron (Fe) standard solution in 0.1 N HCL and Fe standard solution in 0.01 N HCL, 2 M sodium acetate (CH_3COONa) and trace mineral grade. Pepsin, pancreatin, porcine bile extracts, bathophenanthrolin disulfonic acid disodium salt and hydroxhylamine hydrochloride.

(ii) Preparation of solutions for the in-vitro digestion

Pepsin solution was prepared by dissolving 16g of pepsin in 0.1 M HCL to 100g. Pancreatin–bile suspension was prepared by suspending 1g of pancreatin (from porcine pancrease) and 6.5g porcine bile extracts in 0.1 M$NaHCO_3$ to 250ml. Batho reagent was prepared by dissolving 125g of bathophenanthrolin disulfonic acid disodium salt and 50g of hydroxhylamine hydrochloride in 2M sodium acetate to 500ml. Protein precipitant solution obtained by dissolving 50g TCA (trace mineral grade) and 50ml concentrated HCL in distilled water (DI) to 500ml.

(iii) In-vitro gastro intestinal digestion

The sample of sweet potato leaf dish was blended in a warring blender to make a creamy consistency. The pH of the sample was adjusted to 2 ± 0.05 with 6N HCL which is about 2ml. About 20g of the sample was weighed in four 125ml flasks. Pepsin 0.75ml solution was added to each flask. All the flasks were covered and incubated at 37°C for two hours with shaking. One flask was taken from the incubators after 1 hour and 30 minutes for analysis of titratable acidity.

(iv) Determination of titratable acidity

Titratable acidity is defined as the number of equivalents of NaOH required to titrate a 20g aliquot containing 5ml of the pancreatin – bile mixture to pH 7. Pancreatin–bile (5ml) suspension was added to the flask. Through titration by 0.5 N NaOH, pH was adjusted to 7 ± 0.05. The volume (X ml) NaOH was recorded. For one flask, X ml of 0.5N $NaHCO_3$ was added, then diluted with distilled water to 25ml.

(v) Dialysis procedure

A dialysis bag containing an amount of $NaHCO_3$ in 25ml of distilled water equivalent to the previously determined titratable acidity was added to a 20g aliquot of pepsin digest after two hours. The sample was incubated at 37°C with shaking for 30 minutes, during which time the pH increased to 5. About 5ml of pancreatin – bile acid mixture was added to the contents in each digestion vessel outside the dialysis bag. The dialysis bag was removed, rinsed thoroughly by distilled water and emptied of its contents (the dialysate). The dialysate was weighed and analysed by batho method (bathophenanthroline reactive iron).

(vi) Determination of available iron in the dialyzates (Batho method)

5 ml of dialyzate was weighed in 15 ml centrifuge tube. Protein precipitant solution (2.5 ml) was added. The sample was mixed in votex mixer. The tube was placed in boiling water for 10 minutes, then centrifuged at 5000rpm for ten 10minutes. To the supernatant 2.5 ml of batho reagent was added, mixed by votex mixer. The sample was let to stand for 10 minutes for development of red colour. Reading was done at OD 535 by using atomic absorption spectrophotometer (ASS) with a blank 0.01 N HCL. The standards were 0, 0.1, 0.2, 0.3, 0.5, 1, 2 ppm of 1015 ppm Fe.

3.6.2 Calculation of iron bioavailability

The iron bioavailability was expressed as percent of total non-heme in the original aliquot that is present in the dialysis bag at the end of the digestion. Total iron content was determined by atomic absorption spectroscopy (AOAC, 1995). Iron bioavailability (IB) was calculated by using the following formula:

$$\text{IB} = \frac{\text{Available iron } (\mu\text{g/g dish}) \text{ at cooked stage or reheated stage}}{\text{Total iron content } (\mu\text{g/g dish}) \text{ of the precooked stage}} \times 100$$

3.7 Statistical Analysis

3.7.1 The identification of traditional preparation methods

The quantitative data collected were summarized and analyzed by Statistical Package for Social Science (SPSS 9) computer programme. Results on type of vegetable consumed in Singida, Arumeru and Kongwa districts and vegetable preparation methods were presented by tables using percentages. Socio-economic data of the respondents were presented in percentages and frequencies.

3.7.2 Effects of preparation on retention of carotenoids and iron bioavailability in sweet potato leaf dishes

The data were collected in two duplicate samples. The percent retention of carotenoids and in-vitro iron bioavailability in sweet potatoes leaves were summarized by using Excel computer spread sheet. To test the hypothesis that preparation methods have effects on the former, analysis of variance was carried out using Statistical Analysis System (SAS) (SAS, 1991) for completely randomized design. Means were separated by Duncan multiple range test also in SAS computed at probability level of $P = 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Traditional Methods of Preparing Indigenous Vegetables

Out of a total of 156 respondents involved in the survey, 38.5% were from Singida, 35.9% from Kongwa and 25.6% from Arumeru. More than half of them were aged between 18 – 36 years; majority (89.7%) had attained primary education. Details of the socio-demographic characteristics of respondents and the villages visited are shown in appendix 1. Variation of vegetables consumed and methods of preparation among the three districts have been observed.

4.1.1 Type of indigenous vegetables consumed in the three districts

Table 13 presents the distribution of respondents by percent with regard to consumption of eight vegetables. Arumeru district had the highest number of respondents (77% - 100%) who consume most of the vegetables with the exception of jute mallow, which was used by only 45%. Nightshades, African eggplant and amaranth were used by all respondents from Arumeru district and used by the least in Kongwa and Singida. Kongwa district had 58.9%, 76.8% and 67.9% using sweet potato leaves, jute mallow and amaranth respectively. Two vegetables were mostly consumed in Singida, i.e. jute mallow (91.7%) and amaranth (61.7%). Three most consumed vegetables among all districts were identified; amaranth (81.4%), jute mallow (75%) and sweet potato leaf which was consumed by 64.7% of all respondents.

Table 13: The distribution of respondents by percent with regard to consumption of the eight selected vegetables in Singida, Arumeru and Kongwa

District[1]	Percentage of respondents							
	Sweet potato leaves	Jute mallow	Okra	Night-shade	Amar-anth	African eggplant	Pump-kin leaves	Cowpea leaves
Singida	41.7	91.7	15	1.7	61.7	6.7	21.7	26.7
Arumeru	77.5	45	87.5	100	100	100	95	97.5
Kongwa	58.9	76.8	10.7	3.6	67.9	5.4	48.2	48.2
Three Districts[2]	64.7	75.0	34.6	28.8	81.4	29.5	50.6	50.6

[1] Percent within district, [2] Percent among districts

4.1.2 Cooking methods of vegetables

Table 14 presents the distribution of respondents by percentage per district in regard to vegetables cooking methods. Three main cooking methods identified were; boiling, boiling-frying, and stir-frying. Respondents defined boiling as cooking vegetables in water with pot covered or not covered. Soup is normally discarded or reduced depending on the amount of water initially added and the nature of the vegetable. The use of oil is optional in boiling method. Stir–frying was defined as cooking vegetable in small amount of oil while stirring occasionally. In stir–frying method the use of water is limited. Boiling-frying was defined as a combination of the first two methods. In this method vegetables are boiled and soup discarded before they are stir-fried. Boiling was mostly preferred for jute mallow in Singida (91.7%)

and Kongwa (76.8%). Sweet potato leaves were cooked by stir-frying in Singida (31.7%) and Arumeru (67.5%), Kongwa respondents cooked the vegetable mostly by boiling-frying.

Table 14: The distribution of respondents by percent per district with respect to vegetable cooking methods

Districts	Cooking Methods	Vegetables							
		Sweet potato	Jute Mallow	Okra	Night-shade	Amar-anth	Egg-plant	Pump-kin	Cow-pea
Singida	Boiling	6.7	91.7	6.7	-	11.7	3.3	3.3	1.7
	Boil-frying	3.3	-	3.3	1.7	8.3	-	16.7	25
	Stir-frying	31.7	-	5.0	-	41.7	3.3	1.7	-
Arumeru	Boiling	10	32.5	12	22.5	30	27.5	17.5	15
	Boil-frying	-	12.5	5	10	2.5	2.5	17.5	32.5
	Stir-frying	67.5	-	70	67.5	67.5	70	60	50
Kongwa	Boiling	8.9	76.8	5.4	3.6	23.2	3.6	25	26.8
	Boil-frying	30.4	-	-	-	25	1.8	17.9	16.1
	Stir-frying	19.6	-	5.4	-	19.6	-	5.4	5.4

Other vegetables which were commonly available in Arumeru; okra, nightshades, amaranth, African eggplant, pumpkin and cowpea leaves were mostly cooked by stir-frying (50 – 70%). Apart from jute mallow, the remaining vegetables which were available to the least number of respondents in Kongwa and Singida were mostly cooked by boiling methods (Table 14).

4.1.3 Preservation of vegetables by open sun-drying

Table 15 presents the distribution of respondents by percentage with respect to the use of open sun-drying as a preservation method of vegetables by district. Open sun-drying was found to be the main preservative methods for vegetables especially in Singida and Kongwa districts. All respondents from Singida and Kongwa used open sun-drying as a method of preserving vegetables. Only 2.5% of respondents from Arumeru used the method. Majority of respondents (76.3%) used the method in the three districts; among these 38.5% were in Singida, while 35.9% were in Kongwa.

Table 15: The distribution of respondents in each district in respect to the use of open sun-drying as a preservation method

Response	Location (%)			
	Singida	Arumeru	Kongwa	Total
Do	100[1] (38.5)[2]	2.5 (1.9)	100 (35.9)	76.3
Do not	-	92.5 (23.7)	-	23.7

[1] Percent within district, [2] Percent among districts

4.2 The Effects of Traditional Food Preparation Methods on Carotenoids Retention in Sweet Potato Leaf Dishes

4.2.1 Beta-carotene retention

Table 16 presents the effects of traditional preparation methods on beta-carotene (β-carotene) retention in sweet potato leaf dishes. All fresh dishes were statistically significant ($P<0.05$) higher in β-carotene retention than sun-dried dishes, which were 2-3 times lower. The highest β-carotene retention (72.22%) was found in fresh dishes cooked with oil and the lowest (16.07%) in reheated sun-dried dishes cooked with oil.

Table 16: Beta-carotene retention in fresh and dried sweet potato leaf dishes prepared by traditional method

	Beta-Carotene retention (%)[1]			
Treatments	Fresh		Sun-dried	
	Without oil	With oil	Without oil	With oil
Pre-Cooked	62.26^{b}	62.32^{a}	20.90^{d}	20.92^{c}
Cooked	53.54^{b}	72.22^{a}	21.64^{c}	17.60^{c}
Cooked +Reheat	58.96^{a}	64.25^{a}	22.14^{b}	16.07^{b}

Means with the same letters within each row are not statistically significant different at $P<0.05$
[1] Percentage retention calculated on fresh weight basis compared with amount in raw vegetable dish sample

4.2.2 Lutein retention

Table 17 presents lutein retention in fresh and dried sweet potato leaf dishes as affected by traditional preparation methods. As in the case of β-carotene, the highest lutein retention (69.75%) was obtained in fresh leaf dishes cooked with oil and the lowest (34.35%) was in reheated sun-dried dishes with oil. Reheated fresh dishes with oil were second highest in lutein retention (68.70%).

Table 17: Lutein retention in fresh and dried sweet potato leaf dishes prepared by traditional methods

Treatments	Lutein retention (%)[1]			
	Fresh		Sun-dried	
	Without oil	With oil	Without oil	With oil
Pre-Cooked	62.56^{b}	62.26^{a}	51.36^{c}	50.97^{d}
Cooked	60.64^{a}	69.75^{a}	39.48^{b}	38.78^{b}
Cooked +Reheat	59.75^{ab}	68.70^{a}	39.07^{bc}	34.35^{c}

Means with the same letters within each row are not statistically significant different at P<0.05

[1] Percentage retention calculated on fresh weight basis compared with amount in raw vegetable dish sample

4.3 The Effects of Modified Food Preparation Methods on Carotenoids Retention in Sweet Potato Leaf Dishes

4.3.1 Beta-carotene retention

The retention of β-carotene in sweet potato leaf dishes prepared by modified methods is given in table 18. Dishes cooked with oil + tomato were statistically significant ($P<0.05$) higher in β-carotene, than the rest of the dishes.

Table 18: Beta-carotene retention in fresh and dried sweet potato leaf dishes prepared by modified methods

	Beta- Carotene retention (%)[1]					
	Fresh				Oven-dried	
Treatments	With oil	Oil + tomato	soybean	Oil + lemon	With oil	Oil + tomato
Pre-Cooked	100^{c}	100^{c}	100^{c}	100^{c}	100.51^{b}	108.17^{a}
Cooked	72.92^{ab}	75.45^{ab}	70.17^{ab}	60.31^{b}	85.89^{a}	87.73^{a}
Cooked +Reheat	61.95^{b}	79.17^{b}	-	-	80.26^{ab}	99.05^{a}

Means with the same letters within each row are not statistically significant different at $P<0.05$

[1] Percentage retention calculated on fresh weight basis compared with amount in raw vegetable dish sample

In cooking stage, the highest retention (87.73), was found in oven-dried dishes cooked with oil + tomato. Fresh dishes also cooked with oil + tomato were also highest (75.45%) among the fresh cooked dishes. The least retention (61.95%) was observed in fresh dishes cooked with oil alone (Table 18).

4.3.2 Lutein retention

Table 19 presents lutein retention in fresh and oven – dried sweet potato leaf dishes prepared by modified methods. Dishes cooked with oil + tomato were significantly ($P<0.05$) higher compared to other dishes. The highest lutein retention (116.28%) was found in pre-cooked dishes with oil + tomato. The least retention (58.87%) was obtained in dishes cooked with soybean.

Table 19: Lutein retention in fresh and dried sweet potato leaf dishes prepared modified methods

Treatments	Lutein retention (%)[1]					
	Fresh				Oven-dried	
	With oil	Oil + tomato	soybean	Oil + lemon	With oil	Oil + tomato
Pre-Cooked	100^{c}	100^{c}	100^{c}	100^{c}	116.75^{a}	116.28^{b}
Cooked	65.31^{c}	77.36^{bc}	58.87^{cd}	59.70^{cd}	91.89^{ab}	103.10^{a}
Cooked +Reheat	62.07^{bc}	76.32^{ba}	-	-	97.34^{a}	100.73^{a}

Means with the same letters within each row are not statistically significant different at $P<0.05$

[1] Percentage retention calculated on fresh weight basis compared with amount in raw vegetable dish sample.

4.4 Comparison of Carotenoids Retention in Traditional and Modified Preparation Methods of Sweet Potato Leaf Dishes

4.4.1 Beta-carotene and lutein retention in fresh sweet potato leaf dishes

Figure 6 and Figure 7 present comparison of β-carotene and lutein retention respectively in fresh sweet potato leaf dishes prepared by traditional and modified methods. Generally the retention of the carotenoids was lower in traditional methods as compared to modified methods. At cooked stage, modified dishes had a retention range of 70% - 75% β-carotene (Fig. 6) and 58% - 77% lutein (Fig. 7).

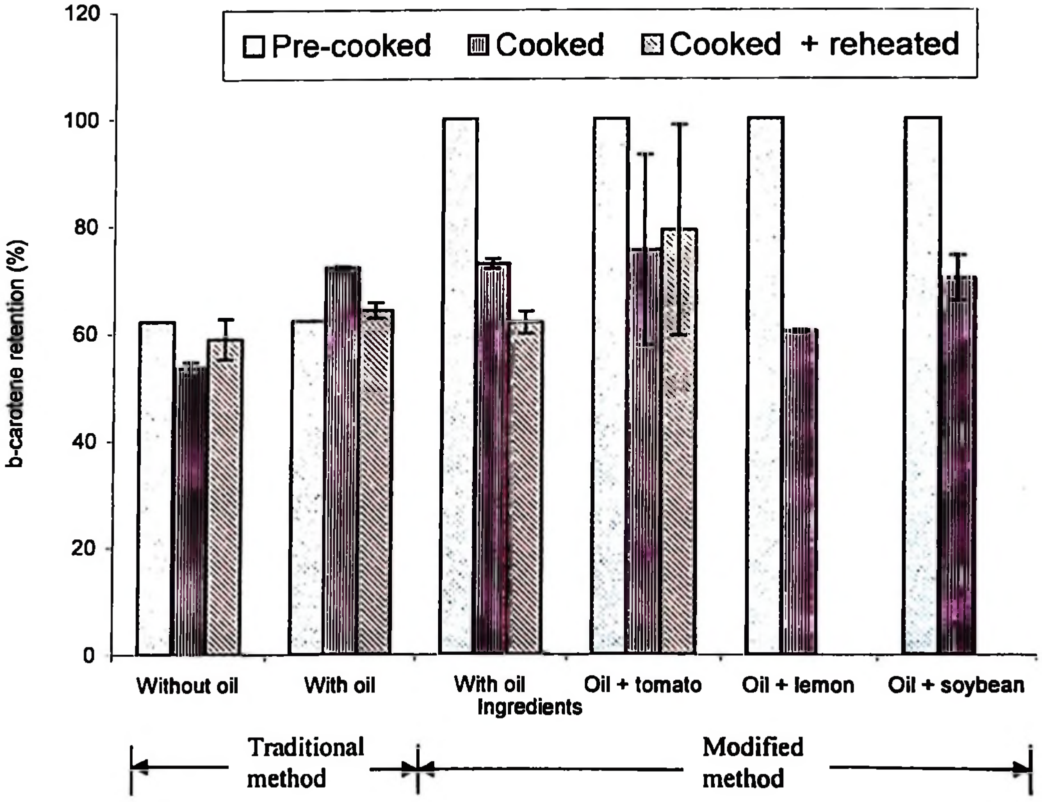

Figure 6: **Comparison of beta-carotene retention in traditional and modified preparation methods of fresh sweet potato leaf dishes (± standard error)**

In the same stage, traditionally prepared dishes had a range of 53% – 72% β-carotene (Fig. 6) and 60% - 69% of lutein retention (Fig. 7). The least retention was found in traditional dishes cooked without oil (53.54% β-carotene and 60.64% lutein).

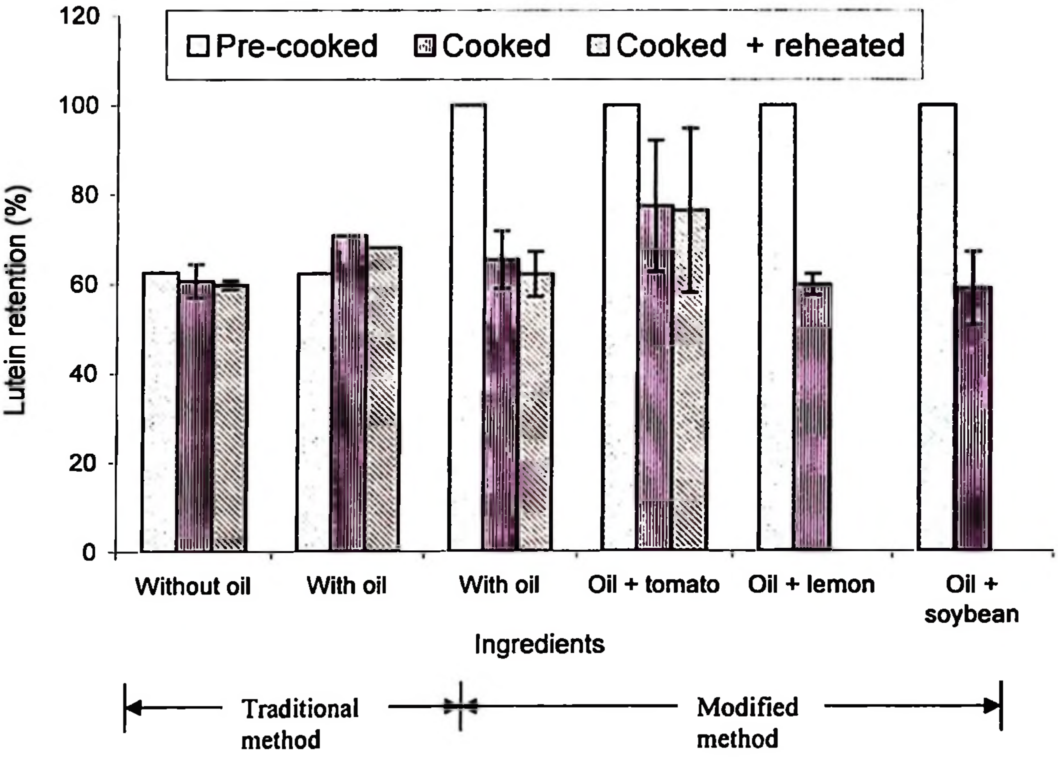

Figure 7: **Comparison of lutein retention in traditional and modified preparation methods of fresh sweet potato leaf dishes (± standard error)**

4.4.2 Beta-carotene and lutein retention in dried sweet potato leaf dishes

The comparison of β-carotene and lutein retention in dried sweet potato leaf dishes is given in figure 8 and 9 respectively. Oven-dried dishes prepared by modified methods retained 2-6 times more carotenoids than sun-dried dishes prepared by traditional methods. At cooked stage, the highest retention (87.73%) of β-carotene was recorded in oven-dried dishes, and the least (17.60%) recorded in sun-dried dishes. Lutein retention was up to 103.10% in cooked oven-dried dishes, while the highest retention for cooked sun-dried dishes was 39.48% of lutein.

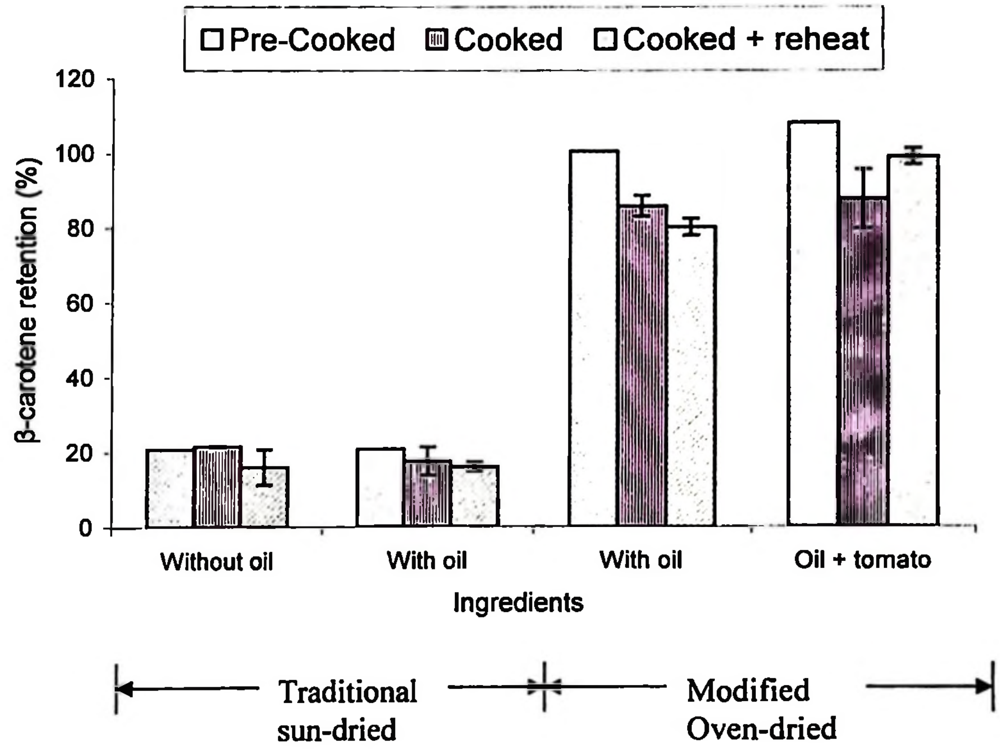

Figure 8: **Comparison of beta-carotene retention traditional and modified preparation methods of dried sweet potato leaf dishes (± standard error)**

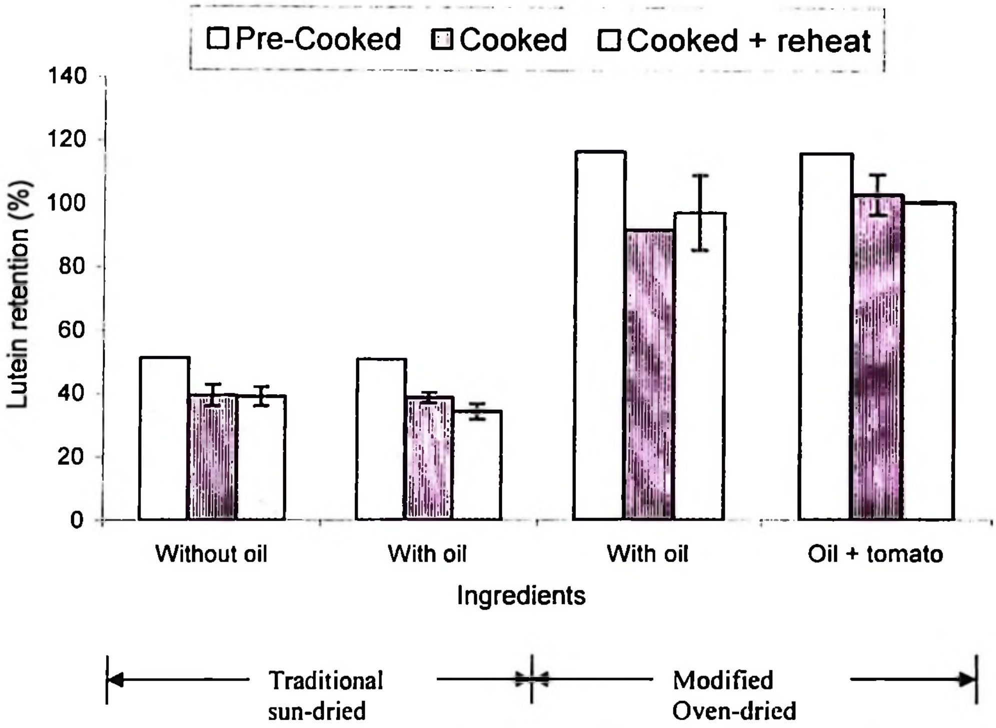

Figure 9: **Comparison of lutein retention in traditional and modified preparation methods of dried sweet potato leaf dishes (± standard error)**

4.5 Effects of Traditional Food Preparation Methods on In-vitro Iron Bioavailability of Sweet Potato Leaf Dishes

The in-vitro iron bioavailability of sweet potato leaf dishes prepared by traditional methods is illustrated in table 20. Sun-dried dishes were generally statistically significant ($P<0.05$) higher in iron bioavailability than fresh dishes. The highest iron bioavailability (3.78%) was recorded in reheated sun-dried dishes cooked without oil, and the lowest (2.12%) in fresh dishes cooked with oil. Reheating increased bioavailability in a range of 0.23% - 0.70% for sun-dried dishes. Contrary observations were found in fresh dishes, where reheating reduced bioavailability in the range of 0.73% to 0.79%.

Table 20: In-vitro iron bioavailability in sweet potato leaf dishes prepared by traditional methods

Treatments	Iron bioavailability (%)			
	Fresh		Sun-dried	
	Without oil	With oil	Without oil	With oil
Cooked	2.90ᵃ	2.91ᵃ	3.08ᵃ	3.19ᵃ
Cooked + Reheat	2.17ᵇ	2.12ᵇ	3.78ᵃ	3.42ᵃᵇ

Means with the same letters within each row are not statistically significant different at P<0.05.

4.6 Effects of Modified Food Preparation Methods on In-vitro Iron Bioavailability in Sweet Potato Leaf Dishes

Table 21 presents in-vitro iron bioavailability in sweet potato leaf dishes prepared by modified methods. Oven-dried dishes cooked with oil + tomato were significantly highest (P<0.05) in iron bioavailability (7.08%) compared to all other dishes. Fresh dishes cooked with oil + tomato were highest (6.26%), among the fresh dishes, followed by dishes cooked with oil + lemon. Reheating reduced the iron bioavailability in a range of 1 – 2% in all fresh and oven-dried dishes compared to cooked dishes.

Table 21: In-vitro iron bioavailability in sweet potato leaf dishes prepared by modified methods

Treatments	Iron bioavailability (%)					
	Fresh			Oven-dried		
	With oil	Oil + tomato	Oil + lemon	Soybean	With oil	Oil + tomato
Cooked	4.79^{c}	6.26^{ba}	5.79^{b}	5.36^{bc}	4.47^{ba}	7.08^{a}
Cooked +Reheat	3.64^{b}	4.55^{ba}	-	-	4.32^{ba}	5.32^{a}

Means with the same letters within each row are not statistically significant different at P<0.05

4.7 Comparison of In-vitro Iron Bioavailability in Traditional and Modified Preparation Methods of Sweet Potato Leaf Dishes

4.7.1 In-vitro iron bioavailability in traditional and modified methods of fresh sweet potato leaf dishes

Figure 10 presents the comparison of in-vitro iron bioavailability in traditional and modified preparation methods of fresh sweet potato leaf dishes. All dishes prepared by modified methods were significantly higher ($P<0.05$) in iron bioavailability than dishes prepared by traditional methods. Modified dishes were 2-3 times higher in iron bioavailability compared to traditional dishes. Modified dishes cooked with oil + tomato were highest in iron bioavailability (6.26%) while the least was observed in traditional reheated fresh leaf dishes cooked with oil (2.12%).

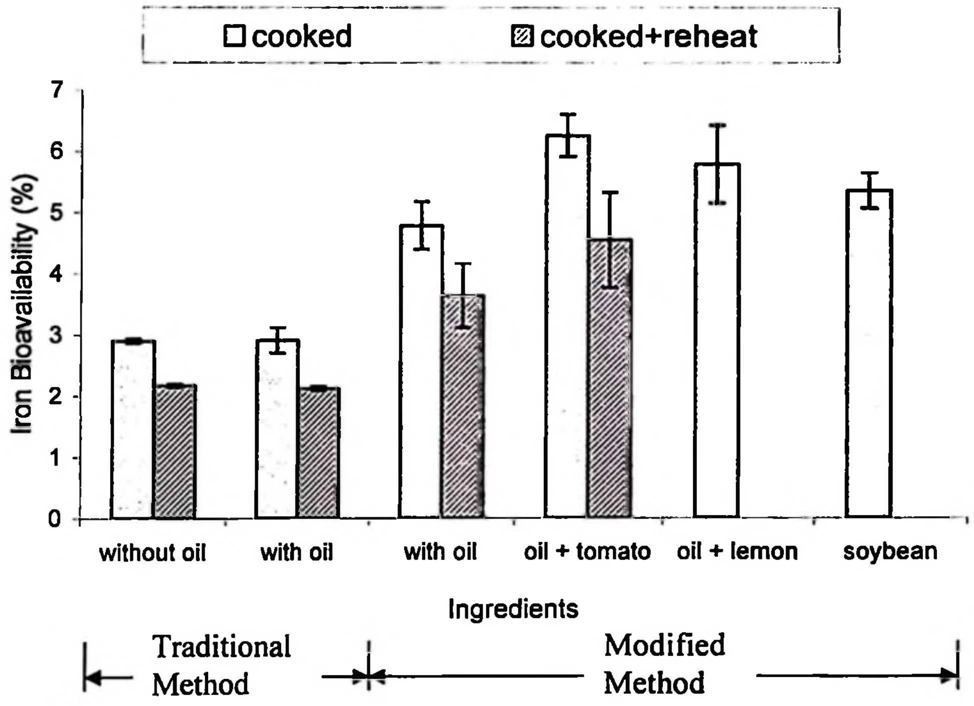

Figure 10: **Comparison of in-vitro iron bioavailability in traditional and modified methods of fresh sweet potato leaf dishes (± standard error)**

4.7.2 In-vitro iron bioavailability in traditional and modified preparation methods of dried sweet potato leaf dishes

Figure 11 presents the comparison of in-vitro iron bioavailability in traditional and modified preparation methods of dried sweet potato leaf dishes. The oven-dried dishes were significantly ($P<0.05$) higher in iron bioavailability than the sun-dried dishes. Oven-dried dishes had highest iron bioavailability of 7.08% and lowest of 4.32%, while in sun-dried dishes the highest iron bioavailability was 3.78% and lowest was 3.19%.

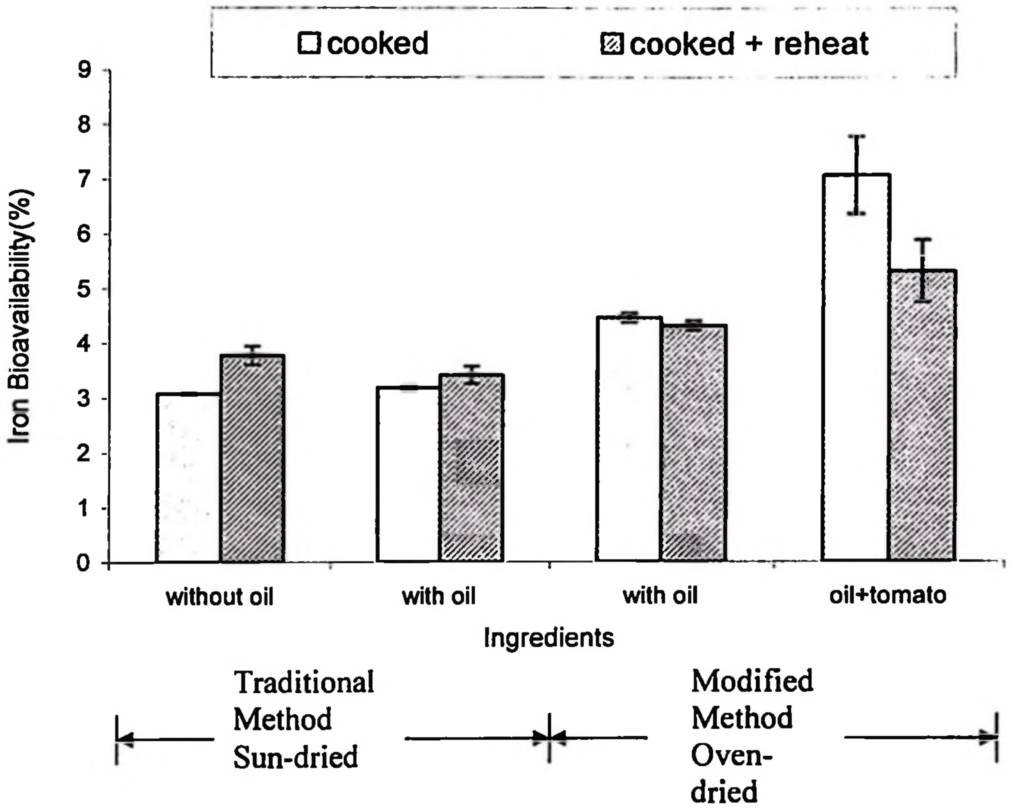

Figure 11: **Comparison of in-vitro iron bioavailability in traditional and modified preparation methods of dried sweet potato leaf dishes (± standard error)**

4.8 Effects of Preparation Stages on Carotenoids Retention and In-vitro Iron Bioavailability in Sweet Potato Leaf Dishes

The effects of processing stages on carotenoids retention and in-vitro iron bioavailability is illustrated in table 22. The results show that cooking and reheating stages are lower in retention of carotenoids compared to pre-cooking stage. Reheating caused lowest β-carotene retention (60.23%), followed by cooking stage (61.74%). Cooking resulted into lowest lutein retention (66.49%), followed by reheating. In the case of iron bioavailability, significant ($P<0.05$) differences were obtained between cooking and reheating stages, whereas lower iron bioavailability (3.66%) was obtained in reheating stage.

Table 22: Effects of preparation stages on carotenoids retention and in-vitro iron bioavailability in sweet potato leaf dishes

Stage	Beta-carotene (%)	Lutein (%)	Iron Bioavailability (%)
Pre-cooking	77.51^{a}	86.06^{a}	-
Cooking	61.74^{b}	66.49^{b}	4.58^{a}
Reheating	60.23^{b}	67.29^{b}	3.66^{b}

Means with the same letters within each row are not statistically significant different at $P<0.05$

CHAPTER FIVE

5.0 DISCUSSION

5.1 Traditional Methods of Preparing Indigenous Vegetables

5.1.1 Indigenous vegetables and cooking methods

Table 13 presented the distribution of respondents within the three districts who consume eight selected vegetables. The variation in the type of vegetables consumed between the three districts could be attributed to climatical factors and the time this survey was conducted. Arumeru district has fertile soils and high amount of bimodal rainfall in the range of 1000 - 2000mm per year (URT, 1999). These conditions favour growth of most of the vegetables. For example, African nightshades and eggplants require high fertile soils and adequate rain for growth (AVRDC, 2003a, b). African nightshade and eggplant are also highly promoted by AVRDC in Arumeru district as target vegetables. These could explain the highest number of respondents who consume most of the vegetables with the exception of jute mallow.

Contrary to Arumeru, Singida and Kongwa districts had least number of vegetable varieties. The main vegetables consumed in these two districts were jute mallow followed by amaranth and lastly sweet potato leaves. This is attributed to the semi-arid climate in the two districts and the high adaptability of the three vegetables to the climate. The two districts have soils which are well-drained but with low fertility, with unimodal type of rainfall and unreliable in the range of 500-800mm per year (URT, 1999). According to Keller (2004) and Mulokozi *et al.* (2004), jute mallow is widely consumed in semi-arid areas. It can easily grow in farms and in the wild. It can be dried and consumed throughout the year, therefore was the main vegetable depended on in Singida and Kongwa districts. Amaranth was the most consumed vegetable and evenly available in all the three districts (Table 13). This could be attributed by the reason that amaranth has high adaptability to a wide range of soils and climate which make it suitable to a wide range of areas (Palada and Chang, 2003). It can also be easily grown in home gardens in the absence of rain. Sweet potato leaves was the third most used vegetable. The vegetable is a relatively hardy crop and therefore can withstand erratic rainfall patterns (Stathers, 2005). The leaves of sweet potato can be harvested throughout the year (Yashimoto *et al.*, 2002; Stathers, 2005). Therefore, it is a dependable source of relish in semi arid areas as well in areas which receive more rains.

The type of vegetables consumed could also be associated with the season this study was conducted. The study was done in November and December, which was the end of a dry season, when very few green plants were available. During the dry and beginning of the rainy seasons vegetables are scarce and households depend mostly on dry vegetables for their daily relishes in the semi arid regions (Lyimo *et al.*, 1991). This could explain why Singida and Kongwa had very few types of vegetables (Table 14).

The variation in preference of cooking methods (Table 14) could be attributed to socio-economic factors, type of vegetables and individual preferences. The results showed that 50 – 70% of respondents from Arumeru preferred stir – frying method for all the vegetables. Arumeru district is an urban district, with many varieties of vegetables including exotic ones (results not shown). In Singida and Kongwa, 91.7% and 76.8% respectively preferred boiling for jute mallow, and less than 50% preferred other cooking methods for other vegetables. The two districts are basically rural districts, with less variety of vegetables especially in the dry season.

The type of vegetables influences the cooking methods. For example, tender leaf vegetables such as amaranth, nightshades, and sweet potato leaves were cooked mainly by stir- frying. An exception is observed with jute mallow, which is tender but cooked by boiling which could be due to its slippery nature. Jute mallow is boiled without oil, groundnuts is added in time of availability; to improve flavor bicarbonate of soda, locally known as *magadi* is added. The second group is fruit vegetables such as okra and African eggplant; which are most preferably cooked by stir–frying method (Table 15). The respondents associated the preference of this method with increased flavor. However, stir-frying has also been associated with loss of nutrients, especially when done under high temperatures (Rodriguez–Amaya and Kimura, 2004).

The third group is vegetables with relatively hard leaves such as pumpkin and cowpea leaves. The main cooking methods for these vegetables were found to be boiling and boiling–frying. An appreciable number of respondents were found to cook amaranth and sweet potato leaves by these methods too. The use of combination of methods has been found to be responsible for nutrients loss as compared to the use of single methods (Rodriguez–Amaya, 2003). In traditional setups, boiling is normally done for long time in a range of 30 – 60 minutes. Several studies have associated prolonged cooking with significant decrease of nutrients loss in foods (Lyimo *et al.*, 1991; Mosha *et al.*, 1997). Respondents reported removal of soup after boiling for a reason of improving taste of vegetables. The discarding of soup is subject to physical removal of nutrients (Reddy and Love, 1999). When the three methods are compared, boiling method is reported to be the best cooking method especially when is done on short time, without removal of soup and in covered pots (Herman and Muhilal, 1995; FAO, 1997; Rodriguez-Amaya, 2003).

5.1.2 Preservation of vegetables by open sun-drying

Table 15 presented the distribution of respondents with respect to the use of open sun–drying as a preservation method by district. The more use of open sun-drying in Singida and Kongwa than in Arumeru as a means of preserving vegetables could be attributed to factors such as: accessibility, climatic and convenience (Svanberg, 2007). In addition, due to long time usage, the method has become a tradition in rural areas (Lyimo *et al.*, 1991). Alternatively, modern methods of drying vegetables such as solar drying and oven drying are reported to improve nutrient contents in dried products and retain higher quantities of the nutrients that are destroyed by traditional methods (Kasimila *et al.*, 1999; Dhewa, 2003; Rensburg *et al.*, 2004). Although modern methods of preservation of vegetables have been introduced, they are not yet feasible in these areas (Badifu *et al.*, 2001). This could be due to the cost involved in acquiring the modern methods, which are not affordable by the rural people.

Secondly, open sun-drying is highly practiced in semi-arid areas which receive short and less rain per year. In these areas, vegetables are plenty during the short rainy seasons, but they become scarce during the dry season. This causes households to dry vegetables close to the end of rain, and use them during the dry season as relish. Almost all vegetables found in the semi-arid areas are open sun-dried, but the processing methods differ among vegetables. For example, cowpea leaves are boiled and drained before dried while sweet potato leaves, jute mallow and amaranth are directly dried without boiling (Mulokozi and Svanberg, 2003).

5.2 The Effects of Traditional Preparation Methods on Carotenoids Retention of Sweet Potato Leaf Dishes

Food preparation practices at home have been associated with lowered retention of carotenoids, though information among studies is difficult to compare due to different methods used in the studies (Booth *et al.,* 1992). Generally changes in carotenoids retention in food processing is caused by physical removal and chemical changes i.e. geometric isomerization, enzymatic and non–enzymatic oxidation (Rodriguez-Amaya, 2004). In the current study, processes such as coiling of leaves, discarding of soup, and draining in sun were recorded to be responsible for 38 – 80% beta- carotene and 38 – 50% lutein losses. The significantly reduced retention of the carotenoids in this study (Tables 16, 17 and 22) due to the mentioned pre-cooking processes could firstly be attributed to physical removal of carotenoids (Ndawula, 2004). For example, during boiling carotenoids may be lost, where soup is discarded which is physical removal. This is because carotenoids are protected by protein layer in plants plastids; during boiling of vegetables the layer denatures and releases the carotenoids in the soup (Rensburg *et al.*, 2004). Coiling and draining of the coiled vegetables in sunlight directly activates chemical reactions such as Maillard reaction and hydrolysis (Lee and Ho, 2002; Severi *et al.*, 1997).

Similarly, the significant loss of β-carotene (79%) and lutein (50%) during sun-drying (Tables 16 and 17) could be attributed to isomerization and oxidation. This is because traditional sun- drying exposes vegetables into direct sunlight and oxygen, which results into chemical changes (Mosquera-Minguez *et al.*, 2002). The changes are facilitated by an extensive system of double bonds (or polyene) in carotenoids, which is responsible for their light absorption ability. The polyene chain makes the carotenoid molecule extremely susceptible to oxidizing and isomerising conditions (Krinsky, 1994). Geometric isomerization (a change of geometry about a double bond) during sun-drying is mainly prompted by heat and light. Isomerization changes the usual configuration trans- forms of carotenoids into cis- forms (Kopsell and Kopsell, 2007). Oxidation is the main cause of carotenoids losses in vegetables. The level of oxidation depends on the extent of exposure to light, packaging material, and presence of oxygen, storage materials, metals, enzymes, unsaturated lipids, antioxidants and physical state of the carotenoids (Rodgruez–Amaya, 2003).

The least retention of carotenoids in sun-dried dishes could also be associated with the degradation of ascorbic acid which could protect the carotenoids from oxidation (Ndawula, 2004). Ascorbic acid (Vitamin C) has a potential role in protecting caroteinoids from oxidation (Sardesai, 1998). Therefore, in the absence of vitamin C, there is a higher possibility of reduction of carotenoids due to oxidation. The findings of this study, concurs with other researchers who found reduction of nutrients as a result of sun-drying. Sun-drying and storage in ventilated containers resulted in a significant ($P<0.05$) decrease in the concentration of total carotenoids, β-carotene and α-carotene for leafy vegetables (Mosha *et al.* 1997). Svanberg (2007) found that amaranth and cowpea leaves lost more carotenoids when they were open sun-dried than when they were solar dried or blanched.

The significantly lower ($P<0.05$) retention values of carotenoids as a result of cooking and reheating of fresh vegetables without oil and sun-dried vegetables (Tables 16 and 17) could be attributed to degradation of carotenoids due to prolonged cooking. Carotenoids are heat stable under normal cooking conditions, but they tend to be altered in higher temperatures and prolonged cooking (Booth *et al.*, 1992; Sardesai, 1998). Traditional cooking which involve prolonged cooking and reheating have been reported to cause significant lose of carotenoids by other researchers. For example, Speek (1988) found 60% losses of carotenoids due to sun-drying followed by cooking leafy vegetables. Mosha *et al.* (1997) reported decreased level of β-carotene with increased time of cooking in sweet potato leaves, amaranth, cowpea and pumpkin leaves. Lyimo *et al.* (1991) reported significant ($P<0.05$) losses of carotenoids due to cooking time of 90 min for cassava leaves, and 50 min for pumpkin and *mwage* leaves.

5.3 Effects of Modified Preparation Methods on Carotenoids Retention of Sweet Potato Leaf Dishes

Retention of carotenoids in vegetables can be significantly improved by reducing the cooking time, washing before cutting, lowering the temperature, avoiding coiling and exposure to direct sun light and shortening the time lag between cutting and cooking (Rodriguez–Amaya, 2003; FAO, 1997). Contrary to the traditional methods, modified pre-cooking processes were shown to have no significant negative effect on the retention of the carotenoids (Tables 18, 19 and Fig. 6 and 7). Similar studies also recorded relatively higher levels of carotenoids in modified methods compared to traditional methods (Mulokozi, 2004; Herman and Muhilal, 1995). Herman and Muhilal (1995) reported high retention of beta-carotene in vegetables cooked by modified methods as compared to Indonesian traditional methods. Modification involved shorter time of cooking, addition of water (i.e., stewing) and the use of lid during cooking. Mulokozi (2004) compared traditional and modified methods of preparation of several leafy vegetables in Tanzania. The traditional preparation method involved cooking by boiling in water without oil for about 20 to 39 minutes in uncovered clay pots. The modification involving reduced cooking time, in pot with lid, and cutting leaves in smaller pieces before cooking. The samples prepared by modified method had higher levels of carotenoids but relatively less compared to the blanched samples. The findings of this study and other similar studies suggest that modified methods are a better alternative to typical traditional preparations methods for improving and retaining the contents of carotenoids in vegetables.

The non-significance differences shown among most fresh and oven-dried dishes (Table 18 and 19) could be associated with reduced isomerization and oxidation during oven-drying. The finding suggests that the contents of carotenoids in oven-dried vegetables are comparable to fresh vegetables. For example, retention of 96% to 98% of β-carotene in two leafy vegetables was reported by Rahman *et al.* (1995) quoted by Rodriguez-Amaya (1997). Koskei (2006) obtained similar levels of lutein and β-carotene when two species of nightshades were oven-dried and compared with fresh leaves.

The results of this study show that all oven-dried dishes have significantly ($P<0.05$) higher retention of carotenoids than sun-dried dishes (Fig. 8 and 9). The findings are similar to Badifu *et al.* (2001) who found that leaves dried by oven had lower loss values compared to sun–dried leaves. These suggest that oven-drying is a better method of vegetable preservation compared to open sun-drying.

The significant higher retention of carotenoids in traditional and modified dishes with oil than in traditional dishes cooked without oil (Table 16 – 19) could be associated with oil soluble nature of carotenoids (Mosquera-Minguez *et al.*, 2002). Carotenoids are soluble in oil and therefore they are more available in hydrophobic environment (Sardesai, 2001). In the current study cooking fresh sweet potato leaves with oil increased the carotenoids retention in a range of 19 – 20% (Tables 16 and 17). Other researchers have also reported low contents of carotenoids in dishes without oil than in dishes with oil. Cooking vegetables with oil increased the level of carotenoids by 1.5 and 1.7 times for β-carotene and lutein respectively (Koskei, 2006). Hedren *et al.* (2002) reported availability of 8 - 29% of the β-carotene content from vegetables cooked without oil and 39 - 94% from leaves cooked with sunflower oil or red palm oil. Mulokozi *et al.* (2004) found that vegetables cooked with oil had 2 - 5 times higher amount of in-vitro available of all-trans-beta-carotene than vegetables cooked without oil. Sunflower oil is the type of oil which was used to prepare the oil dishes although its effectiveness compared to red palm oil has been challenged. For example, adding red palm oil in vegetables instead of sunflower oil resulted in twice as much availability β-carotene due to the high availability of its beta-carotene content (Hedren *et al.*, 2002).

Nutrient contents in foods can be improved by cooking more than one type of food together (FAO, 1997). Modified dishes cooked with soybean had significantly ($P<0.05$) higher carotenoids than traditional dishes cooked without oil (Fig. 6). The retention of carotenoids in dishes cooked with soybean was also not significantly different from dishes cooked with oil (Fig. 6 and 7 and Tables 18 and 19). This could be associated with the higher amount of oil present in soybean. Soybean is a good source of oil ranging between 18 – 20% (Laswai, 2006). The soybean oil is 61% polyunsaturated fat and 24% monounsaturated fat which is comparable to the total unsaturated fat content of other vegetable oils (Rweyemamu, 2007). Soybean oil has shown to prevent degradation of vitamin A during heat treatment. For example, adding vitamin A to soybean oil, made the vitamin to be more available during heat treatment (Dutra, 1998). The findings of this study suggest that soaked soybean can improve the retention of β-carotene and lutein when cooked with vegetables and its effects can be comparable to sun-flower oil.

The highest levels of lutein and β-carotene retention observed in dishes cooked with oil + tomato (Table 18, 19 and Fig. 6 - 9) compared to all dishes could be attributed to the carotenoids oil solubility and the presence of lycopene and other carotenoids in tomatoes. Tomatoes are rich source of lycopene, followed by γ–carotene, phytoene and β–carotene (Howard, 2003, Kristie *et al.*, 2005). The consumption of tomato products with olive oil is reported to improve lycopene content of the plasma (Lee *et al.*, 2000). Studies have associated lycopene with protective role for other carotenoids (Rodriguez-Amaya, 1997). For example, addition of tomato to a recipe enhanced stability of β-carotene (Bhaskarachary *et al.*, 1995).

Reheating fresh and oven-dried dishes cooked with oil + tomato have shown significant increase in carotenoids retention (Tables 18 - 19 and Fig. 8 – 9). The findings could be associated with the high availability of carotenoids in processed tomato products due to denaturation of protein layer during processing (Rensburg *et al.*, 2004). Kirstie *et al.* (2005) found that processed tomatoes have high levels of carotenoids as compared to raw tomatoes. The presence of vitamin C in tomato could also be responsible for high retention of carotenoids (Riso *et al.*, 2004). Vitamin C is a reducing agent (Sardesai, 1997) which could reduce oxidation of carotenoids during cooking. These findings suggest that fresh and oven-dried sweet potato leaves are better source of lutein and β-carotene when cooked with oil + tomato, than with oil alone.

5.4 Effects of Traditional Preparation Methods on In-vitro Iron Bioavailability in Sweet Potato Leaf Dishes

Approaches have been established to estimate iron bioavailability in meals. These include measurement of dialyzable iron and Caco-2 cell uptake, both carried out after in-vitro simulated gastric and pancreatic digestion (Lynch, 2005; Reddy *et al.*, 2000). Data indicate that dialysis and Caco-2 cell uptake are useful for ranking meals and single food items in terms of predicted iron bioavailability, but may not reflect the magnitudes of the effects of factors that influence absorption accurately (Lynch, 2005). The in-vitro iron bioavailability which ranged between 2.12 to 2.90% for fresh dishes (Table 20) is similar to those of Singh *et al.* (2001) who found iron bioavailability of 4% in amaranth and 2.7% in spinach.

The significantly ($P<0.05$) lower levels of iron bioavailability in traditional dishes cooked with oil compared to modified dishes cooked with oil (Table 20, 22 and Fig. 10) could be associated with removal of iron during the pre-cooking stage. Although simple cooking method such as boiling with water has been reported to improve the bioavailability of iron (Yang *et al.*, 2002; Yaday and Sehgal, 2002); processes such as removal of soup after boiling of vegetables lead to physical removal of minerals as well (Lee and Ho, 2006).

Nevertheless, mineral losses due to boiling are relatively low compared to other nutrients during the process (Prasad *et al.*, 2000). The significant decrease of iron bioavailability due to reheating (Tables 20 and 22) could be attributed to degradation of ascorbic acid. Water soluble ascorbic acid is easily lost during washing and cooking. The traditional preparations including prolonged cooking, coiling and exposure of the leaves to sun for drainage could significantly reduce the content of ascorbic acid in the dishes. The degradation of ascorbic acid as an enhancer of iron bioavailability could facilitate the inhibition of iron bioavailability in samples (Naidu, 2003).

Among all the methods of preparations, sun-dried and oven-dried treatments were higher in in-vitro iron bioavailability (Tables 20 and 21 and Fig. 11). This could probably be attributed to the unaccounted moisture and soluble solids losses which could happen during drying. The losses could concentrate and increase the iron composition per unit weight of leaves. The significantly ($P<0.05$) lower level of iron bioavailability in sun-dried compared to oven-dried dishes (Fig. 11) could be associated with high loss of vitamin C (ascorbic acid) during sun-drying. Ascorbic acid is highly unstable during processing; therefore high levels of the acid are lost during drying especially in open sun (Davidsson, 2001; Teucher, 2004). Ascorbic acid promotes non-heme iron absorption by reducing ferric iron to the ferrous state, which is soluble with high pH in the duodenum and small intestine (SCN, 1993). Furthermore, the losses of ascorbic acid during drying could be attributed to increased effects of iron bioavailability inhibitors such as polyphenols present in vegetables due to increased concentration of the inhibitors (SCN, 1993).

5.5 Effects of Modified Methods on In-vitro Iron Bioavailability in Sweet Potato Leaf Dishes

Vegetables and other foods are known to contain polyphenols which are present in the form of phenolic acids, flavonoids and their polymerization products. The compounds form insoluble complexes with iron and may exist as an "iron-tannin" complex and thus inhibit iron absorption (Kannah, 2006). Improvement of iron bioavailability can be achieved by degrading inhibitors that prevent the absorption from the food and by formation of promoting factors uptake (Gibson, 2004). In table 21, the results show that the level of iron bioavailability in modified dishes ranged between 3.64 – 7.08%, which is within the range of 2% to 20% for non-heme iron (Allen and Ahluwalia, 1997). The significant increase of iron bioavailability in modified dishes as compared to traditional dishes (Fig. 10 and 11) could be associated with the presence of iron enhancers as a result of food combinations (FAO, 1997).

The significantly ($P<0.05$) highest level of iron bioavailability in dishes cooked with oil + tomato, followed by dishes cooked with oil + lemon (Table 21 and Fig. 10) could be attributed to the presence of ascorbic acid in tomato and in lemon (Naidu, 2003). Eating acidic foods, especially those rich in ascorbic acid such as lemon, along with iron containing foods can increase absorption three to seven fold (Naidu, 2003). According to Teucher (2004), the promotion of iron absorption in the presence of ascorbic acid is determined by the amount of inhibitors in the meals. Meals containing low to medium levels of inhibitors require less amount of ascorbic acid as compared to meals with high levels of phytic acid. Therefore the acid is more effective when present in high quantities (Davidsson, 2003; Hurrell, 2004). Vijayalakshmi *et al.* (2003) found that tomato was the most effective in enhancing the iron bioavailability of mungbean. The bioavailability of iron was observed to be as high as over 20 µg per 20 g of a mixture of mungbean and vegetables after cooking (Vijayalakshmi *et al.*, 2003). The findings suggest that the use of tomato and lemon as an ingredient in vegetables is an effective method in improving iron bioavailability.

The high iron bioavailability in oil + tomato recipes could also be associated with the high presence of carotenoids in tomato. Of recent, it has been found that vitamin A and ß-carotene can enhance non-heme iron absorption by preventing the inhibitory effect of phytates (Manju *et al.*, 2000). Adding 500IU of vitamin A or β–carotene to a meal of cereal doubles the iron absorption from the gut of human subjects (Graham and Rosser, 2000). Vitamin A or β–carotene will enhance the bioavailability of iron in humans, in presence of high levels of phytate and tannins in the diet (Graham and Rosser, 2000). This suggests that the addition of tomatoes in vegetable dishes might be an effective way of reducing the inhibitory effects of compounds such as phytate and tannins in vegetables and therefore improve iron bioavailability.

The non-significant ($P>0.05$) difference of dishes cooked with soybean and oil dishes (Table 21) could be attributed to high composition of oil in soybean (Rweyemamu, 2007). Soybean proteins and polyphenols have been associated with decreased bioavailability of iron (Sandberg, 2002). However, dishes cooked with soybean were slightly higher in iron bioavailability compared to dishes cooked with oil. This may be explained by the inherently high amount of iron in soybean and soy products (Laswai, 2006; Gibson, 2007; Rweyemamu, 2007). The high iron bioavailability in soybean recipes could also be attributed to soaking of the soybean which was done before cooking in this study. Soaking is effective in reducing phytic acid by activating endogenous phytase enzymes to degrade phytic acid and reduce the amount of polyphenols that inhibit non-heme iron absorption (Svaberg, 1995). Soaking flour for 24 hours increased the amount of soluble iron up to 10 fold (Svaberg, 1995). Even soaking for less than 24 hours could improve the amount of soluble iron. For example, soaking wheat or rye flour for two hours completely decomposed phytic acid (Sandberg and Svanberg, 1991). Similar to the findings of this study, high iron bioavailability were observed when vegetables were cooked with legumes such as soybean and lima bean (Vijayalakshmi *et al,* 2003). Among all treatments done, dishes cooked without oil had significantly lowest iron bioavailability. The findings suggest that, oil has potential for improving iron bioavailability, but the effectiveness increases when vegetables are prepared by modified method in combination with ingredients such as oil + tomato or oil + lemon (Fig. 10) which enhances iron bioavailability .

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Based on the results obtained from this study, the following conclusions can be reached:

(a) The three most cooked vegetables in all the survey districts are amaranth, jute mallow and sweet potato leaves. Jute mallow with boiling method is the most common in Kongwa and Singida. Nightshades, African eggplant and amaranth stir-frying is most common in Arumeru.

(b) Open sun-drying is the most used method of vegetable preservation in Singida and Kongwa.

(c) Lutein, β-carotene retention and in-vitro iron bioavailability are significantly affected by preparation methods, in which modified methods have proved to be superior to traditional methods. Traditional preparation methods retain the carotenoids in a range of only 16 – 70%, whereas modified methods retain carotenoids in a range of 60 – 116%. Iron bioavailability is improved by 1.52 – 3.30% when vegetables are prepared by modified methods.

(d) Cooking and reheating stages are mostly responsible for the significant reduction of carotenoids retention and iron bioavailability respectively in dishes prepared by traditional methods.

(e) Oil has potential for improving the lutein, β-carotene retention and iron bioavailability, but the effectiveness will increase with the use of ingredients with modified methods:

- ➢ Oil + tomato have highest potential for retaining lutein, β-carotene and improving iron bioavailability in vegetable dishes.
- ➢ Oil + lemon dishes are second highest in iron bioavailability; dishes cooked with oil alone are second in terms of carotenoids retention.
- ➢ Iron bioavailability in soaked soybean recipes is comparable to dishes cooked with oil.

In general, this study noted that vegetable dishes which were high in iron bioavailability were also higher in lutein and β-carotene retention suggesting a relationship between the two.

6.2 Recommendations

i. Since modified methods of preparation improved lutein, β-carotene retention and iron bioavailability in sweet potato leaf dishes; simple improved preparations methods such as boiling for a short time, avoiding coiling and draining vegetables in the sun, washing before cutting and retaining of boiled stock or soup are highly recommended.

ii. Oven-drying of vegetables is recommended as a better way of preserving vegetables. Where the method is not feasible, indirect sun-drying methods should be used such as solar cabinet dryers and blanching prior to drying.

iii. Since oil, tomato and lemon have shown to improve lutein, β-carotene and iron bioavailability in sweet potato leaf dishes, the use of a combination of oil + tomato and oil + lemon is recommended.

iv. The use of soaked soybean is recommended as an ingredient in vegetable dishes (its enhancing effect was comparable to that of oil in dishes).

v. Further studies to explain the relationship between carotenoids and iron bioavailability as co-enhancing factors in vegetables dishes are needed.

REFERENCES

Aletor, O., Oshodi, A. A. and Ipinmoroti, K. (2002). Chemical composition of common leafy vegetables and functional properties of their leaf protein concentrates. *Journal of Food Chemistry* 78(1): 63 – 68.

Allen, L. and Gillespie, S. T. (2001). *What Works? A Review of the Efficacy and Effectiveness of Nutrition Interventions.* United Nations Administrative Sub-Committee on Nutrition. Washington DC. 145pp.

Allen, L. H. and Ahluwalia, N. (1997). *Improving Iron Status through Diet.* United States Agency of International Development. John Snow Inc./OMNI Project, Arlington, USA. 120pp.

AOAC (1995). *Official Methods of Analysis.* Association of Official Analytical Chemists International. Arlington, USA. 49pp.

AVRDC (2003a). African eggplant: International cooperators fact sheet. [http://www.avrdc.org./LC/eggplant/home.htm] site visited on 15/7/2007.

AVRDC (2003b). Broad leaved nightshade: International cooperators fact sheet. [http://www.avrdc.org/LC/indigenous/sscabrum.htm] site visited on 15/7/2007.

AVRDC (2005). *Promotion of Neglected Indigenous Vegetable Crops for Nutritional Health in Eastern and Southern Africa.* AVRDC, Shanhua, Taiwan. 29pp.

Azevedo-Meleiro, C. H. and Rodriguez-Amaya, D. B. (2004). Confirmation of the identity of the carotenoids of tropical fruits by high performance liquid chromatography. *Journal of Food Composition and Analysis* 17(6): 749 – 765.

Badifu, G. I., Akpapunam, M. A. and Mqbemere, V. M (1995). The fate of beta-carotene in processed leaves of fluted pumpkin (*Telfairia accidentalis*) a popular vegetable in Nigerian diet. *Plant Foods for Human Nutrition* 48(2): 141 - 147.

Barminas, T. T. (1998). Mineral composition of non-conventional leafy vegetables. *Plant Foods for Human Nutrition* 53: 29 – 36.

Bhaskarachary, K. D., Sankar-Rao, D. S., Deothale, Y. G. and Reddy, V. (1995). Carotene content of some common and less familiar foods of plant origin. *Journal of Food Chemistry* 54: 189 – 193.

Bobat, R., Coovadia, H. and Stephen, C. (2005). Safety and efficacy of zinc supplementation for children with HIV-1 infection in South Africa. *Lancet*, 366(9500): 1862-1867.

Booth, .S. L., Johns T. and Kuhnlein, H. V. (1992). Natural food sources of vitamin A and provitamin A. *Food and Nutrition Bulletin* 14(1): 6 - 19.

Borwankar, R., Sanghvi. T. and Houston, R. (2007). What is the extent of vitamin and mineral deficiencies? *Food and Nutrition Bulletin* 28(1): 174 – 181.

Britton, G. (1995). Structure of and properties of carotenoids in relation to function. *FASEB Journal* 9(15): 1551 – 1558.

Canfiled, L. M., Kaminsky, R. G., Taren, D. L., Shaw, E. and Sander, J. K. (2001). Red palm oil in the maternal diet increases provitamin A carotenoids in breastmilk and serum of the mother. *European Journal of Nutrition* 40(1): 30 – 38.

Codjia, G. (2001). Food sources of vitamin A and provitamin A specific to Africa: A FAO perspective. *Food and Nutrition Bulletin* 22(4): 357 - 360

Davidson, L. (2003). Approaches to improve iron bioavailability from complementary foods. *Journal of Nutrition* 133(5):1560-1562.

Davidson, L., Walczyk, T., Zavaleta, N. and Hurrell, R. (2001). Improving iron absorption from Peruvian school breakfast meal by adding ascorbic acid or Na2EDTA. *American Journal of Clinical Nutrition* 73(2): 283 – 287.

Dewanto, V., Wu, X., Adom, K. K. and Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agriculture and Food Chemistry* 50(10): 3010 – 3014.

Dhewa,C. (April 2003). Crop Post Harvest Programme, Southern Africa. [http:www.new-agri.co.uk/o3/focus/htm] site visited on 30/5/2006.

Dutra, E. J., Favaro, D. M. R., Leonardo, R. J., Jordao, A. A. and Vannucchi, H. (1998). Absorption by humans of beta-carotene from fortified soy bean oil added to rice; effect of heat treatment. *Journal of the American College of Nutrition* 17(14): 361 – 365.

FAO (1997). *Preventing Micronutrient Malnutrition: A Guide to Food–Based Approaches. A Manual for Policy Makers and Programme Planners.* Food and Nutrition Division of Food and Agriculture Organization of the United Nations, Rome, Italy. 70pp.

FAO/WHO (2003). Diet, nutrition and the prevention of chronic diseases. *WHO Technical Series Report no. 916 (TRS 916).* Rome, Italy. 160pp.

Fawzi, W.W. (2003). Micronutrients and human immunodeficiency virus type 1 disease progression among adults and children. *Clinical Infectious Diseases Journal* 37(2):112-116.

Friis, H. (2005). Micronutrients and HIV infection: Evidence, lessons and recommendations. WHO *Report of Consultation on Nutrition and HIV/AIDS in Africa,* 55pp.

Gambling, L. (February 2006). The role of micronutrients in fetal and postnatal development. [http: //www. rowett.ac.uk/divisions/htm] site visited on 5/6/2006.

Gibson, R. (2007). The role of diet and host related factors in nutrient bioavailability and thus in nutrient-based dietary requirement estimates. *Food and Nutrition Bulletin* 28(1): 77 – 100.

Gibson, R. S. (2004). Strategies for preventing micronutrient deficiencies in developing countries. *Asian Pacific Journal of Clinical Nutrition* 13: 54 – 60.

Graham, R. D. and Rosser, J. M. (2000). Carotenoids in staple foods: Their potential in improving human nutrition. *Food Nutrition Bulletin* 21: 401 – 403.

Hedren, E., Diaz, V. and Svanberg, V. (2002). Estimation of carotenoids accessibility from carrots determined by an in-vitro digestion method. *European Journal of Clinical Nutrition* 56(5): 425 – 430.

Hedren, E., Mulokozi, G. and Svanberg, U. (2002). In vitro accessibility of carotenes from green leafy vegetables cooked with sunflower oil or red palm oil. *International Journal of Food Science and Nutrition* 53(6): 445 – 453.

Herman, P. A. and Muhilhal, M. K. (1995). Identifying seasonal vitamin A rich foods and recommended preparation and preservation methods in Indonesia. In: *Empowering Vitamin A Foods. (Edited by Wasantwisut, E. and Attig, A. G.),* Institute of Nutrition, Bangkok. pp. 53 – 60.

Howard, K. (March 2003). Tomatoes and prostate cancer prevention. [http://repositories.cdlib.org/uclabiolchem/nutritionbytes/vol9/iss2/art 4.htm] site visited on 12/5/2007.

Hughes, D.A. (2001). Dietary carotenoides and human immune function. *Journal of Nutrition* 17(10): 823 – 827.

Hurrell, R. (2002). Bioavailability – A time for reflection. *International Journal of Nutrition Research* 72(1): 1- 2.

Hurrell, R. F. (2004). Phytic acid degradation as a means of improving iron absorption. *International Journal of Vitamin Nutrition Research* 74(6):445-452.

Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., Tadokoro, T. and Maekawa, A. (2000). Nutritive evaluation on chemical components of leaves, stalks and stems of sweet potato leaves. *Journal of Food Chemistry* 68: 359 – 367.

Kannah, S. (March 2006). Factors in vegetarian diets influencing iron and zinc bioavailability. [http://xoomer.virgilio.it/tatanone/ironzinc.diets.htm] site visited 19/5/2006.

Kapanidis, A. and Lee, T. C. (1995). Heating cruciferous vegetables increases in-vitro dialyzability of intrinsic and extrinsic iron. *Journal of Food Science* 60: 128 – 131.

Keller, G. (2004). African nightshade, eggplant, spideflower *et al* – production and consumption of traditional vegetables in Tanzania from the farmers' point of view. Dissertation for Award of MSc Degree at Georg–August University, Gottingen, Germany, 217pp.

Khader, V. and Rama, S. (1998). Selected mineral content of common leafy vegetables consumed in India at different stages of maturity. *Plant Foods for Human Nutrition* 53(1): 71 – 81.

Kidmose, U., Yang, R. Y., Thilsted, S. H., Christensen, L. P. and Brandt, K. (2006). Content of carotenoids in commonly consumed Asian vegetables and stability and extractability during frying. *Journal of Food Composition and Analysis* 19(6): 562 – 571.

Kinabo, J., Mnkeni, A., Nyaruhucha, C. N. M., Msuya, J. and Ishengoma, J. (2004). Nutrients content of foods commonly consumed in Iringa and Morogoro regions. In: *Proceedings of the Second Collaborative Research Workshop on Food Security.* 28 – 30 May 2003, Morogoro, Tanzania.

Kirstie, C. A., Campbell, J. K., Zaripheh, S., Jeffery, H. E. and Erdman, W. J (2005). Tomato as a functional food. *Journal of Nutrition* 135: 1226 – 1230.

Kopsell, A. D. and Kopsell, E. D. (2006). Accumulation and bioavalability of dietary carotenoids in vegetable crops. *Trends in Plant Science* 11(10): 499 – 507.

Koskei, K. R. (2006). Effects of preparation and preservation methods on concentrations and bioavailability of iron, caroteniods and phenolic antioxidants in nightshade leafy vegetables. Dissertation for Award of Msc Degree at Egerton University, Egerton, Kenya, 39pp.

Krinsky, N. (1994). The biological properties of carotenoids. *Pure and Applied Chemistry* 66(5): 1003 – 1010.

Laswai, H.S. (2006). *An overview of the use of soy in Tanzania.* Proceedings of Soy Conference on Market Share, Serving Consumer Nutrition Needs with United States Soy Ingredients, Dar- es- salaam. 22 August, 2006. 28pp.

Lee, A., Thurnham, D. I. and Chopra, M. (2000). Consumption of tomato products with olive oil but not sunflower oil increases the antioxidant activity of plasma. *Free Radical Biology and Medicine* 29(10): 1051 – 1055.

Lee, K. (1982). Iron chemistry and bioavailability in food processing. In: *Nutritional Bioavailability of Iron (Edited by Kies, C).* American Chemical Society, Washington DC. pp 26 – 30.

Lietz, G., Henry, C. J., Mulokozi, G., Mugyabuso, J. K., Ballart, A., Ndossi, G. D., Lorri, W. and Tomkins, A. (2001). Comparison of the effects of supplemental red palm oil and sunflower oil on maternal vitamin A status. *American Journal of Clinical Nutrition* 74(4): 501 – 509.

Lyimo, M. H., Nyagwegwe, S. and Mnkeni, A .P. (1991). Investigation on the effect of traditional food processing, preservation and storage methods on vegetable nutrients A case study in Tanzania. *Plant Foods for Human Nutrition* 41(1): 53 – 57.

Lyimo, M., Temu, R. P.C and Mugula, J. K. (2003). Identification and nutrient composition of indigenous vegetables of Tanzania. *Plant Foods for Human Nutrition* 58(1): 85 – 92.

Lynch, S. (2005). The precision of in vitro methods and algorithms for predicting the bioavailability of dietary iron International. *Journal of Vitamin Nutrition Research* 75(6): 436 – 445.

Maberly, G. F., Trowbridge, F .L., Yip, R., Sullivan, K. M. and West, C. E. (1994). Programs against micronutrient malnutrition: Ending Hidden Hunger. *Annual Review of Public Health* 15: 277 - 301.

Madisa, M. E and Tshamekang M. E (March 2006). Conservation and utilization of indigenous vegetables in Botswana. [http://www.ipgri.cgiar.org/publications.htm] site visited on 30/5/2006.

Marcela, C. and Rodriguez-Amaya, D. B. (2004). Optimization of HPLC quantification of carotenoids in cooked green vegetables. Comparison of analytical and calculated data. *Journal of Food Composition and Analysis* 17: 37 – 51.

Mclziniso, P., Hinds, M. J., Bellmer, D. D., Brown, B and Payton, M. E. (2006). Physical quality and carotene content of solar-dried green leafy and yellow succulent vegetables. *Plant Foods for Human Nutrition* 6(1): 13 – 21.

Miller, D. D. and Schricker, B. R. (1982). In vitro estimation of food iron bioavailability. In: *Nutritional Bioavailability of Iron. (Edited by Kies, C.)* American Chemical Society, Washington DC. pp. 11 – 25.

Mosha, T. C., Pace R. D., Adeyeye, S., Mtebe, S. and Laswai, H. (1995). Proximate composition and mineral content of selected Tanzania vegetables and effect of traditional processing on the retention of ascorbic acid, riboflavin and thiamine. *Plant Foods for Human Nutrition* 48(3): 235 – 245.

Mosha, T. C., Pace, R. D., Adeyeye, S., Laswai, H. S., Mtebe, K. (1997). Effect of traditional processing practices on the content of total carotenoids, beta-carotene, and alpha-carotene and vitamin A activity of selected Tanzania vegetables. *Plant Foods for Human Nutrition* 50(3): 189 – 201.

Moy, R. J. D. (1999). Iron deficiency in childhood. *Journal of the Royal Society of Medicine* 92: 234 – 236.

Mudambi, R M and Rajagopal, M. V. (2000). *Fundamentals of Food and Nutrition.* New Age International Limited., New Delhi. 87pp.

Mulokozi, G. and Svanberg, U. (2003). Effect of traditional open sun-drying and solar cabinet drying on carotene content and vitamin A activity of green leaf vegetables *Plant Food for Human Nutrition* 58: 1 – 15.

Mulokozi, G., Heden, E., Svanberg, V. (2004). In vitro accessibility and intake of beta-carotene from cooked green leafy vegetables and their estimated contribution to vitamin A requirements. *Plant Foods for Human Nutrition* 59(1): 1- 9.

Mwajumwa, L. B. S., Kahangi, M. E. and Imungi, J. K. (1991). The prevalence and nutritional value of some Kenyan indigenous leafy vegetables from three locations of Machakos district. *Ecology of Food and Nutrition* 26: 275 – 280.

Naidu, A. (2003). Vitamin C in human health and disease is still a mystery? *Journal of Nutrition* 2(7): 1 – 10.

Ndawula, J., Kabasa, J. D. and Byaruhanga, Y. B.(2004). Alterations in fruit and vegetables beta-carotene and vitamin C content caused by open sun-drying, visqueen-covered and polyethylene-covered solar-driers. *African Health Sciences* 4(2):125 – 130.

O'Brien, M. E., Kupka, R. and Msamanga, G. (2005). Anemia is an independent predictor of mortality and immunologic progression of disease among women with HIV in Tanzania. *Journal of Acquired Immune Deficiency Syndrome* 40(2): 219-225.

Padda, S. M. (2006). Phenolic composition and antioxidant activity of sweet potatoes (Ipomea batatas). Thesis for Award of Degree of Doctor of Philosophy at Lousiana State University, Lousiana, USA, 109pp.

Palada, M. C. and Chang, L. C. (August 2003). Suggested cultural practices for vegetable amaranth: International cooperators guide. [http://www.avrdc.org.tw.htm] site visited on 15/7/2007.

Prasad, N. N., Siddalingaswamy, P. M., Parameswariah, K., Radhakrishna, R. V., Rao, K. R. and Santhanam, K. (2000). Proximate and mineral composition of some processed traditional and popular Indian dishes. *Journal of Food Chemistry* 68: 87 – 84.

Radhika, M. S., Bhaskaram, P., Balakrishna, N. and Ramalakshmi, B.A. (2003). Red palm oil supplementation: a feasible diet-based approach to improve the vitamin A status of pregnant women and their infants. *Food and Nutrition Bulletin* 24(2): 208 – 217.

Raja, T. K., Othman, O. C. and Bahemuka, T. E. (1997). Levels of crude protein and some inorganic elements in selected green vegetables of Dar-es-Salaam. *Journal of Food Science and Technology* 34(5): 419 – 422.

Reddy, B. M., Hurrell, F. R. and Cook D. J. (2000). Estimation of non-heme iron bioavailability from meal composition. *American Journal of Clinical Nutrition* 71(4): 937 – 943.

Reddy, B. M., Hurrell, F. R. and Cook D. J. (2000). Estimation of non-heme iron bioavailability from meal composition. *American Journal of Clinical Nutrition* 71(4): 937-943.

Reddy, M. B and Love, M (1999). The impact of food processing on the nutritional quality of vitamins and mineral. *Advances in experimental medicine and biology* 459: 99 – 106.

Rensburg, W., Netshiluvhi, E., Vorster, H. J. and Ronde, J. A. (2004). Role of indigenous leafy vegetables in combating hunger and malnutrition. *South Africa Journal of Botany* 70(1): 52 – 59.

Riso, P., Brusamolino, A., Scalfi, L. and Porrini, M. (2004). Bioavailability of carotenoids from spinach and tomatoes. *Nutrition Metabolism and Cardiovascular Diseases* 14(3): 150 – 156.

Riso, P., Visioli, F., Erba, D., Testolin, G. and Porrini, G. (2004). Lycopene and vitamin C concentrations increase in plasma and lymphocytes after tomato intake: Effects on cellular antioxidant protection. *European Journal of Clinical Nutrition.* 581(10): 1350 – 1358.

Rodriguez-Amaya, D. B. (1997). *Carotenoids and food preparation: The retention of provitamin A carotenoids in prepared, processed and stored foods.* Opportunities for Micronutrient Interventions (OMNI). Arlington. 99pp.

Rodriguez-Amaya, D. B. (2003). Food carotenoids analysis, composition and alterations during storage and processing of foods. *Forum Nutrition* 56: 35 – 37.

Rodriguez-Amaya, D.B. and Kimura, M (2004). *Harvest Plus Handbook for Carotenoid Analysis.* International Food Policy Research Institute and International Centre for Tropical Agriculture, Washington DC, 63pp.

Ruel, T. M. (2001). *Can Food-Based Strategies Help Reduce Vitamin A And Iron Deficiencies? A Review of Recent Evidence.* International Food Policy Research Institute, Washington DC, 79pp.

Rweyemamu, P. M .L and Halfani, M (April 2007). Development of micronutrient-rich food ingredients from soybean and *Moringa oleifera.* [http://www.moringanews.org/posters.htm] site visited on 5/5/2007.

Sandberg, A. S and Sandberg, U. (1991). Phytate hydrolysis by phytate in cereals. Effects of in-vitro estimation of iron availability. *Journal of Food Science* 56(5): 1330 – 1333.

Sandberg, A. S. (2005). Methods and options, in-vitro dialyzability benefits and limitations. *Journal of Vitamin Nutrition* 75(6): 395 – 404.

Sandberg, A.S. (2002). Bioavailability of minerals in legumes. *Journal of Nutrition* 88(3): 5281 – 5285.

Sanghvi, T., Ross, J and Heymann, H (January 2007). Why is reducing vitamin and mineral deficiencies critical for development? [http://www.unu.edu/unupress/food/supplpdf.html] site visited on 3/4/2007.

Sardesai, V.M. (1998). *Introduction to Clinical Nutrition.* Marcel Dekker, Ltd., New York. 481pp.

Sato, T., Nagata, M. and Engle. M. (2002). Evaluation of antioxidant activity of indigenous vegetables from South and Southeastern Asia, *JIRCAS Annual Report,* Biological Resources Division, Japan. 48 – 50pp.

SCN (1993). *Focus on Micronutrients. A Periodic Review of Developments in International Nutrition.* Administrative Committee on Coordination and Sub-Committee on Nutrition, Washington, DC. 70pp.

Seddon, J. M. (2007). Multivitamin-multimineral supplements and eye disease: age-related mucular degeneration and cataract. *American Journal of Clinical Nutrition* 85(1): 3045 – 3075.

Severi, S., Bedogni, G., Zoboli, G. P., Manzieri, A. M., Poli, M., Gatti, G and Battistini, N. (1998). Effects of home-based food preparation practices on the micronutrient content of foods. *European Journal of Cancer Prevention* 7(4):331-335.

Shi, J. and Maquer, M (2000). Lycopene in tomatoes physical and chemical properties as affected by processing. *Critical Review in Biotechnology* 20(4): 293 – 334.

Singh, G., Kawatra, A. and Sehgal, S. (2001). Nutritional composition of selected green leafy vegetables, herbs and carrots. *Plant Foods for human Nutrition* 56(4): 359 – 364.

Solomons, N. W. (2001). Dietary sources of zinc and factors affecting its bioavailability. *Food and Nutrition Bulletin* 22(2): 138 – 154.

Speek, A. J., Speek–Saichua, S., Schreurs, W. H. P. (1988). Total carotenoids and β–carotene contents of Thai vegetables and the effects of processing. *Journal of Food Chemistry* 27: 245 – 257.

Stathers, T., Namanda, S., Mwanga, R. O. M., Khisa, G., Kapinga, R. (2005). *Manual for Sweet potato Integrated Production and Pest Management for Farmer Field Schools in Sub- Saharan Africa.* International Potato Center. Kampala, Uganda. 168pp.

Svanberg, U. (March 2007). Improving nutritional quality and hygienic safety in supplementary foods for young children. [http://www.sik.se/cth/forsknin/summary 3/ htm] site visited on 20/4/2007.

Takaeka, G. R., Dao, L., Flessa, S., Gillespie, D. M., Jewell, W. T., Huebner, B., Bertow, D. and Ebeler, S. E. (2001). Processing effects on lycopene content and antioxidant activity of tomatoes. *Journal of Agriculture and Food Chemistry* 49(8): 3713 – 3717.

Tanumiharjo, S. A. (2002). Vitamin A and iron status are improved with vitamin A and iron supplementation in pregnant Indonesian women. *Journal of Nutrition* 132: 1902 – 1912.

Teucher, B., Olivares, M. and Cori, H. (2004). Enhancers of iron absorption: ascorbic acid and other organic acids. *International Journal of Vitamin Nutrition Research* 74(6): 403 – 419.

Tontisirin, K., Nantel, G. and Bhatfacharjee, L. (2002). Food-based strategies to meet the challenges of micronutrient malnutrition in the developing world. *Nutrition society* 61(2): 243 – 250.

Turkmen, N. Sari, F. Velioglu, Y. (2005). The effects of cooking methods on total phenolics and antioxidant activity of selected green vegetables *Journal of Food Chemistry* 93(4): 713 – 718.

URT (1999). *Proposed National Action Program to Combat Desertification.* Vice president's office, Dar- es-salaam., 66pp.

Vijayalakshmi, P. S., Amirthaveni, R. P., Devadas, K., Weinberger, S. C. S. and Shanmugasundaram, S. (2003). Enhanced bioavailability of iron from mungbeans and its effects on health of school children. Shanhua, Taiwan *AVRDC—World Vegetable Center Technical Bulletin* No. 30. 32pp.

Walker, S. S., Paton, W .T., Cowan, H. D., Manuel, A. M., Dranitsaris, G. (1989). Bioavailability of iron in oral ferrous sulfate preparations in healthy volunteers. *Canadian Medical Association Journal* 141(6) 543 - 547.

Watkins, M. L., Erickson, J. D., Thun, M. J., Mulinare, J. and Heath, C.W. (2000). Multivitamin use and mortality in large prospective study. *American Journal of Epidemiology* 152(2): 149 – 162.

Weinberger, K. and Msuya, J. (2004). Indigenous vegetables in Tanzania significance and prospects. *AVRDC—World Vegetable Center Technical Bulletin No. 31*. 70pp.

WHO (2001). *Iron Deficiency Anaemia: Assessment, Prevention and Control. A Guide for Programme Managers*. Geneva. 114pp.

Yaday, S. K. and Sehgal, S. (1995). Effects of home processing on ascorbic acid and beta carotene content of spinach (*Spinacia oleracia*) and amaranth (*Amaranthus tricolor)* leaves. *Plant Foods for Human Nutrition* 47(2): 125 - 131.

Yaday, S. K. and Sehgal, S. (2002). Effect of domestic processing and cooking methods on total HCL extractable iron and in vitro availability of iron in spinach and amaranth leaves. *Nutrition Health* 16(2): 113 – 120.

Yang, Y. R., Tsou, S.T.S. and Lee, C. (2002). Effect of cooking on in-vitro iron bioavailability of various vegetables. In: *Bioactive Compounds in Foods: Effects of Processing and storage (Edited by Tung- Ching Lee and Chi-Tang Ho)* American Chemical Society. pp 130 – 142.

Yashimoto, M., Yahara, S., Okuno, S., Islam, S., Ishiguro, K. and Yamakawa, O. (2002). Antimutagenicity of Mono-, Di-, and Tricaffeoylquinic Acid derivates isolated from sweet potato (*Ipomea batatas)* leaf. *Journal of Biotechnology* 66(11), 2336 – 2341.

Zijp, I. M., Korver, O., Tijburg, L. B. (2000). Effect of tea and other dietary factors on iron absorption. *Critical Reviews Food Science and Nutrition* 40(5): 371 – 398.

Zlotkin, S. (2003). The role of nutrition in the prevention of deficiency anemia in infants, children and adolescents. *Canadian Medical Association Journal* 168(1) 59 - 63.

APPENDICES

Appendix 1: List of wards and villages surveyed in Arumeru, Singida rural and Kongwa districts

s/n	Region/district	Ward	Villages	Number of respondents
1.	Arusha/ Arumeru	Mlangarini	Kiseriani	10
		Nduruma	Nduruma	10
		Nkwandua	Moivaro	10
		Kimnyaki	Olevolos	10
2.	Singida/Singida rural	Ilongero	Ilongero	10
			Mwakiti	10
			Itamka	10
		Puma	Nkuninkana	10
		Ihanja	Ihanja	10
			Iseke	10
3.	Dodoma/Kongwa	Mlali	Mlali – Iwegu Manungu	10 10
		Ugogoni	Ibwaga	10
		Sagara	Sagara B	6
		Chamkoroma	Tubugwe	10
		Hogoro	Chamae	10

Appendix 2: Distribution of respondents in the surveyed districts

District	Frequency	Percent
Singida	60	38.5
Arumeru	40	25.6
Kongwa	56	35.9
Total	156	100

Appendix 3: Distribution of respondents in regard to their age, marital status, education level and size of households.

Personal details	Category	Frequency	Percent
Age (years)	Below 18 years	7	4.5
	18 - 36 years	91	58.3
	37 - 54 years	57	36.5
	Above 55	1	0.6
Marital status	Not married	21	13.5
	Married	135	86.5
Level of education	Primary	140	89.7
	Secondary	3	1.9
	Tertiary	1	0.6
	No formal education	12	7.7
Size of households	Less than 5	37	23.7
	5 - 10	114	73.1
	Above 10	5	3.2

Appendix 4: Traditional and Modified Vegetables Processing Methods: Weaknesses and Strengths

Cooking and preparations	**Traditional methods**			**Modified methods**	
	Common feature	**Standardized feature**	**Weakness**	**Modification**	**Strength**
Preparations of fresh vegetables	Vegetables are kept for a long time in open air or sun; after picked in garden, farms or purchased	Sort the vegetables to remove dirt	Exposure of vegetables in open air or sun have been associated with significant lose of nutrients, especially water soluble nutrients and carotenoids (Mosha *et al.*, 1997)	Pick vegetables; sort to remove dirt. Prepare and cook vegetables in a shortest time as possible after picking or purchasing.	Reduced exposure to air and dryness, facilitate nutrients retention
	Cutting in small pieces and washing. Cutting is sometimes done before washing (some vegetables are not cut e.g. sweet potatoes leaves, they are rolled between palms to form coils, then drained on sun before cooking)	Cut in small pieces (1/2 cm or less). Then, wash thoroughly	Significant losses of carotenoids, iron and other nutrients (Rodriguez-Amaya and Kimura, 2004)	Wash the vegetables thoroughly, and then cut big pieces (once or twice per leaf). Tender leaves does not require cutting	Retention of nutrients
Preparations of dry vegetables	Dry on open sun Some vegetables are steamed first before dried on sun e.g. Cowpea leaves.	Steam the vegetables/ boil, discard the water Dry on open sun for 2 to 3 days Store in basket, bags etc	Traditional Sun drying has a remarkable tendency of reducing ascorbic acid, and total carotenoids, beta - carotene and alpha - carotene and other nutrients (Yaday and Sehgal, 1995, Mosha et al, 1997, Lyimo *et al.*, 1991)	Use solar dryer, blanch before drying, dry on sun for a relatively short while and store in dry well covered containers	Retention of nutrients
Boiling	Addition of water	Put pot on	Prolonged	Boil minimum	Minimum water

Cooking and preparations	**Traditional methods**			**Modified methods**	
	Common feature	**Standardized feature**	**Weakness**	**Modification**	**Strength**
	and salt	fire, add vegetables, salt and enough water to boil	time of cooking Vegetables cooked without oil, is reported to have low bioavailability of carotenoids (Hedren *et al.*, 2002)	amount of salted water (95°C – 100°C) in a pot Add cooking oil, or other sources of oils such as soybean	reduces loss of nutrients. Oil enhance carotenoids bioavailability (Rodriguez-Amaya and Kimura, 2004)
	Long boiling time	Boil the vegetables for about 30 to 60 minutes	Long time of cooking enhance nutrients loses (Mosha *et al.*, 1997; Lyimo *et al.*, 1991).	Add vegetables in the boiling salted water, simmer for 5 to 10 minutes or use minimum time to the acceptable texture	Rapid and short time cooking enhance nutrients retention. Loss of nutrients is small if vegetables are added in boiled water (FAO, 1997)
	Lid on pot while boiling is not necessary	Boil without lid on pot	Open boiling is associated with reduction nutrients (Yaday and Sehgal, 1995; Booth *et al.*, 1992),	Keep lid on pot while boiling	Boiling in a covered pan preserves nutrients (FAO, 1997)
	Discard excess stock	Squeeze and drain excess stock	Vitamin C and iodine are water soluble, they are sensitive to losses caused by throwing away cooking water (FAO, 1997)	Use minimum amount of water or keep excessive stock for further use	Retention of nutrients
Stir - frying	Washing, Peeling and chopping	Wash, peel and cut tomatoes in pieces	Physical removal of nutrients (Reddy and Love, 1999)	Wash tomatoes, cut in small pieces	Retention of nutrients
	Vegetables are cooked with or without oil depending on the availability (Mulokozi, 2004, Koskei, 2007)	Heat small amount of oil, add onions, stir – fry till brown color change	Very small amount of oil might not be adequate in improving bioavailability of carotenoids	Heat adequate amount of oil, add onions, stir until a slight color change is noted	Oil increases bioavailability of carotenoids, 8 – 29% with sunflower oil and 39 – 94% with red palm oil (Hedren *et al.*, 2002a; Kidmose *et al.*,

Cooking and preparations	Traditional methods			Modified methods	
	Common feature	Standardized feature	Weakness	Modification	Strength
					2006)
	Tomatoes is added, on basis of availability and affordability	Add tomatoes stir till is soft	Low nutrients	Add tomatoes: increase amount of tomatoes whenever	Tomatoes help to increase contents of nutrients (Yang and Tsou, 2006)
	Lemon is added in very few occasions for flavor or adding tenderness.			Add small amount of lemon juice	Acid in lemon helps to increase bioavailability of iron. (WHO, 2001).
	Serving and reheating Remaining serving is can be reheated after 4 to 24 hours	Serve or keep. Reheat extra before use in meals	Storage at room temperature causes nutrients degradation and also attract food pathogens	Try to cook just enough for a meal. If is necessary to keep some, then cool rapidly before storage. Where possible use refrigerator for storage.	Prevent nutrients degradation