DETERMINATION OF SELECTED TRACE ELEMENTS AND BETA-CAROTENE IN FRESH AND DRIED INDIGENOUS VEGETABLES AS POTENTIAL FOOD SUPPLEMENT FROM KIAMBU, KENYA

By

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DECLARATION

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

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Sign……. Date……..

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

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DEDICATION

I dedicate my efforts in this work to Almighty God who raised me from a humble background and to my dear Mum Jane Waigwe Nyamu who I owe a lot in life for bringing me up. To My brothers Ken, Job and Jeremy. Lastly to my Aunt Esther and my grandmother Phoebe who also contributed to the far I have come.

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ABBREVIATIONS AND ACRONYMS

AAS	Atomic Absorption Spectroscopy
AI	Adequate Intakes
ANOVA	Analysis of Variances
IOM	Institute of Medicine
IU	International Units
OCC	0pen Column Chromatography
RDA	Recommended Dietary Allowances
RE	Retinol Equivalent
UL	Tolerable Upper Intake Levels
UV-Vis	Ultra Violet-Visible
WHO	World Health Organization

ABSTRACT

Population growth, shortage of food due to unpredicted weather coupled with HIV/AIDS has increased nutritional deficiencies in people living in developing world. Use of micronutrients presents a low-cost strategy for achieving large reductions in death caused by malnutrition. The indigenous green leafy vegetables are of much importance among food crops as they provide adequate amounts of vitamins, immune boosting trace elements and anti-oxidants. The nutritional value of the indigenous vegetable remains underutilized due to their seasonal nature and lack of awareness. They are plenty during rainy season but most of it goes to waste during this period because there is no better method of preserving and processing them to stay longer for the dry season. This study was undertaken in 2012 in Lari and Limuru in Kiambu County to determine the levels of Zinc (Zn), Iron (Fe), Manganese (Mn) and Beta-carotene in fresh, solar-dried and open dried indigenous vegetables and to formulate a food supplement. Indigenous vegetables analyzed include *Gynandropsis gnandra* (spider plant), *Corchorus olitorius* (Jute), *Cucurbita maxima* (pumpkin leaves), *Vigna unguiculata* (kunde) and *Symphytum offinale* (mabaki). Beta-carotene was separated using open column chromatography (OCC) and quantified spectrophotometrically. Atomic absorption spectrophotometer (AAS) was used to determine Zn, Mn and Fe. Indigenous vegetables were dried for preservation using open drying and roof top solar drier. A vegetable mix powder was formulated from analyzed indigenous vegetables so as to meet the recommended dietary allowance of vitamin A, zinc, iron and manganese. Growth performance, feed efficiency and hematological parameters were determined by feeding mice with various proportion of the formulated vegetable mix powder added to basal diet. The mice were divided into four groups. One of the groups was fed with a basal diet which served as a control, while the other three were fed diet containing mixture of prepared basal diet (without inclusion of zinc, iron, vitamin A and manganese) with 25%, 50% and 75% of the formulated indigenous vegetable food supplement *ad lib* for 42 days. Beta-carotene levels in indigenous vegetable ranged from 9.61±0.36 mg/100 g to 0.24±0.01 mg/100 g dry weight with highest level being fresh *Gynandropsis gnandra*. Zn levels ranged from 1.62±0.54 mg/100 g to 0.24±0.09 mg/100 g with highest being fresh *Cucurbita maxima*. Mn levels ranged from 12.93±3.98 mg/100 g to 0.15±0.02 mg/100g with highest being fresh *Symphytum offinale*. Fe levels ranged from 90.78±31.34 mg/100 g to 2.45±0.35 mg/100 g with highest being fresh *Gynandropsis gnandra.* There was a significant reduction in the beta-carotene levels in both solar drying and open drying ($p<0.05$) when compared to fresh vegetables. Solar drying had the highest levels of beta-carotene retention with the range of 55% to 82% compared to open drying. There was insignificant decrease in mineral content both in solar dried and open dried indigenous vegetables. The results showed the group fed with 25% of the food mixture had the highest feed efficiency with a value of 6.96%, while the least was the group fed with 75% of the food mixture with a value of 4.42%, however in both cases the change was not statistically significant at $p<0.05$ when compared to control. There was an increase in white blood cells, lymphocytes and monocytes on the group of animals fed with 25% of the indiginous vegetable mixture as compared to control, however the change was not significant different ($p<0.05$). There was a decrease on white blood cells, the lymphocytes, the neutrophyls and the monocytes on the group of animals fed with 75% and the decrease was statistically different ($p<0.05$). Indigenous vegetables contain substantial amount of beta-carotene, zinc, iron and manganese. Solar drying provides a better method of preserving these vegetables as it retains higher level of beta-carotene. A formula of indigenous vegetables food supplement has potential to provide low levels zinc, iron, manganese and beta-carotene.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Indigenous vegetables are plants whose leaves, fruits or roots are used as vegetables (FAO, 1988; Chweya and Eyzaguirre, 1999). They are also those plants that have evolved within and spread throughout an area unassisted by humans. Indigenous vegetables are valuable sources of nutrients especially in rural areas where they contribute substantially to protein, mineral, vitamins, fiber and other nutrients which are usually in short supply in daily diets (Chweya and Eyzaguirre, 1999). Besides, they add flavour, variety, taste, colour and aesthetic appeal to what would otherwise be a monotonous diet (Chweya and Eyzaguirre, 1999; Maundu *et al.*, 1999; Abukutsa-Onyango, 2003). They are compatible to use with starchy staples as well as representing affordable nutrition to the poor by containing more than 100% of the recommended daily allowances for vitamins and minerals and 40% proteins for growing and lactating mothers (Chweya, 1985). They have a potential that has yet to be exploited in the effective management of the HIV/AIDS infected persons and healing stomach related ailments (Chweya and Eyzaguirre, 1999). This emphasizes the need for thorough investigation of nutrition and health properties of these vegetables in providing nutritional therapy.

Although diets have been associated with increased longevity and protection against chronic diseases, the lack of dietary diversity is particularly a severe problem among the poor populations in developing countries such as Kenya, Tanzania and Uganda. This has been attributed to the fact that the diets in such countries are mainly based on starchy staples with little or no fruits and vegetables (Chweya and Eyzaguirre, 1999). Africa region and Kenya in

particular is reported to have the world's lowest intake of micronutrient-rich fruits and vegetables with the mean consumption being less than half the World health organization (WHO) recommended daily intake of 400 g of fruits and vegetables per capita per day in most countries (WHO**,** 2005). Low consumption of fruits and vegetables is the main contributor to micronutrient deficiencies. Children in Africa are at increased risk of micronutrient deficiencies because of the low balanced meal frequency, lack of diversity, reduced maternal attention, and parasitic infections, such as hookworms and schistosoma (WHO**,** 2005). Nutritional deficiencies, particularly deficiencies of iron, zinc and vitamin A, are documented to be the major problems to most children in low income countries (WHO**,** 2005).

Micronutrients are minerals and vitamins that the body needs to maintain good health. People with HIV are more probable to show signs of micronutrient deficiencies, compared to uninfected people WHO**,** 2005). Research done by Tang and Lanzillotti found low levels of vitamin A, zinc and iron in the blood of various populations (Tang and Lanzillotti, 2005). This has been connected with the changes in the rate at which HIV infection progresses to AIDS (WHO**,** 2005). Oxidation resulting to free radicals has been associated with cancer, heart disease, diabetes, cataracts and kidney disease which may be solved by using anti-oxidants from indigenous vegetables (Chweya and Eyzaguirre, 1999). Increased promotion and subsequent use of indigenous vegetables is one way of increasing dietary diversity, and combating the nutritional transition and associated diseases (Tang and Lanzillotti, 2005).

Kiambu district has seven divisions: Lari, Kikuyu, Limuru, Kiambaa, Githunguri, Ndieya and Karai. The district covers an area of 1,324 km2 with 90% being high agricultural potential

land. Rainfall is reliable and ranges from 1,500mm in the highlands to 500mm in the semi-arid areas of Ndeiya and Karai. These two divisions are vulnerable to drought (Republic of Kenya, 2002). Kiambu boarders Nairobi and most vegetables consumed by residence of Nairobi come from these rich agricultural areas of Limuru, Kikuyu, Kiamba, Githunguri and Lari. Most farmers cultivate both exotic and indigenous vegetables in plenty during rainy seasons but most of it go to waste during this time because these vegetables are perishable. There is no better method of processing them so that they can stay longer. If proper measures are taken these vegetables can be processed to increase their lifespan or even increase the ease of their transportation to dry areas of the country such as north eastern and eastern regions. This will help increase the income, motivate farmers, meet the nutritional need of the country, minimize food shortage in Kenya and help in achievement of some of Millennium Development Goals.

Based on these problems and on the fact that indigenous vegetables contains significant amounts of nutrients required by our body, the study determined the amount of beta-carotene, zinc, iron and manganese in fresh, solar dried and open dried indigenous vegetables identified in this region. The indigenous vegetables were solar dried using a rooftop solar drier designed by Thoruwa (2011) and parameters analyzed at Kenyatta University. These will provide information on the levels of nutrients of indigenous vegetables grown in the region that can be able to meet the dietary daily intake and also provide a base line that will motivate farmers utilize solar driers to process this indigenous vegetables to reduce spoilage and also easy in transportation to other areas where they are not grown.

1.2 Problem statement and justification

Kenya and other African countries are facing food and nutritional insecurity (Rosegrant *et al*., 2005). Indigenous green leafy vegetables are of much importance among food crops as they reduce food insecurity, provide adequate amount of vitamins, immune boosting trace element and anti-oxidants (Abukutsa-Onyango, 2003). The nutritional value of the indigenous vegetable remains underutilized due to lack of awareness and appropriate technology for their effective use. In the past, there were few nutritional deficiencies than currently exist. However, due to population increase and economic crisis, the cases of malnutrition have increased in some places in Kenya such as Turkana, Marsabit and Samburu especially in children as high as 37.4 per cent (WHO, 2005). The indigenous vegetables have not received much attention although they grow very fast, can sustain harsh environmental conditions and have high nutritional value (Grivetti and Ogle, 2000). There is need for processing of these vegetable to ensure that they are available all the year even in their low season. The rich nutritive value of these indigenous vegetables need to be utilized to supplement the low levels of vitamin A, zinc and iron in the blood of HIV/AIDS patient (Tang and Lanzillotti, 2005) and others with similar complications. Therefore, this study proposed to determine the levels beta-carotene and immune boosting essential elements such as zinc, manganese and iron in some selected indigenous vegetables before and after processing with solar drying and open drying. This was to establish a better way of preserving these vegetables for use when not in season as a potential food supplement.

1.3 Hypothesis

There is no significant difference between the levels of beta-carotene and immune boosting

trace elements zinc, iron and manganese in fresh, solar dried and open dried indigenous vegetables. There is also no significant difference in weight gain and hematological parameters on mice fed with formulated indigenous vegetables food supplement.

1.4 Objectives

1.4.1 General objective

The general objective of this study was to determine the levels of beta-carotene and elements zinc, iron and manganese in fresh and dried indigenous vegetables, and to prepare a potential food supplement from the analysed vegetables.

1.4.2 Specific objectives

The specific objectives were to:-

i. determine the levels of beta-carotene in fresh, solar dried and open dried indigenous vegetables (*Gynandropsis gnandra*, Corchorus *olitorius, Cucurbita maxima, Vigna unguiculata, Ipomoea batatas, Symphytum offinale, Solanum aethlopicum,* and *Agaricus bisporus*.) in Lari and Limuru in Kiambu county

ii. determine the levels of zinc, iron and manganese in fresh, solar dried and open dried indigenous vegetables (*Gynandropsis gnandra*, Corchorus *olitorius, Cucurbita maxima, Vigna unguiculata, Ipomoea batatas, Symphytum offinale, Solanum aethlopicum* , and *Agaricus bisporus*.) in Lari and Limuru in Kiambu county

iii. formulate a nutritious food diet from the selected analyzed indigenous vegetables that will meet the recommended dietary allowance of beta-carotene, zinc, iron and

manganese.

iv. test the efficacy of the formulated indigenous vegetables food supplement on the weight gain , feeding efficiency and Haematological parameters in mice fed with the formulated indigenous vegetables food supplement (Mice bioassay).

1.5 Significance of the study

The indigenous vegetables have not received much attention although they grow very fast and have high nutritional value (Grivetti and Ogle, 2000). They are plenty during rainy season but most of it goes to waste during this period because there is no better method of preserving them to stay longer for the dry season. Nutritional quality of the diets does improve with consumption of greater food diversity (Onyango *et al*., 1998). Therefore this study provides information on the levels of zinc, iron, manganese and beta-carotene on some of the indigenous vegetables grown in the study area that provide nutrients for the community. It also provide a better method of preserving these vegetables using solar drier and formulation of a vegetable food supplement rich in zinc, iron, manganese and beta-carotene. This will help increase the income, motivate farmers and meet the nutritional needs of the country.

1.6 Scope and limitation of the study

The study focused only on nine varieties of indigenous vegetables locally grown in Lari and Limuru. Seasonal variation and various species were not considered due time and financial constrains. The indigenous vegetables were processed by open drying and solar drying technology. Also the study considered only Fe, Zn and Mn as immune boosting element.

The levels of beta-carotene in indigenous vegetables are influenced by specific variety of vegetables, soil conditions, the stage of leafy maturity and period between harvest and analysis (Amaya, 2001). These conditions could not be controlled since the indigenous vegetables from these areas were not purposefully grown for the study. The initial and final levels of Beta-carotene after processing were considered important and these factors were not likely to influence the outcome of the results significantly.

CHAPTER TWO

LITERATURE REVIEW

2.1 Indigenous vegetables

Indigenous vegetables play an important role in traditional diet in rural and urban Kenya. For the rural Kenyans, the vegetables are inexpensive, easily accessible and excellent source of micronutrients (Maundu *et al*., 1999). Some of these indigenous vegetable include *Gynandropsis gnandra* (spider plant), *Corchorus olitorius* (Jute), *Cucurbita maxima* (pumpkin leaves), *Vigna unguiculata* (kunde), *Ipomoea batatas* (sweet potatoes leaves), *Symphytum offinale* (mabaki), *Solanum aethlopicum* (African egg plant) and *Agaricus bisporus* (button mushroom).

2.1.1 *Solanum nigrum L (*Black nightshade*)*

Black nightshade *(Solanum nigrum* L.), managu (swahili), belonging to the solanaceae family (presented in Figure 2.1), occur in tropical and warm temperate regions from sea level to altitudes over 3500 m. Four varieties of *Solanum* spp have been identified to be commonly used in Kenya (Schippers, 2002). These varieties, belongs to *S. nigrum* (green-berried and purple berried variants) and *S. villosum* (yellow berried and orange berried variants). *Solanum* play a great role in traditional medicine in Africa, whereby it is used to treat a wide range of stomach ailments (Maundu *et al*., 1999), treating toothache, tonsillitis, conjunctivitis and as tonic for babies. The leaves are high in vitamin A and C and moderate source of minerals (FAO, 1998). Kinabo *et al.* (2004) reported contents of Fe in S*olanum nigrum* of 6.10 mg /100 g while Mwajumwa *et al*. (1991) reported contents of Fe of 11.8 mg/100g.

Figure 2.1 *Solanum nigrum*

2.1.2 C*leome gynandra* (Spider plant*)*

Spider plant *(*C*leome gynandra),* cat's whiskers (English) is another indigenous vegetable, it belongs to the family Cleomaceae comprising of 150-200 species, of which 50 of them occur in Africa (Chweya and Eyzaguirre, 1999). Their stems are hairy and oily. The leaves grow on long stalks usually divided into 3, 5 and 7 leaflets. It has white or pink flowers. Since the leaves are bitter they are usually cooked with other vegetables such as amaranth. The leaves are rich in vitamin A and C, with medium levels of calcium, magnesium and iron (FAO, 1998). Figure 1.2 shows C*leome gynandra* plant.

Figure 2.2 *Cleome gynandra*

The levels of beta-carotene reported for a species of spider plant (G*ynandropsis gnandra)* are Gomez (1981) a value of 477 µg/g dry weight while *Nyambaka* (1996) reported a value of 996 µg/g dry weight.

2.1.3 *Cucurbita maxima* (**pumpkin leaves**)

Pumpkin leaves are used in some communities to mash potatoes. It is a climbing plant with stem which have tendrils, and prickly hair (Figure 2.3). Its leaves are hairly, light green, up to 10 cm long. The plant has yellow flowers (Maundu *et al.,* 1999). Pumpkins have an advantage over the other vegetables in that the fruits can be stored for a long time and can play an important role in maintenances of nutritional levels during the long dry seasons when few fresh vegetables are available. The leaves may be dried and stored for upto 6 months (Shippers, 2000). Ogle and Grivetti (1985) reported beta-carotene levels of 360 µg/g dry weight for fresh C*ucurbita maxima*

Figure 2.3 *Cucurbita maxima*

2.1.4 *Corchorus olitorius (***Jute*)*

Jute leaves are consumed in various parts of the world. It is commonly known as 'mrenda' or 'murere' In Kenya (Figure 2.4). It is cooked with cow pea leaves, milk and butter before being

given to lactating mothers among the Luo (Maundu *et al*., 1999). It is an erect woody herb 0.5 to 1.2 meters on cultivation. Its leaves are short staked, ovate to eclipse and margin serrated. It has yellow flowers. The leaves are good source of protein, starch, vitamin A and C and minerals such as calcium, phosphorous and iron (FAO, 1998). Ogle and Grivetti (1985) reported beta-carotene levels of 641 µg/g dry weight for fresh C*orchorus olitorius*

Figure 2.4 *Corchorus olitorius*

2.1.5 *Ipomoea batatas* (Sweet potato leaves)

The sweet potato (*Ipomoea batatas*) is a dicotyledonous plant that belongs to the family *Convolvulaceae*. Its large, starchy, sweet tasting tuberous roots are an important root vegetable (Figure 2.5)

Figure 2.5 *Ipomoea batatas*

The young leaves and vine tips of sweet potato leaves are widely consumed as a vegetable in

West African countries (Guinea, Sierra Leone and Liberia, for example), as well as in northeastern Uganda, East Africa. According to FAO leaflet No. 13 - 1998, sweet potato leaves and shoots are a good source of vitamins A, C, and B2 (Riboflavin).

2.1.6 *Symphytum offinale* (mabaki)

Common comfrey (*mabaki-kikuyu*) is a perennial herb, it has been cultivated in thousands of farmyards and kitchen gardens. It's an easy plant to cultivate and propagate. Comfrey survives rough treatment and especially thrives in moist areas. Flowering stems rise to three feet or more, topped with clusters of dainty down facing buds and the leaves has coarse texture. Figure 2.6 shows *Symphytum offinale* plant.

Figure 2.6 *Symphytum offinale*

2.1.7 *Vigna unguiculata* (kunde-cowpea leaves)

It is an erect, trailing or climbing herb with three leaflets (Figure 2.7). It is cultivated in Kenya. Both leaves and seeds are used as food, cooked alone or with other vegetables. When the leaves are cooked with Corchorus leaves, milk or butter it is served to breast-feeding mothers among the luo. Cowpea leaves may be dried and stored for several months for use

during dry seasons (Maundu et.al., 1999). The beta-carotene levels in fresh *Vigna unguiculata* reported in some studies include Nyambaka (1996) a value of 691 µg/g dry weight, Mwajumwa *et al.* (1991) reported 670 µg/g dry weight, Nawiri *et al.* (2012) reported a value of 806 µg/g while Gomez (1981) reported a value of 632 µg/g dry weight.

Figure 2.7 Vigna unguiculata

2.1.8 *Solanum aethiopicum* (African eggplant-nyaya chungu)

While there are many varieties of the African eggplant, with a range of shapes, sizes and colors, the eggplant most commonly found across sub-Saharan Africa is *Solanum aethiopicum*. This variety has a brilliant red exterior, and is about the size and shape of an egg—giving it the name, garden egg. It is also known as mock tomato, *ngogwe* and *nyanya chungu* (Musinguzi *et al*., 2002). Though technically a fruit, it is usually picked when it is green and is eaten as a vegetable; cooked into stews and sauces or even consumed raw (Figure 2.8). If picked after it is ripe, it can be enjoyed as a fruit—though some varieties are sourer than others. And, even the nutrient-rich leaves have come to be a popular meal—in the markets of Kampala, Uganda, they are the most popular vegetable on sale. Like many other crops indigenous to Africa, the eggplant is easy to grow and high yielding, And even

though the fruit is not well known for its nutritional content (it is 92 percent water) it also provides vitamin B, beta-carotene and vitamin C in addition to calcium, iron and potassium (Musinguzi *et al*., 2002).

Figure 2.8 *Solanum aethiopicum*

2.1.9 *Agaricus bisporus (*button mushroom)

Mushrooms are edible fungi that grow on decaying organic matter, known as a substrate. Unlike vegetables they do not rely on sunlight to grow. Mushrooms start as very small spawns. The spawns will grow in the substrate to produce a fine white fibrous structure called mycelium. From the mycelium the mushroom fruit is produced, this is the part that is harvested. The most common edible mushroom grown and in the market is the white button mushroom (*Agaricus bisporus*). To a lesser extent oyster mushrooms (*Pleurotus ostreatus*) is also grown although it is the easiest of all to grow. Other species grown are wild Reishi mushroom (*Ganoderma lucida*) which is most nutritious and have a high medicinal value of all (Chang and Miles, 2004).

Mushroom cultivation consists of six steps, which is needed to form a production system. The six steps are Phase I composting, II, spawning III, casing IV, pinning V, and fruiting VI.

Mushrooms have a high nutritional value and are high in protein. They are also a good source of vitamins (B1, B2, B12 and C), essential amino acids, and carbohydrates but are low in fat

and fiber and contain no starch. When fresh they have a very high water content of around 90%. Minerals present include phosphorus, potassium, iron, zinc and copper (Chang and Miles, 2004).

2.2 Nutrition and Health of Indiginous Vegetables

2.2.1 Medicinal value of indiginous vegetables

Most studies show the benefits of a varied diet particularly one including vegetables in increasing the longevity of life and reducing rates of chronic degenerative diseases (Johns, 2003). They are useful in the treatment of anaemia, ulcerated conditions of throat and ringworms among others (Musinguzi *et al*., 2002). Many indiginous vegetables especially the bitter or sour ones like spider plant and african nightshades have been reported to heal stomach related ailments (Schippers, 2002). The shift from traditional foods to exotic food products has accelerated the increase of nutrition related diseases and has been directly implicated in the rise of type 2 diabetes, obesity, cancer throughout africa (WHO, 2002).

Potential health related functions of indiginous vegetables include antibiosis, immuno-stimulation, nervous system action, detoxification, anti-inflammatory, anti-gout, antioxidant, gycemic and hypolipidemic properties. For instance, the Luo of western Kenya attributes action against distubanceas of gastrointestinal tract and strong activity against the protozoa to the leafy vegetables that are bitter like *Solunum nigrum* (Johns, 2000).

Nutritional suppliments especially micronutrients rich foods are good for management of HIV/AIDS (WHO, 2005). Taking suppliments rich in zinc, iron, selenium and vitamin helps

in maintaining body's immunocompetence (WHO, 2005). The rich nutritive value of indigenous vegetables need to be utilized to supplement the low levels of vitamin A, zinc and iron in the blood of HIV/AIDS patient (Tang and Lanzillotti, 2005).

2.2.2 Role of Anti-oxidants

Oxidation is a chemical reaction in which atoms lose electrons. Our bodies are constantly breaking down molecules of food and air into their component atoms, and then rearranging these freed atoms to build the different types of molecules our body needs, but during the process it may result to harmful processes (Amaya, 2001). During metabolism, atoms may lose electrons in a process called oxidation because it is fueled by oxygen and may end up forming radicals. Stable atoms have an even number of electrons orbiting in pairs at successive distance from nucleus. When a stable atom loses an electron during oxidation, it is left with an odd number of electrons in its outermost shell. The unpaired electrons pair up with other unpaired electrons forming stable substances, the unpaired electrons may remain unpaired to form free radicals which are unstable (Amaya, 2001).

Free radicals may be formed as by-products of many of our bodies' fundamental physiological processes including metabolic processes such as when our immune system fights infections (Amaya, 2001). Other factors include pollution, overexposure to sun, toxic substances, radiation exposure, tobacco smoke and asbestos. Some of these cause severe damage to our cell, genetic material and other body component (De pee and West, 1996). Damage to these sites disrupts the transport of substances into and out of the cell, alters protein function and disrupts cell function because of defective DNA (deoxyribonucleic

acid). Many of the diseases are linked to free radicals production which includes cancer, heart disease, diabetes cataracts and kidney disease (De pee and West, 1996).

Antioxidants are compounds that protect our cell from the damage caused by oxidation (Amaya, 2001). The body fights free radicals and repairs the damage they cause by use of antioxidants which are the vitamins, minerals and other compounds. This is done by certain antioxidant vitamins by donating their electrons or hydrogen to the free radicals to stabilize them and reduce damage caused by oxidation (Amaya, 2001).

Antioxidant minerals function within complex antioxidant enzyme system that convert free radicals to less damaging substances that are extracted by our bodies (Amaya, 2001). These enzymes also work to break down fatty acids that have become oxidized. In breaking down the fatty acid they destroy the free radicals associated with the oxidized fatty acid. Other compounds such as carotenoids and phytochemicals help stabilize free radicals and prevent damage to cells and tissues (De pee and West, 1996).

2.3 Essential nutrients in indigenous vegetables

2.3.1 Carotenoids

Carotenoids in food are usually carbon 40 tetraterpenoids built from eight carbon 5 isoprenoid units, joined so that the sequence is reversed at the center (Amaya, 2001). Cyclization and other modifications, such as hydrogenation, dehydrogenation and chain shortening result in a myriad of structures (Amaya, 2001). A distinctive characteristic results into a conjugated double-bond system, which serves as the light-absorbing chromophore which causes the orange, yellow, or red color that these compounds impart to many foods.

Hydrocarbon carotenoids which are made up of only carbon and hydrogen are collectively called carotenes. The carotenes include α-carotenes, β-carotenes, γ -carotene and δ-Carotene shown in figure 2.9, 2.10, 2.11 and 2.12 respectively (Amaya, 2001). Those containing oxygen are termed xanthophylls.

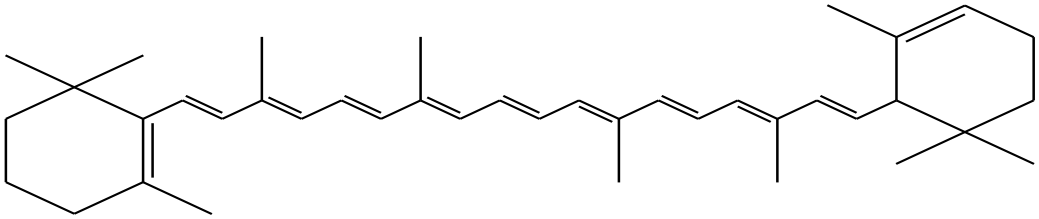

Figure 2.9 α-Carotene

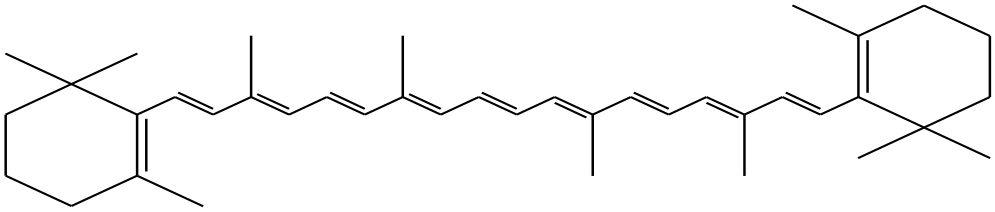

Figure 2.10 β-carotene

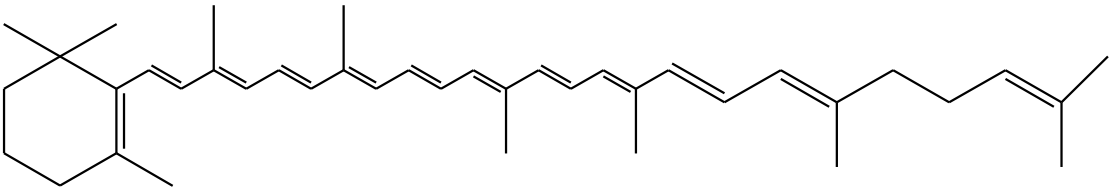

Figure 2.11 γ -carotene

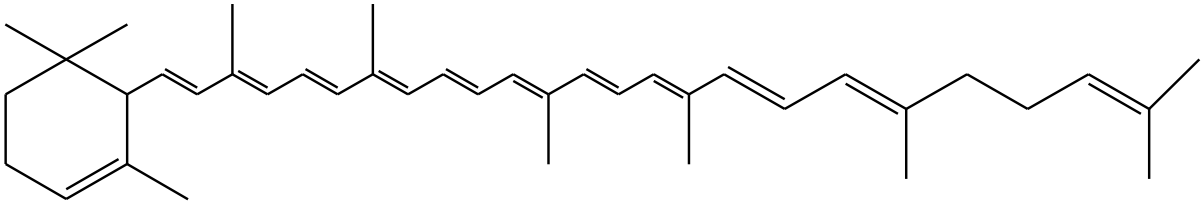

Figure 2.12 δ-Carotene

The carotenoids that are highly unsaturated are prone to isomerization and oxidation. Heat, acids, light and adsorption on an active surface promote isomerization of *trans* carotenoids to the *cis* forms (Amaya, 2001). These factors lead to loss of color and provitamin A activity. Carotenoid also undergoes oxidative degradation, this is where there are wide losses of carotenoids, and it depends on the accessibility of oxygen and is stirred by enzymes, metals, light, and co-oxidation with lipid hydroperoxides (Amaya, 2001). Processing and storage affects carotenoids, the practical consequences being loss of color and biological activity and the formation of volatile compounds that impart desirable or undesirable flavor in some foods (Amaya, 2001).

2.3.1.1 β-carotene

The bicyclic β-carotene (Figure 2.10) is the most widespread of all carotenoids in foods, either as a major or as the manor constituent. The bicyclic α-carotene and the monocyclic γ-carotene sometimes accompany β-carotene, generally at much lower concentrations (Amaya, 2001). Vegetables contain β-carotene which are converted to vitamin A in the walls of small intestine during absorption, making vegetable to have considerable vitamin A activity. Carotenoids which are vitamin A active are referred to as provitamin A. Common provitamin A carotenoids found in foods that come from plants are beta-carotene, alpha-carotene, and beta-cryptoxanthin (De pee and West, 1996). Among these, beta-carotene is most efficiently made into retinol, Alpha-carotene and beta-cryptoxanthin are also converted to vitamin A, but only half as efficiently as beta-carotene (Olson and Kobayashi, 1992).

β-carotene composition is affected by factors such as cultivar or variety; stage of maturity part of the plant consumed; climate or geographic site of production; harvesting and postharvest handling; processing and storage (Amaya, 2001).

2.3.1.2 Role of β-carotene / Vitamin A in health

Vitamin A supplements reduce rates of illness and death among African children living with HIV and the World Health Organization recommend that vitamin A supplements be given every 4-6 months for all young children between 6-59 months old (Friis, 2006). Vitamin A is used in maintenance of normal vision in dim light. The retina of the eyes contain two kind of light receptors; the rods, which produce a photosensitive pigment, rhodopsin or visual purple for vision in dim light and the cones which produces iodopsin or visual violet for vision in bright light and color vision. When the body is deficient in vitamin A, less retinol is available for formation of visual purple.

Vitamin A is essential for the normal skeletal and tooth development. It has been observed for some time that vitamin A deficiency is associated with retarded growth (Robison *et al*., 1986). It has a role in maintaining and formation of healthy functioning of epithelial tissue, which forms the body's primary barrier of infections. The epithelium includes the skin, mucus membrane lining, the ocular and oral cavities and the gastrointestinal, respiratory and genitourinary tracts (Burton and Forster, 1988).

In addition, other biological functions or actions attributed to carotenoids are like prevention of certain types of cancer, cardiovascular disease, and muscular degeneration which are

independent of the provitamin A activity. These have been attributed to an antioxidant property of carotenoids through singlet oxygen quenching and deactivation of free radicals (Palozza and Krinsky, 1992). The ability of carotenoids to quench singlet oxygen is related to the conjugated double-bond system, and maximum protection is given by those having more double bonds (Amaya, 2001).

Recommendations for vitamin A are provided in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine (IOM), 2001. In Table 2.1, Recommended Dietary Allowances (RDA) for vitamin A are listed as micrograms (mcg) of Retinol Activity Equivalents (RAE) to account for the different biological activities of retinol and provitamin .

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

= 0.344 µg retinyl acetate

= 0.6 µg β-carotene

= 1.2 µg other provitamin A carotenoids

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

= 0.6 µg β-carotene

= 12 µg other provitamin A carotenoids

Table 2.1: Recommended Dietary Allowances (RDAs) for vitamin A

Age (years)	Children (mcg RAE)	Males (mcg RAE)	Females (mcg RAE)	Pregnancy (mcg RAE)	Lactation (mcg RAE)
1–3	300 (1,000 IU)				
4–8	400 (1,320 IU)				
9–13	600 (2,000 IU)				
14–18		900 (3,000 IU)	700 (2,310 IU)	750 (2,500 IU)	1,200 (4,000 IU)
19+		900 (3,000 IU)	700 (2,310 IU)	770 (2,565 IU)	1,300 (4,300 IU)

Sourced from Institute of Medicine, 2001

2.3.2 Mineral elements

Mineral elements play a major role in growth and maintaining good health of our body. These include iron, zinc and manganese. The relative concentration of the mineral elements which are essential components of the human body is shown by the table 2.2

Table 2.2 Mineral element in Human body

Element	Approximate amount in a 70-kg man in grams.
Calcium	1,295
Phosphorous	700
Potassium	245
Sodium	
Chlorine	105
Magnesium	105
Iron	35
Manganese	2.8
Copper	0.21
Iodine	0.080
Cobalt	0.028
Fluorine	
Zinc	
Selenium	Very minute quantities

Source Taylor, 1986

2.3.2.1 Iron

Iron is found in several of the proteins at the end of the energy metabolic pathways, where the hydrogen's from energy nutrients are finally donated to oxygen. Iron is also found in many enzymes which oxidize compounds, these reactions are so widespread in metabolism that they seem to occur everywhere. Iron is thus required for making new cells, amino acids, hormones, and neurotransmitters. It is also involved in carrying oxygen from place to place, as part of protein hemoglobin and for holding oxygen ready for use in muscle energy metabolism, as part of the protein myglobin (Eleanor, 1987). Absorption occurs principally in the duodenum and proximal jejunum. Body iron is regulated mainly through changes in absorption (Mason, 2001).

Almost two-thirds of iron in the body is found in hemoglobin, the protein in red blood cells that carries oxygen to tissues. Smaller amounts of iron are found in myoglobin, a protein that helps supply oxygen to muscle, and in enzymes that assist biochemical reactions. Iron is also found in proteins that store iron for future needs and that transport iron in blood. Iron stores are regulated by intestinal iron absorption (Miret *et al*., 2003).

There are two forms of dietary iron: heme and nonheme. Heme iron is derived from hemoglobin, the protein in red blood cells that delivers oxygen to cells. Heme iron is found in animal foods that originally contained hemoglobin, such as red meats, fish, and poultry. Iron in plant foods such as lentils, vegetables and beans is arranged in a chemical structure called nonheme iron (Hurrell, 1997). This is the form of iron added to iron-enriched and iron-fortified foods. Heme iron is absorbed better than nonheme iron, but most dietary iron is

nonheme iron (Miret *et al*., 2003). Healthy adults absorb about 10% to 15% of dietary iron, but individual absorption is influenced by several factors (Miret *et al*., 2003).

Storage levels of iron have the greatest influence on iron absorption. Iron absorption increases when body stores are low. When iron stores are high, absorption decreases to help protect against toxic effects of iron overload (Miret *et al*., 2003). Iron absorption is also influenced by the type of dietary iron consumed. Absorption of heme iron from meat proteins is efficient. Absorption of heme iron ranges from 15% to 35%, and is not significantly affected by diet (Miret *et al*., 2003In contrast, 2% to 20% of nonheme iron in plant foods such as rice, maize, black beans, soybeans and wheat is absorbed (Hunt *et al*., 1994). Nonheme iron absorption is significantly influenced by various food components (Miret *et al*., 2003).

Meat proteins and vitamin C will improve the absorption of nonheme iron (Hunt *et al*., 1994). Tannins (found in tea), calcium, polyphenols, and phytates (found in legumes and whole grains) can decrease absorption of nonheme iron (Hunt *et al*., 1994). Some proteins found in soybeans also inhibit nonheme iron absorption (Miret *et al*., 2003). It is most important to include foods that enhance nonheme iron absorption when daily iron intake is less than recommended, when iron losses are high (which may occur with heavy menstrual losses), when iron requirements are high (as in pregnancy), and when only vegetarian nonheme sources of iron are consumed. Recommendations for iron are provided in the Dietary Reference Intakes (DRIs) developed by the Institute of Medicine (IOM, 2001). Table 2.3 lists the RDAs for iron, in milligrams, for infants, children and adults.

Table 2.3: Recommended Dietary Allowances for Iron for Infants (7 to 12 months), Children, and Adults (mg/day)

Age	Males	Females	Pregnancy	Lactation
7 to 12 months	11	11	N/A	N/A
1 to 3 years	7	7	N/A	N/A
4 to 8 years	10	10	N/A	N/A
9 to 13 years	8	8	N/A	N/A
14 to 18 years	11	15	27	10
19 to 50 years	8	18	27	9
51+ years	8	8	N/A	N/A

Sourced from Institute of Medicine, 2001

The World Health Organization considers iron deficiency the number one nutritional disorder in the world (Stoltzfus, 2001). As many as 80% of the world's population may be iron deficient, while 30% may have iron deficiency anemia (Stoltzfus, 2001). Iron deficiency anemia can be associated with low dietary intake of iron, inadequate absorption of iron, or excessive blood loss (Stoltzfus, 2001).Women of childbearing age, pregnant women, preterm and low birth weight infants, older infants and toddlers, and teenage girls are at greatest risk of developing iron deficiency anemia because they have the greatest need for iron (Stoltzfus, 2001).Women with heavy menstrual losses can lose a significant amount of iron and are at considerable risk for iron deficiency (IOM, 2001).

2.3.2.2 Zinc

Zinc is an essential mineral that is naturally present in some foods, added to others, and available as a dietary supplement. Zinc is also found in many cold lozenges and some over-the-counter drugs sold as cold remedies. Zinc is involved in numerous aspects of cellular metabolism. It is required for the catalytic activity of approximately 100 enzymes (Rink and

Gabriel, 2000) and it plays a role in immune function, protein synthesis, wound healing, DNA synthesis, and cell division (Prasad, 1995). Zinc also supports normal growth and development during pregnancy, childhood, and adolescence (Prasad, 1995) and is required for proper sense of taste and smell. A daily intake of zinc is required to maintain a steady state because the body has no specialized zinc storage system (Rink and Gabriel, 2000).

A wide variety of foods contain zinc (IOM, 2001). Oysters contain more zinc per serving than any other food, but red meat and poultry provide the majority of zinc in the American diet. Other good food sources include beans, nuts, and certain types of seafood (such as crab and lobster), whole grains, fortified breakfast cereals, and dairy products (IOM, 2001).

Phytates—which are present in whole-grain breads, cereals, legumes, and other foods—bind zinc and inhibit its absorption (Sandstrom, 1997). Thus, the bioavailability of zinc from grains and plant foods is lower than that from animal foods, although many grain- and plant-based foods are still good sources of zinc (Sandstrom, 1997). The current Recommended Dietary Allowance (RDAs) for zinc is listed in Table 2.4 (IOM, 2001).

Table 2.4: Recommended Dietary Allowances (RDAs) for Zinc

Age	Male	Female	Pregnancy	Lactation
0–6 months	2 mg*	2 mg*		
7–12 months	3 mg	3 mg		
1–3 years	3 mg	3 mg		
4–8 years	5 mg	5 mg		
9–13 years	8 mg	8 mg		
14–18 years	11 mg	9 mg	12 mg	13 mg
19+ years	11 mg	8 mg	11 mg	12 mg

* Adequate Intake (AI)

Sourced from Institute of Medicine, 2001

Zinc deficiency is characterized by growth retardation, loss of appetite, and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions (Bhuta, 1999). Weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur. Many of these symptoms are non-specific and often associated with other health conditions; therefore, a medical examination is necessary to ascertain whether a zinc deficiency is present (Eleanor, 1987).

2.3.2.3 Manganese

Manganese is found principally in the liver, although appreciable amount are also present in the skin, muscle, and bone. The chief channel of excretion of manganese is through the liver into the bile. Manganese appears to be widely distributed in foods, chiefly in vegetables. Manganese along with other minerals is removed when grains are milled and refined (Rodale, 1998).

Manganese is a very common compound that can be found everywhere on earth. It is one out of three toxic essential trace elements, which means that it is not only necessary for humans to survive, but it is also toxic when high concentrations are present in a human body (Rodale, 1998). The uptake of manganese by humans mainly takes place through food, such as vegetables, grains, tea and herbs. The foodstuffs that contain the highest concentrations are grains and rice, soya beans, eggs, nuts, olive oil, green beans and oysters.

After absorption in the human body, manganese will be transported through the blood to the liver, the kidneys, the pancreas and the endocrine glands. Our bodies contain only a very

small amount of manganese, but this metal is important as a constituent of many key enzymes. The chemical structure of these enzymes is interesting: large protein molecules cluster around a tiny atom of metal. Manganese plays a particularly important role as part of the natural antioxidant enzyme superoxide dismutase (SOD), which helps fight damaging free radicals. It also helps energy metabolism, thyroid function, blood sugar control, and normal skeletal growth (Rodale, 1998).

Manganese effects occur mainly in the respiratory tract and in the brain. Symptoms of manganese poisoning are hallucinations, forgetfulness and nerve damage. Manganese can also cause Parkinson, lung embolism and bronchitis. When men are exposed to manganese for a longer period of time they may become impotent. A syndrome that is caused by manganese has symptoms such as schizophrenia, dullness, weak muscles, headaches and insomnia (Rodale, 1998). The current Recommended Dietary Allowance (RDAs) for manganese is listed in Table 2.5 (IOM, 2001).

Table 2.5: Recommended Dietary Allowances for Manganese

Age	Males (mg/day)	Females (mg/day)	Pregnancy (mg/day)	Lactation (mg/day)
0-6 months	0.003	0.003	N/A	N/A
7-12 months	0.6	0.6	N/A	N/A
1-3 years	1.2	1.2	N/A	N/A
4-8 years	1.5	1.5	N/A	N/A
9-13 years	1.9	1.6	N/A	N/A
19 years and older	2.3	1.8	2	2.6

Sourced from Institute of Medicine, 2001

2.4 Methods of vegetable preservation

One way of mitigating drought and famine caused by climate change is by improving post-harvest handling, processing and subsequent utilization of indigenous vegetables. Traditional vegetables form an important component of the diet especially in situations of drought and famine. The nutritional value of indigenous vegetables is highest when they are fresh. However, it is not always possible to consume fresh vegetables as they are only abundant in the wet season and scarce the rest of the year. Once the vegetables are harvested, they get subjected to a series of physical and biochemical changes that cause loss of nutritional value, flavour, taste and start rotting or spoilage (Kumar *et al.*, 2010). Improving post-harvest handling and processing of the vegetables is one way of overcoming perishability constraints and ensuring continued high quality food supply. Several methods of vegetable preservation are available and these include, sun drying, solar drying, canning, vacuum packing, minimal processing, refrigeration, freezing and irradiation (Kumar *et al.*, 2010).

Drying is one of the longest established methods of food preservation, and one which occurs naturally, for example with seeds (Kumar *et al.*, 2010). The success of any drying operation depends on removing enough moisture from the food to achieve a water activity too low to allow microbiological growth to take place (Kumar *et al.*, 2010). In the process of drying some nutrients such as beta-carotene is known to be susceptible to degradation upon exposure to heat and light (Nawiri *et al.*, 2012). There is need to establish a method of drying with minimal loss. Compared to open drying, solar drying provides higher air temperature that prevents strong radiation reaching the samples directly and also protects them from direct sunlight.

2.4.1 Solar drying

Drying could improve the concentration of both organic and mineral constituents and solar drying method could be useful in preserving indigenous vegetables in a more hygienic way and ensure its all-the-year–round availability (Hassan *et al*., 2007). Solar drying is an improved method of sun drying in which solar energy is collected to heat air in cabinet that in turn dries the food stuff. The figure 2.13 and figure 2.14 shows an improved rooftop solar drier designed by Thoruwa (2011) used for drying. It utilizes the dry air from the solar collector to dry the food.

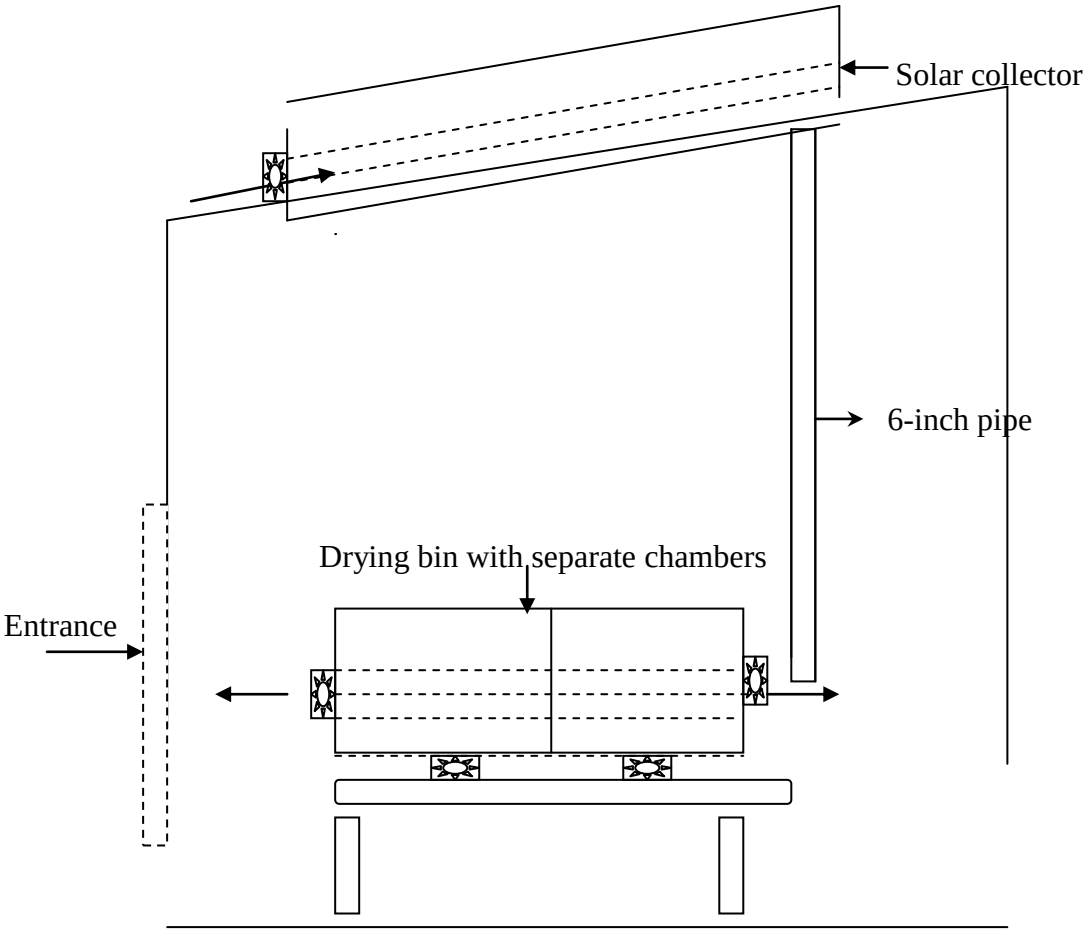

Figure 2.13 Schematic diagram of roof top solar drier

The principles source of energy for drying is derived from the enhancement of sun's radiation

(Kumar *et al.*, 2010). Compared to open drying, solar drying provides higher air temperature that prevents strong radiation reaching the samples directly. This then lower relative humidity which is conducive to improved drying rates and lower final moisture content in the dried crops (Kumar *et al.*, 2010).

Figure 2.14 Photo showing the prototype solar rooftop powered dryer

2.5 Methods of analysis of beta-carotene and essential elements

There are various methods that have been used to determine essential elements and also the carotenoids. For essential elements the most common ones includes flame atomic absorption spectroscopy (FAAS), Inductive coupled plasma mass spectrometry (ICP-MS), Flame atomic emission spectroscopy (FAES) and energy dispersive x-ray fluorescence spectroscopy (EDXRF) (Skoog and Leary, 1992) For carotenoids high performance liquid chromatography (HPLC) and open column chromatography (OCC) are commonly used (Amaya, 2001).

2.5.1 Flame atomic absorption spectroscopy

The technique of flame atomic absorption spectroscopy (FAAS) requires a liquid sample to be aspirated, aerosolized, and mixed with combustible gases, such as acetylene and air or acetylene and nitrous oxide. The mixture is ignited in a flame whose temperature ranges from 2100 to 2800 ^{o} C. During combustion, atoms of the element of interest in the sample are reduced to free, unexcited ground state atoms, which absorb light at characteristic wavelengths (Skoog *et al*., 1998). The method is preferred because it is easily available and affordable.

2.5.1.1 Principle of Flame atomic absorption spectroscopy

It works on the principle of absorption of radiation by the analyte atoms in the ground state, the atomization being achieved by thermal energy of the flame. The wavelength of the radiation absorbed and the extent of the absorption form the basis of qualitative and quantitative determinations (Skoog *et al*., 1998). Atomic absorption is a process involving the absorption by free atoms of an element of light at a wavelength specific to that element. Absorption of light is associated with the process of transition of atoms from one steady state to another. For the case of steady states 0 and P (where 0 is the ground state and P the excited state), with energies E_o and E_p, where $E_p > E_o$, the $0 \rightarrow P$ transition results in the absorption of light with frequency, shown in Equation 2.1

$$\nu op = \frac{Ep - Eo}{h} \quad \text{..... (2.1)}$$

Where; h is the Planks' constant, υ the frequency, 0 is the ground state and P the excited state.

The $0 \rightarrow P$ absorption transition is always stimulated by external radiation. For an unexcited atom, each electron is in the ground state, otherwise it is excited. The proportion of excited to ground state atoms in a population at a given temperature is given by the general statement of the Maxwell-Boltzmann law shown in Equation 2.2

$$\frac{Np}{No} = \frac{gp}{go} e^{\left[\frac{Eo-Ep}{kT}\right]} \quad \text{..... (2.2)}$$

Where; N = the number of atoms in state 0 and P,

g = the statistical weights for states 0 and P,

T = the temperature (in degrees Kelvin)

k = the Boltzmann constant.

E_o = Energy at ground state

E_p = Energy at excited state

The wavelength at which an atom with its valency electrons in the ground state can absorb radiation are called resonance wavelength (Skoog *et al*., 1998). To calculate how much light is absorbed by a cloud of atoms, parallel beams of light at the resonance wavelength for the atoms concerned are considered when striking a cell containing N atoms. If light of intensity I_o enters the cell, the intensity remaining after absorption I_I is given by Equation 2.3.

$$I_{I=} I_o e^{[-kl]} \quad \text{..... (2.3)}$$

Where l is the cell length and k is the absorption coefficient (i.e. the fraction of energy absorbed per unit length). Taking logarithms of both sides, we get Equation 2.4

$$kl = \log \frac{I_o}{I_I} \quad \text{..... (2.4)}$$

The expression $log \frac{I_o}{I_I}$ is defined as absorbance. Since the product kl is proportional to the number of atoms in the cell, so is the absorbance. For this reason, absorption is the preferred readout mode of modern atomic absorption spectrometer, giving a linear relationship between absorbance and concentration as in Equation 2.5.

$$A = \varepsilon cl \quad \text{..... (2.5)}$$

Where; A = absorbance

ε = Molar absorptivity ($Lmol^{-1}cm^{-1}$)

c = Concentration ($moldm^{-3}$)

l = Path length (cm)

2.5.1.2 Instrumentation

Atomic absorption spectrophotometer consists of five main parts:-

Radiation source: Is a light source which emits the sharp line spectrum of the element to be determined. There are two types of radiation sources these are Continuous source and Hollow cathode source. Hollow cathode source is commonly used in AAS instruments and is made up of metallic or alloy of element of interest to be analysed. Hollow cathode lamp consists of a tungsten anode and cylindrical cathode sealed in a glass tube that is filled with neon or argon gas at a pressure of 1-5 torr.

Atomizer: In flame atomizer, temperature is determined by flow rate and the ratio of oxidant and fuel. Solvent is evaporated to produce solid molecular aerosol during dissolvation process. Dissociation leads to atomic gas whereas some of the atoms ionize to give cations and electrons.

Monochromators: They are analyzers that present monochromatic radiation to the detector. They may be filters, prisms or gratings that disperse or separate radiation so that selected wavelength corresponding to a particular energy of the sample is transmitted. Diffraction gratings are preferred to prisms as they offer accuracy over a wide range of wavelengths.

Detectors: They are used to convert radiation energy into electrical signal. They include phototube, photomultiplier tube and photodiode array detectors.

Readout system: These are digital and are interfaced with microprocessors that allow the programming of various aspects, bringing simplicity in operations.

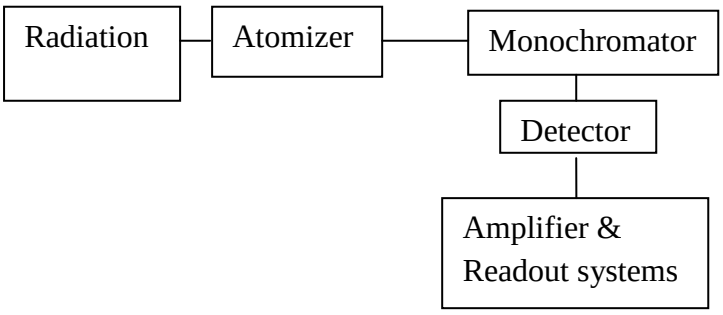

Figure 2.15 Schematic Diagram of Atomic Absorption Spectrophotometer

Source: Skoog *et al*. (1998)

2.5.2 The UV- Visible Spectrophotometry

UV/Visible Spectrophotometry is used to determine the absorption or transmission of UV/ Visible light (180 to 820 nm) by a sample. It can also be used to measure concentrations of absorbing materials based on developed calibration curves of the material (Skoog *et al*., 1998). It is preferred for its affordable price.

2.5.2.1 Principles of UV

Many molecules containing unsaturated linkages and non-bonding electrons absorb ultraviolet or visible light. The absorbance of a solution increases as attenuation of the beam increases. Absorbance is directly proportional to the path length, *b*, and the concentration, *c*, of the absorbing species. Absorption of radiation also obeys the Beer-Lambert law given in equation 2.5. The relationship between absorption of incident radiation and its absorption frequency is the basis behind the use of spectroscopy to identify substances as shown in Equation 2.6

$$E_1 + h_\upsilon \rightarrow E_2 \quad \text{.....(2.6)}$$

Where; E_1 = Low energy state

E_2 = high energy state

h = Plank's constant

υ = Frequency of radiation

Absorption of ultraviolet or visible light electromagnetic radiation causes electron to moves from lower energy levels to a higher energy levels. Ultraviolet-visible absorption spectroscopy measures the absorption of ultraviolet or visible light. Because the spectrum of an atom or molecule depends on its electron energy levels, UV-vis absorption spectra

characteristics are useful for identifying unknown substances.

The UV-vis spectroscopy uses Beer's Law (Beer-Lambert law) to determine the relationship between absorbance and concentration of a substance in a solution. Since absorbance depends on the concentration of the absorbing species, it is possible to quantitatively determine the amount of a given species present (Skoog *et al*., 1998). In general there is a linear relationship between the increasing amount of substance and a decreasing percentage of light transmitted through the target sample. If there is more of a substance to absorb the UV light then less light will pass through to the detector. Correspondingly, less absorption by the target sample allows increased transmission light through the sample.

2.5.2.2 Instrumentation

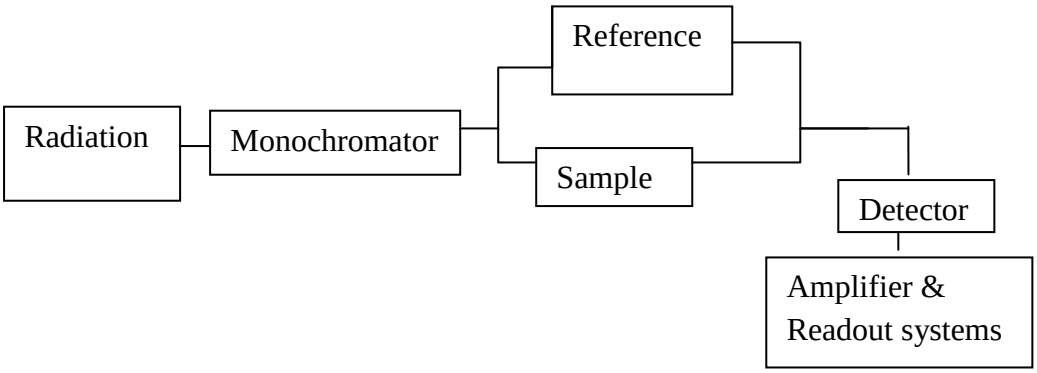

Figure 2.16 Schematic Diagram of Double Beam UV-Vis Spectrophotometer

Source: Skoog *et al*. (1998)

CHAPTER THREE

METHODOLOGY

3.1 Study area

The study was carried out in Lari and Limuru in Kiambu district in Kenya, where 12 sampling stations were identified. These are the regions that receive a lot of rainfall and grow variety of indigenous vegetables. Kiambu district has seven divisions: Lari, Kikuyu, Limuru, Kiambaa, Githunguri, Ndieya and Karai. The county covers an area of 1,324 km^2 with 90% being high agricultural potential land. Rainfall is reliable and ranges from 1,500 mm in the highlands to 500mm in the semi-arid areas of Ndeiya and Karai. These two divisions are vulnerable to drought (Republic of Kenya, 2002).

3.2 Apparatus and instrumentation

Amber colored volumetric flasks (5 x 10mls, 3 x 100mls), volumetric flask beakers (6 x10mls, 6 x 50mls), amber colored vials (20 x 20mls), conical flask (4 x 250mls), Kjeldahl digestion flask (5x 250ml), plastic bottles (400 x 100ml), mechanical blender with stainless steel or glass cup, round bottomed flask (200 and 500ml), Separatory funnel (125ml) with Teflon stopcock, Buchner funnel with Whatman no. 42 filter paper, aluminum foil, suction flask with water aspirator, labels, freezer, rotary evaporator, orbital shaker, chromatographic glass tube (2.5cm i.d x 30cm) tapering at bottom, Atomic absorption Spectrophotometer model AA-10, Electronic balance model AAA (Adam co limited), UV- Visible absorption spectrophotometer (model, Pharmacia Biotech, Novaspec II), pelletor, White blood cell pipette, Polypropylene mice cages and haemocytometry. The table 3.1 shows operating

conditions for the AAS

Table 3.1: The AAS operating conditions for the metals analyzed

Metal	Wavelength (nm)	Slit (nm)	Detection limit (mg/L)	Acetylene flow L/min	Lamp Current (mA)
Fe	248.8	0.2	5.0×10^{-2}	1.5	7.0
Zn	213.9	0.7	2.0×10^{-3}	1.5	2.0
Mn	278.5	0.2	1.0×10^{-2}	1.5	2.0

3.3 Cleaning of apparatus

The glassware to be used for analysis were cleaned with detergent and tap water then rinsed several times with tap water followed by soaking overnight in 10% analytical grade nitric acid water solution. They were finally rinsed with distilled water and dried in oven at 80° C. they were then stored in a clean and dry place. Plastic bottles were cleaned with detergent and tap water, soaked in nitric acid solution and finally rinsed with distilled water. They were later dried in open racks and stored in clean dry drawers waiting to be used.

3.4 Chemicals and reagents

The reagents and chemicals used in this study were of analytical grade. Nitric acid, hydrochloric acid and beta-carotene used were purchased from Thomas Baker chemicals Ltd Mumbai India. Magnesium oxide Hyflosupercel, acetone, petroleum ether, Zinc, Manganese, Iron, sodium sulphate, EDTA, glacial acetic acid, gentian violet stain, methanol and polychromic staining solution were sourced from GmbH Chemical Company, inc. USA.

3.4.1 Preparation of standards

3.4.1.1 Preparation of beta carotene stock solutions

Beta-carotene standard solution was prepared by dissolving 5 mg encapsulated beta-carotene crystals in petroleum ether in 100 ml amber colored volumetric flask. The flask was then filled to the mark with petroleum ether to give 50 µg/ml. The stock solution was protected from light using aluminum foil and stored in a freezer. The stock solutions were diluted to give a series of working standards. This was then used to draw calibration curve of absorbance versus the concentration of the beta-carotene standard solution to determine the concentration of various vegetable samples

3.4.1.2 Preparation of metal stock solutions

Stock solutions were prepared from analytical grade granulated metals of high purity (99.99%). Zinc, manganese and iron stock solutions were prepared by dissolving 0.25 g of each in 50 ml HCl in a 250 ml volumetric flask. It was then diluted to the mark using distilled de-ionized water to give 1000µg/ml. The stock solutions were stored in polyethylene bottles and labeled appropriately. Working standards were freshly prepared from stock solutions each time analysis was to be carried out. This was then used to draw calibration curve of absorbance versus the concentration.

3.4.2 Validation procedure

Reproducibility study was done to determine precision by using known concentration of standard four times using 5ppm beta-carotene, 0.4 µg/ml zinc, 0.35 µg/ml manganese and 2.5 µg/ml iron. Standard deviation and coefficient of variation were calculated to determine

precision. Efficient of extraction and procedure was done using a recovery study. A known amount of beta-carotene (250 µg) was added to finely ground fresh vegetables before extraction. The mixture was then subjected to extraction and quantification procedure. The percentage recovery was then calculated from the amount of beta-carotene predetermined in each sample.

3.5 Sample collection and pretreatment

In the study, two sampling areas were chosen that is Lari and Limuru. 12 farms in the area were identified that grow indigenous vegetables. From the farms 9 types of indigenous vegetables were identified that is *Solanum nigrum* (black night shade), *Gynandropsis gnandra* (spider plant), *Corchorus olitorius* (Jute), *Cucurbita maxima* (pumpkin leaves), *Vigna unguiculata* (kunde), *Ipomoea batatas* (sweet potatoes leaves), *Symphytum offinale* (mabaki), *Solanum aethlopicum* (African egg plant) and *Agaricus bisporus* (mushroom). Samples were randomly picked from various near equidistant sampling points in all 12 sampling sites (farms). Each sampling unit represented one sample. Samples each 1kg was then obtained from the nine types of indigenous vegetables identified in the 12 farms and used for analysis.

The samples were placed in separate perforated self sealing polythene bags to maintain their freshness. They were labeled and taken to laboratory where they were washed with tap water to remove soil and rinsed with distilled water. They were then blanched by submerging in boiling water at a temperature of 90 ° C for two minutes. The samples were divided into three portions, the first portion was dried using solar drier, and the second portion dried in the open under a shade. It was followed by analysis of beta-carotene, zinc, iron and manganese. The

last portion was digested while fresh and analyzed for beta-carotene, zinc, iron and manganese. From the three portions (solar dried, open dried and fresh) 10 g of each sample was analyzed for beta-carotene and 2 g for analysis of zinc, iron and manganese.

3.5.1 Solar drying

Samples for solar drying were allowed to dry between 6-9 hours in an indirect solar dryer (The model is shown in figure 3.1) when temperature ranged between 35-59 ° C. Samples were spread in a wire mesh tray before inserting into the dryer. The dryer was made of metal and covered on top with a transparent glass. The inside was painted black to concentrate the heat and ensure that air inside was heated appropriately. A small opening of 1 inch was left beneath the tray and chimney was inserted in the front to allow free circulation of air dry moisture from solar panels into the dryer. The vegetable samples were spread onto the tray forming a uniform layer. The solar dried samples were stored in polythene bags, nitrogen flashed and then sealed tightly to prevent any oxygen getting in. Figure 3.2 shows part of the samples spread inside the solar drier

Figure 3.1 solar driers

a **b**

Figure 3.2 Samples spread inside the solar drier

3.5.2 Open drying

Samples for open drying were spread on a mat and left to dry under the shade for a day or two depending on the weather. Higher direct air temperature from the sun made the vegetables to dry by loosing their moisture content.

3.6 Determination of moisture content

The moisture content of all the samples was determined following the method of Horwitz, 2001. For the fresh indigenous vegetable it was done immediately while the moisture content for the open drying and solar drying indigenous vegetable was done after drying. The moisture content of fresh, solar dried and open dried types of indigenous vegetable materials was weighed by measuring 5g of each in a crucible. It was then placed in an oven at a temperature of 105 °C for 3 hours. The weight was then taken and moisture content calculated using equation 3.1.

$$\text{Moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100\% \quad \ldots\ldots\ldots\ldots\ldots (3.1)$$

3.7 Determination of Beta- carotene

The extraction procedure was adapted from that given by Amaya (2001). 10 g of the subsample was ground in a mortar and pestle with enough acetone to make a solution. It was then filtered with suction through a Buchner funnel. The process was repeated with the residue two times until it was devoid of any color.

In a separatory funnel, 100 ml of petroleum ether was placed and small portion of the extracted solution of acetone added. Distilled water was then added slowly, by letting it flow in the walls of the separatory funnel to prevent formation of emulsion. The two phases was then separated by discarding the lower aqueous–acetone phase. Another portion of the extract solution was added and the procedure repeated until all the extract had been transferred to petroleum ether. It was then washed four times with distilled water to remove the residue of acetone. The petroleum ether phase was then collected in a beaker and water removed by adding anhydrous sodium sulphate crystals until the crystals become loose. To get small volume to be introduced to chromatographic column, the solution was decant into a round bottomed flask to remove sodium sulfate and the solution concentrated to 10 ml in a rotary evaporation with a temperature of 40° C.

A chromatographic glass tube (2.5cm internal diameter x 30cm) was packed with magnesium oxide Hyflosupercel to a height of 20 cm and 1 cm of anhydrous sodium sulfate added to remove any residual water that may get into adsorbent. A small volume of petroleum ether

was run through the column and the flow rate adjusted to 3- drops per second. The concentrated solution of carotenoid was then poured into the column followed by 50 ml of petroleum ether rinse immediately the carotenoid layer had gone down almost to the surface of sodium sulfate layer. Once, the rinse of petroleum ether had almost reached the surface of anhydrous sodium sulfate, the column was then developed by successfully adding 50 ml each of 1%, 2%, and 5% ethyl ether in petroleum ether. The separation of carotenoid was monitored visually. Beta- carotene orange in color was then collected immediately it started leaving the column in glass vials. Beta-carotene was determined using UV-Visible at 450 nm

3.8 Determination of Zinc, Iron and Manganese

The fresh, solar dried and open dried indigenous vegetables were ground using a kitchen blender. To avoid sample contamination, all handling and preparation steps were carried out on clean benches. They were then homogenized and put in self sealing bags. From this 2.0 g of each was measured by difference using electronic balance model AAA (Adam co. limited). The 2.0 g samples weighed was placed into a 250 ml Kjeldahl digestion flask. Then 10 ml of 68% nitric acid (HNO_3) was added to the sample and left to stand overnight. It was then placed in digestion block and heated for 20 minutes. It was then removed, cooled and 5ml of 30% hydrogen peroxide added. This was then followed by heating for 10 minutes, removed from heat and same amount 5ml of 30% hydrogen peroxide added. The process was repeated until the digest was colorless. The volume of the digest was reduced to about 10 ml by heating and then left to cool. The solution was then transferred to 50ml volumetric flasks and then diluted to the mark with distilled de-ionized water. The digested sample was then transferred into a plastic bottle to be used to determine iron, zinc and manganese (Horwitz, 2001). Zn, Fe, and Mn were analyzed using Flame atomic absorption spectrophotometer.

3.9 Formulation, preparation of experimental diet

A food supplement was formulated from the analyzed indigenous vegetables. This was done by selecting indigenous vegetables that were able to meet the daily recommended allowance of vitamin A, zinc, iron and manganese which are 1.83 mg, 8 mg 16 mg and 2.2 mg respectively (Institute of Medicine, 2001). The amount of each indigenous vegetable that was added to the food formula to provide the RDA of the four immune boosting was determined by linear programming in Excel by Toledo, 1980. The ratio obtained from the mix of *Gynandropsis gnandra*, *Cucurbita maxima*,and *Agaricus bisporus* was 1:3:4 respectively.

The indigenous vegetables were first solar dried and ground into powder form before preparing a vegetable mixture for formulation. The mixture was blended together in the electrical mixer for full homogenization before coming up with a food formula which was later sieved through a silk sieve.

3.10 Experimental animals

Male Mice of the inbred Bagg albino strain, reared in Kenyatta University zoology laboratory, were removed from their mothers when three weeks old and placed in separate, polypropylene cages on soft sawdust bedding at room temperature of 25 ± 2°C.They were fed with basal diet for four days to acclimatize to the new environment. The animals were distributed with the same weight into four (4) groups of five mice each. They were fed with formulated food diets in percentiles together with basal diet that was lacking in Zn, Fe and Mn and vitamin A.

Control- Initial weight 20 g fed Basal diet

Group 1 - Initial weight 15 g (Fed 25% indigenous vegetable mix blend + basal diet)

Group 2 - Initial weight 18 g (Fed 50% indigenous vegetable mix blend + basal diet)

Group 3 - Initial weight 22 g (Fed 75% indigenous vegetable mix blend + basal diet)

The basal diet was formulated according to Reeves *et al,* (1993) and had the constituents shown in Table 3.2

Table 3.2 Formulation content of the basal diet

Constituents	Grams
Corn starch	610.6
Casein	140
Sucrose	100
Corn oil	50
Mineral mixture*	35
Vitamin mixture*	10
Choline bitartrate	2.5

*constituents shown in appendix

The powdered food mixtures were made into pellets shown in figure 3.3 using a pelletor to allow easy feeding of the mice. Mice were given food and water ad libitum for 42 days, measurements of food intakes and body weights being recorded at weekly intervals. An observation period of six weeks duration was chosen because it comprises the time during which the most rapid growth following weaning takes place; differences in growth rate due to sex are not evident and, finally, there is not the excessive fat formation that occurs sometimes in older animals and interferes with the interpretation of increases in body weight.

Figure 3.3 Pelleted Indiginous vegetable mixture

During the period of the experiment, the food intake and the mice were weighed twice weekly. Growth curves were drawn representing the relationship between the mice body weights and time. After the end of experimental period, biological changes; food intake, body weight gain, and food efficiency ratio (body weight gain/total food intake) were calculated and tabulated. Figure 3.4 shows part of the mice in cages.

Figure 3.4 Part of the groups of mice in their cages

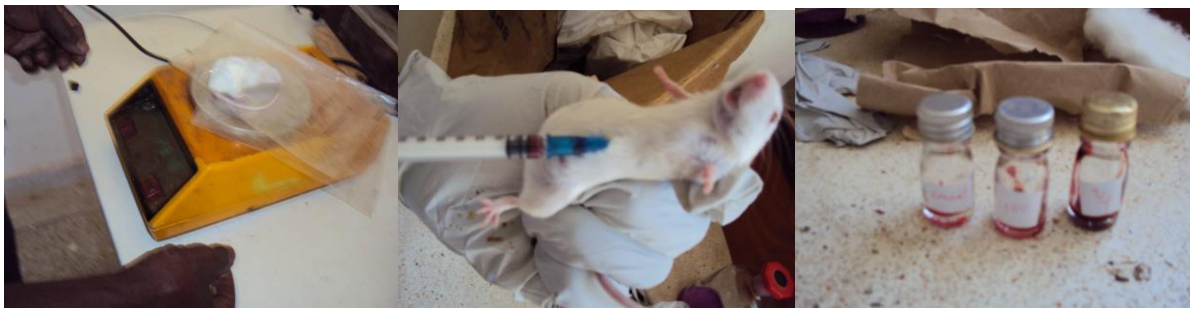

Figure 3.5 weighing and getting sample blood from the mice

3.10.1 Measurement of Hematological parameters.

Blood samples were taken at the beginning of feeding and at the end of feeding the 42nd day. Blood from mice were obtain by cardiac puncture into light-shielded centrifuge tubes and placed into sample bottles containing EDTA (1mg/ml) to be mixed with white blood cell (WBC) diluting fluid that was taken in a watch glass. Blood was drawn upto 0.5 mark of the

WBC pipette and WBC diluting fluid was drawn upto 11 mark. The fluid and blood were mixed well and the first few drops of blood were discarded by holding the pipette vertically. The counting chamber was charged with a drop of blood mixed with diluting fluid. The chamber was left undisturbed for few minutes. The four corners of the chamber was visualised under a low power (10x) objective and the cells were counted in all the four marked corner squares.

$$\text{WBC per cubic mm} = \frac{\text{Number of cells x dilution factor}}{\text{Area counted x depth}} \quad \text{..... (3.2)}$$

Where:

(1) Dilution = 1:20

(2) Area counted = 4 sq mm

(3) Depth of fluid= 1/10mm

$$\text{WBC} = \frac{N \times 20}{0.4} \quad \text{……..... (3.3)}$$

The total leucocyte count was determined by haemocytometry following the method of Gottfried and Gerard (1987). Glacial acetic acid was used to lyses the red cells while the gentian violet was used to slightly stain the nuclei of the leukocyte. The blood specimen was diluted to 1:20 in a WBC (white blood cell) pipette with the diluting fluid which contained 1% acetic acid solution tinged with Gentian violet stain and the cells were counted under low power microscope by using a counter chamber. The numbers of cells in undiluted blood was reported as the number of white cells/cu.mm of whole blood. Calculation was done using equation 3.2 and 3.3

The differential white blood cell count shows the various individual white blood cell types found in peripheral blood of which the predominant circulating leukocyte is the lymphocyte, followed by neutrophils, monocytes, eosinophils, and lastly basophils. The differential count of leucocytes was determined by haemocytometry following the method of Gottfried and Gerard (1987). This was done by examining and categorizing 100 white blood cells on a peripheral smear. To get the percent of each cell type, it was multiplied by the total white blood count to arrive at absolute differential counts for the various cell types. The following solutions were used, the polychromic staining solution (0.15gm of powered Leishman's stain in 133 ml of acetone) which contains Methylene blue and eosin. The basic and acidic dyes induce multiple colours when applied to cells. Methanol which acts as fixative and also as a solvent, the fixative does not allow any further change in the cells and makes them adhere to the glass slide.

The basic component of white cells (cytoplasm) is stained by acidic dye and they are described as eosinophilic or acidophilic. The acidic components (nucleus with nucleic acid) take blue to purple shades by the basic dye and they are called basophilic. The neutral components of the cell are stained by both the dyes. A thin smear was prepared by spreading a small drop blood evenly on a slide by another slide at an angle of 45°. The smear was stained by Leishman stain by placing the slide on a stain rock. It was allowed to react with the stain for 2 to 3 minutes. Then double distilled water was used to wash the excess stain on the slide. It was allowed to dry for 7 minutes and observed under the microscope. The film was examined by moving from one field to the next systematically. About 100 leucocytes were counted to give high degree of accuracy. The WBCs were classified as Lymphocites, neutrophils and monocyte.

3.11 Data analysis

The quantitative data was presented in graphs and tables that showed the levels of beta-carotene, zinc, iron and manganese in fresh, solar dried and open dried indigenous vegetables. Calculation of beta-carotene, zinc, iron and manganese; the mean; standard deviation; correlation coefficient; coefficient of variation and detection limit were done using basic formula (Miller and Miller, 1990). The significance tests were carried out using t- test and F-test at desired confidence levels.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 moisture content

Moisture content of fresh, solar dried and open dried indigenous vegetables were determined in % and the results are given in table 4.1

Table 4.1: Mean (n=12) moisture content of fresh, solar dried and open dried indigenous vegetables

Indigenous vegetable	Fresh	Solar dried	Open dried
S*olanum* nigrum	88.65 ± 0.08	8.24 ± 0.18	7.12 ± 0. 05
G*ynandropsis gnandra*	83.86 ± 0.03	7.24 ± 0.01	7.05 ± 0.08
C*orchorus olitorius*	85.52 ± 0.04	7.26 ± 0.22	6.13 ± 0.22
C*ucurbita maxima*	90.62 ± 0.18	8.88 ± 0.01	7.83 ± 0. 13
V*igna unguiculata*	86.45 ± 0.05	8.25 ± 0.14	8.23 ± 0.02
I*pomoea batatas*	80.95 ± 0.03	8.95 ± 0.08	6.31 ± 0.04
S*ymphytum offinale*	91.84 ± 0.20	10.84 ± 0.20	8.92 ± 0.17
S*olanum aethlopicum*	93.74 ± 0.09	9.40 ± 0.12	9.12 ± 0. 09
Agaricus bisporus	96.87 ± 0.02	10.24 ± 0.04	9.17 ± 0.35

The moisture content of fresh indigenous vegetables ranged from 80.95% to 96.87% with the lowest being G*ynandropsis gnandra and* highest being *Agaricus bisporus*. Moisture content for solar dried indigenous vegetables range between 7.24% to 10.84% and between 6.13% to 9.17 for open drying. The moisture content of the solar dried and open dried samples decreased significantly ($p<0.05$) with corresponding increase in the contents of the dry matter. The results showed that open dried indigenous vegetables had the lowest moisture content compared to the solar dried and the fresh ones. The result also showed that the

moisture content of all the fresh vegetables were above 80%. High moisture content contributes to them being perishable and cannot stay for long due to microbial spoilage. The moisture content in the indigenous vegetable usually constitutes free water molecule held within the tissue matrix and the bound water molecule which is found in the dried samples. For maximum stability of carotenoids in different foods, an optimum water activity is required (Amaya, 2001). According to Nyambaka 1996 beta- carotene is usually stable at moisture level of less than 10%

4.2 Method of validation

4.2.1 Linearity of the UV-visible calibration curves

Regression analysis was used to evaluate the linearity of the UV-visible calibration curves. The results of correlation coefficient r^2 =0.9997 for beta-carotene indicated a highly positive correlation between absorbance and concentration. The calibration curve was used to determine the concentration of beta-carotene in the indigenous vegetable samples. Figure 4.1 shows calibration curve for beta-carotene

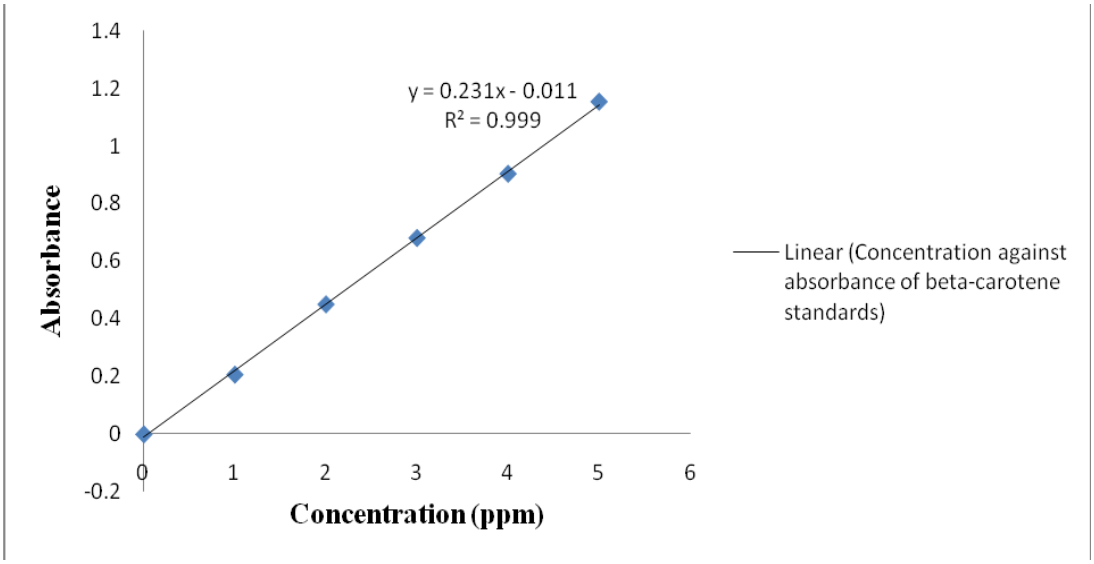

Figure 4.1 showing Calibration Curves for beta-carotene

4.2.2 Precision of UV-visible

Reproducibility study was done to determine precision in determination of beta-carotene by using 5ppm standard beta-carotene four times and determining standard deviation and coefficient of variation. The standard deviation was found to be 0.0026 with coefficient of variation being 0.242%. The results showed that there was no significant difference ($p<0.05$) between successful analysis and the method was reproducible.

4.2.3 Recovery studies of beta-carotene

To determine the efficiency of extraction and analysis procedure, a recovery study was done by adding known amount of beta-carotene standard to finely ground fresh vegetable sample before extraction. Then samples were drawn from the batch of each of the four fresh vegetables varieties that is S*olanum* nigrum, G*ynandropsis gnandra*, V*igna unguiculata and* I*pomoea batatas*. The content was then determined in the samples. The mixture was subjected to the extraction and quantification procedure. The percentage recovery was calculated from the amount of beta-carotene predetermined in each sample. The table 4.2 shows the results.

Table 4.2 Recovery studies of beta-carotene

Indigenous vegetable	Fresh	Beta-carotene added (µg)	After Addition (µg)	Amount recovered (µg)	% Recovery
S*olanum* nigrum	642.0	250.0	877.0	235.0	94.0%
G*ynandropsis gnandra*	961.0	250.0	1113.5	152.5	95.3%
V*igna unguiculata*	495.0	250.0	720.0	225.0	90.0%
I*pomoea batatas*	372.0	250.0	602.0	230.0	92.0%

The percentage recovery was over 90% with the highest recovery being 95.3% from G*ynandropsis gnandra*. The results showed that the extraction process was satisfactory and no significant losses occurred at the various stages of extraction and analysis. The method was therefore good in determining the amount of beta-carotene in the identified indigenous vegetables.

4.2.4 Linearity of AAS calibration curves

The linearity of the calibration curves was determined by regression analysis. Correlation coefficient (r^2) values and the results were calculated by the absorbance readings and concentrations of standards. Results are shown in table 4.3

Table 4.3: Linear Concentration Range of Standard Used and Correlation Coefficient Values of Calibration Curves of Parameters

Parameters	Instrument used	Concentration (mg/L) range of standards used	Correlation coefficient (r^2) values
Fe	AAS	0.0-10.0	0.9997
Zn	AAS	0.0-3.0	0.9994
Mn	AAS	0.0-4.0	0.9998

The results of correlation coefficient r^2 =0.9994 for zinc, r^2 =0.9997 for iron and r^2 =0.9998 for manganese. This showed that linearity of the calibration curves were good and thus results obtained was expected to be accurate

4.2.5 Detection limit of AAS

The detection limit was determined as the lowest concentration obtained by the instrumental signal equal to the black signal plus three times the standard deviation of blank. The results

are presented in table 4.4. It was determined to be 0.0189 µg/g for Manganese, 0.0423 µg/g of iron and 0.0121 µg/g Zinc. The analysis of the results in table 4.4 indicated that there was no significance difference at 95% confidence level of AAS experimental and theoretical detection limits. Therefore, the AAS spectrophotometer used was good to determine Zinc, Iron and manganese in fresh, solar dried and open dried indigenous vegetables.

Table 4.4: The AAS Selected Parameters

Parameters	Experimental detection limits (mg/L)	Theoretical detection limits (mg/L)
Fe	4.23×10^{-2}	5.0×10^{-2}
Zn	1.21×10^{-3}	2.0×10^{-3}
Mn	1.89×10^{-2}	2.0×10^{-2}

4.2.6 Precision of AAS

Standard solution consisting of 0.4 µg/ml Zinc, 0.35 µg/ml Manganese, and 2.5 µg/ml iron was used to determine reproducibility. The standard deviation and coefficient of variation were calculated to determine the precision of the method. The results are shown in the table 4.5.

Table 4.5 reproducibility of AAS method of Zinc, Manganese and Iron

Metal	Standard Conc (µg/ml)	Replicate	Absorbance mean± S.D	Coefficient of variation (C.V)
Zn	**0.4**	**4**	**0.0053±0.00025**	**4.72%**
Mn	**0.35**	**4**	**0.0045±0.00031**	**6.89%**
Fe	**2.5**	**4**	**0.0072±0.00020**	**2.78%**

The standard deviation and coefficient of variation for Zinc was 0.00025 and 4.72% respectively, for manganese was 0.00031 and 6.89% respectively while for iron was 0.002 and 2.78% respectively. From the results it is clear that the reproducibility of AAS for the three elements was satisfactory and the method suffered from minimal interference.

4.3 Levels of beta-carotene in indigenous vegetables

Results of beta-carotene contents of the identified indigenous vegetables are presented in Tables 4.6.

Table 4.6: Mean (n=12) levels of beta-carotene in indigenous vegetables (mg/100 g) and % beta-carotene retention after solar drying and open drying

Indigenous vegetable	Fresh	Solar dried	Open dried
Solanum nigrum	6.42±0.11	5.32±0.09 (82%)	3.98±0.19 (62%)
Gynandropsis gnandra	9.61±0.36	6.32±0.18 (65%)	4.96±0.26 (51%)
Corchorus olitorius	6.02±0.13	4.55±0.08 (74%)	2.94±0.31 (49%)
Cucurbita maxima	5.20±0.27	2.86±0.32 (55%)	2.13±0.10 (41%)
Vigna unguiculata	4.95±0.17	3.83±0.06 (78%)	3.31±0.29 (67%)
Ipomoea batatas	3.72±0.07	2.60±0.13 (70%)	1.93±0.14 (52%)
Symphytum offinale	5.32±0.08	4.04±0.10 (76%)	3.03±0.37 (57%)
Solanum aethlopicum	0.24±0.01	0.11±0.02 (46%)	0.084±0.01 (39%)
Agaricus bisporus	8.62± 0.60	4.23±0.43 (49%)	3.87± 0.26 (45%)

The highest levels of beta-carotene was in fresh *Gynandropsis gnandra*, *Agaricus bisporus*, *Solanum nigrum* and *Corchorus olitorius* with dry weight of 9.61 mg/100 g, 8.62 mg/100 g, 6.42 mg/100 g and 6.02 mg/100 g respectively . *Solanum aethlopicum* contained the lowest levels of beta-carotene with 0.24 mg/100 g dry weight in fresh, 0.11 mg/100 g dry weight in

solar dried and 0.084 mg/100 g. The analysis of beta-carotene in different vegetables species showed significant variation with high standard deviations, this may be due to factors such as cultivar or variety; stage of maturity, climate or geographic site of production; harvesting and postharvest handling (Amaya, 2001).

There was a Significance loss of beta-carotene after solar drying and open drying. Solar drying had the highest levels of beta-carotene retention with the range of 55% to 82%, with the highest retention occurring in S*olanum nigrum* and the lowest in C*ucurbita maxima.* Open drying had percentage retention of between 39% and 67%, with the highest retention occurring in V*igna unguiculata* and the lowest in S*olanum aethlopicum.* There was a significant reduction in the beta-carotene in both solar drying and open drying ($p<0.05$), with the most affected being open drying method compared to fresh vegetables. The reason for much loss in open drying may be due to oxidation and the higher retention in solar drying may be attributed to the indirect exposure of the vegetables to heat and light which contribute to degradation of beta-carotene. During food processing, there is a high risk that the highly susceptible double bonds of carotenoids will undergo oxidation, especially through a free radical process, which is normally minimized by the presence of water (Amaya, 2001). This could partly explain why open sun drying resulted in severe losses to beta-carotene. Solar drying generally seemed better, especially with regard to beta- carotene retention than open drying.

Most of the indigenous vegetable analyzed in this study, the levels of beta-carotene felled within the range of 300-1000 µg/g analysed by others. The levels of beta-carotene in V*igna unguiculata* can be compared to that reported by Nyambaka (1996) with a value of 691 µg/g

dry weight, Mwajumwa *et al* (1991) reported 670 µg/g dry weight, Nawiri *et al* (2012) reported a value of 806 µg/g while Gomez (1981) reported a value of 632 µg/g dry weight. For G*ynandropsis gnandra*, Gomez (1981) obtained a value of 477 µg/g dry weight while *Nyambaka* (1996) reported a value of 996 µg/g dry weight. Ogle and Grivetti (1985) reported a value of 360 µg/g dry weight for fresh C*ucurbita maxima* and 641 µg/g dry weight for fresh C*orchorus olitorius* for which results falls within that range. For C*ucurbita maxima* Mwajumwa *et al*. (1991) in Kenya reported a value of 990 µg/g dry weight.

Table 4.7 shows the mean level of beta-carotene in fresh indigenous vegetable (mg/100 g) in the analyzed samples when compared with the recommended daily beta-carotene intake of average 1.83 mg/day (Institute of Medicine, 2001).

Table 4.7: comparison of the mean levels of beta-carotene (mg/100 g) in indigenous vegetables with average RDA of 1.83 mg/day beta-carotene

Indigenous vegetable	Fresh indigenous vegetable Mean (n=12)	Contribution to RDA value (%)
S*olanum* nigrum	6.42±0.11	350.82%
G*ynandropsis gnandra*	9.61±0.36	525.14%
C*orchorus olitorius*	6.02±0.13	328.96%
C*ucurbita maxima*	5.20±0.27	284.15%
V*igna unguiculata*	4.95±0.17	270.49%
I*pomoea batatas*	3.72±0.07	203.28%
S*ymphytum offinale*	5.32±0.08	290.71%
S*olanum aethlopicum*	0.24±0.01	13.11%
Agaricus bisporus	8.62± 0.60	471.04%

From the results, it can be noted that daily consumption of 100 g of any of the analyzed vegetables would meet the RDA value of 1.83 mg/day except for S*olanum aethlopicum* with a contribution of 13.11%. The highest contribution to RDA is in G*ynandropsis gnandra, Agaricus bisporus,* S*olanum* nigrum and C*orchorus olitorius* with 525.14%, 471.04%, 350.82%, and 328.96% respectively. The highest percentages of beta-carotene will be able to meet the daily requirement of vitamin A absorbed from these vegetables because, Vitamin A in foods that come from plants is not as well absorbed as animal sources of vitamin (De pee and West, 1996).

4.4.0 Levels of metals in vegetables

4.4.1 Iron (Fe) levels in the indigenous vegetables

Results of Fe contents of the identified indigenous vegetables are presented in Tables 4.8. The analysis of Fe in different vegetables species showed significant variation, this may be due to variation in genetic makeup of plant species.

Table 4.8: Mean (n=12) levels of Iron in various indigenous vegetables (mg/100 g)*)*

Indigenous vegetable	**Fresh**	**Solar dried**	**t-value p<0.05**	**Open dried**	**t-value p<0.05**
S*olanum* nigrum	80.34±19.18	77.83±15.56	0.35	76.55±20.19	0.47
G*ynandropsis gnandra*	90.78±31.34	90.24±23.14	0.05	88.75±27.03	0.17
C*orchorus olitorius*	25.86±8.34	25.17±10.13	0.18	23.67±6.45	0.71
C*ucurbita maxima*	23.15 ±5.03	20.86±8.32	0.82	18.16±6.73	2.00
V*igna unguiculata*	46.45±9.38	45.12±10.63	0.32	43.98±7.41	0.72
I*pomoea batatas*	55.75±4.64	52.58±3.61	1.87	53.25±5.17	1.25
S*ymphytum offinale*	18.93±2.28	17.23±4.20	1.23	16.02±4.44	2.01
S*olanum aethlopicum*	2.45±0.35	2.28±0.48	0.99	1.97±0.93	1.67
Agaricus bisporus	3.51±0.48	3.04±0.78	1.78	3.02±0.89	1.68

Gynandropsis gnandra (spider plant) had the highest contents of Fe with 90.78, 90.24 and 88.75 mg per 100 g in both fresh, solar dried and open dried respectively while the lowest was *Solanum aethlopicum* (African egg plant) with 2.45, 2.28 and 1.97 mg per 100g also in fresh, solar dried and open dried respectively.

Using the t-test, it was found that there was no significant difference between iron content in the fresh and solar dried; fresh and open dried indigenous vegetables as expected. By comparing mean levels of Fe in fresh G*ynandropsis gnandra* and the solar dried ones using t-test it was found that the t-calculated value was 0.05. Fresh G*ynandropsis gnandra* and the open dried ones t- calculated was 0.17 which was lower than t-tabulated value of 2.07 at $p<0.05$ in both, although the value was not expected to change after processing. In S*olanum aethlopicum* with the lowest mean of iron there was no significant difference between fresh and solar dried indigenous vegetable, t-calculated value was 0.99 which was lower than t-tabulated value of 2.07 at $p<0.05$. There was also no significant difference between fresh and open dried S*olanum aethlopicum* t-calculated value was 1.67 which was lower than t-tabulated value of 2.07 at $p<0.05$.

Kinabo *et al* (2004) reported contents of Fe in S*olanum nigrum* of 6.10 mg per 100 g edible portion, which is lower than that reported in this study. Mwajumwa *et al*. (1991) reported contents of Fe of 11.8 mg in S*olanum nigrum* which is still low compared to what was obtained in the studies.

Table 4.9 shows the mean level of iron (mg/100 g) in the analyzed samples when compared with the recommended daily Fe intake of average 16 mg/day (Institute of Medicine, 2001).

Table 4.9: comparison of the mean levels of Fe (mg/100 g) in indigenous vegetables with average RDA of 16 mg/day

Indigenous vegetable	Fresh indigenous vegetable Mean (n=12)	Contribution to RDA value (%)
S*olanum* nigrum	80.34±19.18	500.00
G*ynandropsis gnandra*	90.78±31.34	567.38
C*orchorus olitorius*	25.86±8.34	161.62
C*ucurbita maxima*	23.15 ±5.03	144.69
V*igna unguiculata*	46.45±9.38	290.31
I*pomoea batatas*	55.75±4.64	348.44
S*ymphytum offinale*	18.93±2.28	118.31
S*olanum aethlopicum*	2.45±0.35	15.31
Agaricus bisporus	3.51±0.48	21.94

From the results it can be noted that daily consumption of 100 g of any of the analyzed vegetables would meet the RDA value of 16 mg/day except for S*olanum aethlopicum* and *Agaricus bisporus* which provide 15.31% and 21.94% respectively. The highest contributer is S*olanum* nigrum and G*ynandropsis gnandra* which provide 500% and 567.38% respectively. The highest amount of iron in the analysed vegetables will help in fighting deficiency of iron since only 2% to 20% of nonheme iron in plant foods such as vegetables, rice, maize, black beans, soybeans and wheat is absorbed (Monson, 1988). Since there was no significance difference between fresh indigenous vegetables and solar dried indigenous vegetables then taking processed indigenous vegetables by solar drying will also meet the daily requirement if iron to our bodies.

These indigenous vegetables with the highest levels of iron should be recommended to meet Iron deficiency anemia associated with low dietary intake of iron, inadequate absorption of iron, or excessive blood loss (Stoltzfus, 2001).Women of childbearing age, pregnant women, preterm and low birth weight infants, older infants and toddlers, and teenage girls are at greatest risk of developing iron deficiency anemia because they have the greatest need for iron (Stoltzfus, 2001).Women with heavy menstrual losses can lose a significant amount of iron and are at considerable risk for iron deficiency (IOM, 2001).

4.4.2 Zinc (Zn) levels in the indigenous vegetables

Results for Zn contents of the identified indigenous vegetables are presented in Tables 4.10.

Table 4.10: Mean (n=12) levels of zinc in various indigenous vegetables (mg/100 g)

Indigenous vegetable	Fresh	Solar dried	t-value p<0.05	Open dried	t-value p<0.05
S*olanum* nigrum	0.51±0.23	0.46±0.19	0.58	0.42±0.12	1.20
Gynandropsis gnandra	0.39±0.13	0.35±0.09	0.88	0.33±0.17	0.97
Corchorus olitorius	0.24±0.09	0.20±0.05	0.91	0.10±0.06	1.60
Cucurbita maxima	1.62±0.84	1.23±0.51	1.82	0.50±0.14	1.80
Vigna unguiculata	0.83±0.32	0.76±0.42	0.46	0.71±0.19	1.12
Ipomoea batatas	1.34±0.61	0.98±0.37	1.75	0.72±0.27	1.74
Symphytum offinale	0.70±0.09	0.68±0.32	0.21	0.65±0.25	0.66
Solanum aethlopicum	0.56±0.12	0.55±0.27	0.12	0.51±0.17	0.83
Agaricus bisporus	0.91±0.27	0.80±0.43	0.75	0.66±0.35	1.95

It is evident that amounts of Zn found in these vegetables differed with type of vegetable with highest contents of Zn being C*ucurbita maxima* with 1.62, 1.23, and 0.50 mg per 100 g for

fresh, solar dried and open dried respectively. The lowest content of zinc was C*orchorus olitorius* 0.24 mg, 0.20 mg, and 0.10 mg per 100 g for fresh, solar dried and open dried respectively. This variation may be attributed due to variation in genetic makeup of plant species.

Using the t-test, it was found that there was no significant difference between zinc content in the fresh and solar dried; fresh and open indigenous vegetables. For example by comparing mean levels of Zn in fresh C*ucurbita maxima* and the solar dried ones using t-test it was found that the t-calculated value was 1.82 which was lower than t-tabulated value of 2.07 at $p<0.05$. Fresh C*ucurbita maxima* and the open dried ones t- calculated was 1.80 which was lower than t-tabulated value of 2.07 at $p<0.05$ in both, although the value was not expected to change after processing.

In C*orchorus olitorius* with the lowest mean of zinc there was no significant difference between fresh and solar dried indigenous vegetable, t-calculated value was 0.91 which was lower than t-tabulated value of 2.07 at $p<0.05$. There was also no significant difference between fresh and open dried C*orchorus olitorius* t-calculated value was 1.60 which was lower than t-tabulated value of 2.07 at $p<0.05$.

The results are comparable with values reported in the literature of some of the indigenous vegetables analyzed elsewhere. Kinabo *et al.* (2004) reported contents of Zn in C*orchorus olitorius* to be 0.47 mg/100 g edible portion, which is higher than the one reported in this study. He also reported contents of Zn in S*olanum* nigrum to be 0.57 mg/100 g edible portion, which is comparable to those observed in the current study.

Table 4.11: comparison of levels of zinc (mg/100 g) in indigenous vegetables with average RDA of 11 mg/day

Indigenous vegetable	Fresh	Contribution to RDA value (%)
***Solanum* nigrum**	0.51±0.23	4.64
Gynandropsis gnandra	0.39±0.13	3.55
Corchorus olitorius	0.24±0.09	2.18
Cucurbita maxima	1.62±0.84	14.72
Vigna unguiculata	0.83±0.32	7.54
Ipomoea batatas	1.34±0.61	12.18
Symphytum offinale	0.70±0.09	6.36
Solanum aethlopicum	0.56±0.12	5.09
Agaricus bisporus	0.91±0.27	8.27

4.4.3 Manganese (Mn) levels in the indigenous vegetables

Results for Mn contents of the identified indigenous vegetables are presented in Tables 4.12.

Table 4.12: Mean (n=12) levels of manganese in various indigenous vegetables (mg/100 g)

Indigenous vegetable	Fresh	Solar dried	t-value p<0.05	Open dried	t-value p<0.05
***Solanum* nigrum**	8.97±2.61	8.02±4.18	0.68	7.96±3.08	0.87
Gynandropsis gnandra	10.35±3.53	10.07±2.99	0.19	8.76±4.51	0.96
Corchorus olitorius	6.87±2.23	6.33±4.12	0.40	5.17±3.72	1.36
Cucurbita maxima	3.49±1.87	3.23±1.46	0.37	2.67±1.24	1.27
Vigna unguiculata	9.18±2.89	7.84±3.78	0.98	7.38±4.16	1.23
Ipomoea batatas	2.83±0.68	2.27±0.95	1.66	2.31±0.56	2.04
Symphytum offinale	12.93±3.98	10.49±4.89	1.34	9.93±4.12	1.81
Solanum aethlopicum	0.31±0.09	0.30±0.03	0.37	0.27±0.07	1.22
Agaricus bisporus	0.15±0.02	0.13±0.09	0.30	0.12±0.06	1.65

From the table, it is evident that amounts of Mn found in these vegetables differed with type of vegetable with highest contents of Mn being S*ymphytum offinale* with 12.93 mg, 10.49mg, and 8.93 mg per 100 g for fresh, solar dried and open dried respectively. The lowest content Mn was *Agaricus bisporus* 0.15 mg, 0.13 mg, and 0.11 mg per 100 g for fresh, solar dried and open dried respectively. The variation may be due to variation in genetic makeup of plant species.

Using the t-test, it was found that there was no significant difference between manganese content in the fresh and solar dried. For example by comparing mean levels of Mn in fresh G*ynandropsis gnandra* and the solar dried ones using t-test it was found that the t-calculated value was 0.19 which is lower than t-tabulated value of 2.07 at $p<0.05$. There was also no significant difference between Fresh G*ynandropsis gnandra* and the open dried ones t-calculated was 0.96 which was lower than t-tabulated value of 2.07 at $p<0.05$, although the value was not expected to change after processing.

Agaricus bisporus had the lowest mean of Manganese, there was no significant difference between fresh and solar dried indigenous vegetable, t-calculated value was 0.30 which was lower than t-tabulated value of 2.07 at $p<0.05$. There was also no significant difference between fresh and open dried *Agaricus bisporus* t-calculated value was 1.65 which was lower than t-tabulated value of 2.07 at $p<0.05$.

Most of the studied indigenous vegetables, the levels of manganese were comparable to the minimal literature available, Dugo *et al* (2005) reported a value of 3.5 and 3.3 mg/100 g in

dark green leaves of chicory and spinach respectively. The highest value in the study was 12.93 mg/100 g in S*ymphytum offinale*

Table 4.13 shows the mean level of manganese (mg/100 g) in the analyzed samples when compared with the recommended daily Mn intake of average 2.2 mg/day (Institute of Medicine, 2001). The table therefore shows the various percentage contributions of the various indigenous vegetables.

Table 4.13: comparison of the mean levels of manganese (mg/100 g) in fresh indigenous vegetables with average RDA of 2.2 mg/day

Indigenous vegetable	Fresh indigenous vegetable	Contribution to RDA value (%)
S*olanum* nigrum	8.97±2.61	407.72
G*ynandropsis gnandra*	10.35±3.53	470.45
C*orchorus olitorius*	6.87±2.23	312.27
C*ucurbita maxima*	3.49±1.87	158.64
V*igna unguiculata*	9.18±2.89	417.27
I*pomoea batatas*	2.83±0.68	128.64
S*ymphytum offinale*	12.93±3.98	587.72
S*olanum aethlopicum*	0.31±0.09	14.09
Agaricus bisporus	0.15±0.02	6.82

From the table it can be noted that S*ymphytum offinale,* G*ynandropsis gnandra,* V*igna unguiculata* and S*olanum* nigrum provides the highest percentage contribution to RDA value of 587.72%, 470.45%, 417.27% and 407.72% respectively. The lowest was *Agaricus bisporus* with 6.82% followed by S*olanum aethlopicum* with 14.09%. Since the bioavailability of Mn in plant food is low the high percentage contribution of Mn to RDA

value by most indigenous vegetables analyzed will be able to meet Mn deficiency. Also since there was no significance difference between the fresh samples and the solar dried indigenous vegetables, it also shows that taking solar dried indigenous vegetables will also enable meet the daily requirements of Mn.

Including these indigenous vegetables in our daily meal will help meet the required amount of manganese needed which plays a particularly important role as part of the natural antioxidant enzyme superoxide dismutase (SOD), which helps fight damaging free radicals. It also helps energy metabolism, thyroid function, blood sugar control, and normal skeletal growth (Rodale, 1998).

4.5 Indigenous vegetables formulation

Table 4.14 shows indigenous vegetables that were used to formulate vegetable food supplement and then its efficacy tested using mice bioassay. The vegetables were selected to meet dietary allowance of zinc, iron, manganese and vitamin A. *Gynandropsis gnandra, Cucurbita maxima,*and *Agaricus bisporus* were mixed in the ratio1:3:4 respectively.

Table 4.14 Indigenous vegetables used to formulate food supplement (mg/100 g)

Indigenous vegetables	Zinc	Iron	Manganese	Beta-carotene
Gynandropsis gnandra	0.35±0.09	90.24±23.14	10.07±2.99	6.32±0.18
Cucurbita maxima	1.23±0.21	20.86±8.32	3.23±1.46	2.86±0.32
Agaricus bisporus	0.80±0.43	3.04±0.18	0.13±0.09	4.23±0.43

4.6 Feed efficiency and weight gain of mice fed with the formulated vegetable mix

Figure 4.2 shows the feed efficiency of mice fed for 42 days with different proportion of indigenous vegetable mix when compared to a basal diet which served as a control. The group of mice fed with 25% of indigenous vegetable mix had the highest feed efficiency of 6.96% followed by the one fed with basal diet as control with a value of 5.24%. The lowest was the group fed with 75% of the indigenous vegetable mix with a value of 4.42%.

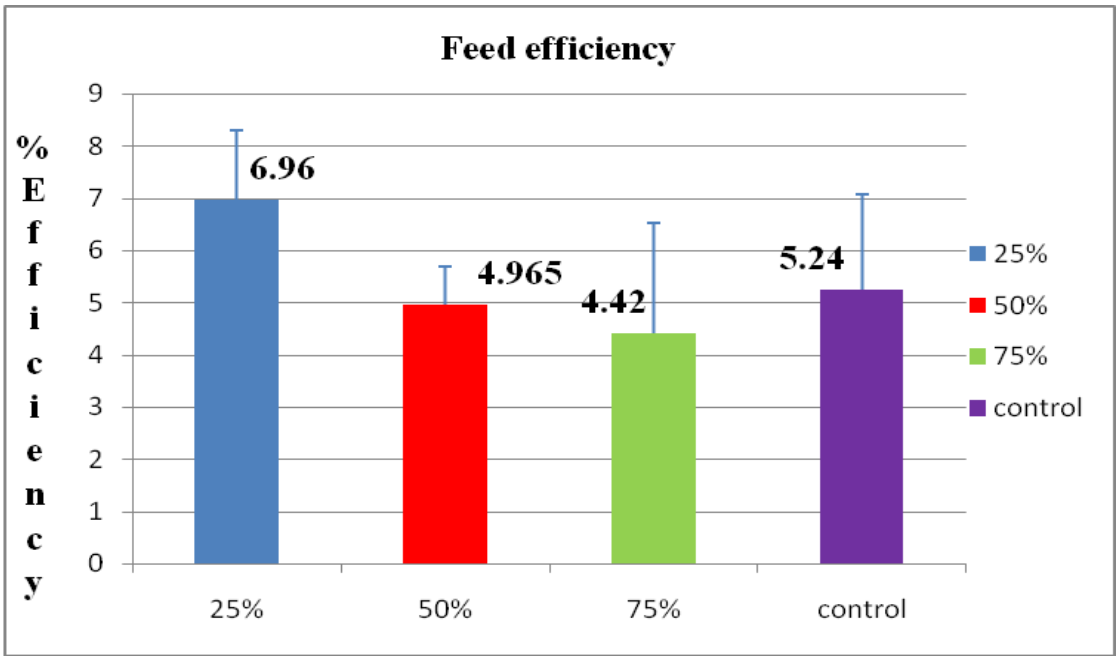

Figure 4.2 Feeding efficiency of four groups of mice fed with 25%, 50%, and 75% of formulated vegetable mix and a control feed.

From the results, it can be stated that increasing the levels of indigenous food supplement in the diet of mice reduced the feeding efficiency while supplementation at the right proportion 25% increased the efficiency above that of the basal diet which served as the control which suggests that the modified diet at this proportion was more accepted to the animals.

The weight gain of mice fed with different proportion of indiginous vegetable mix blend and the ones fed with basal diet which served as control is shown in figure 4.3. At the end of the experiment the highest mean weight gained was attained by the group of mice fed with basal diet with a value of 37.5 g followed by that fed with 25% of indiginous vegetable mix with a mean value of 36.4 g. The lowest were those fed with 50% and 75% of the mix with a mean value of 32.5 g and 31.7g respectively. The result show that the prepared food supplement at the right proportion mix cotribute positively to growth and development as compared to the control group. Therefore this indicates that the prepared food supplement has potential for improving health and immunity if used daily in appropriate proportions

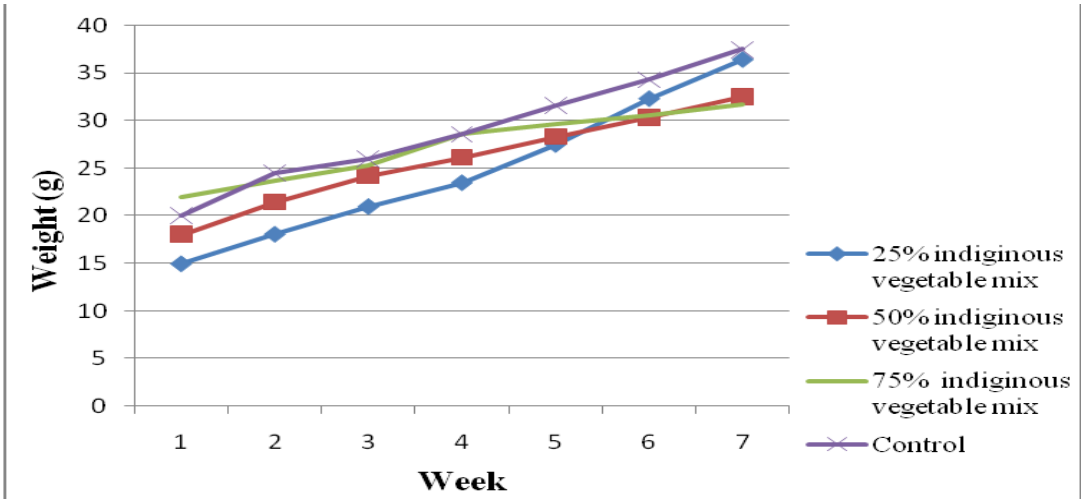

Figure 4.3 a curve of weight gain of mice against time fed

The growth rate of mice fed with the indigenous vegetable mix, shows that mice fed with diets containing 25% had comparable growth rate to the control group. 50% and 75% groups showed significantly lower rate. The rate and pattern of growth as well as absence of illness and mortality can also assess the adequacy of a diet (Guthrie, 1989). During the feeding period animals fed with the formulated indigenous vegetable blend survived, mice fed with

25% of the indigenous vegetable mix had high growth rate and high percentage efficiency ratio and could be attributed to high food intake.

4.7 Hematological parameters

4.7.1 White blood cell count

The mean values of white blood cells, the lymphocytes, the neutrophyls and the monocytes are shown in table 4.15. There was an increase in white blood cells, lymphocytes and monocytes on the group of animals fed with 25% of the indiginous vegetable mixture as compared to control, however the change was not significant different ($p<0.05$). There was a significant decrease on white blood cells and the lymphocytes on the group fed with 50% but neutrophyls and the monocytes of the same group did not decrease significantly ($p<0.05$).

Table 4.15 hematological parameters of mice fed with formulated indigenous vegetable mix

At the end of the experiment				
	Treatment groups			
Parameter	**control**	**25%**	**50%**	**75%**
WBC per cubic mm	5860±521.3	6340±1378^{NS}	4450±1366*	4180±1145*
Lymphocytes per cu.mm	3790±308.8	5257.8±1172^{NS}	2879±936*	2772±894*
Neutrophyls per cu.mm	737±101.6	732.2±156^{NS}	790±115^{NS}	561±114*
Monocytes per cubic mm	232.4±94.6	264±92^{NS}	247±79^{NS}	187±62*

NS-Not significant *significant

There was a decrease on white blood cells, the lymphocytes, the neutrophyls and the monocytes on the group of animals fed with 75% and the decrease was statistically different ($p<0.05$). The results were comparable with that of Adepoju and Adebanjo, 2011 which showed an increase in white blood cell, which was statistically significant when compared

with the control when they fed mice with a formulation of *Cucurbita pepo* seeds. Also they noted that when they increase the doses of the supplement the white blood cell decreased and the results were statistically significant. Hence, its effect on WBC was dose-dependent. The neutrophil levels were reduced at all the dose levels tested and were significantly different from the control.

Figure 4.4 gives a comparison of white blood cells in cubic millimeter of blood obtained at the beginning and at the end of the feeding period with 25%, 50% and 75% of the formulated indigenous vegetable mixture.

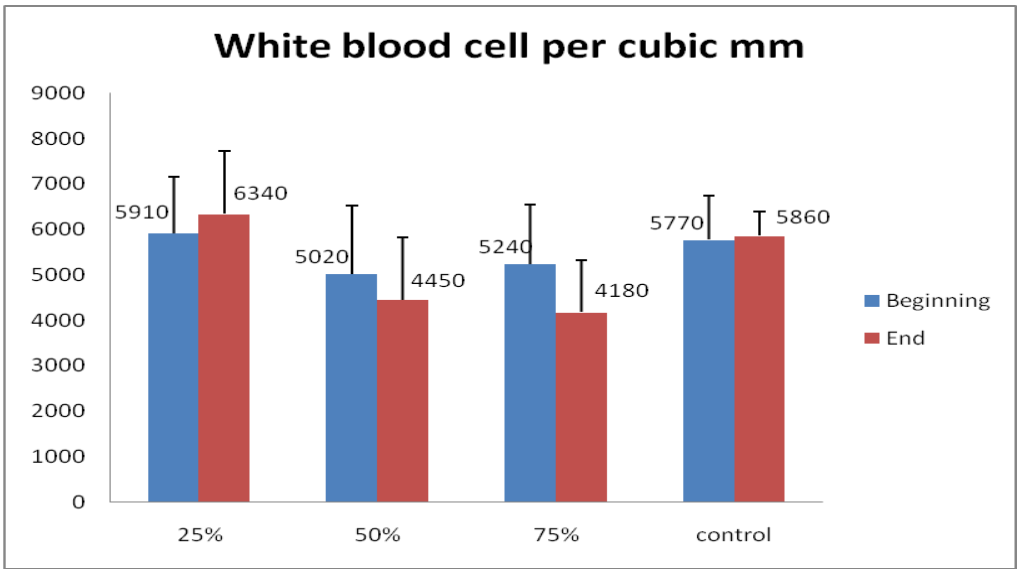

Figure 4.4 a graph of white blood cell in cubic mm obtained at the beginning and at the end of feeding period

There was a statistical significant different at $p<0.05$ in the number of white blood cells on the group fed with 25% and 75% of the feed mixture. The number of white blood increased at the 25% and decreased when the animals were fed with 50% and 75% of the prepared food mixture.

From figure 4.5 the study shows that there was an increase in the number lymphocytes on the group fed with with 25% and a decrease of the lymphocytes on the group fed with 50% and 75% however, the change was not statisticaly significant different ($p<0.05$). Inclusion of formulated indigenous vegetable mix to mice diet tended to improve hematological competence of mice especially when fed with 25%. It was noted that that although inclusion of formulated solar dried indigenous vegetables into the diet increased white blood cells, the effect was dose-dependent and higher increase of the inclusion resulted to a decrease of white blood cell.

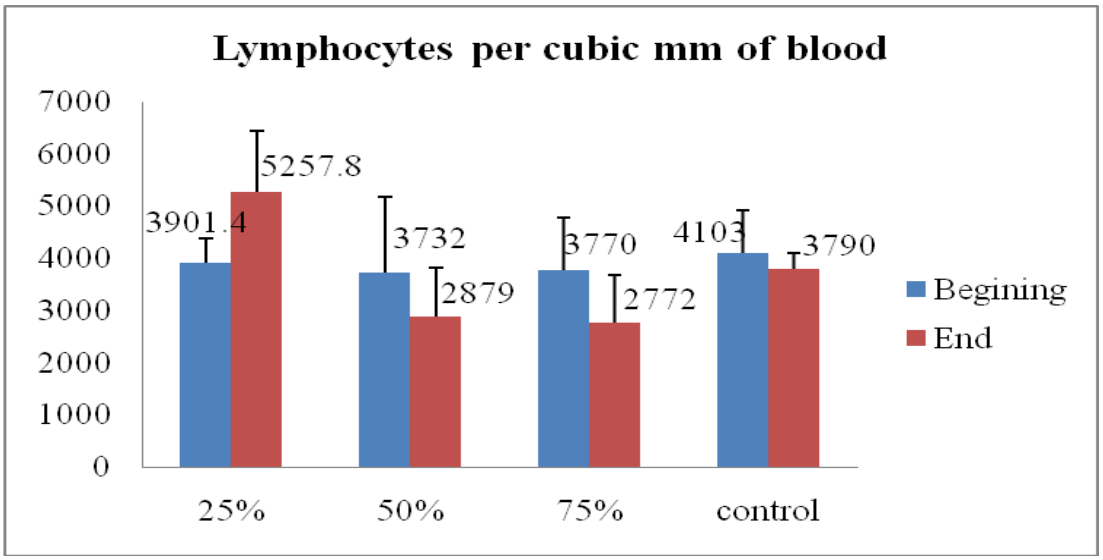

Figure 4.5 a graph of Lymphocytes in cubic mm obtained at the beginning and at the end of feeding period

The results were comparable with that of Adepoju and Adebanjo (2011) which showed an increase in white blood cell, which was statistically significant when compared with the control when they fed mice with a formulation of *Cucurbita pepo* seeds. Also they noted that when they increase the doses of the supplement the white blood cell decreased and the results were statistically significant. Hence, its effect on WBC was dose-dependent.

Figure 4.6 gives a comparison of Neutrophlys in cubic millimeter of blood obtained at the beginning and at the end of the feeding period with 25%, 50% and 75% of the formulated indigenous vegetable mixture. There was no significant change on the group fed with 25% and 75%, however there was a significant change to the group fed with 50% of the food mixture at ($p<0.05$).

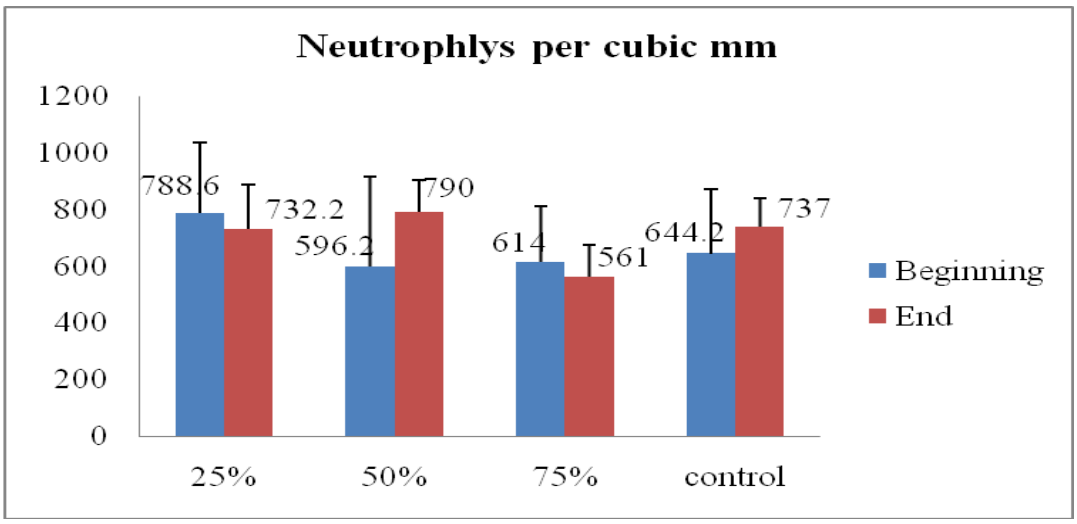

Figure 4.6 a graph of Neutrophlys in cubic mm obtained at the beginning and at the end of feeding period

From the study, figure 4.7 shows the number of monocytes recorded from the beginning and at the end of the feeding period. The group fed with 25% showed an increase in number of monocytes while there was a decrease of monocytes to the group fed with 75% of the feed mixture, this change was statisticaly significant different at $p<0.05$.

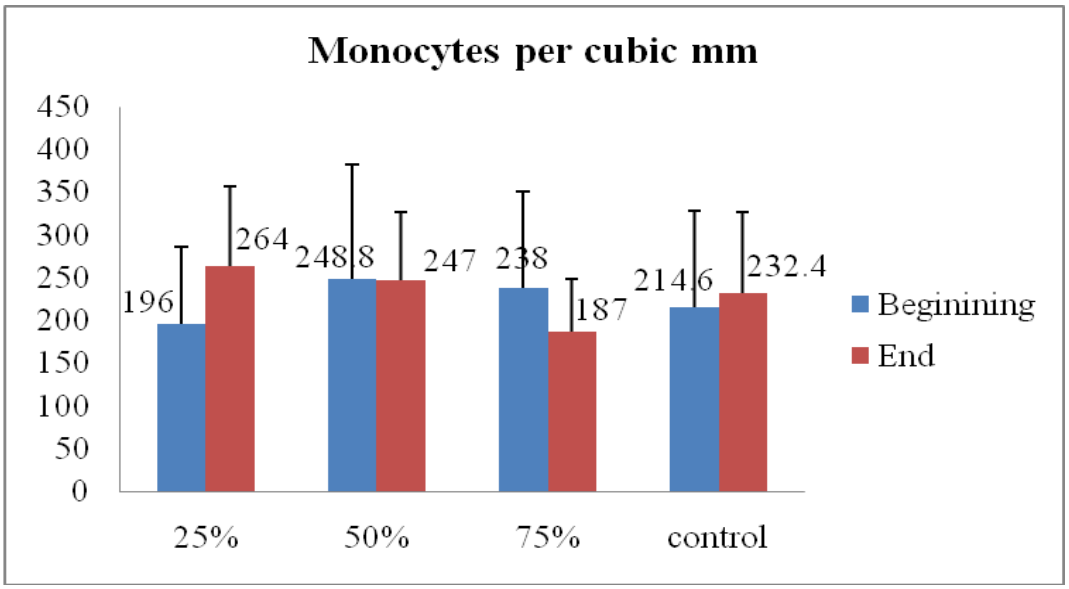

Figure 4.7 a graph of monocytes in cubic mm obtained at the beginning and at the end of feeding period

The result obtained can be compared to the study by Bolu *et al.(2009)* which reported that inclusion of dried pawpaw seed formulation in the diets of broilers tended to improve their hematological competence especially at 5% dietary level. Hematological values are indirect pointers to the health of an individual or an animal, therefore supplementation of solar dried indigenous vegetables into our human diet may help improve our health by increasing our immunity and acting as anti-oxidant.

CHAPTER FIVE

Conclusion and Recommendation

5.1 Conclusion

The indigenous vegetables had enough amount of beta-carotene to meet the daily dietary allowance. The highest levels of beta-carotene was in fresh G*ynandropsis gnandra* , *Agaricus bisporus,* S*olanum nigrum* and C*orchorus olitorius* with dry weight of 9.61 mg/100 g, 8.62 mg/100 g, 6.42 mg/100 g and 6.02 mg/100 g respectively. S*olanum aethlopicum* contained the lowest levels of beta-carotene with 0.24 mg/100 g dry weight in fresh.

The amounts of Zn found in these indigenous vegetables varied with he type of vegetable analyzed. The highest contents of Zn being C*ucurbita maxima* with 1.62 mg per 100 g for fresh and lowest content of zinc was C*orchorus olitorius* 0.24 mg per 100g for fresh. By eating a variety of indigenous vegetable daily one will be able to get the required amount of zinc.

The levels of Mn found in these vegetables varied with type of vegetable with highest contents of Mn being S*ymphytum offinale* with 12.93 mg per 100 g for fresh and the lowest content Mn was *Agaricus bisporus* with 0.15 mg per 100g for fresh. By eating a variety of indigenous vegetable daily one will be able to get the required amount of Mn.

The study indicated high levels of iron in indigenous vegetables, *Gynandropsis gnandra* (spider plant) showed the highest contents of Fe with 90.78 mg per 100g in fresh while the lowest was *Solanum aethlopicum* (African egg plant) with 2.45 mg per 100g in fresh.

It was found that Beta-carotene was susceptible to drying. Open drying proved to be the more destructive of the two drying methods tested in this study. It is noted from table 4.3 that solar dried indigenous vegetables retained high amount of beta- carotene compared to open drying. The minerals were not severely lost in both methods. Most of the indigenous vegetables analyzed in this study were found to contribute high percentages of beta-carotene and essential mineral elements to the daily dietary intake.

There was slight decrease in mineral content both in solar dried and open dried indigenous vegetables although the minerals retained is enough to meet the daily recommended amount.

During the feeding period animals fed with the formulated indigenous vegetable blend survived, mice fed with 25% of the indigenous vegetable mix had high growth rate and high percentage efficiency ratio and could be attributed to high food intake. The neutrophil levels were reduced at all the dose levels tested and were significantly different from the control.

5.2 Recommendation

5.2.1 Recommendations from this study

Based on the above conclusions the following are recommended:

i. People need to be encouraged to grow and eat indigenous vegetables since they have high levels of beta-carotene and essential mineral elements.

ii. The use of solar dryers should be encouraged in drying of vegetables since such food-based approaches remain the only affordable approaches of mitigating malnutrition in the developing countries. This study propose use of solar dying technique for processing indigenous vegetables that retains high levels of nutrients compared to open drying and make the vegetables be available all the year round even during dry season.

iii. From the results, it can also be recommended that when preparing a food supplement, one should make sure the vegetable supplement does not take a major proportion of the food intake as it may not contain all the other required .nutrients

5.2.2 Recommendations for further research

i. More indigenous foods including indigenous fruits growing should be analyzed for their nutritional content to create more awareness of their values.

ii. **A** food supplement should be developed from solar dried indigenous vegetables with high nutritional value so that it can be used to alleviate malnutrition especially to children in areas with prone cases.

iii. Determine the shelf life of solar dried indigenous vegetables from rooftop solar drier.

iv. Determine other natural antioxidants like phenolic compounds and terpenoids in indigenous vegetables especially mushroom that been associated with protection from and/or treatment of chronic illnesses such as heart disease, cancer, diabetes and hypertension.

v. It is also recommended that the levels of other essential minerals,vitamins, proteins,and carbohydrates be determined so that a proper food supplement with all essential nutrients can be prepared and then proportions can be compared with the basal diet

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APPENDICES

Appendix I

IRON ANALYSIS WAVELENGTH 248.3nm						
Std Conc.		Transmittance				
(ppm)		Trial 1	Trial 2	Trial 3	Mean	CV
0		0	0	0	0.0000	0.00
0.5		0.1165	0.1154	0.116	0.1160	0.48
1		0.1508	0.1511	0.1517	0.1512	0.30
1.5		0.21	0.2103	0.2103	0.2102	0.08
2		0.2613	0.2615	0.2619	0.2616	0.12
2.5		0.3182	0.3175	0.3173	0.3177	0.15

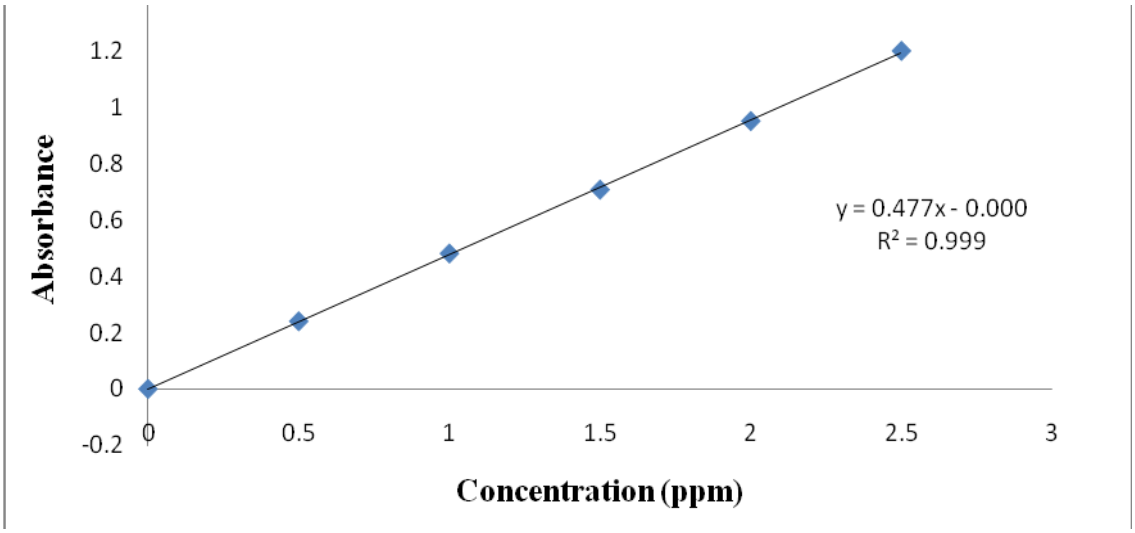
Appendix II

MANGANESE ANALYSIS WAVELENGTH 278.5nm						
Std Conc.		Transmittance				
(ppm)		Trial 1	Trial 2	Trial 3	Mean	CV
0		0	0	0	0.0000	0.00
0.5		0.0231	0.024	0.0239	0.0237	2.08
1		0.0467	0.0464	0.0459	0.0463	0.87
1.5		0.0674	0.0681	0.0672	0.0676	0.70
2		0.093	0.0928	0.0924	0.0927	0.33
2.5		0.1158	0.1156	0.1156	0.1157	0.10

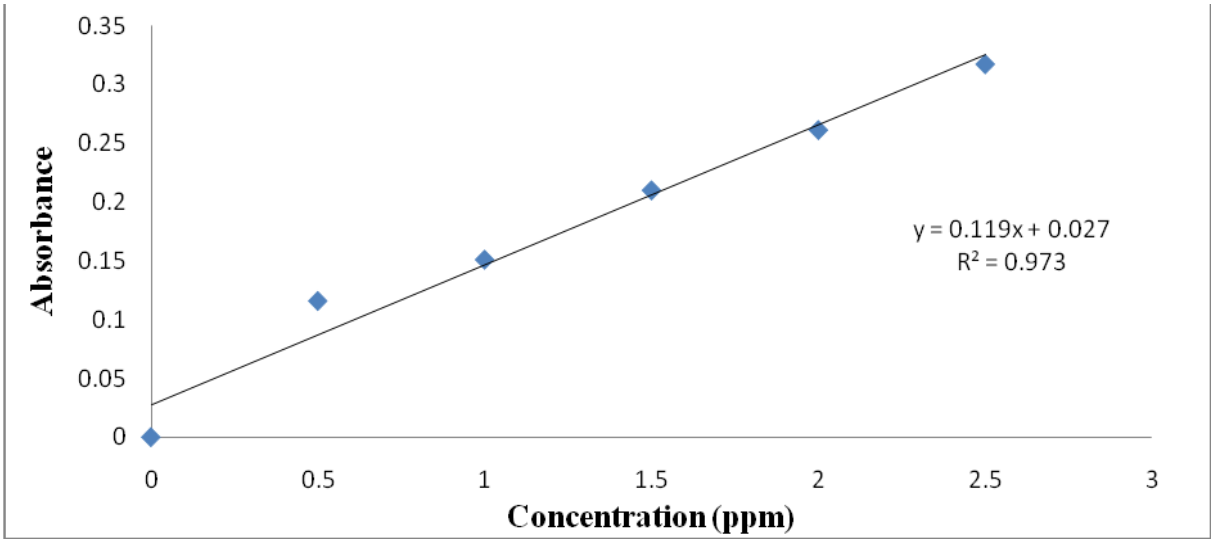
Appendix III

ZINC ANALYSIS WAVELENGTH 213.9nm					
Std Conc.	Transmittance				
(ppm)	Trial 1	Trial 2	Trial 3	Mean	CV
0	0	0	0	0.0000	0.00
0.5	0.2401	0.2399	0.24	0.2400	0.04
1	0.4792	0.4814	0.4812	0.4806	0.25
1.5	0.71	0.7082	0.7021	0.7068	0.59
2	0.9502	0.9497	0.9508	0.9502	0.06
2.5	1.1992	1.1991	1.1994	1.1992	0.01

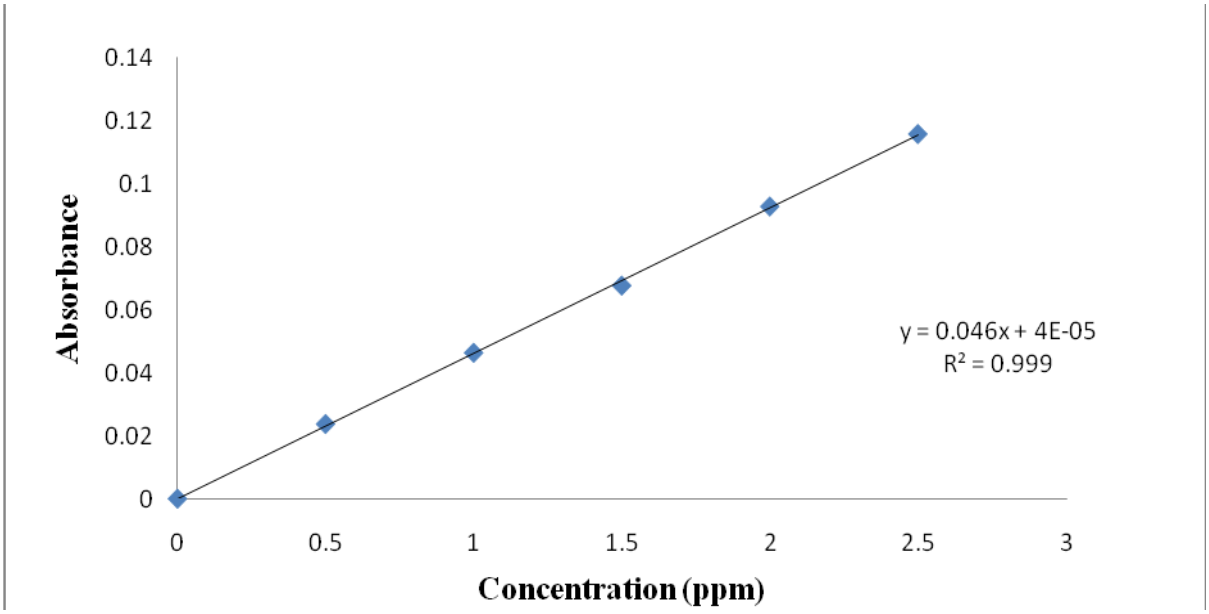
Appendix IV



Calibration Curves for iron

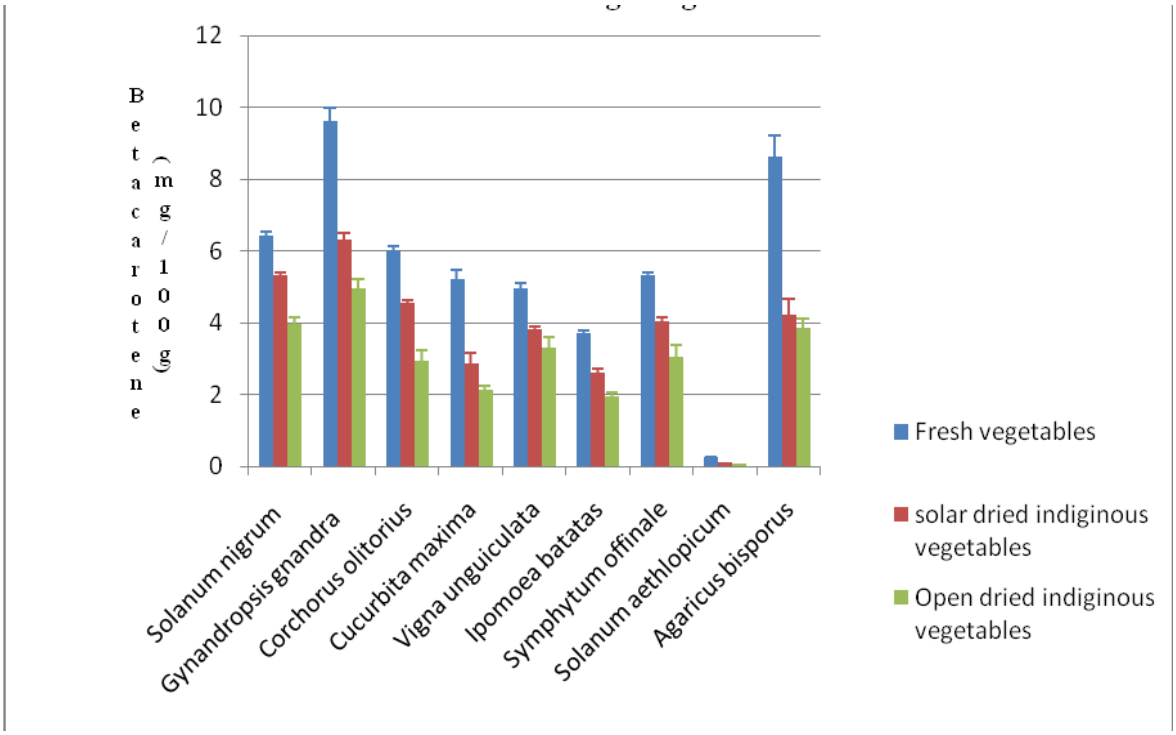


Calibration Curves for manganese



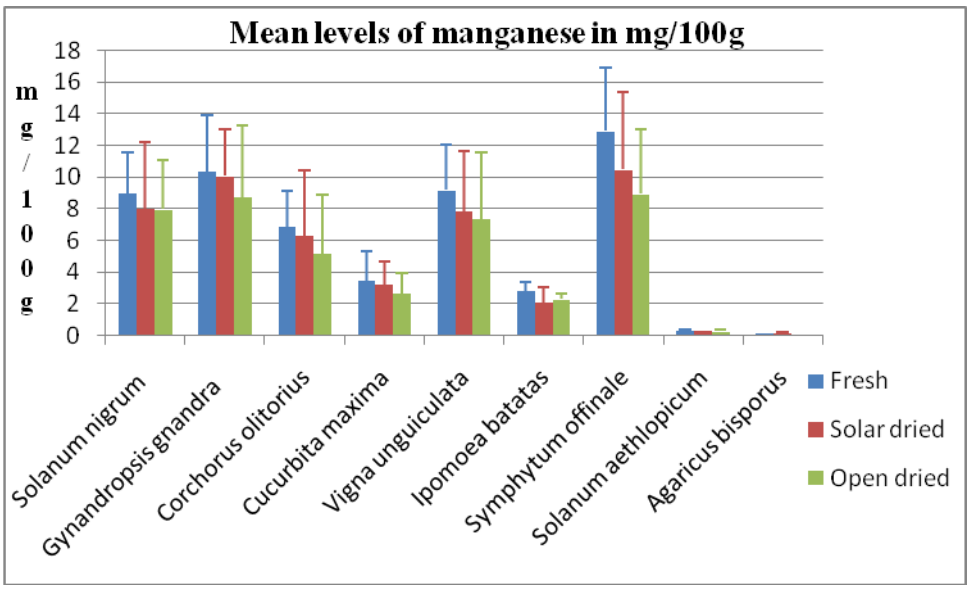
Calibration Curves for zinc

Appendix V



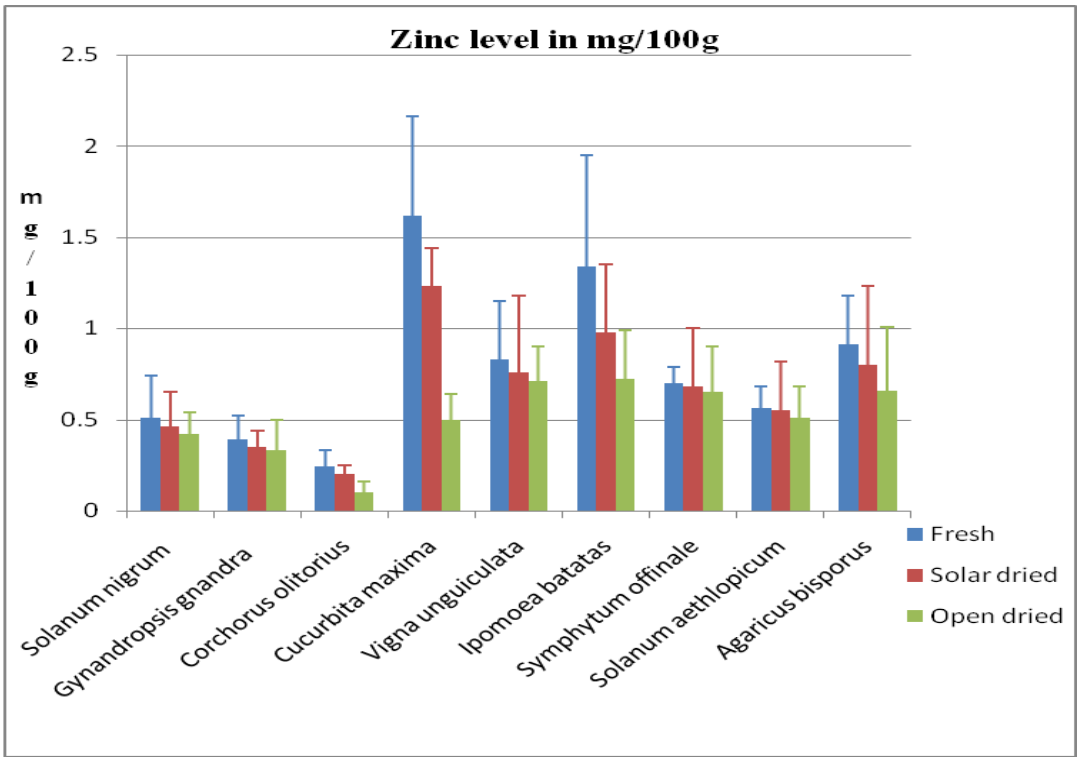
Mean levels of beta-carotene in mg/100 g

Appendix VI



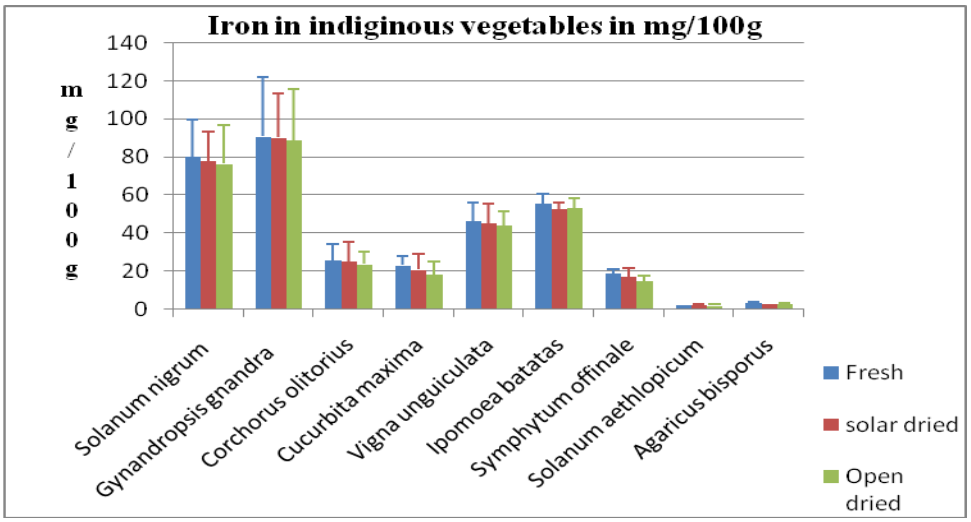
Mean levels of manganese in mg/100 g

Appendix VII



Mean levels of zinc in mg/100 g

Appendix VIII



Appendix IX Mineral mix for rodent diet Formulation

Mineral	Amount g/Kg mix
Calcium carbonate anhydrous, 40.04% Ca	357
Potassium phosphate, monobasic	250
Sodium chloride, 39% Na, 60.66% Cl	74
Potassium sulphate, 44.87%K, 18.39% S	46.60
Magnesium oxide 60.32 Mg	24
Feric citrate, 16.5% Fe	6.06
Zinc carbonate, 52.14% Zn	1.65
Manganous carbonate, 44.79% Mn	0.63

Reeves *et al*, 1993

Appendix X Vitamin mix for rodent diet Formulation (10 g/Kg)

Content	**Amount**
Nicotinic acid	30 mg
Thiamine	5 mg
Riboflafin	6 mg
Folic acid	2 mg
Vitamin K	750
Vitamin A	4000 Iu
Vitamin E	75

Reeves *et al*, 1993

Appendix XI

Table 4.15 Feeding efficiency of mice fed with formulated indiginous vegetable mix

Feeding efficiency							
	Week						
	1	2	3	4	5	6	Mean±SD
25%	6.4	6.52	4.84	8.16	8.44	7.4	**6.96±1.33^{NS}**
50%	5.33	6.22	4.22	4.86	4.4	4.76	**4.965±0.72^{NS}**
75%	5.92	4.88	7.68	2.6	2.7	2.76	**4.42±2.1^{NS}**
Control	8.28	2.66	4.72	5.9	4.8	5.1	**5.24±1.83**

NS-Not significant *significant