NUTRITIONAL COMPOSITION OF SELECTED INDIGENOUS VEGETABLES IN KENDU BAY AREA-HOMA BAY COUNTY

BY

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DECLARATION

Declaration by the student

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This thesis has been submitted for examination with our approval as University supervisors

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DEDICATION

This thesis work is dedicated to my wife Hellen who provided both moral and material support for the success of this study.

ABSTRACT

Nutrient analysis of vegetables plays a crucial role in assessing their nutritional significance. Plant foods provide almost all essential nutrients for human diet. Many stakeholders in human nutrition have emphasized the need for cheap and quality food alternatives. Information on nutrition is used more often by agencies in food production and public health like Food and Agriculture Organization (FAO) to promote consumption of the food products. People are looking for variety in their diets and are aware of the health benefits of fresh vegetables and are keen in food sources rich in antioxidant vitamins (vitamins A, C and E), Ca, Mg, K and fibre. These nutrient requirements can be realized through improved consumption of fresh vegetables. This study aimed at assessing the nutritional and mineral composition of various selected indigenous vegetables, which are commonly consumed in Kendu Bay, Homa Bay County. Consumption of foods that meet nutritional requirements is essential for good health. There is risk of people suffering from malnutrition and related diseases if the nutritional status of the foods they consume is not known. A total of 48 samples of each vegetable variety; *Solanum nigrum, Cleome gynandra, Justicia flava, Amaranthus hybridus, Vigna unguiculata* and *Crotalaria brevidens* together with corresponding soil samples where the vegetables grow were collected from various parts around Kendu Bay and analysed for nutrient contents. Samples were collected from selected plants during flowering stage for purposes of botanical identification. The samples were manually washed with distilled water and residual moisture evaporated at room temperature. Samples were oven dried in paper envelope at 55°C for 24 hours, ground into fine powder using pestle and mortar and sieved through 20-mesh sieve. The sieved samples were weighed and 2.0 g subjected to wet ashing and analysed for K, Mg, Ca, Fe, and Mn. Minerals were extracted from the soil using 10.0 g of the sample and 20 ml of aqua regia. Fresh vegetable samples were macerated for provitamin A and vitamin C analyses. Moisture was analyzed through oven drying. Minerals Mg, Ca, Fe and Mn were analyzed using atomic absorption spectrometry (AAS) while K was analyzed using both AAS and flame photometer. The vitamins; Beta carotene and vitamin C were analyzed using UV-spectrophotometry and titrimetric methods, respectively. The beta carotene contents of the vegetables were used to estimate retinol equivalent. Moisture and ash contents of these vegetable species were determined using Association Official of Analytical Chemists (AOAC) methods. Validity of the instruments was tested by regression, Horwitz ratio and standard recovery method. The results obtained were ash 9.71-19.83 mg / 100 g, moisture 77-87%, all the vegetables had vitamin C contents above recommended daily allowance (RDA) of 40-70 mg , *Vigna unguiculata* gave 64.9-80.8 mg / 100 g vitamin C (wet weight), β-carotene contents were in the range 3.34-9.40 mg / 100 g. Mineral contents varied among species with K 309 mg / 100 g in *Amaranthus hybridus,* Mg 17-24 mg / 100 g, Ca 90-149 mg / 100 g, Mn 5.78-22 mg / 100 g, and Fe 41-77 mg / 100 g. There was a positive correlation between soil and vegetable mineral contents with *Vigna unguiculata* giving a strong positive correlation coefficient (r = 0.952) while *Crotalaria brevidens* gave a weak correlation of 0.26645. The Ca contents were below detectable limits in some of the samples. The findings of the study will provide additional information on the nutritional status of the selected vegetables in Kendu Bay and will be of great interest to the consumers, farmers, the Ministry of Public health and nutritionists in the provision of public awareness.

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DEFINITIONS AND ABBREVIATIONS

AOAC- Association Official of Analytical Chemists

APVMA- Australian Pesticides and Veterinary Authority

ASEAN- Association of Southeast Asian Nations

AVRDC- Asian Research and Development Center

CIPAC- Collaborative International Pesticide Analytical Council

CITAC- Co-operation on International Traceability in Analytical Chemistry

EURACHEM- A collaborative European Working group with the objective of establishing a system for the traceability of chemical measurements and the promotion of good quality practices

INAB- Irish National Accreditation Board

RNI- Referenced Nutrient Intake

UKAS- United Kingdom accreditation service

LIST OF APPENDICES

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Nutrient analysis of vegetables plays a crucial role in assessing their nutritional significance. In order to improve nutrition education programmes, knowledge of nutrient levels of indigenous vegetables is essential (Pretorius and Schonfeldt, 2011; Hussain *et a*l., 2011). Plant foods have almost all of the mineral and organic nutrients established as essential for human nutrition. Vegetables hold an important position in well–balanced diets. Green leafy vegetables are believed to occupy a modest place as a source of trace elements due to their high water content. Nutritional information is used increasingly by public agencies and agricultural industries to promote food products. Today, there is a high interest in nutrient contents in diets and consumers are aware of health benefits. They have special interest in food sources rich in antioxidant vitamins (vitamins A, C and E), calcium, magnesium, and potassium and fibre. Most of these nutrient requirements can be solved by increasing the consumption of fresh vegetables. In addition to meeting nutrient intake levels, better consumption of fruits and vegetables is associated with reduced risk of cardiovascular disease and stroke (Sushanta *et al*., 2008).

The increasing population of the world has doubled the food demands and overwhelmed the available land resources (Hussain *et al*., 2011). In recent years there has been an increasing awareness of the chemical components added to our foods by various food processors in order to enrich it, preserve it and make it more attractive to the consumer. This increasing use of chemicals in foods has become a matter of concern to governmental agencies who feel that there may be some danger to humans in the use of unnecessary or potentially harmful chemicals. Therefore, studies have been undertaken to find naturally-occurring materials which would act as nutrient source (Michalek, 1962).

Alongside other food alternatives, vegetables are considered cheap source of energy. Vegetables are rich sources of essential biochemicals and nutrients such as carbohydrates, carotene, proteins, vitamins, calcium, iron, ascorbic acid and palpable concentration of trace minerals (Hussain *et al*., 2011).

Cheap and qualitative food alternatives have been stressed and recognized by many stakeholders from National governments to international agencies like Food and Agriculture Organization (FAO). Besides these biochemicals, the moisture, fibres, ash contents and the energy values of individual vegetable have also been regarded important to the human health and soil quality (Hussain *et al*., 2011).

Minerals account for approximately 4% of body-weight. Some, such as calcium and phosphorus, are present in the body in relatively large amounts and are known as macronutrients whereas others occur in very small quantities and are known as trace elements (Gaman and Sherrington, 1996).

Vitamin A as such, naturally occurs in animal meat, milk, eggs whereas plants contain vitamin A precursor, beta carotene. Humans and other animals need either vitamin A or beta carotene, which they easily convert to vitamin A. Beta carotene is found in oranges and yellow vegetables as well as green leafy vegetables. A deficiency of vitamin A leads to blindness, failure of normal bone and tooth development in the young, diseases of epithelial cells and membranes of the nose, throat, and eyes, which can decrease the body's resistance to infection (Potter and Hotchkiss, 1995).

1.2 Statement of the problem

In order to be healthy, people need to eat foods that meet nutritional requirements. These food sources should be safe, cheap and readily available, for example, vegetables which are natural sources of nutrients. When nutrient rich foods are consumed in the diet, people are likely to have no problem of malnutrition. But right now, people eat food without caring whether these foods meet nutritional requirements and if at all they care, then the nutritional information is not available. In Kendu Bay, there is frequent use of the indigenous vegetable species by the local people in the diet. When nutrient requirements are not met people must resort to nutrient supplementations. Although this is a reliable nutrient source, it is more expensive and in some cases consumers suffer from side effects of the chemicals used during the manufacturing process.

Consumption of foods without nutritional information increases risks of malnutrition and related diseases which could be costly on both the consumer and the government. It is

expected that the findings of this study will provide additional information of the nutritional status of the vegetables grown in the area.

The study determined provitamin A (β-carotene), vitamin C, calcium, magnesium, potassium, iron and manganese levels in the selected vegetables (*Solanum nigrum, Cleome gynandra, Justicia flava, Amaranthus hybridus, Vigna unguiculata, and Crotalaria brevidens*) using experimental design. The atomic absorption spectrophotometry, uv-spectrophotometry and titrimetric methods were used to determine the nutrient levels in the vegetables.

It was the intention of the study to find out whether these vegetables meet the dietary requirements of the consumers, particularly vitamins and minerals.

1.3 Justification of the study

The presence of nutrients and minerals is affected by the quality of the soil in a given area. In Kendu Bay there is frequent use of indigenous vegetable species by the local people in the diet. It was the intention of the study to find out whether these vegetables met the dietary requirements of the consumers, particularly vitamins and minerals.

There is limited data to show the nutritional status of the indigenous vegetables in Kendu Bay. To ensure that the findings represented the nutritional status of the selected vegetables consumed in Kendu Bay, the study area was divided into two zones to take care of vegetables that could be getting into Kendu Bay area from the neighbouring Oyugis and Homa Bay towns, respectively. Contrary to the previous studies reviewed, this study analysed selected mineral in both the vegetables and soils in the study area. This facilitated comparison of the minerals in the vegetables and soils, an aspect which was lacking in the studies reviewed. It is expected that the findings of this study will provide additional information on the nutritional status of the vegetables grown in the area.

The choice of the vitamins and minerals were based on their importance in human diet. Beta carotene and vitamin C were analyzed since they are found in green leafy vegetables. Mineral analysis involved the elements calcium, magnesium, potassium, iron and manganese since their presence in the vegetable species depended on their presence in the soil.

It was envisaged that the findings of the study will provide additional information on the nutritional status of the vegetables: *Solanum nigrum Cleome gynandra, Amaranthus hybridus Crotalaria brevidens, Vigna unguiculata* and *Justicia flava.* The findings of the study will be of great interest to farmers, the Ministry of Public health and nutritionists in the provision of public awareness. The above factors, therefore, justified the study.

1.4 Objectives of the study

1.4.1 General objective

The main objective of this study was to find out whether the indigenous vegetables that are consumed in Kendu Bay meet the nutritional requirements of the consumers.

1.4.2 Specific objectives

Specific objectives of this study were:

(a) To determine moisture and ash contents of the selected vegetables

(b) To determine concentration levels of beta carotene and vitamin C in the selected vegetables (*Solanum nigrum, Cleome gynandra, Amaranthus hybridus, Crotalaria brevidens, Vigna unguiculata* and *Justicia flava).*

(c) To determine concentration levels of calcium, magnesium, potassium, iron and manganese in the selected vegetables.

(d) To compare concentration levels of the vitamins A and C, calcium, magnesium, potassium, iron and manganese in the selected vegetables with the recommended daily allowances of the vitamins and minerals.

(e) To compare the concentration level of selected minerals in the vegetables with that in the soil.

CHAPTER 2

LITERATURE REVIEW

2.1 Vitamins and their roles in the diet

Vitamins are organic chemicals, other than essential amino acids and fatty acids that must be supplied to an animal in small amounts to maintain health. Vitamins are not usually synthesized in the body and are therefore essential in the diet (Potter and Hotchkiss, 1995).

In the nineteenth century it was assumed that a diet containing carbohydrate, fat, protein and minerals was sufficient to maintain health. However, in 1888, a scientist called Lunin carried out experiments showing that mice could not survive on a diet although they survived on the same diet if they were also given milk (Gaman and Sherrington, 1996). It was apparent that milk contained substances other than carbohydrates, fats, proteins and minerals, which were essential for health.

The effects of vitamin deficiencies had been recognized for centuries but the causes of these diseases were unknown. Scurvy, a disease caused by dietary lack of vitamin C, was common among sailors on long sea voyages in the sixteenth and seventeenth centuries. In his voyage around the world from 1772 to 1775, Captain Cook demonstrated that scurvy could be prevented by the consumption of fresh vegetables (Gaman and Sherrington, 1996).

When the early vitamins were discovered they were named according to the letters of the alphabet. Each vitamin was also given a chemical name when its chemical composition was identified. Letters are still used for vitamins but they are gradually being replaced by chemical names.

Vitamins can be divided into two main groups: fat-soluble vitamins which include vitamins A, D, E and K. Fat soluble vitamins will dissolve in fats and oils but not in water; water soluble vitamins, which include vitamins of the B complex and vitamin C (Potter and Hotchkiss, 1995).

2.1.1 Provitamin A (Beta carotene)

Vitamin A (retinol) as such naturally occurs only in animal materials-meat, milk, eggs, and the like. Plants contain no vitamin A but contain its precursor, β-carotene. Beta carotene is found in orange and yellow vegetables, as well as green leafy vegetables (Potter and Hotchkiss, 1995).

Retinol is a pale yellow solid which dissolves in fats and oils but not in water. Many fruits and vegetables contain carotenes, a group of orange pigments, which can be converted into retinol in the body. The most important of these pigments is beta-carotene. Vitamin A is measured in retinol equivalents; 1µg retinol equivalent is equal to 1 µg retinol. A quantity of 1µg retinol is equal to 6 µg β-carotene.

According to Mustapha and Babura (2009) carotenoids comprise a large group of natural pigments widely distributed in the plants and animal kingdoms. Carotenoids are associated with chlorophyll in higher plants playing important role during photosynthesis by passing on the light energy they absorb to chlorophyll, they also protect chlorophyll from excess light and oxidation. Carotenoids are of two types: carotenes and xanthophylls. The most widespread and important carotene is β-carotene which is found abundantly in some plants. Among the provitamin A carotenoids in food namely beta-carotene, alpha-carotene, gamma-carotenes and beta-cryptoxanthin, beta- carotene is the one that is most efficiently converted to retinol.

Carotenoids have beneficial effects on human health: enhancement of the immune response and reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and muscular degeneration (Yuangsoi *et al*., 2008). The action of carotenoids against diseases has been associated with their antioxidant property, particularly, their ability to quench singlet oxygen and interact with free radicals. Other mechanisms have also been reported: modulation of carcinogen metabolism, inhibition of cell proliferation, enhancement of cell differentiation, stimulation of cell-to-cell communication, and filtering of blue light (Rodriguez-Amaya and Kimura, 2004).

To combat vitamin A deficiency, several countries in South America have passed laws that all sugar for home consumption be fortified with this vitamin (Potter and Hotchkiss, 1995).

Vitamin A deficiency, which now affects 85% of Kenyan children, is the leading cause of child blindness and suppresses the immune system to as great a degree as AIDS. It is a hidden killer that has now seen Sugar Company Mumias fortify its sugar with vitamin A and brought an announcement from the government that vitamin A is in future to be added to Unga, the flour used as a staple food in the Kenyan diet (Pambo *et al*, 2014).

Retinol is essential for growth and metabolism of all body cells; it is required for the formation of rhodopsin (visual purple), a complex substance formed from retinol and protein. Rhodopsin is a pigment found in the retina, a membrane at the back of the eye, and is necessary for the vision in reduced light (Dietz *et al*., 1988; Fikselova *et al*., 2008).

Vitamin A is also essential for the maintenance of healthy skin and surface tissues, particularly the moist mucous membrane such as the cornea at the front of the eye and the lining of the respiratory and digestive tracts, bones, teeth, hair and reproduction (Akubugwo *et al*., 2007; Gaman and Sherrington, 1996). Beta-carotene is thought to act as an antioxidant in the body tissues removing free radicals and a high intake may give some protection against some forms of cancer (Gaman and Sherrington, 1996). Lack of vitamin A in the diet of children reduces the rate of growth. Since without retinol the body is unable to synthesize rhodopsin, vision in reduced light is impaired, causing a condition known as night blindness. If the deficiency is only slight the condition is easily reversed by increasing the intake of vitamin A, although, increasing the intake above the required level will cause no improvement in eyesight (Gaman and Sherrington, 1996).

Vitamin A deficiency affects the health of the skin and resistance to infection is lowered due to the poor condition of the mucous lining of the respiratory tract. In extreme cases the tear gland becomes blocked and the membranes at the front of the eye become dry and inflamed. This condition is known as xerophthalmia. Severe and prolonged deficiency can lead to ulceration of the cornea causing blindness. Vitamin A deficiency usually develops in children after weaning when it is no longer supplied by breast milk (Gaman and Sherrington, 1996; Potter and Hotchkiss, 1995).

Vitamin A is necessary for good health and people who do not get sufficient amount of vitamin A in their diet are more likely to be susceptible to infectious diseases such as AIDS, measles, boils, bronchitis among others (Maina and Mwangi, 2008).

Very large doses of retinol are harmful, hypervitaminosis A causes drowsiness, irritability, skin and bone disorders and an enlarged liver. A relationship has been suggested between high intake of vitamin A during pregnancy and defects of the baby (Gaman and Sherrington, 1996). Pregnant women are, therefore advised not to take vitamin A supplements and not to eat liver or products containing liver during their pregnancy.

Large intakes of carotenes are not similarly harmful since the body will limit the conversion to vitamin A; however, yellow coloration of the skin may result.

In Britain, the Department of Health Referenced Nutrient Intake (RNI) for men is 700 µg retinol equivalents per day and for women is 600 µg retinol equivalents per day. Women need extra 100 µg retinol equivalents per day during pregnancy to meet the needs of the growing baby. During lactation when a woman needs to produce milk containing sufficient vitamin A for her baby, the RNI is 950 µg retinol equivalents per day (Gaman and Sherrington, 1996; Mustapha and Babura, 2009).

In the United States, the recommended allowance for vitamin A activity is 1000 retinol equivalents (RE) for healthy adult male. Because of the smaller size of women, their allowance is 80% of this, but it is increased during lactation (Potter and Hotchkiss, 1995).

2.1.2 Vitamin C (Ascorbic Acid)

Vitamin C, known chemically as ascorbic acid, is an important component of a healthy diet. The history of vitamin C revolves around the history of the human disease scurvy, probably the first human illness to be recognized as a deficiency disease. As early as 1536, Jacques Cartier, a French explorer, reported the miraculous curative effects of infusion of pine bark and needles used by Native Americans. These items are now known to be good sources of ascorbic acid (Pamplona and Roger, 2008).

Vitamin C is the most important vitamin for human nutrition that is supplied by fruits and vegetables. L-Ascorbic acid (AA) is the main biologically active form of vitamin C. Vitamin C is widely required in the metabolism process as human body cannot produce ascorbic acid nor synthesize it during metabolic process (Hussain *et al*., 2011).

Vitamin C has been widely used in the pharmaceuticals, chemicals, cosmetics, and food industries by its bioactivity and antioxidant properties. Ascorbic acid is rapidly oxidized to

dehydro ascorbic acid (DHA) due to the presence of two hydroxyl groups in its structure. Oxidation reactions can be induced by exposure to increased temperatures, high pH, and light, presence of oxygen or metals and enzymatic action. This reaction is reversible and a principle step in the antioxidant activity of ascorbic acid (Novakova *et al*., 2008).

Ascorbic acid has been found to prevent tissue damage and sperm agglutination thereby enhancing male fertility. In Nigeria vitamin C has been prescribed for cold, cough, sores, wounds among other ailments (Ogunlesi *et al*., 2010).

Vitamin C functions as anti- inflammatory and therefore assists the body to fight inflammatory diseases like chronic fatigue, constipation, joint pain, and sore throat and urinary tract infections. It also aids the body to absorb iron hence reduces iron deficiency and anaemia (Maina and Mwangi, 2008).

Vitamin C is required in the formation of healthy collagen which is the protein that forms connective tissues like skins, cartilage, bones and teeth. Collagen fibres keep bones and blood vessels strong, and assist to anchor teeth to the gums. It also needed for the repair of blood vessels, bruises and broken bones. Without vitamin C, collagen formation is disrupted resulting in scurvy, the vitamin C deficiency disease. Scurvy is a process that disrupts the body's ability to manufacture collagen and connective tissues (English and Cass, 2013; Matthias and Pauling, 1992).

Vitamin C presence is necessary for the activity of dopamine β-hydroxylase, as well for the synthesis of collagen which is the main protein of connective tissue. It is also necessary for the prevention of coronary heart diseases and cancer (Gaman and Sherrington, 1996; Novakova *et al.,* 2008). Ascorbic acid aids the absorption of iron from the intestine and healing of wounds. A person existing on a diet containing insufficient ascorbic acid will eventually develop the condition known as scurvy. Since vitamin C cannot be stored in the body, a regular intake is essential. In Britain it has been shown that 10 mg per day is sufficient both to prevent and cure scurvy.

The Referenced Nutrient Intake (RNI) for adults is 40 mg per day. During pregnancy this is increased to 50 mg and during lactation to 70 mg per day to meet the needs of the baby (Gaman and Sherrington, 1996).

2.2 Minerals

The mineral elements are those chemical elements, which are required by the body. Those present in the body in relatively large amounts are known as macro- minerals whereas those present in very small quantities are known as trace elements (Gaman and Sherrington, 1996).

Table 2.1 gives a list of mineral elements required by the human body. The mineral elements are in two categories, those required by the body in large quantity (macro-minerals) and those that are required in small quantity (trace-elements).

Table 2. 1: Mineral elements required in the body

Macro-minerals	**Trace elements**
Calcium	Chromium
Chlorine	Cobalt
Magnesium	Copper
Phosphorus	Fluorine
Potassium	Iodine
Sodium	Iron
Sulphur	Manganese
	Molybdenum
	Selenium
	Zinc

Source: Gaman and Sherrington (1996)

2.2.1 Calcium

Calcium and phosphorus are the minerals that humans require in the greatest amounts. Deficiencies result chiefly in bone and teeth diseases. Calcium is required in the body for strong teeth, bones and connective tissue (Pravina *et al*., 2013). It enhances healthy digestion through the production of hormones and enzymes, assists nerves pass the electrical messages needed for the contraction of the heart and other muscles in the body as well as normal blood clotting (Pravina *et al*., 2013; Maina and Mwangi, 2008).

Calcium is one of the essential factors required for the clotting of blood. Absorption of calcium increases when the diet is low in calcium. Also, a higher proportion is absorbed by young people during periods of growth and by women during pregnancy. Since Vitamin D is essential for calcium absorption the effects of a dietary deficiency of calcium are the same as a deficiency of vitamin D. Severe calcium deficiency causes rickets in children and osteomalacia in adults. There is considerable concern over another bone condition known as osteoporosis in which the bone deteriorates and there is an increased rise in fractures. This occurs in older people, particularly in women after menopause. Calcium is gradually lost from the bones which become porous and fracture more readily (Theobald, 2005; Gaman and Sherrington, 1996).

The foods that naturally contain calcium include milk, green leafy vegetables, seafood, nuts and dried beans. The average adult skeleton contains about 1200 g of calcium, present in the form of hydroxyapatite, an inorganic crystalline structure made of calcium and phosphorus [$Ca_{10}(OH)_2(PO_4)_6$] (Theobald, 2005).

Table 2.2 contains a list of daily intake for calcium recommended for various age groups and gender by the Department of Health Reference Nutrient Intake, in Britain.

Table 2. 2: Referenced Nutrient Intake for calcium

Age	mg calcium per day
0 -1 year	525
1-3 years	350
4-6 years	450
7-10 years	550
11-18 years, males	1000
11-18 years, females	800
Adults	700
Women, lactation	1250

Source: Gaman and Sherrington (1996)

2.2.2 Magnesium

Magnesium is found together with calcium and phosphorus in bones and teeth. It is also needed in the body for functioning of some enzymes. It is found in the chloroplasts of green plants and therefore green vegetables are a good source. Recommended daily intake is 350 mg and doses larger than 400 mg may cause stomach problems and diarrhoea (Gaman and Sherrington, 1996). Magnesium prevents the formation of blood clots, reduces pressure, prevents diabetes related complications, helps in maintaining bone strength, minimizes the risk of heart disease and reduces the risk of free radical damage. It functions as a cofactor of many enzymes involved in energy metabolism, protein synthesis, RNA and DNA synthesis and maintenance of the electrical potential of nervous tissues and cell membranes. It regulates potassium fluxes and is involved in the metabolism of potassium (Maina and Mwangi, 2008; Jan and Tae, 2008).

The Dietary Guidelines for the Americans describes a healthy diet as one that emphasizes a variety of fruits, vegetables, whole grains and fat-free or low-fat milk and milk products. Whole grains and dark-green leafy vegetables are good sources of magnesium. Severe magnesium deficiency can result in hypocalvemia or hypokalemia because mineral homeostasis is disrupted (Malter, 2008; Faryadi, 2012).

2.2.3 Potassium

Potassium is the principal intracellular cation and with sodium helps regulate osmotic pressure and pH equilibria. It is also involved with the cellular enzyme function. Potassium is found in a wide variety of foods, particularly fruits (especially bananas) and vegetables. Deficiency is rare but symptoms include mental confusion and muscular weakness. The effect on the heart muscle can lead to heart failure. It is thought that taking fairly high intakes of potassium may counteract the effects of a high sodium (salt) intake and reduce the likelihood of developing high blood pressure. Current guidelines, therefore, recommend increasing potassium intake in adults from an average of 3 g to 3.5 g per day (Potter and Hotchkiss, 1995).

Potassium acts as an electrolyte and it is required for keeping the heart, brain, kidney, muscle tissues and other important organs of the human body in good condition. Potassium chloride is the main compound of the element potassium. It is considered a perfect stress buster (Bhaskarachary, 2011).

2.2.4 Iron

Iron is required as a component of blood hemoglobin, which carries oxygen, and muscle myoglobin, which stores oxygen. Of all required nutrients, shortage of iron may be the most inadequacy in the diets of the industrialized world. Copper aids in utilization of iron and in hemoglobin synthesis. The need for iron and copper is related to the rate of growth and blood loss (Potter and Hotchkiss, 1995).

Iron is found in cereals but about half of this is lost in milling if the bran and germ are discarded. Potatoes and other vegetables are moderately good sources. Only between 5% and 20% of the iron a person eats is absorbed. Non-haem iron is present as trivalent (ferric) Fe^{3+} but it is absorbed more readily in the divalent (ferrous) Fe^{2+} form and therefore

reduction of the ferric form is necessary. Ascorbic acid aids the reduction and therefore aids iron absorption. Eating iron-rich foods with a source of vitamin C is therefore sensible. Iron deficiency causes anaemia, in which the number of red blood corpuscles is reduced and the amount of oxygen carried to the tissue is also reduced. Anaemia is much more common in women than in men due to blood loss during menstruation. Iron is an essential micronutrient for normal functioning of the central nervous system as well as in the oxidation of carbohydrates, proteins and fats (Akubugwo *et al*., 2007; Gaman and Sherrington, 1996).

Expectant women require extra iron to prevent premature delivery; athletes also need extra iron to keep the blood and oxygen pumping to their heart and other muscles as they contract (Maina and Mwangi, 2008). The most common symptoms of iron deficiency anaemia are tiredness and weakness due to the inadequate oxygen supply to the body's cells, and paleness in the hands and eyelids due to deoxygenated hemoglobin. There is also possibility of iron-overload and if severe the condition is diagnosed as hemochromatosis. This can result in serious damage to the body's tissues, including cirrhosis of the liver, heart failure, diabetes, abdominal pain and arthritis (Nanadur *et al*., 2008). In a study by Weinberger and Msuya (2004) amaranth leaves were found to contain the highest iron content of 37.05 mg / 100 g of edible portion while *solanum spp.* had 15.90 mg / 100 g. A research work carried out by Okonya and Maass (2014) found iron content in the range of 17.60 - 38.70 mg / 100 g. In a research work by Abukutsa-Onyango *et al.* (2010) found nightshade and cowpea vegetables to contain high iron levels which provided 100 percent of the recommended daily allowances of iron. A study on nutritional evaluation of indigenous foods found *Amaranthus hybridus* to contain moisture 84.78 %, ash 16.19 mg / 100 g, Ca 264.50 mg / 100 g, Fe 45.85 mg / 100 g, Mg 1320.00 mg / 100 g, beta carotene 4.29 mg / 100 g and vitamin C 62.93 mg / 100 g (Kunyanga *et al*., 2013).

Table 2.3 shows the recommended daily intakes for iron. The intake is dependent on factors like age and gender. Young children need a lower quantity of iron than older ones. It shows that adolescent females require more iron than their male counterparts. Expectant and lactating mothers require the highest amount of iron in the daily intakes.

Table 2. 3: Recommended Intake for Iron

Age	mg iron per day
4-6 years	6.1
7-10 years	8.7
Males: 11-18 years 19-50 years	11.3 8.7
Females: 11-50 years Above 50 years	14.8* 8.7
Pregnancy	14.8
Lactation	14.8

* Insufficient for women with high menstrual losses (iron supplement recommended)
Source: Gaman and Sherrington (1996)

2.2.5 Manganese

Manganese is actively absorbed by plants. The formation of plants mass depends on the manganese and molybdenum supply. Manganese and molybdenum take part in a number of important physiological and biological processes such as in the nitrogen metabolism, photosynthesis, breathing and maintaining the needed oxidation - reduction conditions in the cells (Kostova *et al*., 2008).

The principal route of intake for manganese is through food consumption. It is required for normal amino acid, lipid, protein, and carbohydrate metabolism. It is needed for normal immune function, regulation of blood sugar and cellular energy, reproduction, digestion and bone growth (Aschner and Aschner, 2005).

Manganese is chiefly found in the liver, kidneys, pancreas, lungs, prostate gland, adrenals, brain and bones. It is used in the metabolism of carbohydrates, and in strengthening tissue and bone. Manganese functions as an essential component of bone structure, for reproduction and for the normal functioning of the nervous system; it is part of the enzyme system (Adeyeye and Omolayo, 2011).

The National Academy of Sciences has officially stated that no one has observed a manganese deficiency in humans. In laboratory experiments with animals, an induced manganese deficiency produced restricted growth, glandular disorders and defective reproductive functions. Manganese is found in significant quantities in leafy vegetables, bananas, beets, carrots, cucumbers among the many (Aschner and Aschner, 2005). Recommended dose for manganese is 5 mg per day. Excess manganese hinders iron adsorption (Clark *et al*., 2007). A study by Odhav *et al*. (2007) reported recommended daily allowance (RDA) for the minerals as: calcium 1000 mg, magnesium 400 mg, manganese 7 mg and iron 8 mg.

2.3 Vegetables and Nutrients

The word "vegetable" has been defined in various ways. Longman dictionary of contemporary English defines it as "part of a plant that is grown for food to be eaten in the main part of a meal, rather than with sweet things". According to Asibey-Berko and Tayie (1999) "vegetable" is defined as follows: "an edible part of a plant that is used for human food and is usually eaten cooked or raw as the main part of a meal rather than a dessert".

The local names of the indigenous vegetables analysed in Kendu Bay are shown in table 2.4 as follows:

Table 2. 4: Names of vegetable samples

Botanical Name	**Local Name**	**Common Name**
Amaranthus hybridus	Ododo	Pig weed
Justicia flava	Atipa	Yellow Justicia
Crotalaria brevidens	Mitoo	Slenderleaf
Vigna unguiculata	Boo	Cowpea
Solanum nigrum	Osuga	Black nightshade
Cleome gynandra	Akeyo	Cat's whisker/ Spider plant

Leafy vegetables are high in water and cellulose and low in calories and proteins. They add valuable amounts of minerals and vitamins to the diet, although they do not contain large

amounts of most of these nutrients. They are usually rich in iron and provitamin A, and often in the B complex vitamins (Meyer, 1960). Indigenous vegetables grow wild and are readily available in the field as they do not need any formal cultivation. They have been used in Africa for a long period to supplement the diets (Odhav *et al*., 2007).

Alongside other food alternatives, vegetables are considered cheap sources of energy and are rich sources of essential biochemicals and nutrients such as carbohydrates, carotene, protein, vitamins, calcium, iron, and palpable concentration of trace minerals (Hussain *et al.*, 2011; Lintas, 1992). Nutritional quality is determined by analyzing minerals, vitamins, fibres and anticarcinogens. In addition to meeting nutrient intake levels, better consumption of fruits and vegetables is associated with reduced risk of cardiovascular disease and stroke (Watada, 1995; Sushanta *et al*., 2008).

In July 2008, the strategy unit in the United Kingdom cabinet office released the report Food Matters: Towards a strategy for the 21st century, in the report the government acknowledges that a healthier, more sustainable diet would need to contain less animal products than those typically eaten today and also presents several strategies for increasing fruit and vegetable consumption (Morgan, 2007).

Despite their exceptional nutritional qualities and preferential environmental profiles compared with animal-based products, fruits and vegetables are consistently undervalued by government and consumers (Morgan, 2007). Plant foods can contribute significantly to human nutrition and health, as they consist of almost all essential nutrients required by the human body. Need for diet has been regarded as the major source of human exposure to microelements. Macro and microelements influence biochemical processes in the human body and are responsible in combating a variety of human ailments and diseases (Subramanian *et al*., 2012).

Indigenous vegetables are undervalued in favour of the introduced exotic vegetables and their availability has reduced drastically due to excessive cultivation of field crops in which chemical elimination of wild vegetable is employed. There is lack of awareness among young people about the availability of the nutritionally rich indigenous vegetables. The World Health Organization (WHO) has reported that some 200 million people are affected by chronic under-nutrition, in sub-Saharan Africa (Odhav *et al*., 2007).

A report in Australia focused specifically on fruits and vegetables due to their role in promoting personal, population and planetary health- the goal of the New Nutrition Science project. Most recent findings from 2007 showed that it is probable that: Green leafy vegetables, fruits and carotenoid containing foods reduce the risk of mouth, pharynx, larynx, lung, oesophageal cancers. The Mediterrenean diet, an eating pattern high in fruit and vegetables, has been found to have protective benefits against cancer (Morgan, 2007).

A study carried out in Nigeria on *Clerodendrum volubile,* an indigenous vegetable, showed high contents of sulphur, chlorine, manganese, and zinc. The results also showed high contents of ascorbic acid (Erukainure *et al*., 2011). It is important to rotate consumption of vegetables as each contains varying level of each mineral. Despite the nutritional contribution of indigenous vegetables to local diets, and their health maintenance and protective properties, little effort has been put to their exploitation. High moisture content in vegetables makes them easy to digest as the body does not need some of its own water to digest them. This implies that the body consumes less energy in digesting these vegetables. According to a study in Ghana, moisture contents of indigenous vegetables ranged from 72.93 to 91.83%; Iron 1.00 mg / 100 g to 40.50 mg / 100 g (Kwenin *et al*., 2011).

Cleome gynandra has many medicinal applications such as antioxidant and anti-carcinogenic. It has important mineral content and free radical scavenging properties. It has moisture content between 81.8 to 89.6% (Mishra *et al*., 2011). Its nutritional value depends on soil fertility, age, among other factors. The vegetable is rich in vitamins (A and C) and minerals (calcium and iron). Increased soil fertility increases the crude protein, but reduces β- carotene, ascorbic acid and iron contents of the leaves. Eating *Cleome gynandra* leaves is said to reduce dizzy spells in pregnant women (Chweya and Mnzava, 1997).

A study by Chweya and Mnzava (1997) on the nutritional and chemical composition of *Cleome gynandra* found moisture to be 81.8-89.6 %, total ash (dry weight) 2.1-3.0 %, potassium 410 mg, calcium 213- 434 mg, magnesium 86 mg, iron 1-11 mg, β- carotene 6.7- 18.9 mg and ascorbic acid 127- 484 mg. Nutritional analysis of the leaves of *Cleome gynandra*, *Fleurya aestuans* and *Solanum nigrum*, from Kumasi area in Ghana, showed that the leaves of the three indigenous leafy vegetables contained nutritionally significant

amounts of calcium, copper, iron, magnesium, manganese, molybdenum and zinc, but were devoid of detectable selenium (Glew *et al*., 2009).

Research work on some vegetables from Delta state, Nigeria, showed that these vegetables contained an appreciable amount of nutrients, mineral elements and low levels of anti-nutrients and could be included in diets to supplement our daily allowance needed by the body (Agbaire and Emoyan, 2012). According to Olaiya and Adebisi (2010) biochemical analysis of ten green leafy vegetables from south–western Nigeria showed moisture contents ranging from 75% to 91.5%. The leafy vegetables were rich in fibre with values between 0.8 g / 100 g in *Cucurbita pepo* and 9.5 g / 100 g in *Amaranthus coudatus*. The ash content of the vegetables ranged from 0.20 g/100 g in *Basella alba* to 3.9 g / 100 g in *Amaranthus spinosus*.

A study on mineral composition of non- conventional leafy vegetables showed that mineral contents depended on the type of vegetables. The mean daily intake of Mg, Ca, and Fe were lower than their recommended daily allowances (RDA). However, the manganese daily intake was found not to differ significantly from the RDA value (Barminas *et al*., 1998). Production and consumption of African leafy vegetables (ALVs) is generally low in central Kenya, yet micronutrients and vitamin malnutrition in some parts are high particularly among young children and women in childbearing age. They are worst hit by proteins, calcium, iron, vitamins A and C malnutrition yet these nutrients are richly found in ALVs (Muthoni *et al*., 2010).

Serious malnutrition is associated with less use of micronutrients which leads to poor intellectual development, anaemia, poor vision and death among children. Indigenous vegetables have ensured food security and nutrition in many African countries. The vegetables have many advantages and potentials that are yet to be exploited (Abukutsa, 2010).

A study carried out in Tarime, Tanzania, showed that *Crotalaria brevidens* contained high amounts of beta-carotene. The importance of green leafy vegetables as sources of nutrients in societies where consumption of animal based food products is low is well recognized (Christian, 1991). Although plant sources of pro-vitamin A are abundant worldwide, vitamin A deficiency is still a major nutritional and public health concern in a number of

developing countries. Cooking methods are aimed at removing the unacceptable bitter taste contained in the vegetables. Studies have documented high losses of the water soluble vitamins, minerals, thermal decomposition and isomerisation of the naturally occurring trans-carotenoids to cis-isomers which have lower vitamin A activity. There is a growing concern with regard to nutrient losses as a result of these cooking methods. Increased consumption of green leafy vegetables and their cultivation is one of the strategies promoted for the control of vitamin A deficiency in developing countries (Fikselova *et al*., 2008; Christian, 1991).

African leafy vegetables such as amaranthus, black night shades, spider plants, pumpkin leaves and cassava leaves are easy to grow and can do well with minimal inputs in the marginal areas with low rainfall and poor soils. They are more resilient to pests and diseases than exotic vegetables (Muthoni *et al*., 2010). The World Health Organization's (WHO) global initiative on increased consumption of fruits and vegetables can only be realized through African indigenous and traditional leafy vegetables (Smith and Eyzaguire, 2007).

In a study carried out in Nyang'oma area, Bondo district, it was found that most traditional leafy vegetables, domesticated and wild, generally contained higher levels of calcium, iron and zinc compared with the exotic varieties (Orech *et al*., 2007). A study carried out at the University of Lagos to determine vitamin C content of tropical vegetables by titrimetric method found vitamin C content of *Amaranthus hybridus* as 60.12 mg / 100 g; *Amaranthus globe* 97.49 mg / 100 g (Ogunlesi *et al*., 2010). A research work by Adeyeye and Omolayo (2011) on *Amaranthus hybridus* found nutrient contents were; potassium 11.6 mg / 100 g, calcium 50.2 mg / 100 g, magnesium 11.6 mg / 100 g, iron 5.2 mg / 100 g, manganese 0.08 mg / 100 g and total ash 17. 2 g / 100 g based on dry matter.

Solanum nigrum (African black nightshade) is generally consumed during times when other foods are scarce or as a medicinal treatment in tropical Africa. The plant is a weed that grows wild in the fields as well as other locations, although it is occasionally cultivated. Its uses in Africa and Asia include treatment for the following conditions: itching, burns, cuts, skin diseases, heart disease, fever and eye disease (Glew *et al*., 2009).The raw fruit is chewed and swallowed for treatment of stomach ulcers or for

general abdominal upsets leading to continued stomach ache. Unripe fruits are also squeezed onto aching teeth or on babies' gums to ease pain during teething. The infusion of the leaves and seeds is rubbed onto the gums of children in cases of crooked teeth (Kokwaro, 2009; Kokwaro and Johns, 1998). The leaves and seeds have significant levels of protein, fibre and carbohydrate. It is a good source of magnesium, phosphorus and the water soluble vitamins. *Solanum nigrum* is rich in fibre due to its seedy nature. High fibre foods usually provide loose stools; reduce stool transit time in the intestines and lower chances of colon cancer (Asibey-Berko and Tayie, 1999).

A study by Maina and Mwangi (2008) on nutritional composition of *Amaranthus hybridus* and *Solanum nigrum* found: moisture 88.9% and 87.8%, calcium 270 mg / 100 g and 200 mg / 100 g, magnesium 130 mg / 100 g and 0 mg / 100g, iron 3 mg / 100 g and 0.3 mg / 100 g, respectively. Ascorbic acid was 42 mg / 100 g for amaranth and *solanum nigrum* had no quantity recorded. Carotene was 1725 µg / 100 g and 3700 µg / 100 g for *Amaranthus hybridus* and *Solanum nigrum*, respectively. A research study by Subramanian *et al*. (2012) on mineral composition of *Solanum nigrum* found that: Fe 31.19 mg / 100 g, Mg 18.59 mg / 100 g, Mn 9.84 mg / 100 g. A study in Ghana on proximate analysis of *Solanum nigrum* showed moisture 80.4 ±1.3%, ash 4.1±0.5 mg / 100g (Asibey-Berko and Tayie, 1999). Another study in Ghana on mineral content of *Solanum nigrum* showed the following results: Fe 14.59 mg / 100 g, Ca 42.92 mg / 100 g and K 528.72 mg / 100 g (Tayie and Asibey-Berko, 2001). According to a study by Oduse *et al.* (2012) *Solanum nigrum* had ash 4.47 g/ 100 g, moisture 88.47%, Ca 12.93 mg / 100 g, Fe 0.75 mg / 100 g, Mg 0.323 mg/ 100 g.

A study in Nigeria on the nutritional and chemical value of *Amaranthus hybridus* found that moisture was 83.48% on wet weight basis, ash 13.80 mg / 100 g, potassium 54.20 mg / 100 g, calcium 44.15 mg / 100 g, magnesium 231.22 mg / 100 g and iron 13.58 mg / 100 g on dry weight basis. Beta-carotene was 3.29 mg / 100 g and ascorbic acid 25.40 mg / 100 g on wet weight basis (Akubugwo *et al*., 2007). *Amaranthus hybridus* (pig weed) is the most common of several edible species of Amaranthus. Amaranth is a Greek word meaning immortal or not withering. The Luo call it Ododo, Kikuyu Terere, Luhya Omboga. It is rich in essential micronutrients like carotene, vitamin C, iron and calcium. It has high levels of vitamins A, B, and E and contains more proteins than maize or sorghum. Its seed

has higher fat content (7-8%). It grows wildly and does not require any organized planting (Maina and Mwangi, 2008). *Amaranthus hybridus* is reported to be grown for its leaves which are rich in beta-carotene, calcium, iron and vitamin C. It is also used in the treatment of eye diseases (Kwenin *et al*., 2011; Mishra *et al*., 2011).

Cleome gynandra is found throughout tropical Africa. In Africa and Asia, the leaves, young shoots and sometimes the flowers of the plants are consumed as a side dish or in soup. In addition to its use as a food, it has medicinal properties. Examples of its medicinal use include the consumption of the leaves by mothers before and after birth, for treatment of blood loss and for treatment of anaemia. In Ghana it is used in treatment for constipation and ear infections (Glew *et al*., 2009). In Taiwan *Cleome gynandra* is used to treat dysentery, gonorrhoea, malaria and rheumatoid arthritis. It is also used to treat severe thread-worm infection and to relieve chest pains. Moisture content in *Cleome gynandra* was found to range between 81.8 to 89.6 percent, total ash 2.1-3.0 mg / 100 g and potassium 410 mg / 100 g, calcium 213-434 mg, magnesium 86 mg / 100 g, iron 1-11 mg / 100 g, β- carotene 6.7- 18.9 mg / 100 g and ascorbic acid 127- 484 mg /100 g (Mishra *et al*., 2011). The young softer leaves are pounded and the resulting liquid squeezed into aching ears, nostrils and eyes in cases of epileptic fits. Roots are boiled and the decoction drunk to facilitate birth (Kokwaro and Johns, 1998; Mishra *et al*., 2011).

*Crotalaria brevidens l*eaves have a bitter taste and are used as vegetable. In Siaya, leaves are said to be good in treating gastrointestinal problems (Kakwaro and Johns, 1998).

A study in Tarime, Tanzania showed that *Crotalaria brevidens* contained high amounts of β-carotene (Christian, 1991). *Justicia flava l*eaves are used as vegetable, roots are cooked in water and the extract used as a medicine for stomachache and diarrhoea and chewed for coughs (Kokwaro, 2009).

Vigna unguiculata (Cowpea) evolved certainly in Africa as wild cowpeas only exist in Africa and Madagascar and its *l*eaves are used as vegetable. The critical role played by cowpea in the lives of people in Africa and other developing countries cannot be gainsaid. It adapts to high temperatures and drought than other crop species and is more tolerant to soil of low fertility due to its high rate of nitrogen fixation. The plant can be consumed at all stages of growth as vegetable crop and the leaves have significant nutritional value.

Since grain legume starch is digested rather slowly than starch from cereals and tubers, the cowpea consumption produces less abrupt changes in blood glucose levels (Timko *et al*., 2007). It is also used if the afterbirth is retained. The patient may chew the root without any preparation; or may be pounded, mixed with cold water and the concoction drunk (Kokwaro, 2009). A research work by Owolabi *et al*. (2012) on mineral contents of *Vigna unguiculata* showed Fe 0.48 mg / 100 g, Ca 17.15 mg / 100 g, Mg 9.00 mg / 100 g and K 18.69 mg / 100 g.

A study carried out by Odhav *et al.* (2007) in Kwazulu- Natal, South Africa, to determine the nutritional value of traditional leafy vegetables found moisture content(wet weight) as: *Amaranthus hybridus* 83%, Justicia flava 84%, *Solanum nigrum* 85%; Ash content (g / 100 g) as 4.91, 3.32 and 2.24 for *Amaranthus hybridus*, *Justicia flava* and *Solanum nigrum*, respectively. Mineral levels were (mg / 100 g dry weight): *Amaranthus hybridus*; Ca -2363 mg, Mn - 24 mg, Mg -1317 mg, Fe – 21 mg. *Justicia flava*; Ca -2073 mg, Mn - 8.4 mg, Mg – 1409 mg, Fe – 16 mg. *Solanum nigrum*; Ca – 2067 mg, Mn – 3 mg, Mg – 277 mg, Fe – 85 mg.

According to results of a study by Pretorius and Schonfeldt (2011) on nutrient contents of *Vigna unguiculata* and *Cleome gynandra;* moisture contents for *Vigna unguiculata* and *Cleome gynandra* were 87.6 and 84.2 percent, total ash 1.42 mg / 100 g and 2.77 mg / 100 g, Iron 3.9 mg / 100 g and 14.3 mg / 100 g, magnesium 54.7 mg / 100 g and 146 mg / 100 g, calcium 221 mg / 100 g and 393 mg / 100 g , β- carotene 2249 µg / 100 g and 4117 µg / 100 g, respectively.

The findings of a study by Acipa et al. (2013) on nutritional profile of some food plant showed *Crotalaria brevidens* had vitamin C 530 mg /100 g, β- carotene 60.07 mg / 100 g and calcium 437.99 mg / 100 g; *Cleome gynandra* had vitamin C 430 mg / 100 g, β- carotene 28.54 mg / 100 g and calcium 294.18 mg / 100 g; *Solanum nigrum* had vitamin C 1050 mg / 100 g, β- carotene 69.58 mg / 100 g and calcium 447.16 mg / 100 g.

In phytoevaluation of the nutritional values of green vegetables, the dried samples were pulverized and calcium, iron, magnesium, phosphorus and vitamin contents determined by atomic absorption spectrometry. Potassium and sodium were determined by flame photometry (Olaiya and Adebisi, 2010).

According to a study by Hussain *et al.* (2011) vegetable species sampled were manually washed with distilled water and accurately weighed in a crucible and subjected to ashing in a furnace for 4 hours at 550 °C. Work by Sushanta *et al.* (2008) on elemental analysis of vegetables, ash content was determined by combusting the plant materials and the ash were used for estimation of calcium by flame photometry method. The standard colorimetric methods were employed for magnesium, phosphorus and iron. Pigment extraction for β-carotene analysis was carried out according to the method of the Association Official of Analytical Chemists (AOAC) (Mustapha and Babura, 2009).

A study in Bangladesh used UV-spectrophotometric method for the determination of vitamin C contents in various fruits and vegetables. To determine contents of total vitamin C in food samples, a well established method is the 2, 4-dinitrophenylhydrazine method (DNPH). This is a simplified method for the simultaneous determination of the total vitamin C; employed coupling reaction of 2, 4-dinitrophenylhydrazine dye with vitamin C and followed by spectrophotometric determination (Rahman *et al*., 2006). Olaiya and Adebisi (2010) used the Association Official of Analytical Chemists method in the determination of moisture, ash, fibre, fat, proteins and carbohydrates from green leafy vegetables.

A study carried out in Kwazulu- Natal, South Africa, to determine the nutritional value of traditional leafy vegetables used oven drying method to determine moisture content. The sample was placed in an oven at 105 ^{o}C and dried for 3 hours. Ash content was determined by the incineration of a 4 g sample in a muffle furnace at 600 ^{o} C for 6 hours (Odhav *et al*., 2007).

2.4 Ashing of vegetables

The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific organic components present within a food, such as Ca, Na, K and Cl (Yang *et al*., 2013).

Determination of ash and mineral content of foods is important for a number of reasons and these are:

Nutritional labelling in which the concentration and type of minerals present must often be stipulated on the label of a food. Quality of many foods depends on the concentration and

type of minerals they contain, including their taste, appearance, texture and microbiological stability, in which, high mineral contents are used to retard the growth of certain microorganisms. Nutrition of some minerals is essential to a healthy diet (for example, calcium, phosphorus, potassium and sodium) whereas others can be toxic (for instance, lead, mercury, cadmium and aluminium). It is often important to know the mineral content of foods during processing because this affects the physicochemical properties of foods (Prapasri *et al*., 2011; Jacobs, 1999). There are three main types of analytical procedure used to determine ash contents of foods. These are dry ashing, wet ashing and low temperature plasma dry ashing. The method chosen for a particular analysis depends on the reason for carrying out the analysis, the type of food analyzed and the equipment available.

2.4.1 Dry ashing of vegetables

Dry ashing procedures use a high temperature muffle furnace capable of maintaining temperatures of between 500 and 600 ^{o}C. Water and other volatile materials are vaporized and organic substances are burned in the presence of the oxygen in air to CO_2, H_2O and N_2. Most minerals are converted to oxides, sulphates, phosphates, chlorides or silicates (Enders and Lehmann, 2012).

Although most minerals have fairly low volatility at these high temperatures, some are volatile and may be partially lost, for example, iron, lead and mercury. If an analysis is being carried out to determine the concentration of one of these substances then it is advisable to use an alternative ashing method that uses lower temperatures (Enders and Lehmann, 2012; Jacobs, 1999).

The food sample is weighed before and after ashing to determine the concentration of ash present. The ash content can be expressed on either dry or wet weight basis as illustrated below (Enders and Lehmann, 2012).

% ash (dry) = M_{ash} /M_{dry} x 100

% ash (wet) = M_{ash} /M_{wet} x 100

Where

M_{ash} = mass of ashed sample

M_{dry} = mass of original dry sample

M_{wet} = mass of original wet sample

Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried, ground food sample is usually weighed into a flask containing acids and oxidizing agents (for instance. nitric, perchloric and / or sulphuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidising agents used. Typically a digestion takes from 10 minutes to a few hours at temperatures of about 350 ^{o}C. The resulting solution can then be analyzed for specific minerals (Enders and Lehmann, 2012).

2.4.2 Low temperature plasma ashing

A sample is placed into a glass chamber which is evacuated using a vacuum pump. A small amount of oxygen is pumped into the chamber and broken down to nascent oxygen, [O] by application of an electromagnetic radio frequency field. The organic matter in the sample is rapidly oxidized by nascent oxygen and the moisture is evaporated because of the elevated temperatures. The relatively cool temperatures (< 150 ^{o} C) used in temperature plasma cause less loss of volatile minerals than other methods (Bond and Giroux, 2013).

2.5 Moisture contents of vegetables

The moisture content of the food material is important because it affects the physical, chemical aspects of the food which relates with freshness and stability for the storage of the food for a long period of time and the moisture content determines actual quality of the food before consumption and to the subsequent processing in the food sector by the food producers. Importance of moisture analysis in food includes:

Legal and labelling requirements whereby legal limitations regarding the amount of water present in the food is necessary for producing some of the specific products.

Economically important requirements in which moisture present in the food material is related with some type of food in dealing with economical values and the big business

among food industries, therefore, food moisture analysis plays significant role in the modern world. The shelf life of the food and food products is dependent on the moisture content of the food as microbial activity of the food materials favour with the moisture availability in the food. Moisture rich foods are easily susceptible to the microbial attack (Jacobs, 1999).

The quality of the food is determined in terms of the food texture, taste, and appearance, but the moisture content of the food is a determining factor of the quality and the stability of the processed food products. Food processing operations are involved with the amount of moisture content present in the food item which is going to be processed for a specific purpose (Yeshajahu and Clifton, 1994).

The moisture determination methods include: dry methods, direct distillation methods, electrical methods and chemical methods.The method chosen is influenced by the following: amount of water in food, kind of apparatus used, type of food handled, how quick results are needed, reason for moisture content determination and accuracy of the results needed (Rodriguez-Amaya, 2001).

2.5.1 Dry technique for moisture determination

The percentage weight loss of water is calculated after removal of water by heating and after standardization. This technique is known as loss on drying (LOD). In this technique a sample of material is weighed, heated in an oven for an appropriate period, cooled in the dry atmosphere of desiccators and then reweighed. If the volatile content of the solid is primarily water, this technique gives a good measure of moisture content. The advantages of this method are that: it is an easy procedure, no skill, no complex apparatus and no risk due to chemicals (Reeb and Milota, 1999).

Moisture and total solids contents of foods can be calculated as follows using oven drying procedures:

Moisture content on wet weight basis (wwb) is given as weight of wet sample less weight of dry sample. Percent moisture content is obtained by dividing the result obtained by weight of wet sample then multiplied by 100.

Percent moisture content on dry matter basis (dmb) is obtained by multiplying the difference in weight between wet sample and dry sample by 100, then dividing by weight of the dry sample. Percent total solid (weight/weight) is attained by dividing weight of dry sample by that of wet sample and then multiplying by 100 (Reeb and Milota, 1999).

2.5.2 Electrical technique for moisture determination

Because the manual laboratory method is relatively slow, automatic moisture analyzers have been developed that can reduce time necessary for a test from a couple of hours to just a few minutes. These analyzers incorporate an electric balance with a sample tray and surrounding heating element. Under microprocessor control, the sample can be heated rapidly and a result computed prior to the completion of the process, based on the moisture loss rate, known as a drying curve. Water content of certain foods can be determined by measuring the change in capacitance or resistance to an electric current passed through a sample (Fredriksson, 2010).

2.5.3 Direct distillation technique for moisture determination

In direct distillation with immiscible solvents of higher boiling point than water, the sample is heated in mineral oil or liquid with a flash point well above the boiling point for water. Other immiscible liquids with boiling point only slightly above water can be used for example, toluene, xylene and benzene. Example here is the Brown-Duvel method developed in 1907 and was used with certain modifications for moisture determination in cereals (Yeshajahu and Clifton, 1994).

In this method a 100 g sample of whole grain is heated in the flask with 150 ml of nonvolatile oil to a specified cutoff temperature (180 ^{o}C) for wheat. The amount of water that is distilled into a graduated cylinder is read in millilitres and reported as percent moisture content (Yeshajahu and Clifton, 1994).

2.5.4 Chemical technique for moisture determination

An accurate method for determining the amount of water is the Karl Fischer titration method, developed in 1935 by the German Chemist, Karl Fischer. (Yeshajahu and Clifton, 1994).

The procedure involves the reduction of iodine by sulphur dioxide in the presence of water:

$$2H_2O + SO_2 + I_2 \longrightarrow H_2SO_4 + 2HI$$

In a modified procedure, methanol and pyridine are used in a four-component system to dissolve iodine and sulphur dioxide.

The basic reaction takes place in two steps:

$$C_5H_5NI_2 + C_5H_5N.SO_2 + C_5H_5N + H_2O \longrightarrow 2C_5H_5N.HI + C_5H_5N.SO_3$$

and

$$C_5H_5N.SO_3 + CH_3OH \longrightarrow C_5H_5(H)SO_4CH_3$$

These reactions show that for each mole of water, 1 mol iodine, 1 mol SO_2, 3 mol of pyridine, and 1 mol of methanol are used.

The Karl Fischer Reagent (KFR) is added directly as the titrant if the water in the sample is accessible. However, if water in a solid sample is inaccessible to the reagent, the moisture is extracted from the food with an appropriate solvent (for example. methanol).Then the methanol extract is titrated with KFR.

Before the amount of water found in a food sample can be determined, a KFR water equivalence (KFR_{eq}) must be determined. The KFR_{eq} value represents the equivalent amount of water that reacts with 1ml of KFR. Standardisation must be checked before each use because the KFR_{eq} will change with time (Jacobs, 1999).

The KFR_{eq} can be established with pure water, a water-in-methanol standard, or sodium tartrate dihydrate. Pure water is a difficult standard to use because of inaccuracy in measuring the small amounts required. The water-in-methanol standard is premixed by the manufacturer and generally contains 1mg H_2O per ml of solution. This standard can change over prolonged storage periods by absorbing atmospheric moisture (Bruttel and Schlink, 2003; Yeshajahu and Clifton, 1994).

Sodium tartrate dihydrate, is a primary standard for determining KFR_{eq} ($Na_2C_4H_4O_6.2H_2O$)

The KFR_{eq} is calculated as follows using sodium tartrate dihydrate:

KFR_{eq} (mg H_2O) = (36 g / mol $Na_2C_4H_4O_6.2H_2O$ x Sx1000) / 230.08 $gmol^{-1}$ x A

Where:

KFR_{eq} = Karl Fischer Reagent water equivalence

S = weight of sodium tartrate dihydrate (g)

A = ml of KFR required for titration of sodium tartrate dihydrate

According to Yeshajahu and Clifton (1994), once the KFReq is known, the moisture content of the sample is determined as follows;

% H_2O = KFR_{eq} x Ks x 100 / S

Where:

KFR_{eq} = Karl Fischer Reagent water equivalence

Ks = ml of KFR used to titrate sample

S = weight of sample (mg)

2.5.5 Calcium carbide technique for moisture determination

It is a simple but rapid method that requires no elaborate apparatus. It is a gas production procedure:

$CaC_2 + 2H_2O \longrightarrow Ca(OH)_2 + C_2H_2$

In this carbide and water reaction, one needs to accurately collect the generated gas, acetylene. The amount of gas collected is proportional to the amount of water present (Yeshajahu and Clifton, 1994).

2.5.6 Conductivity technique for moisture determination

The conductivity method functions because the conductivity of an electric current increases with the percentage of water in the sample (Fredriksson, 2010).

2.6 Effect of soil minerals on vegetable minerals

Soil is a complex mixture of mineral particles, organic matter, water and air. The mineral particles come from the breakdown of rocks and as a result potassium and other minerals are released and may become available to plants. Minerals in the soil have a profound influence on the chemical characteristics and dynamic behaviour of soils and the environment (April and Richard, 2008).

2.7 Determination of nutrient contents in the soil and vegetables

Mineral analysis is performed using various methods such as complexometric titration, colorimetric method, gravimetric method, atomic absorption spectrometry, and flame photometry among others. Vitamin contents are analyzed by spectrophotometry, high performance liquid chromatography, titrimetry, bioassay, just to name a few (Prapasri *et al*., 2011).

2.7.1 Summary of nutrient analysis of soils and vegetables

The methods for determining nutritional contents of foods differ according to the type of reaction being investigated. Instrumental methods of analysis are mostly based on measurement of an intensive chemical property which is proportional to the concentration of the substance. It is important to have a selective analytical method by which the concentration of single species can be determined in the presence of others, independent of the solution (Lusweti, 2000).

2.7.2 Flame photometry

The underlying principle of flame photometry may be explained when a liquid sample containing a metallic salt solution under investigation is introduced into a flame, the following steps normally take place in quick succession;

The solvent gets evaporated leaving back the corresponding solid salt which undergoes vaporization and gets converted into its respective gaseous state. This is followed by the progressive dissociation of either a portion or all of the gaseous molecules to give rise to free neutral atoms. The resulting neutral atoms are excited by the thermal energy of the flame in which they are fairly unstable, and hence instantly emit photons and eventually return to the ground state. The resulting emission spectrum caused by the emitted photons

and its subsequent measurement forms the fundamental basis of flame emission spectroscopy (Yeshajahu and Clifton, 1994).

Upon their return to a lower or ground electronic state, the excited atoms and molecules emit radiations that are characteristic for each. The emitted radiation passes through a monochromator that isolates the desired spectral feature which is then registered by a photodetector whose output is amplified and read on a meter or recorder (Willard *et al*., 1986).

2.7.3 UV Spectrophotometry

The UV-Vis spectra are obtained by measuring the intensity of the absorption of monochromatic radiation across a range of wavelengths passing a solution in a cuvette. The practical wavelength region for UV range extends from 190-400 nm and Vis range is 400-780 nm. In a typical experiment, a light beam of intensity $I_\circ$ strikes a sample consisting of a quartz or glass cell containing a solution. After passing through the cell, the light beam has a reduced intensity, I, due to reflection losses at the cell windows, absorption in the sample and, eventually, by scattering at dispersed particles. Only absorption losses are caused by the dissolved sample. The run is repeated using an identical cell containing only solvent to compensate for reflection and scattering losses, and the transmittance, T, is calculated by using the following equation (Willard *et al*., 1986):

$$T = I / I_\circ = I_{solution} / I_{sovent}$$

The presentation of transmittance T, as a function of wavelength λ, is the required spectrum of the sample. Absorbance is proportional to the number of absorbing species in the illuminated part of the cell. Absorbance, A, is defined by the equation,

$$A = -\log T = \log I_\circ / I$$

and is proportional to the cell thickness, d (cm), the concentration of the solution c (mol/L); and a substance-specific proportionality constant ε called molar absorptivity, (L/mol.cm)

$$A = \varepsilon c d$$

For a given system, a linear relationship exists between A and the sample concentration, but usually only for dilute solution ($c \leq 0.1$mol/L).

Solvents used in spectrophotometry must meet certain requirements to assure successful and accurate results. The solvent chosen must dissolve the sample, yet be compatible with cuvette materials. The solvent must also be relatively transparent in the spectral region of interest (Willard *et al*., 1986).

To determine content of total vitamin C in food samples, a well established method is the 2,4- dinitrophenyl hydrazine dye method (DNPH). This is a simplified method for the simultaneous determination of total vitamin C employed in coupling reaction of 2,4-dinitrophenylhydrazine dye with vitamin C and followed by spectrophotometric determination (Rahman *et al*., 2006; Qasim *et al*., 2009).

2.7.4 Atomic Absorption Spectrophotometry

Atomic absorption spectroscopy (AAS) in terms of analytical method is a recent technique, having been described by Walsh in 1955. The first atomic absorption observations were of the Fraunhofer lines in the solar spectrum and were made in 1802 by Wollaston. The flame was first used as a source of atomization and was available in the early 1960s, whereas the graphite furnace was described in the 1960s by L'vov and Massman and made commercially available in 1970 (Kellner *et al.*, 1998).

Principle: Atomic absorption spectrophotometry is based upon the absorption of radiation by free atoms, usually in the ground state. By selecting a wavelength for a given element that corresponds to an optical transition between atoms in the ground state and atoms in an excited level, the absorption of the radiation leads to a depopulation of the ground state. The value of the absorption is related to the concentration of the atoms in the ground state, and therefore, to the concentration of the element (Kellner *et al*., 1998).

By measuring the amount of radiation absorbed, a quantitative determination of the amount of analyte can be made. An atomic absorption spectrometer will therefore consist of a primary radiation source which produces the radiation to be absorbed, a source of free atoms with an associated sample introduction system, an optical dispersive system, a detector, and electronics for data acquisition, processing, and editing. The presence of free

atoms must be obtained in the path between the primary radiation source and the detector (Willard *et al*., 1986).

The absorption obeys Beer's law. The most commonly-used primary radiation sources are the hollow-cathode lamp (HCL) and the electrodeless discharge lamp (EDL). They both belong to low pressure discharges. The hollow-cathode lamp consists of a hollow cathode made of a highly pure metal whose spectrum is to be produced with an inner diameter in the 2-5 mm range. A high voltage and a current of up to 30 mA are to produce a discharge which takes place entirely in the hollow cathode. The fill gas is either argon or neon. Neon is preferred for elements with high ionization potential. A transparent silica window is used for light transmission. The success of the AAS method is clearly related to the availability of HCLs (Kellner *et al*., 1998).

2.7.5 Titrimetry

Vitamin C can be determined in food by use of an oxidation-reduction reaction. The reduction-oxidation reaction is preferable to an acid-base titration because a number of other species in the juice can act as acids, but relatively few interfere with the oxidation of ascorbic acid by iodine.

Potassium iodate (KIO_3) is used as a titrant and when added to an ascorbic acid solution that contains strong acid and potassium iodide (KI), the KIO_3 reacts with KI, liberating molecular iodine (I_2) as below (indirect titration method):

$$KIO_3 + 5KI + 6H^+ \longrightarrow 3I_2 + 6K^+ + 3H_2O \qquad (2.1)$$

During the titration, as long as the solution contains ascorbic acid, the iodine (I_2) produced in equation (2.1) is used up in a rapid reaction with ascorbic acid, AA, (equation 2.2), during which dehydro ascorbic acid, DHA, ($C_6H_6O_6$) and iodide ion (I^-) are formed:

$$C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2I^- + 2H^+ \qquad (2.2)$$

AA DHA

Once all the ascorbic acid has been consumed, any excess iodine (I_2) will remain in solution. This excess iodine reacts with starch to form an intensely blue coloured complex indicating that the endpoint is reached (Spinola *et al*., 2012).

Alternatively, a known amount of iodine is generated by the reaction between iodate, iodide and sulphuric acid (back titration method):

$$IO_3^- + 5I^- + 6H^+ \longrightarrow 3I_2 + 3H_2O$$

A measured amount of sample solution is added. The ascorbic acid in the sample reacts quantitatively with some of the iodine as the iodine is in excess:

$$C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2I^- + 2H^+$$

AA DHA

The excess iodine is then titrated against standard sodium thiosulphate solution:

$$I_2 + 2S_2O_3^{2-} \longrightarrow S_4O_6^{2-} + 2I^-$$

From the titration results the amount of iodine that reacts with the sodium thiosulphate solution can be found. Since the total amount of iodine originally formed is known, the amount that reacts with ascorbic acid is found by difference. Therefore the amount of ascorbic acid that reacts with this amount of iodine can be found (Sigmann and Wheeler, 2004).

2.8 Validation of nutrients determination techniques

Method validation is the process by which the capabilities of a method are assessed. It involves confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled. Analytical measurements require methods and equipments which have been tested to ensure that they are fit for purpose. Suitable, well maintained and calibrated equipment is important for the success of any analytical measurement (Eurachem / Citac, 1998; Njapau *et al*., 2008; Wong, 2009). Instrument response can be assessed using quality control samples and calibration standards and where there is doubt that interferences are present; results obtained from test-portions using external standard calibration can be checked by spiking test portions

with known amounts of the analyte of interest. In method validation, parameters such as: limit of detection (LOD), limit of quantification (LOQ), sensitivity, repeatability and reproducibility are assessed (INAB, 2012). Other validation methods include: taking part in inter-laboratory comparison tests and performing a limited number of control analyses at a different test laboratory (Eurachem / Citac, 1998; APVMA, 2004). According to Eurachem / Citac (2011) and UKAS (2007) method validation studies give data on the overall performance and on individual influence factors which can be used to estimate the uncertainty associated with the result of the method in normal use and emphasis is on identifying and removing (rather than correcting for) significant effects .

2.8.1 Limit of detection and quantification of analytical instruments

Limit of detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value while limit of quantification (LOQ) of an individual analytical procedure is the lowest amount of analyte in a sample that can be determined quantitatively with suitable precision and accuracy (Yuangsoi *et al*., 2008).

$LOD = 3S_o$

$LOQ = 10\ S_{o.}$

Where: $S_o = SD / S$

SD = the standard deviation of the response near LOD or LOQ and S is the slope of the linearity curve near LOD or LOQ.

2.8.2 Repeatability and reproducibility of analytical methods

Repeatability is the closeness of the agreement between the results of successive measurements of the same measurand carried out by the same procedure, same analyst, same instrument, same condition, and same laboratory within a short interval of time.

Reproducibility is the closeness of agreement between test results of measurements of the same measurand, where the measurements are performed under different conditions such as different laboratories, different analysts, different instrument, different time interval (AOAC, 2007; CIPAC, 2003).

2.8.3 Horwitz equation and Horwitz ratio for the reproducibility of results

Horwitz Equation is among the first empirical parameters used as a reference value for the laboratory. It is reported by Rivera and Rodriguez (2011) that in 1980, Horwitz, Kamps and Boyer examined the results of over 50 inter-laboratory collaborative studies conducted by AOAC on various commodities for numerous analytes and realized they showed a relationship between the mean coefficient of variation expressed as a power of 2, with the mean concentration, measured, expressed as powers of 10, independent of determinative methods. $RSD_R\ \% = 2^{(1-\ 0.5 \log c)}$

Where c is the concentration of analyte expressed as a dimensionless mass fraction (numerator and denominator have the same unit) and relative standard deviation, RSD_R, is the coefficient of variation, CV, under reproducibility conditions. Horwitz Ratio (HorRat) is the ratio of the RSD_r, in percent, calculated from the data under repeatability conditions, to the relative standard deviation (RSD) predicted from the Horwitz Equation, RSD_R.

$HorRat\ (r) = RSD_r / RSD_R$

Standard deviation, $SD = (\Sigma d^2 / n)^{0.5}$

$RSD = SD / \overline{x}$

$\%\ RSD_r = RSD \times 100$

Where: n = number of replicate measurements

$\overline{x}$ = mean of measurements

Australian accreditation body, National Association of Testing Authorities (NATA) and Codex Alimentarius Commission recognize the Horwitz equation as a source of variance on analytical methods and they accept the expression of uncertainty by its values (Rivera and Rodriguez, 2011). Horwitz Equation is limited to analytical methods that express measurands as concentration of mass and therefore excludes moisture, ash and fibre determination. It is now widely used as the bench mark for the performance of analytical methods through HorRat value (CIPAC, 2003; McClure and Lee, 2003).

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

The experimental research design was employed in this study and involved determination of nutritional composition of green leafy vegetables using atomic absorption spectrophotometry, flame photometry, UV-spectrophotometry, titrimetry, ashing and moisture methods. Nutritional analysis involved quantitative determination of specific minerals and vitamins; ash and moisture contents in selected vegetables, whereas soil analysis involved quantitative determination of specific minerals.

3.2 Area of the study

This study was carried out in Kendu Bay area, Rachuonyo North District, Homa Bay County in Western Kenya (figure 3.1). Rachuonyo North District in Nyanza province lies between 34˚25'E, and 35˚0'E longitudinally and between 0˚5'S and 0˚45'S latitudinally. It covers an area of 428 km^2 and borders the following districts; Nyakach to the north, Rachuonyo South to the east and Homa Bay to the south and Lake Victoria to the west. The region was divided into two zones consisting; Kendu Bay and Homa Bay zones respectively. Kendu Bay zone included East Karachuonyo and the neighbouring Oyugis town while Homa Bay zone included west Karachuonyo and the neighbouring Homa Bay town.

In Kendu Bay region, rainfall is highly variable. A major part falls within the low rainfall range. The soil is a mixed loam and sand and a small portion has clay soil. Vegetation is basically savannah grassland with scattered short trees and thickets. The main economic activities in the region include cattle rearing, small scale farming of drought resistant crops such as cassava, sorghum and to a small extent maize and beans; fishing and sand harvesting. The region is predominantly inhabited by the Luo community. Figure 3.1 shows the map of the study area.

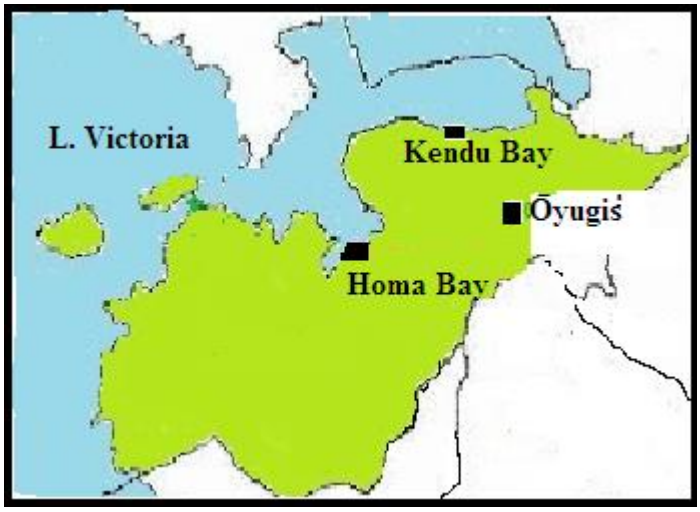

Figure 3. 1: Homa Bay county map

Key:

- Targeted study areas

- Homa Bay County

Source: Nyanza counties wikimedia

3.3 Sampling and pretreatment

3.3.1 Vegetable sample

Vegetable samples were collected from fields and gardens in various parts of Kendu Bay. The area was divided into two zones; Kendu Bay and Homa Bay. Kendu Bay consisted of East Karachounyo and the neighbouring Oyugis town while Homa Bay consisted of West Karachuonyo and the neighbouring Homa bay town. From each zone was obtained 24 samples for each of the vegetable varieties. Samples were collected from selected plants in flowering and fruiting stage for correct botanical identification. Healthy and disease-free edible parts of vegetables were selected to assess nutritional composition.

For β-carotene analysis, samples from the two zones were mixed together and thoroughly homogenized to give a composite laboratory sample, which were quartered to get a representative test sample for each vegetable. The samples for mineral determination were manually washed with distilled water and residual moisture evaporated at room temperature. These were further dried in an oven until a constant weight was obtained and

were stored in plastic bags free from moisture, ready for mineral analysis. Samples for vitamin and moisture analysis were not washed with water but were wiped with dry tissue paper and weighed for immediate analysis.

3.3.2 Soil sample

Soil samples were collected from the same fields as the vegetables and a total of 24 soil samples were collected from at a depth of 0 to 15 cm from farms in each of the two regions of the study area. The samples were stored in plastic bags and transported to the laboratory for mineral extraction and analysis. Minerals in the soil were determined according to the method described by McCarthy (1997). Soil samples were dried in the oven at 100 ^{o}C for 24 hours, stored in desiccators until cooled. The samples were mixed and homogenized by spreading it on a cloth and pulling each corner in succession over its diagonal partner. The spreading and pulling was repeated three times. The sample was then spread and quartered until a sample of 100.000 g was obtained. The sample was weighed in a sample bottle and stored for later analysis.

3.3.3 Extraction of minerals from the soil sample

Minerals were extracted from the soil by placing a 10.000 g of the sample in an Erlenmeyer flask and 20 ml of an extracting solution (1 M HCl plus 1 M nitric acid-analar grades) added. The sample was then placed in a mechanical shaker for 15 minutes and then filtered through Whatman No. 2 filter paper into a 100 ml volumetric flask. This was diluted to the mark with the extracting solution. Mineral contents of the sample were determined by AAS, although potassium used flame photometer as well.

3.4 Quantitative determination of nutrients

Minerals were determined by atomic absorption spectrophotometry (AAS) and flame photometry methods.

Atomic absorption spectrophotometric method was used to analyze potassium, calcium, magnesium, manganese and iron elements

Flame photometric method was used to analyze potassium element.

UV-spectrophotometry was used to analyze provitamin A whereas vitamin C was analyzed by titrimetric method.

Total ash content was determined by dry ashing method. Wet ashing was used for samples for specific elemental analysis while moisture content was determined by oven drying method.

3.4.1 Moisture determination by drying method

The moisture contents of the vegetables were determined according to the method described by Jacobs (1999): The percentage weight loss of water was calculated after removal of water by heating and after standardization. This technique is known as loss on drying (LOD). In this study a sample of material was placed in a clean, dry pre-weighed crucible and weighed using analytical balance (Mettler 160). The sample was heated in an oven (Universal Hot Air Oven, Vitco- India) until a constant weight was achieved, cooled in the dry atmosphere of a desiccator and then reweighed. Moisture and total solid contents of foods are calculated as follows using oven drying procedures:

Moisture content on a wet weight basis (wwb) was given as weight of wet sample less weight of dry sample. Percent moisture content was obtained by dividing the result obtained by weight of wet sample then multiplying by 100.

Percent moisture content on dry matter basis (dmb) was obtained by multiplying the difference in weight between wet sample and dry sample by 100, then dividing by the weight of the dry sample.

Percent total solid (weight/weight) was attained by dividing the weight of dry sample by that of wet sample and then multiplying by 100 (Jacobs, 1999).

3.4.2 Titrimetric method for vitamin C determination

Vitamin C contents in the vegetables were determined by titration with potassium iodate according to the method described by Spinola *et al*. (2012). Potassium iodate was used as a titrant and when added to an ascorbic acid solution that contained strong acid and potassium iodide (KI), the KIO_3 reacted with KI, liberating molecular iodine (I_2) as below:

$$KIO_3 + 5KI + 6H^+ \longrightarrow 3I_2 + 6K^+ + 3H_2O \qquad (3.1)$$

During the titration, as long as the solution contains ascorbic acid, the iodine (I_2) produced in equation 3.1 was used up in a rapid reaction with ascorbic acid, AA, (equation 2), during which dehydroascorbic acid, DHA, ($C_6H_6O_6$) and iodide ion (I^-) were formed:

$$C_6H_8O_6 + I_2 \longrightarrow C_6H_6O_6 + 2I^- + 2H^+ \quad (3.2)$$

AA DHA

Once all the ascorbic acid had been consumed, any excess iodine (I_2) remained in the solution. This excess iodine reacted with starch to form an intensely blue coloured complex indicating that the endpoint was reached.

A 25.000 g blended sample was homogenized with about 50 ml of 5% metaphosphoric acid - 10% acetic acid solution. This was quantitatively transferred into a 250 ml beaker and filtered using a funnel and glasswool into a 250 ml volumetric flask. The residue in the funnel was washed using the extracting solvent. The filtrate and the washing were shaken gently for a homogeneous solution. It was then diluted up to the mark by the 5% metaphosphoric acid - 10% acetic acid solution. The solution was used to determine the content of vitamin C in the sample.

A 5.000 g of potassium iodide (KI) was dissolved in a 500 ml volumetric flask and filled to the mark with distilled water to make 0.06 M potassium iodide solution.

A 0.268 g of potassium iodate (KIO_3) was dissolved in a 500 ml volumetric flask and filled to the mark with distilled water to make 0.0025 M potassium iodate solution.

A volume of 4 ml of 3 M sulphuric acid was used for each titration to provide acidic condition.

Vitamin C standard solution: 0.250 g of vitamin C was dissolved in 100 ml of distilled water and diluted to volume in a 250 ml volumetric flask with distilled water.

A 1% Starch solution: 1.000 g of soluble starch was accurately weighed using analytical balance and made with a little water to form a paste. The paste was then poured with constant stirring into a 100 ml beaker containing boiling water and boiled for one minute. The solution was then allowed to cool and followed by the addition of 2.000 g of potassium iodide. It was finally transferred into a 100 ml volumetric flask and kept stoppered.

Standardisations of potassium iodate solution with the vitamin C standard solution:

A 25.0 ml of vitamin C standard solution was pipetted into an Erlenmeyer flask. A 4 ml of 3 M sulphuric acid and 5 ml of 0.06 M potassium iodide solution was added followed by 10 drops of 1% starch solution. A burette was rinsed with 10 ml of potassium iodate solution and then filled with the potassium iodate solution. The solution in the Erlenmeyer flask was titrated against potassium iodate solution until the end point was reached (the first sign of blue colour that remains after at least 20 seconds of swirling). This titration was repeated two more times and final volume recorded.

Molarity of potassium iodate was calculated as follows:

Molarity of iodate = (Ma x Va) / 3Vt

Where:

Ma = molarity of ascorbic acid

Va = volume of ascorbic acid pipetted (aliquot)

Vt = volume of iodate titrated (titre)

The standardized iodate solution was used in titration of analytical sample.

Titration of juice samples: A 25.0 ml of vegetable sample was pipetted into a 125 ml Erlenmeyer flask and treated as vitamin C standard above and then titrated against potassium iodate. The titration was repeated two more times and results recorded.

Concentration of vitamin C was determined by the formula:

Where: V = volume of titrant (L)

M = molarity of titrant (mol L^{-1} = 0.0025)

Mw = molar mass for ascorbic acid (mg / mol = 176000 mg mol^{-1})

d = dilution factor = 10

S = sample weight = 25.000 g

3.4.3 UV-Spectrophotometric determination of provitamin A

Provitamin A contents in the indigenous vegetables were determined by the spectro-photometric method as described by Mustapha and Babura (2009) and Fikselova *et al*. (2008).

A sample of each vegetable was washed with distilled water and ground to a fine pulp using pestle and mortar. The operation was done under dim light to reduce the rate of carotene oxidation. A 10.000 g of macerated sample was weighed using analytical balance (Mettler PE 160) for β-carotene analysis. The procedure was as follows:

Into a conical flask containing 50 ml of 95% ethanol, 10 g of the macerated sample was placed and maintained at a temperature of 70-80 ^{o}C in a water bath for 20 minutes with periodic shaking. The supernatant was decanted, allowed to cool and its volume measured by means of a measuring cylinder and recorded as initial volume. The ethanol concentration was brought to 85 % by adding 15 ml of distilled water into a 100 ml volumetric flask and topping to the mark with absolute ethanol. This ethanol mixture was further cooled in a container of ice cold water for about 5 minutes. The sample mixture was transferred into a separating funnel and 25 ml of petroleum ether added and the cooled ethanol poured over it. The funnel was swirled gently to obtain homogeneous mixture and latter allowed stand until two separate layers were obtained. The bottom layer was run off into a beaker while the top layer was collected into a conical flask. The bottom layer was transferred into the funnel, re-extracted with 10 ml petroleum ether for 5-6 times until the extract became fairly yellow.

The entire petroleum ether was collected into a 250 ml conical flask and transferred into separating funnel for re-extraction with 50 ml 80% ethanol. The final extract was measured and poured into amber sample bottles for further analysis.

The absorbance of the extract was measured using a spectrophotometer (UV/VIS Spectro Scan 30 Biotech Engineering Management Co. Ltd, UK) at a wavelength of 450 nm. A cuvette containing petroleum ether (blank) was used to calibrate the spectrophotometer to zero point. Samples of each extract was placed in cuvettes and readings recorded.

The concentration of β-carotene was calculated using Beer- Lambert's law:

Where:

A = Absorbance

d = dilution = 10

w = sample weight (g) = 10.000

v = volume of sample solution (L) $= 50 \times 10^{-3}$

ε = Extinction coefficient for beta carotene in petroleum ether (2592×10^{-4} $Lmg^{-1}cm^{-1}$)

The vitamin A content was estimated by dividing the beta carotene content by 6.

3.4.4 Ashing method

Dry ashing of the vegetables for the determination of the total ash was carried out using the method described by Yang et al. (2013) and Jacobs (1999): An empty crucible was weighed using analytical balance (Mettler PE 160) and a 5-10 g sample put into the pre-weighed crucible and reweighed. This was ignited in a muffle furnace at a temperature of 550 °C until free from carbon. Muffle furnace was turned off and opened when the temperature dropped to below 250 °C. The door was carefully opened to avoid losing ash that may be fluffy. The crucible and its contents were removed from the muffle furnace and allowed to cool for a moment and placed in desiccators until cooled. The cold crucible and its content was transferred from the desiccators and reweighed.

Total ash = (weight of crucible + sample after ashing) - weight of empty crucible

% Ash on wet weight basis = (Total ash / weight of original sample) x 100

% Ash on dry weight basis = {Total ash / (original sample weight x dry matter coefficient)} x 100

Wet ashing of the vegetable samples was carried out using the method described by Jacobs (1999): A dried, ground 2.0 g sample was accurately weighed (Mettler PE 160) into a 150 ml Griffin beaker. A 10 ml concentrated HNO_3 (analar grade) was added and allowed to

soak. If the material had a high fat content, it would be allowed to soak overnight. A 3 ml of 60% perchloric acid, $HClO_4$ (analar grade) was added (caution!) and slowly heated on a hot plate until frothing stopped and HNO_3 almost evaporated. Boiling was continued until perchloric reaction occurred (copious fumes), and then watch glass placed on beaker, sample should become colourless or light straw in colour. (Do not let liquid in beaker reduce to dryness). Beaker was removed from hot plate and let to cool. Watch glass was washed with a minimum of distilled deionized water and 10 ml of 50% HCl added. This was transferred to appropriate volumetric flask (50 ml) and diluted with distilled, deionized water. This was ready for elemental analysis.

3.4.5 Flame photometric method for the determination of potassium

Potassium content of the vegetables and soil was determined according to the method described by Ashutosh (2005). All the chemicals and reagents used were analar grade.

Standard potassium, 1000 ppm: 1.900 g of dried KCl was accurately weighed into a 1000 ml volumetric flask, dissolved in deionized water and diluted to the mark.

Radiation buffer for potassium determination:

A saturated solution was prepared with reagent-grade NaCl, $CaCl_2$, $MgCl_2$, in that order.

Preparation of calibration curves:

A 5 ml of radiation buffer was transferred to each series of 100 ml volumetric flasks. A volume of 10 ml of the standard solution was added to the volumetric flask and deionized water added to the mark. This formed a 100 ppm standard solution from which volumes, 0, 2, 4, 6, 8 and 10 ml, respectively were measured and transferred to six clean empty 100 ml volumetric flasks. These were diluted to 100 ml with deionized water and mixed well. These made standard solutions of 0, 2, 4, 6, 8 and 10 ppm, respectively.

First, the digital flame photometer (model-Jenway PFP 7) was switched on followed by the air compressor with the required value. The gas cylinder was opened after the instrument had warmed up for 10 minutes. Initially the deionized water was allowed to aspirate into the flame and calibrate the instrument. The intensity of the standard solutions was measured by taking at least three readings for each sample and the instrument adjusted to

give absorbance value of 0.000 for blank and 100.0 for the maximum concentration of 10 ppm. Between each set of measurements, deionized water was aspirated through the burner. The average values were corrected for background luminosity, and a working curve prepared from these data. Concentration of the analyte was worked out as follows:

Conc. (mg / 100 g) = {(A-c) x V x d x 100} / S x m

Where:

A = absorbance

c = intercept of absorbance axis

V = volume of analyte solution = 0.1 L

S = sample weight (g) = 10.000 g

m = slope of the linear graph (L mg^{-1})

d = dilution factor = 10

Analysis of sample solution: An aliquot portion of the sample was prepared as described in the above preparation of working curve. The emission intensity was measured for the unknown. After correcting the data for background, the concentration of the unknown was determined by comparison with the working curve.

3.4.6 Atomic absorption spectrophotometric method for analysis of K, Mg, Ca, Fe and Mn

The minerals K, Mg, Ca, Fe and Mn contents in the vegetable and soil samples were determined according to the AAS method described by Bartram and Ballance (1996)

Preparation of standard solutions:

To prepare the standards, a 100 ppm stock solution was prepared for potassium, magnesium, calcium, iron and manganese.

Calcium: 0.249 g of calcium carbonate was suspended in water and dissolved with a minimum of 1+1 nitric acid. 10 ml of concentrated nitric acid was added then made up to 1000 ml with deionized water.

Iron: A 0.700 g of $FeSO_4(NH_4)_2SO_4.6H_2O$ was dissolved in a mixture of 10 ml 50% (v / v) hydrochloric acid and 3 ml of concentrated hydrochloric acid and made up to 1000 ml with deionized water.

Magnesium: A 0.165 g MgO, was dissolved in a minimum of 50% (v / v) HNO_3, 10 ml concentrated HNO_3 added and made up to 1000 ml with deionized water.

Manganese: A 0.100 g of manganese metal in 10 ml concentrated hydrochloric acid mixed with 1 ml concentrated nitric acid and made up to 1000ml with deionized water.

Potassium: 0.191 g of KCl was dissolved in deionized water and made up to 1000 ml with deionized water.

Preparation of modifier, lanthanum chloride 11.3% (w/v)

To prepare the modifier, a 113.000 g of lanthanum oxide was added to 500 ml deionized water in a 1000 ml volumetric flask. A 250 ml of 11% hydrochloric acid was carefully added and made to the mark with deionized water.

To six clean 100 ml volumetric flasks, 0, 1, 2, 3, 4 and 5 ml of standard stock solution were added, followed by 10 ml of lanthanum chloride solution, respectively and made up to the mark using deionized water. This was done for all the elements under determination. The resultant solution concentrations were 0, 1, 2, 3, 4 and 5 ppm, respectively.

Hollow cathode lamp for the element being determined was installed and the instrument (Shimadzu AA-6300 GFA- EX7i) switched on and allowed to warm for 20 minutes.

Air and acetylene gas were switched on and flow rate adjusted to the recommended rate, ignited and allowed a few minutes for the flame to stabilize.

A blank of deionized water was aspirated and the instrument zeroed. The standard solution was aspirated and aspiration rate adjusted to obtain maximum sensitivity. This was done for all the working standard concentrations for each element whose level was being determined. Absorbance for each standard solution was recorded.

For each element, a calibration curve (Appendix II) was prepared by plotting absorbance of the standards against their concentrations.

Analysis of samples:

The nebulizer was rinsed by aspirating with deionized water containing 1.5 ml nitric acid per litre, the blank was atomized and the instrument zeroed. Sample was treated as the standards, atomized and absorbance reading recorded. Hollow cathode lamps were changed and the procedure repeated for each element.

The analyte concentration was determined using regression equation:

Absorbance = m Concentration + c

Conc. (mg / 100 g) = {(A-c) x V x d x 100} / S x m

Where:

A = absorbance

c = intercept of absorbance axis

V = volume of analyte solution = 0.1 L

S = sample weight (g) = 2.000 g

m = slope of the linear graph (L mg^{-1})

d = dilution factor = 1

3.5 Soil mineral analysis

Minerals were determined by atomic absorption spectrophotometry (AAS) and flame photometry methods. Atomic absorption spectrophotometric method was used to analyze potassium, calcium, magnesium, manganese and iron elements.

Flame photometric method was used to analyze potassium element. This facilitated the comparison between AAS and flame photometry methods.

Concentration of the elements was determined by the aid of the predetermined standard graph.

The analyte concentration was determined using regression equation:

Absorbance = m Concentration + c

Conc. (mg / 100 g) = {(A-c) x V x d x 100} / S x m

Where:

A = absorbance

c = intercept of absorbance axis

V = volume of analyte solution = 0.1 L

S = sample weight (g) = 10.000 g

m = slope of the linear graph (L mg^{-1})

d = dilution factor = 10

3.6 Reproducibility tests for nutrients analysis methods.

Reproducibility test was done by analyzing standards for elements K, Mg, Ca, Fe and Mn as well as vitamin C and beta carotene. The mean, standard deviation, % recovery, repeatability and relative standard deviations (RDS_r and RSD_R) and Horwitz ratio (HorRat) were calculated.

3.7 Analysis of the samples results.

Results obtained were expressed as mean, standard deviation (SD), % relative standard deviation (RSD_r), Howitz predicted % relative standard deviation (RSD_R). Correlation coefficients were determined by using MS Excel. The standard deviation of the instrument response was determined by using linest function of MS Office, Excel 2007 and this deviation was used to calculate limit of detection (LOD).

LOD = 3SD / S

Where:

SD = Standard deviation of instrument response

S = Slope of the regression line.

Horwitz equation

$RSD_R = 2^{(1 - 0.5\log c)}$

Where:

C = mean concentration of analyte in sample as a decimal fraction.

The value obtained from the above equation was used to calculate the Horwitz ratio (HorRat).

HorRat value = RSDr / RSD_R

The HorRat values and % recovery values were used for result acceptability.

CHAPTER 4

RESULTS

4.1 Introduction

The indigenous vegetables were analyzed for their nutritional composition. Soil was analyzed for specific mineral contents which were also analysed in the vegetables. The results of the nutrients K, Ca, Mg, Fe, Mn, vitamin C, beta carotene, moisture and total ash analysed in six selected indigenous vegetables were reported in tables and figures.

4.2 Method validation of the test instruments

Performance of instruments used in the determination of nutrients in the soil and selected vegetables was tested using regression analysis and reproducibility of the instrument.

4.2.1 Regression analysis of AAS instrument

Linear response of the instrument to the standard mineral concentration is illustrated by calibration graph. Figure 4.1 shows a sample of calibration curve for element manganese.

Figure 4. 1: Calibration curve for manganese

The slope of the calibration curve was taken as the sensitivity of the analytical instrument.

The limit of detection (LOD) was determined by multiplying the standard deviation (SD) of the response by 3 then dividing by the slope of the calibration curve. SD was obtained from a linear regression function (LINEST) in the MS Excel. Table 4.1 gives a summary of the results.

Table 4. 1: Correlation coefficients, limit of detection and regression equation

Parameter	Instrument	r	LOD (ppm)	Regression equation
Potassium	AAS	0.9997	0.1230	y = 0.823x + 0.648
Magnesium	AAS	0.9092	2.5080	y = 0.615x + 1.410
Calcium	AAS	0.9940	0.6011	y = 0.902x + 0.383
Iron	AAS	0.9991	0.2340	y = 0.972x + 0.101
Manganese	AAS	0.9999	0.0336	y = 0.953x + 0.171
Potassium	FP	0.9968	0.8746	y = 10.7x - 2.4

AAS - Atomic absorption spectrophotometer

r - Correlation coefficient

FP - Flame photometer

LOD - Limit of detection

4.2.2 Reproducibility of the test instrument

This was carried out by a standard recovery in which three replicate blanks were spiked with standard solution to make a 3 ppm solution for each parameter and three readings from the instrument recorded. The mean, standard deviation, % recovery, repeatability relative standard deviation (RSD_r), reproducibility relative standard deviation (RSD_R) and Horwitz Ratio (HorRat) were determined and tabulated in table 4.2.

Table 4. 2: Reproducibility test

Parameter	Method	Mean conc (ppm)	SD	% Recovery	RSD_r	RSD_R	HorRat value
Potassium	AAS	3.1618	0.1766	105	5.5854	13.45	0.415
Magnesium	AAS	3.1221	0.6343	104	20.3164	13.48	1.507
Calcium	AAS	3.0335	0.1392	101	4.5887	13.54	0.33
Iron	AAS	3.0967	0.1478	103	4.7728	13.50	0.35
Manganese	AAS	3.1740	0.1577	105	4.9685	13.44	0.36
Potassium	FP	3.0903	0.1588	103	5.1386	13.50	0.38

AAS- Atomic absorption spectrophotometer

FP- Flame photometer

4.3 Total ash determination of the vegetables

The ash contents of the selected indigenous vegetables were analysed using dry ashing technique and the results obtained were as shown in table 4.3.

Table 4. 3: Total ash Content (g / 100 g)

Vegetable	KB Zone Sample g / 100 g (dry)	HB Zone Sample g / 100 g (dry)	Mean Ash content g / 100 g (dry) ± SD
Amaranthus hybridus	18.73	19.48	19.10 ± 0.53
Justicia flava	19.87	19.80	19.83 ± 0.05
Crotalaria brevidens	9.80	9.62	9.71 ± 0.12
Vigna unguiculata	12.67	12.49	12.58 ± 0.12
Solanum nigrum	12.95	12.84	12.90 ± 0.08
Cleome gynandra	15.27	15.06	15.17 ± 0.15

KB-Kendu Bay; HB- Homa Bay

4.4 Moisture contents of the selected vegetables

Moisture contents of the selected vegetables were determined by oven drying method and results were as shown in table 4.4.

Table 4. 4: Moisture contents (% wet weight basis)

Vegetable	Kendu Bay zone Sample % (wet)	Homa Bay zone Sample % (wet)	Mean ± SD
Amaranthus hybridus	76.63	78.10	77.37 ± 1.04
Justicia flava	78.91	85.22	82.07 ± 4.46
Crotalaria brevidens	75.47	81.49	78.48 ± 4.26
Vigna unguiculata	83.00	83.33	83.17 ± 0.23
Solanum nigrum	86.03	87.46	86.75 ± 1.01
Cleome gynandra	84.69	83.96	84.33 ± 0.52

4.5 Vitamin C contents of the selected indigenous vegetables

The vitamin C contents of the vegetables were determined by titrimetric method and table 4.5 gives a summary of results of vitamin C contents of the selected indigenous vegetables in mg / 100 g (wet matter basis).

Table 4. 5: Vitamin C contents (mg / 100 g wet weight basis)

	Cleome gynandra	*Vigna unguiculata*	*Justicia flava*	*Solanum nigrum*	*Amaranthus hybridus*	*Crotalaria brevidens*
Kendu Bay	60.7	80.8	79.2	52.8	116.2	70.2
Homa Bay	61.2	64.9	52.8	63.4	59.7	73.9

4.6 -carotene determination

Beta carotene contents of the vegetables were analysed by UV-VIS spectrophotometric method. Results for β-carotene contents in the indigenous vegetables in mg / 100 g (wet weight) were as shown in table 4.6.

Table 4. 6 -carotene contents (mg / 100 g wet weight basis)

Vegetable	Absorbance	β-carotene	Retinol (vitamin A)
Cleome gynandra	0.7710	7.44	1.24
Vigna unguiculata	0.5133	4.95	0.83
Justicia flava	0.9360	9.03	1.51
Solanum nigrum	0.9750	9.40	1.57
Amaranthus hybridus	0.3460	3.34	0.56
Crotalaria brevidens	0.6452	6.22	1.04

4.7 Mineral analysis

To determine mineral contents in the vegetable and soil samples, both the AAS and flame photometric methods were used. The concentration of K, Mg, Ca, Fe and Mn from six indigenous vegetable species (*Amaranthus hybridus, Cleome gynandra, Justicia flava, Solanum nigrum and Vigna unguiculata*) from Kendu Bay and Homa Bay zones and soils from where the vegetables were collected was determined.

4.7.1 AAS mineral analysis

To determine mineral nutrients in the vegetable and soil samples, the AAS method was used for the minerals; K, Mg, Ca, Fe and Mn on dry weight basis (mg /100g) .The findings were as shown in tables 4.7.

Table 4.7: Mineral contents in vegetable and soil samples (mg / 100 g dry weight)

	K		Mg		Ca		Fe		Mn	
	KB	HB	KB	HB	KB	HB	KB	HB	KB	HB
Cleome gynandra	267.4	240.3	20.4	20.1	131.9	149.3	55.6	55.1	8.5	5.8
Vigna unguiculata	37.6	206.1	22.2	18.2	142.6	ND	61.9	41.2	22.0	14.0
Justicia flava	296.9	40.6	17.4	20.7	ND	13.3	73.0	76.4	15.0	13.9
Solanum nigrum	262.2	306.1	21.7	21.8	134.0	132.4	58.5	64.5	14.6	11.8
Amaranthus hybridus	309.2	243.8	21.3	24.4	ND	104.7	73.6	51.8	17.4	8.7
Crotalaria brevidens	264.5	39.1	21.4	20.9	90.8	136.5	77.3	51.8	16.5	8.6
Soil	11.4	27.1	4.6	4.5	26.8	ND	16.9	22.2	14.2	16.1

ND- Not detectable, KB- Kendu Bay

HB- Homa Bay

4.7.2 Flame photometer determination of potassium

To compare the validity of results obtained by AAS instrument, flame photometer was used to determine potassium contents in the vegetables and soils. The results obtained using the flame photometer, on dry matter basis (mg / 100 g) were as indicated in table 4.8.

Table 4. 8: Potassium contents by flame photometer (mg / 100 g dry weight basis)

	Kendu Bay Zone	Homa Bay Zone
Cleome gynandra	274.82	250.70
Vigna unguiculata	48.22	226.62
Justicia flava	318.23	43.39
Solanum nigrum	270.00	323.05
Amaranthus hybridus	265.19	298.94
Crotalaria brevidens	245.90	43.69
Soil	13.46	30.28

4.8 Minerals in soil and vegetables

The concentration of K, Mg, Ca, Fe and Mn in the selected vegetables and the soils from which they were obtained were compared using correlation coefficients. The samples from the two sampling zones were treated separately as KB (Kendu Bay) and HB (Homa Bay). Specific mineral in the soil was marched with its content in the vegetable. The correlation coefficients were calculated using MS Office, Excel 2007. The findings were as given in table 4.9.

Table 4. 9: Correlation of mineral contents in vegetable and soil

Vegetable sample	Correlation Coefficient, r	
	KB	HB
Amaranthus hybridus	0.43618	0.70902
Cleome gynandra	0.38852	0.72597
Crotalaria brevidens	0.26645	0.60617
Justicia flava	0.44972	0.56645
Solanum nigrum	0.39706	0.72878
Vigna unguiculata	0.95164	0.71834

KB- Kendu Bay HB- Homa Bay

Comparison of the minerals in the sampled vegetables and soil in this study was carried out and a sample of correlation graph is shown in figure 4.2.

Figure 4. 2: Correlation graph for soil and Vigna unguiculata **mineral content (KB)**

CHAPTER 5

DISCUSSION

5.1 Introduction

The study was to find out the concentration levels of beta-carotene, vitamin C, K, Mg, Ca, Fe, Mn, moisture and ash in the selected indigenous vegetable. The study was also to compare the concentration levels of the selected vitamins and minerals in the selected vegetables in Kendu Bay with the recommended daily allowances. The study was as well to compare concentration level of the selected minerals in the vegetables to that in the soils where the vegetables are grown. Results were analyzed by arithmetic means, standard deviations, percentages, range, correlation coefficient, percent recovery and the Horwitz Ratio. Results from different species were compared for a given analyte. Minerals and vitamin levels in selected vegetable species were compared with the recommended daily intakes. The findings of this study have also been compared to those of other researchers. Findings and significance of the findings were discussed as follows:

5.2 Method validation

The reliability of the instruments and procedures used in the determination of nutrients in the selected vegetables was tested by regression analysis and reproducibility of the results obtained.

5.2.1 Regression

The response of the instruments to the standard solutions of the analytes was recorded (appendix A) and correlation coefficients calculated. The correlation coefficient (r) for potassium (r = 0.9997), magnesium (r = 0.9092), calcium (r = 0.9940), iron (r = 0.9991), manganese (r = 0.9999) under AAS and potassium (r = 0.9968) under flame photometer showed linearity (appendix II) in response of the instruments to concentration of standards. These values agreed with the acceptable values of best fit.

The limit of detection of potassium (0.1230), magnesium (2.5080), calcium (0.6011), iron (0.2340) and manganese (0.0336) proved the reliability of the instruments as low levels of the analytes (ppm) were detectable (Table 4.1 and Figure 4.1).

5.2.2 Reproducibility test

The Horwitz Ratio was used for acceptability of the results. The Horwitz Ratio for all the parameters measured was between 0.33 – 1.50 (Table 4.2).

The values are within accepted values of 0.30 to 1.33 except magnesium as outlined by AOAC (2012). Under reproducibility conditions, values of between 0.50 and 2 are accepted (Rivera and Rodriguez, 2011).

5.3 Total ash

The findings of the total ash contents of the vegetables analysed in this study varied. The mean ash contents on dry matter were between 9.71 g / 100 g to 19.83 g / 100 g. *Justicia flava* had the highest value of 19.83 g / 100 g, *Amaranthus hybridus* 19.10 g / 100 g, *Vigna unguiculata* 12.58 g / 100 g, *Cleome gynandra* 15.17 g / 100 g, *Solanum nigrum* 12.90 g / 100 g and *Crotalaria brevidens* 9.71 g / 100 g (Table 4.3). A similar research work by Adeyeye and Omolayo (2011) on *Amaranthus hybridus* recorded 17.2 g / 100 g total ash. A research work by Pretorius and Schonfeldt (2011) recorded much lower value of 1.42 g/ 100 g and 2.77 g / 100 g for *vigna unguiculata* and *Cleome gynandra*, respectively. In a study by Odhav *et al*. (2007) *Amaranthus hybridus*, *Justicia flava* and *Solanum nigrum* recorded 4.91, 3.32 and 2.24 / 100 g ash contents, respectively. The difference could be attributed to different soils and climatic conditions of the study areas.

5.4 Moisture contents

The results of the moisture contents of the analysed vegetable species in the study were in the range 75- 87% and there was minimum variation among the species as well as between the two sampling zones. *Amaranthus hybridus* recorded moisture content of 77.37%, *Justicia flava* 81.93%, *Crotalaria brevidens* 78.48%, *Vigna unguiculata* 83.17%, *Solanum nigrum*, 86.75% and *Cleome gynandra* 84.33% (Table 4.4). A similar study by Pretorius and Schonfeldt (2011) reported moisture content on *Cleome gynandra* 84.2% and *Vigna unguiculata* 87.6%, which was in agreement with the findings of this study. The similarity in moisture contents could be attributed to similar adaptation features to the environment by same vegetable species. Contrary to the findings of this study, Maina and Mwangi (2008) reported 88.9% and 87.8% moisture contents for *Amaranthus hybridus* and *Solanum nigrum*, respectively.

5.5 Vitamin C contents in the selected vegetables

The findings of this study on vitamin C contents in the vegetables analysed were between 52 and 116 mg / 100 g in the two sampling zones of the study area. *Amaranthus hybridus* recorded the highest content (116.2 mg / 100 g) while *Justicia flava* had the lowest value of 52.8 mg / 100 g (Table 4.5). All the sampled vegetables recorded vitamin C contents that met the recommended daily intake of 40-70 mg if consumed in large quantity and when the vitamin C degradation is minimized. A research work by Maina and Mwangi (2008) on *Amaranthus hybridus* recorded 42 mg / 100 g. A similar study on *Amaranthus hybridus* reported vitamin C concentration of 60.12 mg / 100 g (Ogunlesi *et al*., 2010). Chweya and Mnzava (1997) recorded vitamin C contents in the range 127-484 mg / 100 g for *Cleome gynandra*. The differences in vitamin C contents obtained in this study and the other studies could be attributed to different climatic conditions in the study areas.

5.6 Beta carotene contents in the vegetables

The analysis of beta carotene contents in the indigenous vegetables in the study gave results in the range of 3.34-9.40 mg / 100 g (wet). This when converted to retinol, ranged between 0.56 to 1.57 mg / 100 g. *Solanum nigrum* recorded the highest β-carotene content of 9.40 mg / 100 g, whereas *Amaranthus hybridus* had the lowest of 3.34 mg / 100 g (Table 4.6). A research study by Pretorius and Schonfield (2011) on *Vigna unguiculata* and *Cleome gynandra* recorded 2.23 and 4.12 mg / 100g, respectively. In a similar study by Chweya and Mnzava (1997) on *Cleome gynandra* value of 6.7-18.9 mg / 100 g β-carotene was recorded. The vitamin contents of the vegetables met the recommended daily allowances of 0.7-1.0 mg except *Amaranthus hybridus*. There are also losses of β-carotene attributed to oxidation during the cooking process (Pretorius and Schönfeldt, 2011; Fikselova *et al*., 2008; Chweya and Mnzava, 1997).

5.7 Mineral nutrients in the vegetables and soil

The study analysed mineral contents of the vegetables and findings varied among the species as well as from one place to another within the study area. Since the vegetables were collected from different places that had different soil characteristics, nutrient variations were expected. The mineral contents in vegetables varied according to mineral availability and the specific mineral uptake ability of the plant.

5.7.1 Potassium

The results of potassium contents in the vegetable species in this study varied from one zone to another and among vegetable varieties. From Kendu Bay, potassium contents were in the range 37.6-309.2 mg / 100 g with *Amaranthus hybridus* recording the highest content of 309.2 mg / 100 g and *Vigna unguiculata* the lowest value of 37.6 mg / 100 g. From Homa Bay, *Crotalaria brevidens* recorded the lowest potassium content of 39.1 mg / 100 g compared to *Cleome gynandra* which gave 306.1 mg / 100 g using AAS (Table 4.7). Flame photometer gave potassium contents that were between 48.22 to 318.23 mg / 100 g from Kendu Bay. *Justicia flava* had 318.23 mg / 100 g and *Vigna unguiculata* 48.22 mg / 100 g. In samples from Homa Bay *Solanum nigrum* recorded the highest value of 323.05 mg / 100 g and *Justicia flava* gave the lowest value of 43.39 mg / 100 g (Table 4.8). Similar work on nutritional and chemical values of *Amaranthus hybridus* yielded a lower value of 54.20 mg / 100 g (Akubugwo *et al*., 2007). Chweya and Mnzava (1997) reported a value of 410 mg / 100 g on the potassium content of *Cleome gynandra*. Potassium contents did not meet the recommended daily allowance of 3500 mg; however, if the vegetables are consumed in large quantities and frequently, they make a significant contribution to the diet. Potassium content in the soil reported a value of 11.4 mg / 100 g in Kendu Bay and 27.1 mg / 100 g in Homa Bay samples. Determination of potassium content in soil samples by flame photometer reported values of 13.4579 and 30.284 mg / 100 g for Kendu Bay and Homa Bay samples, respectively (Table 4.8).

5.7.2 Magnesium

Findings of this research work on magnesium contents in the indigenous vegetables analysed were in the range 17.4-24.4 mg / 100 g (Table 4.7). The concentrations were relatively constant among all the sample varieties. A similar study by Adeyeye and Omolayo (2011) on chemical composition and functional properties of leaf protein concentrates reported a value of 11.6 mg / 100 g. Subramanian *et al*. (2012) reported Magnesium content of 18.59±0.612 mg / 100 g in their study on analysis of minerals and heavy metals in some medicinal plants (*Solanum nigrum*) collected from local market. In this study *Solanum nigrum* reported a value of 21.7 mg / 100 g. The magnesium concentrations in the vegetables were below the recommended daily allowance of 350 mg. Research work by Odhav *et al*. (2007) on preliminary assessment of nutritional value of

traditional leafy vegetables in Kwazulu-Natal, South Africa found Mg concentration in *Amaranthus hybridus* was 1317 mg / 100 g, *Justicia flava* 1407 mg / 100 g and *Solanum nigrum* 277 mg / 100 g which were very high compared to the results of this study. This could be due to differences in soil characteristics and the mineral absorption by the vegetables. In this study *Cleome gynandra* recorded magnesium content of 20.4 mg / 100 g and 20.1 mg / 100 g for Kendu Bay and Homa Bay samples, respectively. This was not significantly different from findings (20.8 mg / 100 g) reported by Mibei *et al*. (2011) on *Cleome gynandra*. Soil samples reported Magnesium value of 4.5- 4.6 mg / 100 g (Table 4.7).

5.7.3 Calcium

The results for calcium contents in the analysed vegetables and soils varied in this study. There were no detectable contents for *Justicia flava* and *Amaranthus hybridus* from Kendu Bay as well as *Vigna unguiculata* and soil from Homa Bay. For other samples calcium contents ranged from 90.8 to 149.3 mg / 100 g. *Cleome gynandra* from Homa Bay recorded the highest value of 149.3 mg / 100 g. *Crotalaria brevidens* from Kendu Bay gave 90.8 mg / 100 g (Table 4.7). The contents of calcium in the selected vegetables were below the recommended daily allowance of 700-1000 mg. However, the findings of the study showed that the vegetables are potential sources of nutrients in the diet. Study on *Solanum nigrum* by Asibey-Beko and Tayie (1999) reported calcium content value of 42.92 mg / 100 g. A study by Mishra *et al*. (2011) on *Cleome gynandra* reported calcium contents of between 213 mg / 100 g and 434 mg / 100 g. In this study calcium content in the soil was 26.8 mg / 100 g of Kendu Bay sample while Homa Bay sample content was not detected (Table 4.7).

5.7.4 Iron

The mineral contents in the sampled vegetable species were in the range 41.2-77.3 mg / 100 g. *Crotalaria brevidens* from Kendu Bay recorded the highest value of 77.3 mg /100 g, whereas the same species from Homa Bay recorded a value of 51.8 mg / 100 g. From Homa Bay, high iron contents were recorded for *Justicia flava*, *Solanum nigrum* and *Cleome gynandra* whereas from Kendu Bay the order was *Crotalaria brevidens*, *Amaranthus hybridus* and *Justicia flava*. A similar study in South Eastern Nigeria on determination of the vitamin and mineral composition of common leafy vegetables

reported 64.81±0.96 mg / 100 g for *Solanum nigrum* (Achikanu et al., 2013). A research work by Akubugwo *et al*. (2007) on *Amaranthus hybridus* recorded 13.58 mg / 100 g of iron. The variation in iron contents could be due to differences in the mineral distribution in soils within the different study areas. All the vegetable species fulfilled the recommended daily allowance for iron of 8-14.8 mg. Soil samples recorded iron value of 16.9 mg / 100 g and 22.2 mg / 100 g for Kendu Bay and Homa Bay samples, respectively (Table 4.7).

5.7.5 Manganese

This was the least abundant mineral among those analyzed in the vegetable samples. The content ranged between 5.8-22.0 mg / 100 g with *Cleome gynandra* recording the lowest in both zones while *Vigna unguiculata* recorded the highest concentration in the two zones (Table 4.7). There was minimum variation in contents among the sampled vegetables. A study by Odhav et al. (2007) reported values for *Amaranthus hybridus* as 24 mg / 100 g, *Justicia flava* 8.4 mg / 100 g and *Solanum nigrum* 3 mg / 100 g. A research study by Adeyeye and Omolayo (2011), *Amaranthus hybridus* reported very low concentration of 0.08 mg / 100 g. Subramanian et al. (2012) reported manganese content of 9.843±0.367 mg / 100 g for *Solanum nigrum*. Recommended daily allowance for manganese is 5-7 mg and the sampled vegetables met the daily requirements of the mineral. The sampled soil recorded manganese content value of 14.2 mg / 100 g and 16.1 mg / 100 g for Kendu Bay and Homa Bay, respectively (Table 4.7).

5.8 Minerals in soil and vegetables

The findings showed that there was a positive correlation between minerals in the soil and those found in the vegetables. *Vigna unguiculata* recorded the highest correlation coefficient of 0.95164 in Kendu Bay and 0.71834 in Homa Bay (Table 4.8). The result showed that plants accumulate minerals after absorbing them. This was illustrated by soil from the field where a plant was obtained showing content below detectable limit while the plant itself recorded good amount of the mineral. This explained why sample from Homa Bay had calcium content below the limit of detection yet only *Vigna unguiculata* recorded no detection for calcium. In a similar study by Ndlovu and Afolayan (2008) on nutritional analysis of the South African wild vegetable, there was no detection made for calcium. Since the study area is composed of sandy soils which have been reported in a study by

University of Hawaii (2007-2014) to have lower cation exchange capacities (CECs) which decreased their ability to hold and retain nutrients. The results could be attributed to sandy soils.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This research study was to assess the contents of nutrients; potassium, magnesium, calcium, iron, manganese, vitamin C and Vitamin A as well as moisture and total ash in selected varieties of indigenous vegetables. Based on the findings of this study, it can be concluded that;

- Total ash was between 9.71 g /100 g and 19.83 g / 100 g with *Justicia flava* having the highest ash content of 19.83 g / 100 g. The total ash content in the vegetables implied the presence of oxides of minerals including those that were not studied in this work.
- Moisture content on wet matter basis was; *Solanum nigrum*, 86.75%, *Cleome gynandra* 84.33%, *Vigna unguiculata* 83.17%, *Justicia flava* 82.07%, *Crotalaria brevidens* 78.48% and *Amaranthus hybridus* 77.37%. This high moisture implies more water in the plants which aids in nutrient uptake through mass flow and diffusion.
- Vitamin C varied among the vegetable varieties and also from one sampling zone to the other. *Solanum nigrum* had the highest vitamin C value of 116.2 mg /100 g and the contents recorded from all the sampled varieties were within the recommended daily allowance.
- Beta carotene contents were as follows: *Solanum nigrum* 9.40 mg / 100 g, *Justicia flava* 9.03 mg / 100 g, *Cleome gynandra* 7.44 mg / 100 g, *Crotalaria brevidens* 6.22 mg / 100 g, *Vigna unguiculata* 4.95 mg, *Amaranthus hybridus* 3.34 mg / 100 g. These values when converted to retinol equivalents gave 1.57, 1.51, 1.24, 1.04, 0.83 and 0.56 mg retinol (vitamin A) in that order. The vitamin contents of the vegetables met the recommended daily allowance (RDA) of 0.7-1.0 mg except *Amaranthus hybridus*. Most of these values were very close to the RDA.
- Mineral levels varied from region to region due to different soils with different characteristics, and it also depended on plants ability to absorb these minerals from the soil.

- Potassium was the most abundant mineral among the sampled varieties with *Amaranthus hybridus*, *Cleome gynandra*, and *Solanum nigrum* giving higher contents. These contents, 49-310 mg /100 were below the recommended daily intake of 3000-3500 mg.
- Magnesium contents were in the range 17- 24 mg / 100 g. The quantities recorded in this study failed to meet the nutritional requirement of the mineral in the diet.
- Calcium contents were recorded in the range 90.8-149.3 mg / 100 g among most varieties sampled and the contents were also below recommended daily allowances.
- Iron levels were 41.2-77.3 mg / 100 g in all the vegetable varieties and that the levels were above the recommended daily allowance hence met the nutritional requirement of the diet.

- There was a positive correlation between the mineral content in the soil where the vegetables were collected and the vegetables. Vegetables from Homa Bay gave strong correlation with values between 0.566-0.728. Samples from Kendu Bay recorded the highest correlation from *Vigna unguiculata* with a correlation coefficient of 0.951.

6.2 Recommendations

Consumption of indigenous vegetables as sources of vitamin C, beta carotene and minerals such as potassium, iron and manganese should be encouraged to help alleviate nutrient deficiency related diseases. The results of this study showed that vitamin A in the analysed vegetable met recommended daily allowances of 0.7- 1.0 mg except *Amaranthus hybridus* and therefore, vitamin A supplementations in foods like flour and sugar should be encouraged. This is necessary because conversion of beta carotene to vitamin A varies among individuals due endogenous activity of the digestive enzymes. It is also not possible to practically have complete conversion of all of the ingested beta carotene to vitamin A.

In this study, soil sample from Homa Bay showed calcium content below detection limit yet all the vegetables analysed except *Vigna unguiculata* recorded some calcium contents. This was contrary to the expectation since vegetables derive their minerals from the soil where they grow. Since soil samples in the study were collected during the rainy season,

investigation should therefore be carried out to assess if seasonal variation has effect on calcium availability in the soil and by extension vegetables.

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APPENDICES

Appendix I: Mineral analysis

Appendix I 1: Sample codes

Sample ID	Name and source
W150	Soil from Kendu Bay
W151	Amaranthus from Homa Bay
W152	Justicia flava from Homa Bay
W153	Crotalaria brevidens from Homa Bay
W154	Vigna unguiculata from Kendu Bay
W155	Crotalaria brevidens from Kendu Bay
W156	Soil from Homa Bay
W158	Solanum nigrum from Kendu Bay
W159	Solanum nigrum from Homa Bay
W160	Amaranthus from Kendu Bay
W161	Justicia flava from Kendu Bay
W162	Cleome gynandra from Kendu Bay
W163	Cleome gynandra from Homa Bay
W164	Vigna unguiculata from Homa Bay

Appendix I 1: Results for element potassium (AAS)

K						
Action	Sample ID	True Value	Conc (ppm)	Abs	Actual Conc	Date
BLK				0.0020		22/01/2014
STD		1	1.4855	0.0752		22/01/2014
STD		2	2.2539	0.1141		22/01/2014
STD		3	3.1566	0.1598		22/01/2014
STD		4	3.9310	0.1990		22/01/2014
STD		5	4.7626	0.2411		22/01/2014
UNK1	W150		11.3702	0.5756	11.3702 ppm	22/01/2014
UNK2	W151		48.7598	2.4684	48.7598 ppm	22/01/2014
UNK3	W152		8.1148	0.4108	8.1148 ppm	22/01/2014
UNK4	W153		7.8284	0.3963	7.8284 ppm	22/01/2014
UNK5	W154		7.5222	0.3808	7.5222 ppm	22/01/2014
UNK6	W155		52.9002	2.6780	52.9002 ppm	22/01/2014
UNK7	W156		27.0526	1.3695	27.0526 ppm	22/01/2014
UNK8	W158		52.4340	2.6544	52.4340 ppm	22/01/2014
UNK9	W159		61.2263	3.0995	61.2263 ppm	22/01/2014
UNK10	W160		61.8367	3.1304	61.8367 ppm	22/01/2014
UNK11	W161		59.3833	3.0062	59.3833 ppm	22/01/2014
UNK12	W162		53.4711	2.7069	53.4711 ppm	22/01/2014
UNK13	W163		48.0527	2.4326	48.0527 ppm	22/01/2014
UNK14	W164		41.2179	2.0866	41.2179 ppm	22/01/2014

Appendix I 2: Results for element magnesium (AAS)

Mg						
Action	Sample ID	True Value	Conc (ppm)	Abs	Actual Conc	Date
BLK				-0.0113		23/01/2014
STD		1	1.6663	0.7427		23/01/2014
STD		2	2.6314	1.1729		23/01/2014
STD		3	3.9809	1.7744		23/01/2014
STD		4	3.8898	1.7338		23/01/2014
STD		5	4.1137	1.8336		23/01/2014
UNK1	W150		4.5537	2.0297	4.5537 ppm	23/01/2014
UNK2	W151		4.8754	2.1731	4.8754 ppm	23/01/2014
UNK3	W152		4.1360	1.8435	4.1360 ppm	23/01/2014
UNK4	W153		4.1766	1.8616	4.1766 ppm	23/01/2014
UNK5	W154		4.4449	1.9812	4.4449 ppm	23/01/2014
UNK6	W155		4.2851	1.9100	4.2851 ppm	23/01/2014
UNK7	W156		4.4862	1.9996	4.4862 ppm	23/01/2014
UNK8	W158		4.3455	1.9369	4.3455 ppm	23/01/2014
UNK9	W159		4.3664	1.9462	4.3664 ppm	23/01/2014
UNK10	W160		4.2668	1.9018	4.2668 ppm	23/01/2014
UNK11	W161		3.4707	1.5470	3.4707 ppm	23/01/2014
UNK12	W162		4.0707	1.8144	4.0707 ppm	23/01/2014
UNK13	W163		4.0108	1.7877	4.0108 ppm	23/01/2014
UNK14	W164		3.6336	1.6196	3.6336 ppm	23/01/2014

Appendix I 3: Results for element calcium (AAS)

Ca						
Action	Sample ID	True Value	Conc (ppm)	Abs	Actual Conc	Date
BLK				0.0060		24/01/2014
STD		1	1.3955	0.0970		24/01/2014
STD		2	2.0543	0.1428		24/01/2014
STD		3	3.1750	0.2207		24/01/2014
STD		4	3.7922	0.2636		24/01/2014
STD		5	5.0395	0.3503		24/01/2014
UNK1	W150		26.7641	1.8604	26.7641 ppm	24/01/2014
UNK2	W151		20.9391	1.4555	20.9391 ppm	24/01/2014
UNK3	W152		2.6672	0.1854	2.6672 ppm	24/01/2014
UNK4	W153		27.3064	1.8981	27.3064 ppm	24/01/2014
UNK5	W154		28.5264	1.9829	28.5264 ppm	24/01/2014
UNK6	W155		18.1683	1.2629	18.1683 ppm	24/01/2014
UNK7	W156		-14.4711	-1.0059	-14.4711 ppm	24/01/2014
UNK8	W158		26.8043	1.8632	26.8043 ppm	24/01/2014
UNK9	W159		26.4749	1.8403	26.4749 ppm	24/01/2014
UNK10	W160		-8.8015	-0.6118	- 8.8015 ppm	24/01/2014
UNK11	W161		-14.4696	-1.0058	-14.4696 ppm	24/01/2014
UNK12	W162		26.3771	1.8335	26.3771 ppm	24/01/2014
UNK13	W163		29.8585	2.0755	29.8585 ppm	24/01/2014
UNK14	W164		-14.4711	-1.0059	-14.4711 ppm	24/01/2014

Appendix I 4: Results for element iron (AAS)

Fe						
Action	Sample ID	True Value	Conc (ppm)	Abs	Actual Conc	Date
BLK				-0.0023		24/01/2014
STD		1	1.0922	0.0573		24/01/2014
STD		2	2.0662	0.1084		24/01/2014
STD		3	2.9144	0.1529		24/01/2014
STD		4	4.0657	0.2133		24/01/2014
STD		5	4.9539	0.2599		24/01/2014
UNK1	W150		16.8574	0.8844	16.8574 ppm	24/01/2014
UNK2	W151		10.3519	0.5431	10.3519 ppm	24/01/2014
UNK3	W152		15.2715	0.8012	15.2715 ppm	24/01/2014
UNK4	W153		10.3653	0.5438	10.3653 ppm	24/01/2014
UNK5	W154		12.3724	0.6491	12.3724 ppm	24/01/2014
UNK6	W155		15.4545	0.8108	15.4545 ppm	24/01/2014
UNK7	W156		22.1601	1.1626	22.1601 ppm	24/01/2014
UNK8	W158		11.7053	0.6141	11.7053 ppm	24/01/2014
UNK9	W159		12.9023	0.6769	12.9023 ppm	24/01/2014
UNK10	W160		14.7226	0.7724	14.7226 ppm	24/01/2014
UNK11	W161		14.6063	0.7663	14.6063 ppm	24/01/2014
UNK12	W162		11.1239	0.5836	11.1239 ppm	24/01/2014
UNK13	W163		11.0114	0.5777	11.0114 ppm	24/01/2014
UNK14	W164		8.2343	0.4320	8.2343 ppm	24/01/2014

Appendix I 5: Results for element manganese (AAS)

Mn						
Action	Sample ID	True Value	Conc (ppm)	Abs	Actual Conc	Date
BLK				-0.0015		22/01/2014
STD		1	1.1310	0.1105		22/01/2014
STD		2	2.0678	0.2020		22/01/2014
STD		3	3.0277	0.2958		22/01/2014
STD		4	3.9970	0.3905		22/01/2014
STD		5	4.9325	0.4819		22/01/2014
UNK1	W150		14.2029	1.3876	14.2029 ppm	22/01/2014
UNK2	W151		1.7360	0.1696	1.736 ppm	22/01/2014
UNK3	W152		2.7759	0.2712	2.7759 ppm	22/01/2014
UNK4	W153		1.7216	0.1682	1.7216 ppm	22/01/2014
UNK5	W154		4.3890	0.4288	4.3890 ppm	22/01/2014
UNK6	W155		3.2928	0.3217	3.2928 ppm	22/01/2014
UNK7	W156		16.0310	1.5662	16.0310 ppm	22/01/2014
UNK8	W158		2.9171	0.2850	2.9171 ppm	22/01/2014
UNK9	W159		2.3593	0.2305	2.3593 ppm	22/01/2014
UNK10	W160		3.4873	0.3407	3.4873 ppm	22/01/2014
UNK11	W161		3.0021	0.2933	3.0021 ppm	22/01/2014
UNK12	W162		1.6919	0.1653	1.6919 ppm	22/01/2014
UNK13	W163		1.1566	0.1130	1.1566 ppm	22/01/2014
UNK14	W164		2.7912	0.2727	2.7912 ppm	22/01/2014

Appendix I 6: Results for element potassium (Flame photometer)

Sample ID	True value (ppm)	Conc.(ppm)	Abs	Actual Conc (ppm)	Date
			0.000		23/4/2014
	2	2.0934	20		23/4/2014
	4	3.7757	38		23/4/2014
	6	6.299	65		23/4/2014
	8	7.7009	80		23/4/2014
	10	10.1308	106		23/4/2014
HB Cleome		50.841	52	50.841	23/4/2014
KB Cleome		55.514	57	55.514	23/4/2014
HB Vigna		46.1682	47	46.1682	23/4/2014
KB vigna		11.5888	10	11.5888	23/4/2014
HB Justicia		10.6542	9	10.6542	23/4/2014
KB Justicia		63.9252	66	63.9252	23/4/2014
HB Solanum		64.8598	67	64.8598	23/4/2014
KB Solanum		54.5779	56	54.5779	23/4/2014
HB Amaranth		60.1869	62	60.1869	23/4/2014
KB Amaranth		53.6448	55	53.6448	23/4/2014
HB Crotalaria		10.6542	9	10.6542	23/4/2014
KB Crotalaria		49.9065	51	49.9065	23/4/2014
HB Soil		30.2804	30	30.2804	23/4/2014
KB Soil		13.4579	12	13.4579	23/4/2014

Appendix II: Calibration

Appendix II 1: Calibration curve for element K

Abs = 0.050624 Conc. + 0.0000 r = 0.9997

Conc.	Abs.
1.0000	0.0752
2.0000	0.1141
3.0000	0.1598
4.0000	0.1990
5.0000	0.2411

Appendix II 2: Calibration curve for element Ca

Abs = 0.071032 Conc. + 0.0000 r = 0.9940

Conc.	Abs
1	0.0970
2	0.1428
3	0.2207
4	0.2636
5	0.3503

Appendix II 3: Calibration curve for element Fe

Abs = 0.052464Conc + 0.0000 r = 0.9991

Conc.	Abs
1.0000	0.0573
2.0000	0.1084
3.0000	0.1529
4.0000	0.2133
5.0000	0.2599

Appendix II 4: Calibration curve for element Mn

Abs = 0.097698Conc + 0.0000 r = 0.9999

Conc.	Abs
1.0000	0.1105
2.0000	0.2020
3.0000	0.2958
4.0000	0.3905
5.0000	0.4819

Appendix III: MS Excel Linest function

Appendix III 1 : Output data from linest function

Output from linest obtained by the function:

= LINEST (start Y:end Y, start X:end X, TRUE,TRUE)

Y = mX + b	
m	b
slope	intercept
SD of m	SD of b
R^2	SD of y
F- stat	degree of freedom

Appendix III 2: Linest output for element K

0.82313	0.64853
0.010744	0.035635
0.999489	0.033976
5869.258	3
6.77543	0.003463

Appendix III 3: Linest output for element Ca

0.90259	0.38353
0.057204	0.189725
0.988093	0.180895
248.9583	3
8.146687	0.098169

Appendix III 4: Linest output for element Fe

0.97229	0.10161
0.023991	0.079568
0.998177	0.075865
1642.527	3
9.453478	0.017266

Appendix III 5: Linest output for element Mg

0.61532	1.41046
0.162677	0.539539
0.82666	0.51443
14.30702	3
3.786187	0.793915

Appendix III 6: Linest output for element Mn

0.95324	0.17144
0.003384	0.011224
0.999962	0.010702
79337.35	3
9.086665	0.000344

Appendix IV: Ashing

Appendix IV 1: Ashing results for Homa bay samples

	Amaranthus hybridus	Justicia flava	Crotalaria brevidens	Vigna unguiculata	Solanum nigrum	Cleome gynandra
Crucible+sample (g)	35.830	30.906	19.777	31.391	33.008	30.273
Mass of Crucible (g)	28.248	24.978	13.735	25.000	25.921	24.065
Mass of dry sample (g)	7.582	5.928	6.042	6.391	7.087	6.208
Mass of crucible + residue (g)	29.725	26.152	14.316	25.794	26.831	25.000
Mass of Ash (residue) (g)	1.477	1.174	0.581	0.794	0.910	0.935

Appendix IV 2: Ashing results for Kendu Bay samples

	Amaranthus hybridus	*Justicia flava*	*Crotalaria brevidens*	*Vigna unguiculata*	*Solanum nigrum*	*Cleome gynandra*
Crucible+sample (g)	35.830	30.906	19.777	31.391	33.008	30.273
Mass of Crucible (g)	28.248	24.978	13.735	25.000	25.921	24.065
Mass of dry sample (g)	7.582	5.928	6.042	6.391	7.087	6.208
Mass of crucible + residue (g)	29.730	26.156	14.327	25.810	26.839	25.013
Mass of Ash (residue) (g)	1.482	1.178	0.592	0.810	0.918	0.948

Appendix V: Moisture determination

Appendix V 1: Moisture results for Homa Bay samples

	Amaranthus hybridus	*Justicia flava*	*Crotalaria brevidens*	*Vigna unguiculata*	*Solanum nigrum*	*Cleome gynandra*
Mass of wet sample (g)	2.10	3.79	3.08	2.88	3.51	3.18
Mass of dried sample (g)	0.46	0.56	0.57	0.48	0.44	0.51
Moisture mass (g)	1.64	3.23	2.51	2.40	3.07	2.67
% moisture	78.10	85.22	81.49	83.33	87.46	83.96

Appendix V 2: Moisture results for Kendu Bay samples

	Amaranthus hybridus	*Justicia flava*	*Crotalaria brevidens*	*Vigna unguiculata*	*Solanum nigrum*	*Cleome gynandra*
Mass of wet sample (g)	4.42	3.84	2.12	4.06	3.15	4.05
Mass of dried sample (g)	0.90	0.81	0.52	0.69	0.44	0.62
Moisture mass (g)	3.52	3.03	1.60	3.37	2.71	3.43
% moisture	76.63	78.91	75.47	83.00	86.03	84.69

Appendix VI: Titration

Appendix VI 1: Standardisation of potasium iodate

Titrand: 0.0057 M ascorbic acid

Titrant: KIO_3

Aliquot: 25 ml

	I	II	III
Final burette reading (ml)	19.1	18.9	19.0
Initial burette reading (ml)	0.0	0.0	0.0
Volume of titrant used (ml)	19.1	18.9	19.0

Average titre : 19.0 ml

Molarity of KIO_3 from the above results: 0.0025M

Appendix VI 2: Titration of vegetable samples

Titrant : 0.0025M KIO_3

Aliquot: 25 ml

Macerated sample : 25.0 g

Kendu Bay and Homa Bay samples

	VOLUME OF TITRANT USED (ml)							
VEGETABLE SAMPLE	TEST 1		TEST 2		TEST 3		Average titre	
	KB	HB	KB	HB	KB	HB	KB	HB
Cleome gynandra	1.6	1.2	1.2	1.1	1.1	1.2	1.15	1.16
Vigna unguiculata	1.6	1.3	1.5	1.3	1.5	1.1	1.53	1.23
Justicia flava	1.5	1.1	1.6	1.0	1.4	0.9	1.50	1.00
Solanum nigrum	1.0	1.2	1.0	1.2	1.0	1.2	1.0	1.20
Amaranthus hybridus	2.3	1.1	2.2	1.1	2.1	1.2	2.2	1.13
Crotalaria brevidens	1.3	1.4	1,3	1.4	1.4	1.4	1.33	1.40

Appendix VII: Reproducibility

Appendix VII 1: Instrument reproducibility tests

Parameter	Method	Actual Conc. (ppm)	Conc. (ppm)	Abs	Mean Conc.	SD
K	AAS	3.0	3.1566	0.1598		
		3.0	3.3808	0.1711	3.1618	0.1766
		3.0	2.9482	0.1492		
Mg	AAS	3.0	3.9809	1.7744		
		3.0	2.4682	1.1001	3.1221	0.6343
		3.0	2.9173	1.3003		
Ca	AAS	3.0	3.1750	0.2207		
		3.0	3.0814	0.2142	3.0335	0.1392
		3.0	2.8441	0.1977		
Fe	AAS	3.0	2.9144	0.1529		
		3.0	3.0993	0.1626	3.0967	0.1478
		3.0	3.2766	0.1719		
Mn	AAS	3.0	3.0277	0.2953		
		3.0	3.1014	0.3030	3.1740	0.1577
		3.0	3.393	0.3315		
K	FP	3.0	3.0280	30		
		3.0	2.9345	29	3.0903	0.1588
		3.0	3.3084	33		

Appendix VII 2: Horwitz Ratio and % Recovery

Parameter	Method	Mean conc (ppm)	SD	% Recovery	RSD_r	RSD_R	HorRat value
Potassium	AAS	3.16180	0.1766	105	5.5854	13.45	0.415
Magnesium	AAS	3.1221	0.6343	104	20.3164	13.48	1.500
Calcium	AAS	3.0335	0.1392	101	4.5887	13.54	0.33
Iron	AAS	3.0967	0.1478	103	4.7728	13.50	0.35
Manganese	AAS	3.1740	0.1577	105	4.9685	13.44	0.36
Potassium	FP	3.0903	0.1588	103	5.1386	13.50	0.38

Appendix VII 3 : Correlation graph for AAS Vs Flame photometer for Homa Bay samples

AAS	FP
240.26	250.7
206.09	226.62
40.57	43.39
306.13	323.05
243.8	298.94
39.14	43.69
27.053	30.2804

0.993476