NUTRIENT AND ANTINUTRIENT CONTENT IN LEAVES OF SELECTED COASTAL KENYA CASSAVA VARIETIES AS AFFECTED BY MATURITY STAGE, LEAFAGE AND PREPARATION METHOD

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DEDICATION

This work is dedicated to my loving husband, Isaac Kaptum Boit; my children, Graham, Hollyne and Griffin and to my parents, Mr. and Mrs. Jimmy Waluchio. God bless you all.

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LIST OF ABBREVIATIONS

CLM___ Cassava Leaf Meal

DW____ Dry Weight

FAO___ Food Agricultural Organization

FGD___ Focused Group Discussion

GOK__ Government of Kenya

HCL__ Hydochloric Acid

HCN___ Hydrocyanic Acid

IDA___ Iron Deficiency Anaemia

IDD___ Iodine Deficiency Disorders

KALRO___ Kenya Agricultural Livestock and Research Organization

KCN___ Potassium Cyanide

KEMRI___ Kenya Medical Research Institute

MOH___ Ministry of Health

NACOSTI ___ National Commission for Science, Technology and Innovation

NIH___ National Institute of Health

RDA__Recommended Daily Allowances

SPSS ___Statistical Package for Social Sciences

UNICEF__ United Nations Children's Emergency Fund

UON___University of Nairobi

VAD___ Vitamin A Deficiency

WHO___ World Health Organization

OPERATIONAL DEFINITIONS

Anti- nutrients_ they are natural or synthetic compounds that interfere with the absorption of nutrients**.** They include; cyanide, oxalates, tripsin inhibitors, phytates and phenolic compounds among others.

Bioavailability- is the degree to which food nutrients are available for absorption and utilization in the body. It is the ease with which any nutrient can make its way from the food you eat into your body.

Cassava_ is any of several tropical American plants belonging to the genus Manihot, of the family Euphorbiaceae and species esculenta or dulcis. They exist in varieties as *Manihot esculenta* (bitter cassava) and *Manihot dulcis* (sweet cassava) cultivated for their tuberous roots, which yield important food products.

Cultivar_ A cultivar is a plant or grouping of plants selected for desirable characteristics that can be maintained by propagation (e.g. via stem cuttings).

Cyanide_ is a salt or ester of hydrocyanic acid, containing the anion CN^- or the group —CN. The salts are generally extremely toxic.

Harvesting stage_ this is the period when the crop/leaves are ready for consumption.

Leaf age- Position of the leaf on the stem i.e. from the apex downwards as the plant matures.

Maturity stage_ this is the age in months reached by the plant as at the time of harvesting.

Nutrients_ substances in food required by the body for energy, growth and development, and protection.

Utilization_ it is the making use of something and in this case it is how use of cassava leaves are used.

GENERAL ABSTRACT

In Kenya, different cassava varieties have been bred for high root yields and low cyanide content, and plant drought and disease resistance for improved food security. However, despite the high protein, vitamin and mineral contents of cassava leaves, limited information exists on utilization and the level of the nutrients and anti-nutrients in the leaves of different cassava varieties' at different plant maturity stages and leafages. This study was conducted to determine the current mode of utilization and preparation of cassava leaves, effect of plant maturity stage, leaf age and preparation methods on nutrients (β carotene, Vitamin C, Zinc, Iron and Calcium) and anti-nutrient (Cyanide, Nitrates, Oxalates, Phytates and Tannins) content in raw and prepared cassava leaves of selected coastal Kenya varieties. A survey was conducted to determine the households' utilization and preparation of the cassava leaves. The 2^{nd}, $3^{rd,\ 4th}$ and 5^{th} leaves of the three popular varieties: - *Kibanda Meno, Karembo and Tajirika* were harvested at 3, 6 and 9 months after planting and separately analyzed for nutrients and anti-nutrients. The survey indicated that cassava leaves are popular vegetables in coastal region and all the types of cassava varieties are used. There were no significant differences ($p > 0.05$) in Crude protein, Zinc, Iron, Cyanide, Oxalates and Nitrate content of raw cassava leaves at different leafages; however there were significant differences ($P<0.05$) in protein content among the three cassava varieties. The Crude protein, Zinc and Iron content were higher at 3 months plant maturity stage; while Vitamin C and Calcium contents were higher ($P<0.001$) at 9 months. The Crude protein, β carotene, Zinc, Iron, Vitamin C and Calcium content ranged from 20% to 35%, 9.07 to 22.09, 11.7 to 135.2, 21.8 to 203.8, 27 to 1087 and 124 to1545mg/100g, respectively. The Cyanide, Oxalates, Tannins and Nitrates content ranged from 324.6 to 1849 mg/kg, 29.54 to 49.04g/100g, 1208 to 3474, 21.2 to 72.7 mg/100g, respectively for all the three varieties and leaf ages.

The Crude protein, β carotene, Zinc and Iron in the leaves decreased as cassava plants matured while the cyanide, tannins, oxalates and nitrates increased with plant maturity. Tajirika and Kibanda Meno varieties exhibited high levels of nutrients and low levels of anti-nutrients, hence most preferred. The most appropriate harvesting stage was at 6 months compared to 3 and 9 months. The raw cassava leaves traditional preparation methods of pounding, fermentation and blanching/solar drying significantly ($p<0.05$) lowered the nutrient and anti nutrient content. The Cyanide content ranged 170-380 for blanched/solar dried/boiled, 260-410 for fermented/boiled and 150-320 mg/kg for pounded/boiled. The average losses of the anti-nutrients Cyanide, Tannins, Nitrates, Oxalates and Phytates were: 83, 76, 46, 16 and 88 % through pounding-boiling and 72, 85, 66, 48 and 54% through solar drying/boiling, respectively while the losses were 63, 86, 26, 59 and 23% through fermentation/boiling. Average Vitamin C and β carotene retention were 18% and 61% in blanched/solar drying, respectively while retention of Vitamin C and β carotene in pounded leaves were 52 and 63%, respectively. Iron and Calcium levels slightly increased to 109 and 159%, 112 and 114% with fermentation and solar drying, respectively. The best preparation method was pounding which reduced the Cyanide, Tannins and Phytate content followed by solar drying. Therefore, reducing the anti-nutrient toxicity is thus essential to encourage consumption of cassava leaves for their nutritional value.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Food security and poverty mitigation are some of the most important factors that the country must deal with in order to achieve vision 2030 (Mwan'gombe, 2013). The emphasis by the Kenyan Government through the Ministry of Agriculture, Livestock and Fisheries to improve food security through use of high yielding, drought and disease resistant crops like cassava is a strategy towards achieving vision 2030. There has been wide promotion of cassava (*Cassava esculenta* Crantz) production and utilization in tropical countries, Kenya included. This has promoted the production and utilization of cassava in many parts of Kenya; with about 60% of all the cassava produced originating from Coast, Western and Eastern regions (Karuri, 2001). Cassava (*Cassava esculenta* Crantz) is mostly grown by small scale farmers for household use and to a limited extent for sale.

The cassava is known to perform better than cereals such as maize and sorghum under environmental stresses of poor soil conditions, unreliable rainfall and poor crop husbandry. (Githunguri, *et al*., 2007). According to (Chavez, 2000), approximately 500 million people use cassava roots as the main product in their basic diet. Cassava roots are mostly harvested either in piecemeal or uprooting of whole plant, with minimal harvesting of the leaves. The leaves although utilized by some communities, are left to drop off as waste. In some parts of Kenya, cassava leaves are harvested from the third through to the eighteenth month and utilized as vegetables. The use of cassava leaves gives a positive nutritional balance since they have higher contents of protein, vitamins, minerals and fibre (Wobeto *et al*., 2006).

Even though the leaves have high vitamin and mineral content they have been shown to have considerable levels of anti-nutrients. These anti-nutrients bind both minerals and nutrients making them indigestible by humans (Wobeto, *et al*., 2007).

Although cassava roots are basic diet by majority households, they are of low in nutritional value (1.4% proteins and ash 0.6%). Therefore, households that continuously consume cassava roots, as staple food, are exposed to nutrient malnourishment, especially protein and mineral deficiency. Cassava leaves and roots have been reported to have anti-nutrients such as phytates, tannins, and oxalates that bind micronutrients like zinc, iron and calcium making them unavailable and contributing to micronutrient deficiencies and malnutrition. Further, most cassava consuming African countries can substitute the roots as a staple food with the use of leaves as vegetables due to their high nutrient density (Chavez, 2000). This can ensure household food and nutrient security (Chavez, 2000). The levels of these nutrients and anti-nutrients in commonly grown in Kenyan cassava varieties and how they vary with maturity of the cassava plant and harvesting stage is not known. However, limited information exists on the utilization of the leaves and the level of the anti-nutrients in different cassava varieties' leaves grown in Kenya. This study was conducted to establish the content of anti-nutrient and nutrients in leaves of selected coastal Kenya cassava varieties as affected by maturity stage, leaf age and preparation methods.

1.2 Statement of the Problem

In Kenya, during dry season there is food insecurity and rural communities solve this problem by producing drought resistant crops like cassava. However, most of the cassava producing communities utilize only the roots that are deficient in micronutrients and proteins, hence the possible increase in micronutrient deficiency diseases.

Access to green leafy vegetables during the dry season is made difficult due to the unavailability and the expense involved. A few communities use cassava leaves as vegetables, however the problem with cassava leaves is the presence of anti nutrients that bind the essential minerals like iron, calcium and zinc making them unavailable. Levels of nutrients and anti nutrients vary as the leaf matures and also with the plant maturity. Research has confirmed that levels of anti nutrients and nutrients may decrease or increase with plant maturity. In Kenya, cassava is grown for tubers and not for leaves hence no data exists on nutrients and anti nutrient levels in different cassava varieties.

Several communities who utilize the leaves use different methods to prepare the cassava leaves. Boiling, sun-drying and fermentation are common processes that can reduce anti-nutrients in green leafy vegetables like cassava leaves. However, the extent of reduction for cassava leaves is not known. In Kenya, roots are uprooted during harvesting and the leaves are utilized fresh. This may increase food insecurity thus there is need for preservation of the cassava leaves. However, little information exists on the utilization of the leaves, levels of the nutrients and anti-nutrients and means of preservation of Coastal Kenya varieties as affected by maturity and preparation methods.

1.3 Justification

Due to climate change phenomenon there is need to improve food security through use of drought resistant crops like cassava. Kenyans have been reported to have a very low dietary diversity, hence the need for alternative sources of food and nutrients. There are more than thirty different cassava varieties grown in Kenya. These cassava cultivars are utilized mainly for their roots because they provide much needed calories. The roots however, are deficient in protein, minerals and vitamins (Montagnac, 2009).

A few communities especially in Coastal Kenya utilize the leaves as vegetables despite the scare of the high "cyanide" content. In some cases, these leaves are left to drop off and rot in the field. Levels of cyanide and other anti- nutrients may differ relative to the part of the plant, maturity stage at harvesting time; hence the need to establish the best time for harvesting of the leaves in order to harness the nutritional benefits. Establishing the effects of the current common preparation methods on reduction of anti-nutrient contents while retaining adequate nutrients and micronutrients in cassava leaves will contribute towards improving their utilization. Further, encouraging the use of cassava leaves as vegetables can only be necessary only when data on nutrient and anti-nutrient content are known. Minerals and protein malnutrition are a major public health concern thus promotion and utilization of cassava leaves that are high in minerals, especially the micronutrients and proteins; as green vegetables may contribute to the fight against micronutrient malnutrition.

1.4 Aim

The study aimed to contribute towards improving utilization of cassava in Kenya.

1.5 Purpose

This study was conducted with an intention to create awareness on importance of cassava leaves in community nutrition, contribute to policy on the best and safe use of cassava leaves and help to reduce micronutrient deficiencies thus promote cassava utilization in Kenya.

1.6 Hypothesis

1. Nutrient and anti-nutrient levels in cassava leaves of different cultivars' at different maturity stages and leaf age are not significantly different.

2. The mode of utilization and preparation of cassava leaves among communities of coastal Kenya is not well documented.
3. Preparation methods used by Coastal residents do not reduce levels of nutrients and anti-nutrients in cassava leaves.

1.7 Objectives

1.7.1 General Objective

To determine the anti-nutrients and nutrient content in leaves of selected coastal Kenya cassava varieties as affected by maturity stage, leaf age and preparation methods.

1.7.2 Specific objectives

1. To determine the effect of cassava plant maturity stage and leaf age on nutrients (Beta carotene, Vitamin C, Iron, Zinc and Calcium) and anti-nutrients (Cyanide, Nitrates, Oxalates, Phytates and Tannins) content in raw cassava leaves of selected popular coastal Kenya varieties.
2. To assess the modes utilization and preparation methods of cassava leaves among communities of coastal Kenya.
3. To determine the effect of pounding, solar drying and fermentation on nutrients (β-carotene, Vitamin C and minerals (Iron, Zinc and Calcium) and anti-nutrients (Cyanide, Nitrates, Oxalates, Phytates and Tannins) content of prepared cassava leaves.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview of Cassava

Cassava is an important, adaptable and unexploited crop in Kenya and is known as Manihot *esculenta* Crantz (Olsen and Schaal, 1999). It is also known as mandioca, yuca, and manioc by the rural populations in various countries (UNICEF, 2007). Cassava (Manihot *esculenta* Crantz) originated from Brazil (Olsen and Schaal, 1999) where it has been cultivated for more than five hundred years, since its introduction by the Indians in Latin America. The introduction of this crop later to Asia and Africa proved to be highly beneficial to the poor populations who formed the majority in these continents.

Cassava, *Manihot esculenta Grantz* (Euphorbiaceae), is a drought tolerant crop that successfully grows on soils that are considered as marginal providing harvests in areas that do not favour the growth of other crops (Hahn et al. 1987; Hahn and Keyser 1985). Some of the conditions that support the growth of cassava include annual rainfall that ranges between fifty and five thousand millimetres, 18 to 25°C in temperature, and a soil pH of between 4 and 9 (Balagopalan *et al.*, 1988). The roots of Cassava are an important carbohydrate source for over 800 million individuals across the globe (Mbanzibwa *et al.,* 2011). More than 70 million individuals globally get 500 calories of their daily caloric intake from cassava roots (Westby, 2008). In Africa, cassava is considered to be third among the major sources of carbohydrates, while it is second to maize in terms of the most popular food crops in coastal and western Kenya (Karuri *et al.,* 2001). The marginal and semi-arid areas of Kenya use cassava as a staple food that has the potential to provide a solution to the problem of food insecurity that these areas face as the crop is tolerant of drought.

Cassava is able to withstand prolonged periods of drought and pest with an annual production close to 662,405 MT fresh roots against a 1,204,800MT annual demand (FAOSTAT, 2014). Cassava leaves have also proven to be highly nutritious, providing minerals, proteins, and vitamins (Eggum, 1970; Gomez and Vadivieso, 1985; Ravindran and Ravindran, 1988). In addition, the roots and leaves are available all year round (Ntawuruhunga and Legg, 2007). This makes cassava a highly dependable crop for food security, especially areas that are prone to droughts (Westby, 2008). Eighty percent (80%) of the land mass in Kenya constitutes of agro-ecological and semi-arid areas, supporting almost 95% of the population of poor people (Mwan'gombe *et al*., 2013)**.**

2.2 Nutritional contents and utilization of cassava

Cassava leaves and roots are nutritionally rich and hence provide a reliable source of food (FAO, 2004). Cassava root is basically a carbohydrate source and is rich in calcium and vitamin C. Between 80 percent and 90 percent of the dry weight (DW) of the cassava roots is made of carbohydrates, 80 percent of which is starch, while a small portion comprises of sugars (Gil and Buitrago, 2002; Tewe and Lutaladio, 2004). Contrary to this, the amount of vitamins and minerals in cassava roots are low, with a high quantity of phosphorus and calcium. In addition, the amount of proteins and lipids found in the roots is lower than that found in the leaves (Montagnac *et al*., 2009). More of the protein in the roots is found in the peel, which is lost upon peeling of the product during preparation (Jacquot, 1957). As compared to a fresh white egg, there is a high level of protein in cassava leaves, with the amino-acid profile being well balanced except for lysine, methionine, and isoleucine (Jacquot, 1957).

Furthermore, cassava leaves have a higher essential amino acid profile as compared to the soybean protein as recommended by the Food and Agriculture Organization's (FAO) (West *et al*., 1988). Some of the minerals found in cassava leaves in high quantities include zinc, iron, magnesium, manganese, and calcium, while vitamins include carotenoids, vitamin C, and vitamins B1 and B2 (Wobeto *et al*., 2006; Adewusi and Bradbury, 1993). Nevertheless, little information exists on the nutritional benefits of the cassava leaves and the concentration of proteins in this product.

2.3 Importance of Traditional green leafy vegetables

Both urban and rural populations in Africa mainly depend on indigenous green leafy vegetables for their daily dietary needs considering their availability and their nutritional quality (Muchoki *et al*., 2010). These vegetables have high quantities of minerals and vitamins including vitamins A, B, and C (Mnzava, 1997; Keding *et al*., 2009). Some of the traditional green leafy vegetables have also proven to have medicinal properties that are important to the local communities (Hilou, 2006). These vegetables highly contribute to food security (Yiridoe and Anchirinah, 2005). Most African developing countries face a challenge curbing nutrition and food insecurity, which largely affects children under the age of five, women, and lactating and pregnant mothers (Andersen, 2003). From a cultural perspective, most of the traditional vegetables play different roles among the African ethic groups, who consume different types of these vegetables for various purposes, including their medicinal and therapeutic properties (Mensah *et al*., 2008; Roberts and Tyler, 1999).

Non-starchy vegetables for instance, provide individuals with high amounts of fiber, which is applied in the treatment of diabetes, obesity, gastrointestinal disorders, and cancer (Iniaghe *et al*., 2009). Owing to their richness in micronutrients, green leafy vegetables have a major task in curbing the prominence of micronutrient deficiency across tropical Africa (Ejoh *et al*., 2005).

Some of the areas that cultivate cassava use the leaves as traditional vegetables considering their richness in vitamins and minerals (Rivandran, *et al.,* 1988). These leaves are more convenient as compared to the cassava roots owing to their capacity to be stored in dry form and their low content of water, which makes it cheaper to dry them (Dahniya, 1983). Like other green leafy vegetables, cassava leaves are nutritionally valuable as they have high quantities of Vitamin C, Vitamin A, protein, Calcium, and Iron (Latham, 1979). As such, consumption these leaves allow African communities to compensate for the unavailability of some of the minerals, vitamins, and proteins in the cassava roots.

The popularity of young cassava leaves is higher than that of other vegetables due to their high contents of proteins, minerals and vitamins (Wargiono *et al*., 2002). Thus, various conditions that affect millions of individuals in the tropical and subtropical regions such Vitamin A deficiency, anaemia, and protein deficiency, can be averted through consumption of young cassava leaves as a form of vegetable (Hidajat and Wargiono, 2002). Most of the diets consumed by the poor communities in Africa are majorly starchy, hence, consumption of cassava leaves would supplement these diets with its high content of vitamins and proteins. Cassava leaves form an important consideration in fights against nutritional deficiency and micronutrient undernourishment considering their richness in vitamins, proteins, and minerals (Aletor and Adeogun, 1995; Brown and Kane, 1994; Hidajat and Wargiono, 2002).

One of the major challenge faced in the promotion of cassava leaves consumption as a vegetable is the high content of cyanide in this product as compared to the roots (>200mg HCN equivalents/kg), which occurs as cyanogenic glucoside (Uyoh *et al*., 2007; Etonihu *et al*., 2011). The content in the leaves is between 5 and 20 times higher than in the roots (Bokanga, 1994). In addition to cassava leaves' nutritional valued is also impeded by the presence of tannin, phytin, and cyanide (Reeds *et al*., 1982).

Despite these limitations, there have been various publications that have suggested the consumption of cassava leaves and their nutritional benefits in providing minerals, proteins, and vitamins, as much as they are prepared properly (Akinwale *et al*., 2010; Ayodeji, 2005; Mulokozi et al., 2007).

Cassava leaves are important vegetable that compares with other commonly used traditional vegetables.

Table 1: Comparison of nutrients in (100g edible portion) of cassava leaves with sweet potato and peanut leaves.

Nutrient	Cassava	Sweet-potato	Peanut
Protein (g)	28.1	30.6	26.6
Beta-carotene (mg)	88	75	113.3
Vitamin C (mg)	90.2	141.7	293.3
Iron (mg)	16.7	14.7	16
Zinc (mg)	5.08	3.33	5.33
Manganese (mg)	14.1	-	-
Magnesium (mg)	229.3	493.3	676.7
Calcium (mg)	1509.4	623.3	1236.7

Source: Wobeto *et al*. (2006).

2.4 Micronutrient Deficiency

Besides the obvious problem of food security faced by African countries, these countries also have a challenge of nutritional insecurity especially due to the high rate of micronutrient deficiencies (Falade *et al*., 2003). According to Lonnerdal and Sandstorm (2001), micronutrients could be defined as nutrients that the body needs in small quantities for it to effectively carry out its functions. They include vitamins and minerals, which are important for various body functions including reproduction, growth and development, immune function, and the functioning of the brain, among others (Lonnerdal and Sandstorm, 2001). Most developing countries such as Nigeria have named Vitamin A, iodine, iron, and zinc as the highly significant micronutrients in view of public health (UNICEF, 2007).

This is because of the extent and significance of their deficiencies and effects on health, learning capacities and productivity of affected people. Micronutrient deficiencies increase morbidity and mortality rates not only in expectant and lactating mothers and under fives, who are more vulnerable but also to the general population including vibrant adolescents (Black, 2005). The prevalence of malnutrition and micronutrient deficiencies increase rapidly in under-five children because of rapid growth and development, thus the deficiency of these nutrients endangers the normal health, growth and development of the child. Children may look healthy and their diets may provide adequate energy and protein but are lacking in micronutrients. This is referred to as "hidden hunger" (Black, 2003).

Societies that experience prevalence of micronutrient malnutrition incur high costs in terms of the loss of life, the cost of disease management, hampered economic productivity among the affected, and low quality of life (Shetty, 2011). Close to 2 billion individuals globally experience micronutrient deficiencies such as, anaemia related to iron deficiency, iodine and Vitamin A deficiency, all of which are conditions of major concern to public health, threatening the health of the affected **(**ILSI/FAO, 1997). According a survey done in Kenya back in 1999 to assess the level of micronutrient deficiencies across the country, it was established that a large number of the population is affected by micronutrient deficiency, with 60% and 16% being iron and iodine deficient respectively. On the other hand, 61.2% and 14.7% of children and 29.6% and 9.1% of mothers across the country are moderately and acutely Vitamin A deficient, respectively. At least 50% children, women, and men risk developing Zinc deficiency although, the magnitude of zinc deficiency remains unclear in Kenya as a result of the insufficiency of the data that is presented from national nutritional surveys (MOH/KEMRI, 2009).

In Africa, people have utilized cassava for the reasons that it is drought and disease resistant and also rich in nutrients like; protein, minerals and vitamins.

It comes in many varieties of different cultivars and is cheaply grown from stems (Mwan'gombe *et al.,* 2013). The emphasis by the Government through the ministry of agriculture to improve food security through use of drought and disease resistant crops is a strategy towards achieving vision 2030 (Mwan'gombe *et al.,* 2013). This has promoted the utilization of cassava in many parts of this nation namely; the Coast, Western and Eastern. However, most of the cassava used is the roots hence leaving the leaves to drop off and rot in the field. Not much has been said on the utilization of the cassava leaves yet they possess the same nutrients as are found in the roots. In some areas within the country individuals utilize cassava leaves harvested between the third and the eighth months as vegetables. The cassava leaves also possess ant nutrients just like the roots and hence the need to determine the levels in different varieties of cultivars and if there is a difference in levels as the plant grows older (unpublished information).

2.5 Nutrients

As earlier noted, cassava leaves have high amounts of minerals including magnesium, calcium, manganese, iron, and zinc (Ravindran, 1988). Further, they have high quantities of Vitamin B2, Vitamin C, and Vitamin A (Rivandran, 1988). Calcium forms an important component I the bone formation process, with a daily requirement of 1–2.5 g per day (FDA, 1979) while zinc is vital in the synthesis of nucleic acid and protein, proper bio-membrane integrity, normal development, carbohydrate metabolism, pregnancy and delivery. On the other hand, zinc (Zn) and iron (Fe) are highly important parts of the diet whose deficiencies form a main concern for public health on a global scale (Kayodé APP, 2006). Among all micronutrients, Zn and Fe malnutrition are is of great concern across the whole world, not only due to the seriousness of the health ramifications, but also as a result of the prevalence of deficiencies in these nutrients in Africa and the world at large (Kayodé APP, 2006).

The quality of plant-based diets is also limited by the presence of nutrient inhibitors such as polyphenols, phytates, and oxalates (Gibson, 1994).

2.5.1 Iron

Iron plays different important roles in the body including the formation of myoglobin and haemoglobin. Most of the iron in the body (65%-75%) forms part of the haemoglobin, a red blood cells component responsible for carrying oxygen from the heart across the body (Anderson, 2010). Myoglobin, on the other hand, transports oxygen to muscle cells (Darshan, 2010). In addition, iron is an important component in the energy producing reactions that takes place in the body. Iron occurs either as non-heme or heme iron, with the former found in both plant and animal tissues while the latter is only found in animal tissues products (Darshan, 2010). The heme iron from animals is highly bioavailable with the absorption rate being between 25 and 35 % as compared to that of heme iron, which only has an absorption rate of 3%. Unfortunately, the non-heme iron is the dominant in the diet among poor people from developing countries, including Kenya. Iron deficiency could range from mild depletion of body iron stores with no health or functional effects, to anaemia related iron deficiency which has an impact on the functioning of various organs within the body (Darshan, 2010).

The World Health Organization acknowledges iron deficiency anaemia as being among the most infamous nutrient deficiencies across the globe, which is contributed towards by various reasons, including low consumption of iron in the diet, impaired absorption of iron, or high losses of blood (Darshan, 2010). Several groups including pregnant women, children, women who have reached puberty, adolescents, older adults, and athletes are more exposed to iron deficiency. Some of the foods that are high in iron include; legumes, whole grains, green leafy vegetables, meats and eggs (Darshan, 2010).

2.5.2 Calcium

Calcium level is high in the human body with the recommended daily intake of 1–2.5 g per day. It is required in humans for bones and teeth formation, clotting of blood, enzyme function, nerve transmission and cellular metabolic controls. Some of the foods that provide high quantities of calcium include dried legumes, dark green leafy vegetables, milk and milk products (Nutrition and Dietetics clinical reference manual, 2010). Deficiency of calcium can result into dental carries, stunted growth, rickets and osteoporosis (Walker, 1972; Imungi and Potter, 1983). Calcium bioavailability from vegetables is dependent on the specific vegetable itself while vitamin D is efficient for calcium absorption (Hegsted, 1973). Oxalic acid is the main constituent in some foods that can limit calcium utilization.

2.5.3 Zinc

Zinc forms part of most of the enzymes found in the body and is an important component in metabolism. Zinc deficiency and the related impact in developing countries has not been effectively explored, an aspect that raises a major concern considering the implications that such deficiency may have in terms of pregnancy complications, impaired immune functionality, low birth weight, impaired growth among infants and children, and infant and maternal morbidity and mortality (Shankar, *et al.,* 1998). The manifestation of zinc deficiency is in the form of foetal mal-development and stunted growth among children, which indicates its value to children and women in countries that are developing. Zinc can be found in foods like shellfish, organ meats, dairy products, and eggs. The mineral is also found in plant sources. Nevertheless, plant sources are rich in dietary fibre and phytic acid, which are inhibitors of zinc absorption (Lonnerdal and Sandstrom, 2001).

Of these anti-nutrients, phytic acid (Phytates), which is the main form in which phosphorus is stored in leguminous seeds, legumes, and cereals, is a major Zn absorption inhibitor as it leads to the formation of solute chelates that are insoluble (Lonnerdal and Sandstrom, 2001).

2.5.4 Vitamins

2.5.4.1 Vitamin A (Beta- carotene)

Vitamin A exists as a precursor known as beta-carotene in plants. It is a constituent of rhodopsin which is necessary in the maintenance of epithelial tissues. Vitamin A deficiency is among the most widely, spread nutritional disorder especially in developing countries (Ricardo, 1993). Vitamin A deficiency leads to xerophthalmia, night blindness and eventually complete blindness. It decreases body's resistance to infections (FAO, 1995). Vitamin A is prominent in red and yellow coloured fruits, fortified margarine, milk, and carrots. Fats, protein and zinc are essential and absorption of Vitamin A thus a low diet in these nutrients can lead to vitamin A deficiency.

2.5.4.2 Vitamin C

All compounds that show the biological properties of L-ascorbic acid (LAA) that are needed for maintenance of a healthy skin, prevention of scurvy, and promoting the health of the blood vessels and the gums is can be categorized as Vitamin C. (Harris, 1996). It is used in maintenance of intercellular matrix of cartilage, bone and teeth. Vitamin C facilitates iron absorption. Deficiencies in Vitamin C lead to scurvy and delayed wound healing (FAO, 1995). Vitamin C has various biological functions, including inorganic iron absorption, collagen formation, plasma cholesterol level reduction, immune system enhancement, inhibition of the formation of nitrosoamine, and iron reaction with free radicals such as singlet oxygen. This vitamin is an antioxidant; hence it plays an integral role in reduction of the risk of cardiovascular diseases, arteriosclerosis, and a few cancers (Harris, 1996).

Vitamin C can be found in various sources including vegetables and fruits. Ascorbic acid can be destroyed through cooking, food processing, and storage, hence proper considerations ought to be made during these processes (FAO, 1995).

2.5.5 Proteins

One of the more outstanding nutritional needs of people in the tropics is for higher protein levels in diet. The dry weight of cassava leaves has high amounts of crude protein, of between 29.3% and 32.4% (Eggum,1970), higher than Amarathus, a conventional vegetable, whose protein content forms only 19.6% of the dry weight (Awoyinka *et al*., 1995). Other plant protein sources include; beans peas, green grams among others. Levels of some nutrients in different Kenyan indigenous and local vegetables are as shown in Table 2.

Table 2: Nutritional values of some Kenyan indigenous and local vegetables, per 100g fresh edible portion.

Scientific name	Common name/ Local	Crude protein (g)	β-carotene (mg)	Vitamin C (mg)	Calcium (mg)	Iron (mg)	Dry matter (g)
Vigna unguiculata	Cowpeas/ kunde[1]	4.2	6 -8	70 -100	200 -400	10-15	15-20
Solanum nigrum	Black nightshade /mnavu[1]	3-6	8-10	40-140	250	5-17	18-22
Gynandropsis gynandra	Spider flower/ saget[1]	10-13	6-19	130-180	434	11-15	15-20
Corchorus olitorus	Jute mallow/ mrere[1]	8	4-8	170-210	270	8	20-23
Amaranthus spp	African spinach/ terere[1]	4-5	5-10	90-160	800	5-15	11-15
Clotalaria brevidens	Sunhemp[1]	4-5	3-9	110-130	270	4	-
Basella alba	Indian spinach[1]	5	4	100	250	4	15
Cucurbita	Pumkin leaves[1]	3-5	2-6	170-175	400	9-11	20-25
Brassica oleraceae var.Acephala	Kales[1]	5	2-6	100	250	4	15
Lactuca sativa	lettuce[1]	1.4	0.2-0.6	15	35	1	6
Ipomea batatas	Sweet potato[2]	3.2	2.7	20	85	4.5	13.3
Manihot esculenta	Cassava leaves[2]	28.1	88	90.2	1509.4	16.7	19

Source: Onyango *et al.,* (2005)[1] and Wobeto *et al.,* (2006)[2].

Recommended dietary allowances are as shown in Table 3.

Table 3: Recommended daily intakes by World Health Organization (WHO) for some nutrients

	Age	Vitamin C (mg)	Retinol Equivalents(µg)	Calcium(mg)	Iron(mg)
Children	1	20	300	500-600	5-10
	1-3	20	250	400-500	5-10
	3-5	20	300	400-500	5-10
Boys	5-7	20	300	400-500	5-10
	7-10	20	400	400-500	5-10
	10-12	20	575	600-700	5-10
	12-14	27.5	725	600-700	8-16
	14-16	30	750	600-700	8-16
	16-18	30	750	500-600	5-9
Girls	5-7	20	300	400-500	5-10
	7-10	20	400	400-500	5-10
	10-12	20	575	600-700	5-10
	12-14	27.5	725	600-700	10-20
	14-16	30	750	600-700	13-26
	16-18	30	750	500-600	14-28
Men	18+	30	750	400-500	5-9
Women	18+	30	750	400-500	14-28
Pregnancy Last 3 months		30	750		14-28
Lactation first 6 months		30	1200		14-28

Source: WHO/FAO (2001)

2.6 Anti- nutrients in cassava leaves

Anti-nutrients are also referred to as nutritional stress factors. These factors may either be in the form of synthetic or natural compounds and they impede nutrient absorption. The commonly occurring anti nutrients in plants includes; cyanide, Phytates, nitrates and nitrites, Phenollic compounds and oxalates among others. As much as cassava contains various beneficial nutrients, it also has anti-nutritional and toxic substances, which impair nutrient uptake and absorption of nutrients (Wobeto *et al.*, 2007). However, it has been documented in that various processing methods reduce the levels of some of these toxic substances in vegetables (Ogbadoyi *et al*., 2006).

2.6.1 Phytates

Phytate (inositol hexakisphosphate) is an anti nutrient that controls the intracellular signalling and forms the phosphate storage part in plant seeds; although it binds proteins and minerals in the gastrointestinal tract making them unavailable for absorption and utilization by the body (Rhou and Erdman, 1995). In particular, phytate has a binding effect on multivalent metal ions, including zinc, iron, and calcium, all of which are important nutrients. This leads to formation of salts that are highly insoluble and minerals that are less bioavailability (Rhou and Erdman, 1995).

2.6.2 Tannins/Phenollics

Flavonoids form a set of compounds that are referred to as polyphenolic, such as tannins, which are anti-nutritional agents. Data on polyphenols found in cassava leaves is expressed by researchers as tannin equivalents while employing a non-specific assay (Wobeto *et al*., 2007; Fasuyi, 2005). Polyphenols, which are antioxidants, bind various minerals in food, reducing its bioavailability. In addition, they impair the functionality of digestive enzymes, thus slowing digestion and in some cases cause proteins precipitation (Beecher, 2003). The levels in plants vary and may be influenced by factors like; germination, storage and processing time. Increase phenolic compounds levels are known to decrease fertility among women of reproductive age by altering the levels of hormones, hence affecting the early pregnancy stages (Greenwell, 2000). However, processing by cooking reduces tannin content in green leafy vegetables.

2.6.3 Cyanide

The cyanide, which occurs as cyanogenic glucosides, is a toxic compound that has been associated with adverse health outcomes among humans. The level of cyanide in cassava surpasses 10 mg/kg dry weight, which is the recommended maximum consumption level by the World Health Organization and the Food and Agricultural Organization (FAO/WHO, 1991). This makes cassava leaves highly toxic for consumption by humans. The content of cyanide in cassava roots is much lower (10 times lower) as compared to the leaves, an aspect that explains its utilization for methodological standardization (Haque and Bradbury, 2004). The level of cyanide is cassava is defined by the type of cassava of reference, with each variety exhibiting different levels of this toxic compound. Excessive intake of cyanide is known to cause cretinism and goitre which are associated with iodine deficiency (Nhassico *et al*., 2008). This is as a result of the production of thiocyanate as a by-product of cyanide metabolism, which restricts the uptake of iodide by thyroid gland (Ermans *et al*., 1980). As such, prior to consumption, it is important for cassava leaves to be properly processed in view of reducing the content of cyanide (Gomez and Valdivieso, 1985).

2.6.4 Oxalates

Oxalates are di-carboxylic acids [$(COO)_{22}-$] that are present in plant-based foods such as cassava, which have a negative impact on the bioavailability of magnesium and calcium (Massey *et al*., 2007). These anti-nutritional agents bind calcium, leading to formation of crystals or excretion through urine. The crystals that form (calcium oxalate) majorly contribute to kidney stones. It is highly advisable to reduce oxalates intake and promote the intake of calcium among individuals who are risk of kidney stones (Massey *et al*., 2007). Cassava leaves have an oxalate concentration of between 1.35 and 2.88 g/100 g of total dry weight (Wobeto *et al*., 2007).

Less attention had been given towards the importance of the levels of oxalates in foods until recently, as it was believed that only 10% of the calcium excreted daily was due to dietary calcium (Massey *et al*., 2007). The impact that oxalates have on the health of humans is highly dependent on the calcium available and the oxalate levels consumed. According to Wobeto *et al*. (2007), cassava's calcium-to-oxalate ratio is a high of 5, which surpasses the recommended 0.44%, below which calcium uptake is endangered. As such, the level of oxalates in cassava leaves have no negative impact on calcium uptake. Nevertheless, groups that consume cassava leaves should consider breeding different varieties of cassava to obtain types that have lower levels of oxalates and enhanced calcium. Other anti-nutrients including nitrates, phytates, oxalates, polyphenols, and saponins also reduce the bioavailability of nutrients. The anti nutrient compounds also act as antioxidants and anti-carcinogens depending on amounts consumed.

2.6.5 Nitrates and nitrites

Nitrates occur naturally in most soils and water sources; hence they are taken up by growing plants (Mohri, 1993). Leafy vegetables are the main contributors of nitrates in diets, and contributes about 75% of the total foods ingested (Mohri, 1993). Nitrates in themselves are not toxic at the levels present in most foods but the toxicity occurs when the nitrates are reduced to nitrites (Mohri, 1993). When high levels of nitrates in vegetables are ingested, they are changed to nitrite. This can result in the development of blue-baby disease, metheamoglobinemia, or cancer (Oguchi *et al*., 1996; Takebe and Yoneyame, 1997; Macrae, *et al*., 1997; Gupta *et al*., 2011). However, since nitrates and nitrites are water soluble, some amounts may be lost through leaching during the preparation process. Further, most of the nitrites present are oxidized to nitrate and upon cooking, they leach out of the vegetable (Ricardo, 1993).

Green leafy vegetables with increased levels of nitrates include; spinach, radishes, lettuce, beets, and celery, among others, (Prasad and Chetty, 2008).

Levels of anti-nutrients in some African vegetables are as summarized in Table 4.

Table 4: The anti-nutrient levels of some of the leafy vegetables found in Nigeria in mg/100 g of wet weight

Vegetable	Common Name	Phytates	Oxalates	Tannins	Cyanide	Nitrates
Talinum triangulae	Water leaf	210.54	28.93	1.01	23.81	5.5
Amaranthus hydridus	Green leaf	155	47.35	0.67	24.36	4.99
Manihot esculenta	Cassava leaf	191.25	15.74	0.65	25.69	3.58
Telfariria. occiedetails	Pumkin leaves	84.72	48.17	0.89	25.4	7.88
Solanum nigrum	Night blackshade	97.21	2.99	0.16	24.18	4.08
Crassocephalum crepidiodes	-	249.16	13.2	9.58	24	3.93
Cindosculus aconitifolis	-	313.67	23.11	0.76	28.71	5.1
Moringa oleifera		21		0.012		

Source: Ilelaboye *et al*., 2013

2.7 Preparation of cassava leaves

Cassava leaves are prepared using different techniques by different nations that consume cassava leaves. Both Tanzanian and Congolese communities use cassava leaves as vegetables (Achidi *et al*., 2005). According to a study conducted by Collaborative Study on Cassava in Africa (COSCA), Congolese farmers choose the varieties of cassava that have a large leaf canopy, while Tanzanian farmers cultivate "mpiru" a tree cassava, which produces leaves (Nweke *et al.* 2002). In Rwanda, the leaves are prepared both traditionally and industrially on large scale to allow for preservation. Case in point, cassava leaves could be prepared by plucking seven of the topmost leaves and crushing them using a mortar and pestle until they are soft and liquid oozes from the mixture.

The leaves are then boiled in enough water for 2-3 hours then fried and spiced to desired taste with addition of coconut milk (unpublished work). Further, Indonesia prepares the leaves as follows; the cassava leaves are washed in running water and drained on a coriander (Ngudi *et al*., 2003b). They are then stack in 10 to 15 leaves, rolled and cut into 2 mm wide pieces then put in water in a cooking pot covering them by 1 inch and cooked by a high flame for 10 minutes. The partially cooked leaves are then drained and put in another fresh water covering the leaves and are then boiled on medium heat for 30-40 minutes and until they are tender. The cassava leaves are then added in spicy coconut milk. Food processing techniques for example boiling, fermentation, and sun-drying have been reported to improve palatability, nutrient availability and reduce levels of anti-nutrients (Nyirenda, 2011).

2.7.1 Fermentation

Fermentation has proven to be a highly effective method through which food is prepared and preserved. It has been known to improve palatability, taste, and texture, improve nutritional value and improve food safety of food products (Chelule, 2010). The fermentation process is known to be very effective in elimination of a number of anti-nutritional factors in food. It improves digestibility, utilization of proteins and fatty acids, improves solubility of minerals and reduces gasro-enteric upsets (Chelule, 2010). Food fermentation can be used as a tool in alleviating nutritional defects at household level during food preparation and processing (Sasson, 1988).

2.7.2 Pounding and boiling

Boiling cannot effectively remove cyanide as high temperatures of 100˚C can only denaturing of linamarase, a β-glucosidase that is heat labile, which can no longer be hydrolysed to form cyanohydrins (Cooke and Maduagwu, 1978).

Boiling for about 25 minutes reduces bound glucosides to a range of 45% to 50% (Cooke and Maduagwu, 1978). Cassava leaves have a two-step boiling process as the only safe way to process them. In the first step, cyanogenic glucosides (toxin) are removed from the leaves through pounding, and in the second step the leaves are cooked until tender.

2.7.3 Solar-drying

Drying is the deliberate removal of water from food products. Solar-drying is the oldest method of drying food at a low cost using the sun's ultraviolet rays that can also inhibit growth of microorganisms (Mehas and Rodgers, 1989). The temperatures of food during sun-drying are usually 5^0-15^0C above the ambient temperatures and it takes 3-4 days depending on the product and the prevailing weather conditions. In drying of vegetables, enzyme systems must be inactivated prior to drying and this is usually by blanching.

2.8 Effect of cassava leaves preparation on nutrients and anti-nutrients

Fermentation and solar drying are the most effective methods through which the leaves of cassava can be processed and roots that have an effect on anti-nutrients levels such as those of phenols, cyanide, oxalates, and nitrates, in cassava leaves used as vegetables (Munyoki, *et al*. 2010). The principle behind the different processing methods is to reduce the anti-nutrient levels because reduction of the anti-nutrients in plant foods allows for an increase in the nutrients bioavailability, thus improving the quality if the foods (Munyoki, *et al*. 2010).

Cassava roots are traditionally prepared using different methods, which result into different products that are used variedly in accordance with the local preferences and customs. Some communities found in some of the developing countries use cassava leaves as vegetables, with the roots being used to process different traditional foods (Hahn, 1997).

Studies have confirmed that cassava fermentation results in an increased level of iron and calcium, and a reduced level of magnesium, while the level of zinc remained constant in cassava that has been grated (Adewusi, 1999). Further, the minerals in amaranthus vegetable were reduced by blanching, yet the same process increased the bioavailability of minerals found in cassava leaves.

Blanching and fermentation of cassava vegetables are highly important processes as they increase the availability of minerals. Hence, it is important for such methods to be promoted in order to reduce the level of micronutrient deficiency in developing countries (Adewusi, 1999). Cassava leaves and roots are bulky and highly perishable and they rot within 3-4 days of harvest hence require processing (Hahn, 1997). Cassava leaves and roots have different levels of cyanide that is highly toxic for both animals and humans. In addition, uncooked leaves and roots are unpalatable thus it is important for cassava to be adequately processed so as to reduce their toxicity and to increase the shelf-life of cassava products (Hahn, 1997).

2.9 Gaps in Knowledge

- Variation in levels of nutrient and anti nutrients as affected by cassava plant maturity stage and preparation methods not elucidated.
- Little information exists on the safest or best way to utilize the cassava leaves in Kenya bearing in mind they have both nutrients and anti- nutrients

CHAPTER THREE

EFFECT OF PLANT MATURITY STAGE AND LEAF AGE ON NUTRIENT AND ANTI-NUTRIENT CONTENT IN LEAVES OF SELECTED POPULAR COASTAL KENYA CASSAVA VARIETIES.

3.1 Abstract

In sub-Saharan Africa, cassava is an essential food crop that provides households' daily energy requirements. However, the potential for cassava crop to economically improve nutrition and economic status of rural households is not fully realized. Cassava roots are low in nutritional value (1.4% proteins, and 0.6% ash) as compared to cassava leaves that contain high protein (29.3%), mineral (6.4%) and dietary fiber (26.9%) yet they are rarely utilized in households' diets because of the presence of anti nutrients. The anti nutrients content in different cassava varieties at different maturity stages and leafage are unknown and may increase or decrease with plant or leaf maturity. This study was conducted to determine the effect of maturity stage and leaf age on nutrients (β-carotene, Vitamin C, iron, zinc and calcium) and anti- nutrient (nitrates, cyanide, oxalates and tannins) contents in cassava leaves. The 2^{nd}, $3^{rd,}$ 4^{th} and 5^{th} leaves of three popular varieties: - Tajirika, Kibanda Meno and Karembo were sampled and harvested at 3, 6 and 9 months from Kenya Agricultural and Livestock Research Organization Centre in Mtwapa. The leaves were separately analyzed using proximate and chemical analysis for specific nutrients and anti nutrients at the Chemistry laboratory, University of Nairobi. There were no significant differences ($p > 0.05$) in Crude protein, Zinc, Iron, Cyanide, Oxalates and Nitrate content of raw cassava leaves at different leafages; however there were significant differences ($P<0.05$) in protein content among the three cassava varieties. The Crude protein, Zinc and Iron content were higher at 3 months plant maturity stage; while Vitamin C and Calcium contents were higher ($P<0.001$) at 9 months.

The Crude protein, β carotene, Zinc, Iron, Vitamin C and Calcium content ranged from 20% to 35%, 9.07 to 22.09, 11.7 to 135.2, 21.8 to 203.8, 27 to 1087 and 124 to1545mg/100g, respectively. The Cyanide, Oxalates, Tannins and Nitrates content ranged from 324.6 to 1849 mg/kg, 29.54 to 49.04g/100g, 1208 to 3474, 21.2 to 72.7 mg/100g, respectively for all the three varieties and leaf ages. The Crude protein, β carotene, Zinc and Iron in the leaves decreased as cassava plants matured while the cyanide, tannins, oxalates and nitrates increased with plant maturity. Tajirika and Kibanda Meno varieties exhibited high levels of nutrients and low levels of anti-nutrients, hence most preferred. The most appropriate harvesting stage was at 6 months compared to 3 and 9 months.

Key words: Cassava leaf, nutrients, anti-nutrients, Cassava cultivars, micronutrient, leafage, maturity stage

3.2 Introduction

Cassava roots are consumed as a basic starchy food in Africa (Nweke *et al*., 2002). Some communities for instance in Congo, consume the leaves as green vegetables (Achidi *et al*., 2005). In Coastal Kenya region, cassava is grown as the second main staple food crop (Munga, 2009). The crop is basically grown by small scale farmers for food and to a limited extend for sale. The crop is known to perform better than cereals such as maize and sorghum under environmental stresses such as poor soil conditions, unreliable rainfall and poor crop husbandry (Montagnac *et al*., 2009). In Kenya, more than 30 different cassava varieties are grown in different regions in the country for their roots although some communities utilize the cassava leaves as vegetables. In most areas, the leaves are considered as residues hence left drop off as waste. Even though cassava leaves have high vitamin and mineral content compared to the roots, they have anti-nutrients. These toxic substances bind the nutrients and make them indigestible (Massey, 2007). The anti-nutrient content in fresh cassava leaves and roots vary according to the maturity stage of the plant, and are also dependent on locality, variety, climate and other agro-conditions (Fasuyi and Aletor, 2005).

Young leaves particularly have high content of amino acids and vitamins and are regularly consumed by some communities (FAO, 2007). However, anti-nutrients (Oxalates, Tannins, Phytates and Nitrates) are reported to bind micronutrients like zinc, iron and calcium making them unavailable thereby contributing to micronutrient deficiencies and malnutrition (Wobeto *et al.*, 2006). In most developing countries Kenya included, the major concern is about the nutritionally inadequate diets that result in malnutrition (Black, 2003). Worldwide, micronutrient malnutrition posses a major problem that is much bigger than hunger hence it is commonly referred to as ‘hidden hunger’ (Black, 2003). It imposes huge costs on societies in terms of poor health, deaths, reduced economic productivity and poor quality of life (Shetty, 2011).

Micronutrient deficiencies harmfully impact on health and economic productivity of individuals. It is estimated that half (50%) of the population in Sub-Saharan Africa suffers from iron deficiency while 33% and 90% from zinc and vitamin A deficiencies, respectively (Micronutrient Initiative, 2004). These deficiencies present as anemia, eye disease, poor immunity, impaired neuropsychological development, and stunting (FAO, 2004). The objective of this study was to determine the effect of different plant maturity stages and leafage on nutrients (β- carotene and Vitamin C), minerals (Iron, Zinc and Calcium) and anti-nutrients (Cyanide, Oxalates, Phytates Nitrates, and Phenollic compounds) content in cassava leaves.

3.3 Materials and Methods

3.3.1 Study site

The Coastal region of Kenya has six Counties namely; Mombasa, Kwale, Kilifi, Taita Taveta, Tana River and Lamu. The area covers approximately 83,603 km² with a population of 3,325,307 inhabitants (KNBS 2009 census). The Coast region's largest area is found along the Indian Ocean and is famous for its warm humid weather good for cultivating cassava due to its favourable climate. Kwale County (Kinango and Msambweni Sub- counties) and Kilifi County (Kaloleni and Kilifi Sub-counties) are the major cassava cultivating counties while in Mombasa County cassava is grown in parts of Likoni. Both the leaves and roots are utilized domestically and for sale. A map of the region and specific counties are as indicated. Figure 1.

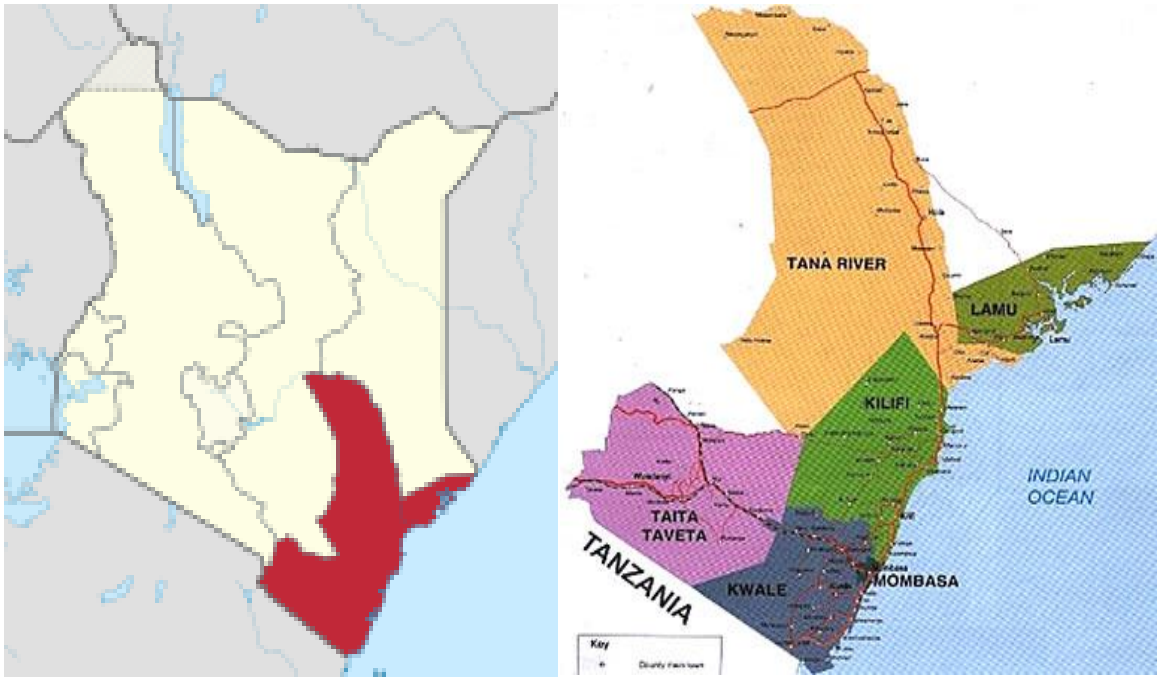

Figure 1: Geographical location of Coastal region in Kenya and its specific Counties

3.3.2 Study design and sample collection

The study was a split-split plot design with three varieties being the main plot, the three month maturity stages being the first split and leaf age as sub-split of maturity stage. Cassava leaves were harvested from Eastern Africa Agricultural Productivity Project (EAAPP) planted cassava fields at Kenya Agricultural and Livestock Research Organization (KALRO), Mtwapa. The 2nd, 3rd, 4th and 5th leaves were harvested from Three popular cassava varieties:-Kibanda Meno, Karembo and Tajirika after 3, 6 and 9 months of planting. Each leaf age was packaged in separate Ziploc bags, stored in cooler boxes with ice packs and transported to the Department of Food Science Nutrition and Technology, Chemistry laboratory at University of Nairobi for analysis within 24 hours after sampling.

3.3.3 Sample Preparation and Analysis

On arrival at the Department of Food Science Nutrition and Technology laboratories the cyanide and moisture contents of the leaves were immediately analyzed due to loss of moisture and volatile cyanide on storage. The crude protein, β- carotene, vitamin C, iron, zinc and Calcium and anti-nutrients (nitrates, oxalates and Phenollic compound) samples were preserved at 8-10^0 C in environmental chamber until analysis. All samples were analyzed in duplicate.

3.3.3.1 Determination of Moisture Content

Moisture content was determined according to AOAC method No. 2001.12 (AOAC, 2005). Approximately 5 gm of raw cassava leaves were weighed using an analytical balance (AR3130E max cap 310) into a previously weighed aluminum moisture dish and dried in a thermostatically controlled hot oven at 105^{0}C to constant weight. The moisture content was obtained by subtracting the empty dish from the residue and aluminum moisture dish weight and dividing over the original sample weight multiplied by 100.

% moisture = difference in moisture evaporated/weight of sample x 100.

3.3.3.2 Total Ash Determination

Total ash was determined by AOAC method No.923.03 (AOAC, 1990). Approximately 2gm of the dried leaves sample was weighed accurately in a porcelain dish previously dried in a hot oven at 105^{0}C and cooled. The cooled tarred dishes were held in a muffle furnace at approximately 600^{0}C for 4hrs, cooled in a desiccator to room temperature and weighed.

The weight of total ash was obtained as the difference between the weight of the empty crucible and the weight of the ash plus crucible. The percentage total ash was calculated by dividing residue weight by original sample weight then multiplied by 100.

3.3.3.3 Determination of Crude Proteins

Protein content was determined as total nitrogen by the Semi micro Kjeldahl method No.955.04 (AOAC, 1990). The total nitrogen was converted to crude protein by multiplying with a factor 6.25.

3.3.3.4 Determination of Ascorbic Acid (Vitamin C)

The ascorbic acid content was determined by indophenols method No.985.01 (AOAC, 1990) and titration with 2, 6 dichlorophenolindophenol dye.

3.3.3.5 Determination of β- Carotene

β- Carotene was determined by the method described by Musa (2010) and Astrup *et al.* (1971). Two (2) grams of the leaves sample was mixed with approximately 0.5 grams of sea sand and was ground in a mortar and pestle in a mixture and the β carotene contents extracted completely with acetone. The homogenate was filtered through glass wool and rewashed with acetone and collected in a 50ml volumetric flask and until the filtrate was colourless. About 25ml of the extract was evaporated in a rotary vacuum evaporator in a water bath at 65^0C. The chromatographic column was prepared by packing silica gel to 15cm depth and two (2) drops of ethanol and petroleum ether each was added to remove any moisture and activate the silica gel. The top of the column was lined with 1mm of anhydrous sodium sulphate to remove any traces of water in the sample.

The evaporated sample was dissolved in 2ml of petroleum ether (boiling point 40^0C-60^0C), then poured into the chromatographic column, and separated through petroleum spirit. The first yellow eluate was collected in a 25ml flask and made to the mark with petroleum spirit. β- carotene fraction was measured at 450nm using a CE 440 UV/VIS Double Beam Scanning Spectrophotometer (Cambridge, England). The spectrophotometer was calibrated with pure petroleum spirit. The absorbance was converted to β-carotene equivalents using the formula. B-carotene equivalent = 0.4/ 0.12 X Absorbance X dilutions/sample weight X 100 (expressed as mg/100g of leaves on dry matter basis).

3.3.3.6 Determination of minerals: Calcium, zinc and Iron

The mineral elements iron (Fe), zinc(Zn) and Calcium(Ca) of samples were determined according to the method of Ezeonu *et al*., (2002) using an Atomic Absorption Spectrophotometer 500 (AAS 500) PG Instruments Limited, Alma Park Wibtoft Leicestershire, England LE175BE. About 2gm of dried ground leaves sample was weighed into a pre-weighed crucible and ashed for 8hrs at 550^0C. The ash was cooled to room temperature and the residue dissolved in 20ml of 20% hydrochloric acid by boiling. Twenty (20) milliliters of distilled water were added and boiling continued until the sample was clear. The contents were filtered through (GE Healthcare Life sciences, Whatman CAT No.1441-150) into 100 ml volumetric flask and 1ml of nitric acid added to the extracts to prevent phosphorus interference. The filtrate was filled to 100 ml mark with distilled deionised water and mixed thoroughly. The extract was then used to determine calcium, zinc and iron levels using a calibrated AAS 500. The amount of elements was calculated against their standards as indicated. Absorbance (ppm)/sample weight X100 = mg/kg = ppm

3.3.3.7 Determination of Cyanide

The cyanide content was determined with method number 915.03B. (AOAC, 1990). Cassava leaves were finely chopped into small pieces to increase surface area to volume ratio and to enable easy cyanide extraction. About 10g of sample was weighed and dissolved in 100ml distilled water in boiling tubes. The mixture was kept at room temperature for 2 hours and distilled in distillation unit until 200mls of the distillate was obtained. The distillate was divided into 2 portions of 100ml each. The Eight (8) mls of 5% potassium iodide (KI) was added to each of the 100ml portions of the distillate and the solutions titrated with 0.02N Silver nitrate ($AgNo_3$) until the solution turns light blue (turbidity). The titre value was obtained and the cyanide content for the sample was calculated as equivalent to 1.08mg of HCN/5 g and then expressed as HCN mg/kg of sample of fresh leaves.

3.3.3.8 Determination of Total Phenolic Compounds (Tannins):-

Total phenolic compounds were determined as tannins by Folin-Denis method (Burns, 1963). About 100g Sodium tungstate, 20g Phosphomolybdic acid and 50ml Orthophosphoric acid were mixed in 750ml water to prepare the Folin-Denis Reagent. The prepared mixture was refluxed for 2hrs, cooled and diluted to 1 litre. About 35g of anhydrous Sodium carbonate was dissolved in 100ml of water at 70^0C -80^0C to prepare Saturated Sodium carbonate solution. The mixture was cooled overnight and then seeded with crystals of hydrated Sodium carbonate and filtered through glass wool before crystallization. A fresh mixture of Follin-Denis reagent and saturated Sodium Carbonate solution were prepared for daily analysis.

About 1-10ml aliquots of the standard tannic acid solution prepared by dissolving 100g tannic acid in one (1) litre distilled water were measured into 100ml flasks containing 75ml of distilled water, five (5) mililitre of Folin-Denis reagent and 10ml saturated Sodium carbonate solution. The different mixtures were diluted to volume (100 mls) with distilled water, mixed thoroughly and left to stand at room temperature for 30 mins. Optical densities were read at 760nm using the CE 440 UV/VIS Double Beam Scanning Spectrophotometer (Cambridge, England). The absorbance was plotted against mg tannic acid/100ml to obtain a standard curve.

An extract was prepared from 0.5g ground leaves sample in a mortar and pestle with 50ml distilled water and filtered. One (1) milliliter of the filtrate was pipetted into 100ml flask containing 75ml distilled water and five (5) milliliter of Folin-Denis reagent and 10ml of saturated Sodium carbonate solution added. The mixture was made to a volume of 100 milliliters, mixed thoroughly and then absorbance read at 760nm using the CE 440 UV/VIS Double Beam Scanning Spectrophotometer (Cambridge, England) after 30mins incubation. Milligram tannic acid per 100g of sample was calculated from the standard curve.

3.3.3.9 Determination of Oxalates

Both soluble and total oxalates content of the samples were determined by titrimetric method as described (AOAC, 1999). About 3mg of Sodium oxalate was dissolved in 10ml of 0.5M Sulphuric acid to prepare the Standard oxalate solution followed by a titration with 0.1M Potassium permanganate at 60^0C using a micro-burette to obtain a faint purple colour that was stable for at least 15 seconds and a standard curve plotted. Approximately 0.1g of the dried sample was extracted with 30ml of 1M Hydrochloric acid in a water bath and boiled for 30 mins. The mixture was cooled and filtered through (GE Healthcare Life sciences, Whatman CAT No.1441-150) Whatman filter paper.

The pH of the filtrate was adjusted to between 8.0- 9.0 with 8M Ammonium hydroxide. The pH of mixture was again re-adjusted to pH between 5.0-5.2 with 6M Acetic acid. About 0.4ml of 5% Calcium chloride was added to a 10ml aliquot and shaken thoroughly. The mixture was left standing at room temperatures (28^0C-30^0C) for 16 hours and then centrifuged at 3000 rpm for 15 mins. The supernatant was discarded and the pellet rinsed twice with 2ml of 0.35M Ammonium hydroxide and drip dried. The drip dried pellet was dissolved in 10ml of 0.5M Sulphuric acid followed by titration with 0.1M Potassium permanganate at 60^0C using a micro-burette to a faint purple colour that was stable for at least 15 seconds. Oxalates content of the sample was calculated from the standard curve prepared earlier as mg/100g of sample.

3.3.3.10 Determination of Nitrates

The nitrate content of the samples was determined by the method described by Cataldo *et al*. (1975). Different concentrations (0, 12.5, 25, 37.5, 50 and 62.5 with standards containing ~0 to 60 µg NO3-N in a 0.25 ml aliquot) of Potassium nitrate were used to prepare the nitrate standard curve and nitrates calculated as equivalent mg/100g sample. The previously dried sample was ground and re-dried overnight in a hot air oven at 70^0C. Approximately 0.1g of the dried sample was suspended in 10ml of distilled water in a beaker and incubated at 45^0C for 1hr to extract the nitrates. The mixture was filtered using (GE Healthcare Life sciences, Whatman CAT No.1441-150) Whatman filter paper and about 0.2ml of the filtrate pipetted into a 50ml beaker. About 0.8ml of 5% (w/v) Salicyclic acid in Sulphuric acid was added and mixed thoroughly and the mixture allowed to stand for 20 min at ambient temperatures (28^0C- 30^0C). About 19ml of 2N Sodium hydroxide was added to the mixture and allowed to cool for 30 mins. The absorbance reading was obtained using the CE 440 UV/VIS Double Beam Spectrophotometer (Cambridge, England) at 410nm.

A blank was prepared using distilled water and the nitrate content expressed as mg/100g of dry sample using a constant determined from the standard curve.

3.3.3.11 Determination of Phytates (phytic acid)

Phytates content was determined by the method No.925.10 (AOAC, 1990). Approximately 0.5g of sample was weighed into a centrifuging tube and 10 ml of petroleum ether added and kept in ultra-sonic bath for 30 min to defat the sample. The mixture was centrifuged at 13,000rpm for 5 min, the supernatant discarded and the residue air-dried. The residue was extracted with 10ml of 2.4% Hydrochloric acid (HCL) by centrifuging for 10 min and the same process repeated 3 times for 5 min. All the supernatants were pooled together and made up to known volume (100mls) with distilled water. About 2 ml of the extract was measured in a test tube and 4ml of distilled water added to it. The wade reagent (2ml of 0.03% hydrated Ferrous chloride ($FeCl_3.6H_20$) and 2ml of 0.3% sulphosalicylic acid) was added and the contents vortexed and centrifuged at 3500 rpm for 5mins. The absorbance of the supernatant was read using the CE 440 UV/VIS Double Beam Scanning Spectrophotometer (Cambridge, England) at 500nm. Distilled water was used as the blank. The phytic acid content was calculated by using the standard curve prepared with different concentrations (0, 0.05, 0.25, 0.5, 0.75 ml) of phosphorus standard solution in distilled water.

N/B. The calculation of phytic acid content assumes that the amount of phosphorus measured is completely released from phytic acid and comprises 28.2% of phytic acid expressed in g/100g on dry matter basis.

Phytic acid (g/100g) = Phophorus [g/100g]/0.282

3.4 Statistical Data Analysis

The data were analyzed using analysis of variance (ANOVA) and separation of means was done using Least Significant Difference (LSD) test calculated at 95% confidence interval using Gen stat 15th edition.

3.5 Results and Discussions

The results for moisture, total ash and crude protein content at different leaf age and maturity stages are shown in Table 5. There were no significant differences ($p>0.05$) in moisture content of the leaves of different cassava varieties' at different leaf ages. Moisture content ranged from 76.2 to 69%, 73.01 to 68 and 72.5 to 69% in Kibanda Meno, Tajirika and Karembo, respectively. The 2nd and 3rd leaves had slightly higher moisture levels as compared to the 4th and 5th leaves. The results are in agreement with Ravindram and Ravindram, (1988) who sampled cassava leaves at different leaf ages and established a similar trend. The implication of the higher moisture levels means high perishability and thus the need for preservation of the leaves.

The ash content ranged between 5.3 and 7.1% in all the leafages of different cassava varieties. There were no significant differences ($p>0.05$) in ash content of the leaves of different cassava varieties' at different leaf ages although a slight increase was observed as the leaves matured. This indicates that mature cassava leaves have a higher mineral composition than younger leaves. The results are in agreement with the study by Ravindram and Ravindram (1988) who sampled cassava leaves at different leaf ages and established an increase in the ash levels with leaf maturity. In addition, the topmost four leaves fall in the same category of young leaves hence the insignificance. Therefore, since mature leaves have higher ash levels, there is need to mix the young and older leaves during preparation to harness all the nutritional benefits.

Table 5: Moisture, Ash and Crude Protein Content of different cassava varieties at different leafage and maturity stages (expressed as percent of edible portion on dry matter basis).

Variety	Month	Leaf age	Moisture (%)	Ash (%)	Crude Protein (%)
Kibanda Meno	3	2	77.96±0.2^{d}	6.63±0.04^{cde}	31.44±0.07^{e}
		3	75.43±0.1^{cd}	6.52±0.1^{cd}	29.76±0.1^{de}
		4	73.43±0.1^{bc}	6.22±0.6^{bcd}	25.33±0.9^{bc}
		5	66.41±0.5^{a}	6.77±0.1^{defg}	21.74±0.6^{a}
	6	2	72.87±0.7^{bc}	7.29±0.3^{fg}	29.64±0.5^{de}
		3	75.2±0.5^{cd}	6.05±0.2^{abc}	26.73±0.6^{cd}
		4	72.67±0.5^{bc}	5.55±0.1^{a}	26.53±0.2^{cd}
		5	72.49±0.6^{bc}	7.26±0.4^{efg}	25.37±0.4^{abc}
	9	2	77.84±0.9^{d}	7.41±0.05^{g}	26.31±0.2^{c}
		3	76.18±0.4^{cd}	6.65±0.09^{cdef}	31.26±0.7^{e}
		4	69.42±0.6^{ab}	6.35±0.01^{bcd}	28.34±1.1^{cde}
		5	69.79±0.9^{ab}	5.74±0.09^{ab}	22.36±0.5^{ab}
Tajirika	3	2	70.45±0.5^{b}	6.51±0.1^{bc}	22.22±0.3^{a}
		3	69.71±0.3^{ab}	6.03±0.1^{ab}	21.52±0.4^{a}
		4	69.83±0.2^{ab}	6.43±0.03^{b}	21.27±0.2^{a}
		5	69.6±0.7^{ab}	6.53±0.2^{bc}	22.15±0.7^{a}
	6	2	73.75±0.5^{c}	6.48±0.03^{bc}	20.51±0.5^{a}
		3	67.03±0.4^{a}	6.67±0.1^{bc}	21.74±o.8^{a}
		4	68.78±0.7^{ab}	6.24±0.1^{ab}	21.63±0.5^{a}
		5	70.05±0.4^{ab}	6.09±0.03^{ab}	23.28±0.6^{ab}
	9	2	74.83±0.1^{c}	7.15±0.1^{c}	22.54±0.3^{b}
		3	67.75±0.7^{ab}	6.52±0.1^{bc}	20.66±0.5^{a}
		4	69.09±0.4^{ab}	5.66±0.1^{a}	20.45±0.8^{a}
		5	67.93±0.3^{ab}	6.15±0.08^{ab}	20.51±0.6^{a}
Karembo	3	2	77.17±0.2^{fg}	5.62±0.07^{abc}	30.81±0.1^{d}
		3	74.9±0.3^{ef}	5.74±0.1^{abc}	26.77±0.4^{bcd}
		4	67.62±0.7^{ab}	5.40±0.2^{ab}	22.87±0.5^{abc}
		5	66.04±0.5^{a}	5.68±0.1^{abc}	20.62±0.5^{a}
	6	2	67.31±0.3^{ab}	6.39±0.2^{c}	25.33±0.6^{bc}
		3	70.54±1.0^{bcd}	5.54±0.3^{abc}	26.79±0.4^{cd}
		4	68.83±0.5^{abc}	5.15±0.09^{a}	23.28±0.4^{abc}
		5	79.03±0.3^{g}	5.36±0.08^{ab}	25.67±1.1^{bc}
	9	2	73.3±1.0^{de}	6.19±0.1^{bc}	22.71±2.4^{ab}
		3	71.91±0.2^{cde}	5.57±0.1^{abc}	26.52±0.7^{bc}
		4	70.75±0.2^{bcd}	5.44±0.04^{ab}	26.32±0.4^{bc}
		5	69.77±0.1^{abcd}	5.24±0.03^{a}	25.52±0.5^{bc}

*Values expressed as percentage edible portion on dry weight basis in crude protein and Total ash

**Values are mean ± Standard Deviation

The Protein content of different leaf ages ranged from 33.5 to 21.35%, 25.5 to 20.1% and 35.02 to 20.8% for Kibanda Meno, Tajirika and Karembo, respectively. The crude protein content was not significantly different ($p>0.05$) at all the leafages in the three cassava varieties but there was a slight decrease in the content of crude protein as the leaf matures. The results are in agreement with Ravindram and Ravindram, (1988), who sampled cassava leaves at different leaf ages and concluded that crude protein levels decrease with leafage. The implication of these results is that during harvesting, there is need to pick the young leaves to be able to obtain adequate protein to alleviate protein malnutrition. The variations could be attributed to variety differences or sampling procedures used.

The results for β- carotene, Vitamin C, Iron, Zinc and Calcium content of leaves of different cassava varieties at different maturity stage and leafage are as shown in **Table 6**.

The β- Carotene content in cassava leaves at different leaf ages ranged from 5.02 to 20.18mg/100g, 10.5 to 27.3 mg/100g and 9.1 to 20.7 mg/100g while the Vitamin C content ranged from 39.5 to 400.8, 130 to 1146 and 42.1 to 1244 mg/100g for Kibanda Meno, Tajirika and Karembo, respectively. β- Carotene and Vitamin C contents were not significantly different ($p>0.05$) at different leafages of different cassava varieties. It is therefore important that during harvesting the leaves are mixed to access the β- carotene and Vitamin C. The mineral profile of cassava leaves compared closely with those reported for other tropical leafy vegetables. The Zinc, Iron and Calcium contents ranged from 11.7 to 135.2, 21.8 to 203.8, and 124 to1545mg/100g at all the leafages in all the three varieties. Zinc content decreased while calcium and iron contents slightly increased with leaf age for the different varieties. These results are in agreement with studies by Ravindran and Ravindran (1988) who sampled cassava leaves at different ages and established similar trends.

The results demonstrate the need to mix the cassava leaves i.e. the topmost five leaves to access the nutrients and micronutrients in the cassava leaves.

3.5.2 Nutrient levels at different plant maturity stages for different cassava varieties

The moisture content of the leaves of the three varieties at different plant maturity stages ranged between 68.98 and 76.56 %, 67.10 and 71.12%, and 69.81 and 72.62% for Kibanda Meno, Tajirika and Karembo, respectively. Moisture content did not differ significantly ($p>0.05$) with the stage of maturity or the cassava variety. This indicates that moisture levels were significantly high in all the three maturity stages of cassava plants These indicates that moisture content is high and thus makes cassava leaves highly perishable and hence the need for appropriate preservation.

The ash content of cassava leaves ranged between 4.0% and 7.7% in all the three cassava varieties and at the different maturity stages. Ash content were significantly ($p<0.05$) lower in the 9 months than in 3 and 6 months cassava leaves. Higher ash levels were observed in the 3^{rd} month as compared to the 6^{th} and 9^{th} months. These values are within the reported ranges of 4.6% to 7.8% (FAO, (1990) and 6.3% to 7.7% (Udoetok, 2012). High ash content indicates a high concentration of minerals as reported by Kendall (2010). In comparing the ash content in cassava leaves with other green leafy vegetables, the ash content in this study were considerably lower than ash content of 12.1% in cowpeas as reported by (Muchoki, *et al*.,2010), of 12.87% in sweet potatoes as reported by (Lwasai, 2012) and of 17.8% in amaranth leaves as reported by (Aduwesi, 1999). The differences could be attributed to the differences in soils, maturity stage or the climatic conditions (Wobeto *et al*., 2006).

Crude protein content of leaves from the different cassava varieties at 3 different maturity stages ranged 20 % to 31.6 %. The crude protein content was significantly higher ($p<0.05$) at 3 months in different cassava varieties as compared to 6 and 9 months. The protein content at 6 and 9 months of plant maturity were however not significantly different ($p>0.05$) for all the cassava varieties. These results agree with the study by Wobeto, *et al*., (2006) who sampled cassava leaves at 12, 14 and 17 months plant maturity and reported that crude protein decreased with the plant maturity. The protein content in this study were within the ranges of 14-40 % and 20-35% as reported in other studies Eggum, (1970) and (Adewusi and Bradbury, 1993), respectively while Awoyinka, (1995) reported protein content of between 29.3% and 32.4% in young leaves from Nigeria. The protein content of the cassava leaves are similar to protein content of other local green leafy vegetables like sweet potato leaves at 24.85% (Antia, 2006) and 30.6% (Wobeto *et.al*., 2006), and cowpeas leaves at 31.8% (Muchoki, *et al*.,2010). Therefore this study concludes that protein content in cassava leaves can be utilized to substitute protein needs in the predominantly cassava roots diet.

The β carotene content in cassava leaves at different plant maturity stages ranged between 12.4mg to 16mg/100g, 13.8mg to 24.2 mg/100g, 9.6mg to 15.6 mg/100g in Kibanda Meno, Tajirika and Karembo, respectively. There was no significant difference ($p>0.05$) in β carotene content at different plant maturity stages except for slight numerical differences. The results indicate that β-carotene content of cassava leaves decrease with increase in plant maturity stage. Similar trends were reported by Wobeto *et al.,* (2006) who sampled cassava leaves at the ages of 12, 14 and 17 and established that β carotene decreased with maturity stage. However, Simao *et al.,* (2013) who sampled cassava leaves at 10, 12 and 14 month of plant maturity stage and indicated the reverse trend. Younger leaves have a high concentration of β-carotene and the content decrease as the plant matures (Simao *et al.,* 2013).

Table 6: β- carotene, Vitamin C, Iron, Zinc and Calcium content of leaves of different cassava varieties at different leafage and plant maturity stages (expressed as mg/100g leaves dry matter basis).

Variety	Month	Leaf age	β-carotene mg/100g	Vitamin C mg/100g	Zinc mg/100g	Calcium mg/100g	Iron mg/100g
Kibanda Meno	3	2	11.55±0.3^{abc}	38.9±0.3^{a}	75.1±0.2^{f}	957.0±0.3^{b}	63.23±0.4^{d}
		3	12.44±0.1^{abc}	59.5±0.5^{b}	67.79±2.0^{e}	1060.0±1.3^{bc}	57.59±0.5^{c}
		4	12.79±0.1^{bc}	77.1±0.8^{c}	53.25±0.7^{c}	1254.0±1.2^{de}	59.66±0.8^{cd}
	6	2	12.61±0.4^{bc}	167.4±0.5^{e}	28.87±0.8^{a}	1379.0±0.9^{e}	72.88±0.4^{e}
		3	18.86±1.0^{e}	170.1±0.7^{ef}	71.79±0.3^{ef}	402.0±22^{a}	57.53±0.8^{cd}
		4	9.42±0.4^{ab}	163.3±0.6^{e}	100.82±1.3^{g}	974.0±4^{b}	54.19±1.0^{c}
		5	9.63±0.4^{ab}	152.7±4.1^{d}	46.99±1.5^{b}	1185.0±33^{cd}	50.91±0.1^{c}
	9	2	12.21±0.5^{abc}	157.9±0.8^{de}	71.4±0.4^{ef}	1375.0±40^{e}	21.07±1.4^{a}
		3	9.07±0.7^{a}	267.9±3.6^{g}	60.69±0.8^{d}	1016.0±50^{b}	24.24±0.5^{a}
		4	16.92±0.5^{de}	285.5±1.0^{gh}	63.22±1.0^{d}	1076.0±19^{bc}	37.17±0.4^{b}
		5	12.32±0.6^{abc}	300.2±0.9^{gh}	53.15±0.1^{c}	1275.0±25de	36.03±0.3^{b}
Tajirika	3	2	20.08±0.2^{cd}	231.5±3.8^{a}	133.29±1.1^{e}	74.3±0.9^{a}	203.8±0.8^{gh}
		3	18.54±0.7^{bc}	272.3±0.3^{a}	112.66±4.9^{d}	55.5±1.3^{a}	138.4±3.1^{e}
		4	21.38±0.4^{cd}	345.4±0.8^{b}	109.12±2.1^{d}	660.7±0.3^{c}	166.3±3.7^{f}
		5	13.2±0.6^{a}	350.7±0.7^{bc}	135.23±5.5^{e}	743.7±0.5^{d}	185.5±4.6^{g}
	6	2	21.93±0.4^{cd}	475.6±2.9^{cd}	82.95±1.3^{b}	750.8±1.3^{d}	119.8±2.5^{d}
		3	20.82±0.3^{cd}	440.4±1.2^{c}	61.5±1.0^{a}	378.3±5.9^{b}	102.9±0.9^{c}
		4	15.54±0.6^{ab}	462.9±1.9^{cd}	94.86±0.6^{c}	400.8±4.8^{b}	125.3±2.4^{de}
		5	14.9±0.8^{a}	386.1±1.4^{bc}	66.8±0.4^{a}	678.5±0.9^{c}	119.3±2.2^{d}
	9	2	22.09±1.2^{d}	453.0±5.0^{cd}	95.43±0.7^{c}	968.9±18.5^{e}	74.0±1.0^{b}
		3	14.84±0.5^{a}	496.0±4.1^{d}	88.09±0.3^{bc}	1277.5±22.3^{g}	54.5±1.5^{a}
		4	22.88±0.4^{d}	755.6±4.7^{e}	65.08±0.4^{a}	1426.4±9.5^{h}	80.2±0.5^{b}
		5	13.38±0.6^{a}	623.6±6.8^{d}	83.55±0.7^{b}	1085.7±6.6^{f}	71.1±0.4^{b}
Karembo	3	2	16.29±0.3^{de}	11.25±0.5^{a}	15.9±0.3^{bc}	104.7±0.1^{a}	31.44±0.4^{de}
		3	13.01±0.5^{abcd}	42.17±1.0^{c}	13.32±0.8^{ab}	168.5±0.7^{c}	34.91±0.5^{ef}
		4	11.27±0.4^{ab}	31.30±0.5^{bc}	11.73±0.2^{a}	131.8±0.8^{b}	29.31±0.6^{cd}
		5	15.51±0.7^{cde}	23.56±0.6^{b}	18.26±0.5^{bc}	170.3±3.9^{c}	37.0±0.6^{f}
	6	2	15.31±0.5^{cde}	62.54±0.9^{de}	22.03±0.8^{d}	178.2±6.5^{c}	34.75±1.4^{ef}
		3	12.85±0.4^{abcd}	56.97±1.0^{d}	17.55±0.5^{c}	167.5±2.5^{c}	23.61±0.5^{ab}
		4	11.97±0.5^{abc}	66.54±0.4^{de}	15.76±0.6^{abc}	213.9±2.9^{de}	26.72±0.4^{bc}
		5	15.96±0.9^{de}	70.45±0.5^{de}	13.87±0.8^{abc}	231.8±2.6^{e}	32.43±0.3^{def}
	9	2	9.48±0.5^{a}	646.67±3.1^{f}	17.36±0.5^{bc}	181.2±0.5^{c}	32.97±0.5^{def}
		3	13.99±0.6^{bcd}	787.67±3.5^{g}	16.82±0.5^{bc}	227.9±0.8^{e}	25.13±0.8^{abc}
		4	17.82±0.4^{e}	1145.63±2.6^{h}	17.61±0.4^{c}	202.3±0.6^{d}	21.85±0.7^{a}
		5	14.79±0.3^{bcde}	1244.32±1.9^{h}	17.43±0.3^{c}	398.5±1.0^{f}	22.41±0.4^{ab}

*Values expressed as mg/100g edible portion on dry weight basis in β carotene, Vitamin C, Iron, Zinc and Calcium content

**Values are mean ± Standard Deviation

The β-carotene content of leaves in this study was lower than the values obtained in other studies. Wobeto *et al*. (2006) reported values of between 55.72 to 64.12mg/100g while Simao *et al.*, (2013) reported 294.77 and 310.88mg/100g. The differences could be due to plant ages, climatic changes, or the fertilizers used (Simao *et al.*, 2013). The β-carotene levels in cassava leaves are comparable with β-carotene of other conventional vegetable leaves, like sweet potato whose values for β-carotene were 75mg/100g (Wobeto, 2006) and 8.99 mg/100g (Mosha, 1997), while (Muchoki,*et al*., 2010) and (Mosha, 1997) reported levels of 33mg/100g and 14.72mg/100g in cowpeas, respectively. Amaranthus leaves in Tanzania and Kenya were reported to have 19.12mg/100g and 5.75mg/100g by Mosha (1997) and Chege, (2014), respectively. These reported values compare well with the cassava leaves hence the β carotene levels in cassava leaves can meet the Recommended Dietary Allowances (RDA's) of 900µg retinol equivalent and 250 - 300µg retinol equivalent for adults and children, respectively**.**

The vitamin C content ranged between 46.5 and 281.6 mg/100g, 151.2 and 1017.6 mg/100g, 27 and 981.1 mg/100g for Kibanda Meno, Tajirika and Karembo, respectively. This indicates that Vitamin C content in cassava leaves, increase with plant maturity. Vitamin C content was significantly high (p=0.001) for all the plant maturity stages in all the varieties with levels being highest in 9 months cassava leaves. These results compare with studies from both (Wobeto *et al*., 2006) and (Simao *et al*., 2013) who reported an increase in vitamin C levels of cassava leaves between 10 and 17 months. Increase in vitamin C with maturity has been reported in other studies with other vegetables. The increase in the vitamin C concentration during heading in *Amaranthus cruentus* agreed with the submission of Chweya, (1993) and Chweya and Nameus (1997) that vitamin C content increased significantly with plant age in *Gynandropsis gynandra* and *Cleome gynandra* respectively.

According to Barros et al. (2007b) Vitamin C content in *Lactarius piperatus* was highest at maturity and lowest in immaturity. These can be attributed to many factors. First, the higher the intensity of light during the growing season, the greater is vitamin C content in plant tissues. This is because (Ascorbic acid) AA is synthesized from sugars supplied through photosynthesis in plants hence the outside fruit exposed to maximum sunlight contains higher amount of vitamin C than inside and shaded fruit on the same plant(Harris,1975). In addition, Nitrogen fertilizers at high rates tend to decrease the vitamin C content in many fruits and vegetables and also Vitamin C content of many crops can be increased with less frequent irrigation. The vitamin C content in the varieties studied was high compared to values reported for other vegetables. Montagnac (2009) studied levels of Vitamin C in cassava leaves and indicated that levels ranged from 60 to 370 mg/100g.

Comparing vitamin C levels in cassava leaves with other vegetables such as peanut leaves and sweet potato, the levels were 293.3 and 308 mg/100g, respectively (Wobeto *et al*,. 2006) while cowpeas leaves had 303 mg/100g (Muchoki, 2007). At 9 months, the vitamin C levels were high in Kibanda meno, Tajirika and Karembo in decreasing order. It can therefore be concluded that since vitamin C levels increase with maturity, then the cassava leaves can be harvested at all the ages of the plant to maximize the benefits. The average levels in raw cassava leaves at 3 months are adequate.

Calcium content in cassava leaves ranged between 342 and 1545, 124 and 1153, 520 and 1086 mg/100g for Kibanda Meno, Tajirika and Karembo varieties, respectively. Results indicated that Calcium levels increased with plant maturity. The reasons could be attributed to the levels of this nutrient in the soils. The levels are high at all the three maturity stages with the levels being highest at 9 months for all the varieties. There were significant differences in calcium levels ($p=0.001$) at all ages in all the varieties. Even though higher levels were observed at 9 months, there were no significant differences in the 6 and 9 months.

The results compare with the study by (Wobeto *et al*., 2006) who sampled cassava leaves at 12, 14 and 17 and established that Ca (Calcium) levels were highest at 17 months. According to Chavez (2000), Calcium levels are between 40 to 1630 mg/100g. The slight differences could be due to differences in soils, varieties, fertilizers or climatic conditions (Wobeto *et al*., 2006).

Results obtained from analysis of cassava leaves showed that zinc values ranged between 48.7 and 121.7 mg/100g, 22.26 and 163.32 mg/100g, 12.2 and 35.69 mg/100g for Kibanda Meno, Tajirika and Karembo, respectively. The levels were significantly higher ($p=0.001$) at 3 months and lower at 9 months. This indicates that zinc levels in cassava leaves decreased with plant maturity. The reason for the decrease could be the same as the findings of (Noggle and Fritz, 2006) that during fruit initiation and development, some metabolites for cellular synthesis and growth substances are translocated from the leaves, stems, and roots to the developing fruits. The results partially agree with the study by Wobeto *et al.*,(2007) who sampled cassava leaves at 12, 14 and 17 months and identified the same trend. According to (Ravindran, 1992), zinc levels ranged from 30 to 63.7 mg/kg DM which are lower than the values in this study. The differences in levels can be attributed to varieties, fertilizers, soils, maturity stage among others.

Determination of iron content showed that values ranged between 270 and 1780 mg/kg for 3, 6 and 9 months plants of different varieties. Iron levels in 3 and 6 months were significantly higher ($p<0.05$), than those in 9 months in the different cassava varieties. There were no significant differences ($p>0.05$) in the 3rd and 6th months cassava leaves even though higher levels were observed in the 3rd month. The levels decreased with plant maturity implying that young plants have high concentrations of iron than older plants. The reasons for the decrease in iron levels may have been attributed to the rapid uptake of mineral by plants during early growth and the gradual dilution that occurs as plant matures (Lanyasunya *et al*., 2007).

These results agree with those of Wobeto *et al*., (2006) who indicated that iron levels in cassava leaves of plants aged 12, 14 and 17 months decreased with maturity. Iron levels in Cassava leaf meal ranged from 61.5 to 270 mg iron/kg DM (Madruga and Câmara, 2000) and hence the results obtained in this study were slightly higher which could be attributed to the difference in cultivars, the soils or the maturity stage. In comparing cassava leaves with other foods like, liver and eggs (Montragnac, 2009) reported levels of 121 mg/kg FW and 58.7 mg/kg FW, respectively. Other vegetables like Amaranthus and spinach have iron levels of about 34.14 and 26.54 mg/100g, respectively (Yadav *et al.*, (2002). Cassava leaves can therefore be utilized as vegetables because their mineral composition is higher than other traditional vegetables.

3.5.3 Anti-nutrients levels at different maturity stages and leafage for different cassava varieties'

The major drawback to the widespread use of cassava leaves as food is "cyanide scare" as its content of cyanogenic glucosides could, depending on the variety, be 6 times higher than in the roots (Reeds *et al*., 1982) Apart from cyanide, tannin and possibly phytin (Reeds *et al*., 1982) may limit the nutritional value of cassava leaves.

The presence of cyanide in all the studied leaves confirmed the earlier reports that all cassava cultivars contain cyanogenic glucoside, in wide disparities according to varieties (CIAT, 2007).

3.5.3.1. Effect of leafage and maturity stage on anti-nutrient levels

The cyanide, oxalate, nitrate and phenollic compound results are as shown in Table **7:**
In determination of cyanide, oxalate ,nitrates and phenollic compounds contents as affected by leaf age results showed that the levels were not significantly different($p>0.05$) in all the varieties.

However, there were slight numerical decreases in anti-nutrient contents as the leaves matured. These observations compare with studies by (Reeds *et.al*., 1982) who sampled cassava leaves and established similar trends. This indicates a concentration of the anti-nutrient levels in young leaves than mature leaves hence the need for proper preparation. According to (Ravindram and Ravindram, 1988) the five topmost leaves from the apex are in the same category and that could be the reason why there were no much significant differences in the anti-nutrient content in relation to leafage.

Cyanide content in the three varieties at the different ages ranged from 409.3 to 633.3, 829.3 to 324.6 and 1849 to 843mg/kg in Kibanda Meno, Tajirika and Karembo, respectively. The cyanide content was significantly higher ($p<0.05$) at 9 months than at both 3 and 6 months with highest levels being observed in Karembo variety. Cyanide levels increased with plant maturity. These results slightly compare with (Wobeto *et al.*, 2007) who sampled cassava leaves at 12, 14 and 17 months and found that cyanide content increased with plant maturity. Cyanide values obtained were within the ranges reported by other researchers, from 189 to 2466 mg HCN/kg fresh weight basis (Fukuba *et al*., 1982) and 800 to 3200 mg HCN/kg dry matter (Ravindran, 1995). Cyanide is known to be distributed throughout the cassava plant, with highest levels in leaves (Etonihu *et al*., 2011). Other studies have confirmed that, cyanide content in leaves ranges from 53 to 1300 mg cyanide (HCN) equivalents/kg DW (Siritunga and Sayre, 2003); Wobeto *et al*., 2007). The levels obtained were high above the recommended 10mg/kg dry matter basis (FAO/WHO, 1991) in relation to levels of cyanide toxicity. The high levels could because of period of harvesting (dry or wet), plant age, climate or variety (Ravindram, *et al*,.1987) the implication of these results is that proper preparation methods are required to reduce the levels to acceptable levels before consumption.

Table 7: Cyanide, Oxalates, Nitrates and Tannins compound content of different cassava varieties at different leaf age and maturity stages (expressed in mg/100g edible portion on dry matter basis).

Variety	Month	Leaf age	Oxalates g/100g	Cyanide mg/100g	Nitrates mg/100g	Tannins mg/100g
Kibanda Meno	3	2	47.8 ± 1.1^{ab}	29.21 ± 3.0^{bc}	67.76 ± 1.0^{gh}	2950 ± 54.9^{de}
		3	35.6 ± 0.2^{a}	42.58 ± 1.3^{d}	63.91 ± 0.4^{fg}	2865 ± 10^{d}
		4	57.1 ± 1.5^{b}	23.22 ± 0.7^{a}	75.11 ± 0.5^{i}	2924 ± 49.6^{de}
		5	69.0 ± 0.5^{b}	22.15 ± 0.5^{a}	71.92 ± 0.5^{hi}	1604 ± 0.6^{a}
	6	2	118.5 ± 0.3^{d}	34.05 ± 3.5^{c}	47.21 ± 0.4^{d}	1809 ± 49^{a}
		3	98.5 ± 0.4^{c}	33.21 ± 0.5^{bc}	62.52 ± 0.6^{ef}	2371 ± 48.6^{bc}
		4	102.0 ± 0.4^{c}	41.78 ± 1.9^{d}	54.7 ± 0.4^{e}	2539 ± 51^{c}
		5	98.1 ± 0.5^{c}	29.36 ± 0.3^{bc}	49.63 ± 0.8^{d}	2255 ± 45.1^{b}
	9	2	148.5 ± 0.7^{f}	53.19 ± 0.6^{ef}	40.84 ± 0.3^{c}	2820 ± 31.5^{d}
		3	117.2 ± 0.4^{d}	49.89 ± 0.4^{e}	41.92 ± 0.4^{c}	3220 ± 48^{f}
		4	154.7 ± 0.5^{f}	55.95 ± 2.5^{f}	36.32 ± 0.6^{bc}	3474 ± 32.7^{g}
		5	136.3 ± 0.6^{e}	48.82 ± 0.6^{e}	30.36 ± 0.5^{a}	3087 ± 43.6^{ef}
Tajirika	3	2	58.8 ± 0.1^{e}	28.02 ± 0.8^{a}	35.24 ± 0.3^{cd}	2574 ± 65^{bc}
		3	52.92 ± 0.8^{d}	48.51 ± 1.1^{efg}	40.09 ± 0.5^{de}	2875 ± 13^{de}
		4	48.95 ± 0.5^{cd}	50.83 ± 1.0^{ef}	43.18 ± 0.4^{ef}	2682 ± 41^{bcd}
		5	44.17 ± 0.3^{bc}	39.32 ± 1.2^{bc}	72.68 ± 1.2^{g}	2479 ± 6^{b}
	6	2	69.54 ± 0.6^{f}	45.32 ± 0.4^{de}	48.07 ± 0.9^{f}	2040 ± 14.2^{a}
		3	42.47 ± 0.4^{b}	26.78 ± 0.6^{a}	37.12 ± 0.3^{cd}	2419 ± 29.1^{b}
		4	48.24 ± 1.9^{cd}	46.52 ± 0.8^{e}	26.25 ± 1.2^{b}	2462 ± 44^{b}
		5	44.6 ± 2.1^{bc}	58.06 ± 0.4^{g}	32.56 ± 0.6^{c}	2625 ± 57^{bcd}
	9	2	45.47 ± 0.5^{bc}	51.74 ± 1.1^{f}	25.49 ± 0.7^{b}	2529 ± 25^{bc}
		3	40.44 ± 0.7^{b}	36.14 ± 0.8^{b}	37.5 ± 0.3^{cd}	2748 ± 5^{cd}
		4	88.35 ± 0.6^{g}	42.65 ± 0.5^{cd}	21.2 ± 0.4^{b}	3293 ± 43^{f}
		5	30.59 ± 0.3^{a}	46.14 ± 0.4^{de}	12.62 ± 1.1^{a}	3104 ± 39^{ef}
Karembo	3	2	101.3 ± 1.3^{b}	28.48 ± 0.5^{cd}	59.22 ± 0.3^{ef}	2349 ± 8.3^{e}
		3	114.9 ± 0.4^{c}	38.3 ± 0.8^{fg}	39.24 ± 0.4^{d}	2264 ± 22.5^{e}
		4	144.4 ± 0.5^{f}	17.79 ± 0.3^{b}	42.42 ± 1.1^{de}	1394 ± 3.5^{b}
		5	71.7 ± 1.2^{a}	34.11 ± 1.0^{ef}	41.75 ± 0.5^{de}	1208 ± 3.8^{a}
	6	2	129.5 ± 1.3^{d}	35.73 ± 1.1^{f}	44.59 ± 0.6^{e}	2836 ± 40^{fg}
		3	150.4 ± 0.4^{f}	30.69 ± 0.8^{de}	34.32 ± 0.7^{c}	2976 ± 2.6^{fg}
		4	136.5 ± 0.4^{e}	26.46 ± 1.4^{cd}	39.63 ± 0.5^{d}	1946 ± 4.7^{d}
		5	132.3 ± 0.5^{de}	25.81 ± 0.3^{bc}	29.55 ± 0.7^{bc}	1856 ± 14.9^{cd}
	9	2	131.8 ± 0.4^{de}	20.39 ± 0.6^{b}	28.42 ± 0.5^{b}	2408 ± 46.9^{ef}
		3	130.7 ± 0.3^{e}	30.93 ± 1.2^{de}	21.22 ± 0.4^{a}	2022 ± 7^{cd}
		4	189.0 ± 0.6^{gh}	35.33 ± 0.4^{f}	34.21 ± 0.1^{c}	1735 ± 4.03^{c}
		5	178.4 ± 0.2^{g}	12.04 ± 1.5^{a}	29.72 ± 0.3^{bc}	2250 ± 41.9^{e}

**Values expressed as mg/100g edible portion on dry weight basis in moisture, protein and ash

*Values are mean ± Standard Deviation

Oxalate content in the three cassava varieties at different maturity stages ranged between 29 and 49 g/100g with levels increasing with plant maturity. Oxalate content for different cassava varieties at different ages were not significantly different ($p>0.05$). Although there were no significant differences in all the varieties at all ages, oxalate levels were slightly lower in 3 months as compared to 6 and 9 months. These levels slightly compare with (Wobeto *et al*., 2007) who sampled the cassava leaves at 12, 14 and 17 months and found that oxalate content was not significantly different in the three ages although the levels were lowest in 12 months cassava leaves. Oxalate content ranged from 1.35 to 2.88 g/100 g DM for cassava leaf meal (Corrêa, 2000; Wobeto *et al*., 2007) in Brazil. The levels in this study are slightly higher than the reported values. The differences could be because of differences in the plant age, soil composition or edaphic conditions (Simao *et al.*, 2013). Oxalates reduce calcium and magnesium utilization by binding them and making them indigestible although the negative effect of oxalates on humans depends on the levels (Massey, 2007). Oxalate content in cassava leaves were high compared with other vegetables. Cowpeas for instance had 18.89 g/100g (Muchoki *et.al*., 2010) while sweet potato leaves had 33.16 g/100g (Lwasai, 2011). With the high levels in cassava leaves, there is need for proper preparation to reduce the oxalate levels in order to harness the benefits of the mentioned nutrients.

The nitrate content in the varieties studied ranged between 80 and 26.2 mg/100g in all the cultivars for all the ages studied which indicate a decrease with the maturity of the plant. There were no significant differences ($p>0.05$) between the 3^{rd} and 6^{th} months even though higher levels were observed in the 3^{rd} month. Nitrate levels were significantly ($p<0.05$) lower in the 9 months than in 3 and 6 months cassava leaves. This results obtained also slightly compare with the studies by Correa (2000) and Wobeto *et al*., 2007) who also reported a decrease in nitrate levels as the plant matured. Nitrate levels were 43 -310 mg/100g in Brazil (Correa, 2000); (Wobeto *et al*., 2007).

The values in this study are slightly lower. However, considering the varieties studied, Kibanda Meno and Karembo had higher levels than Tajirika making the Tajirika variety most preferable. Cassava-eating populations that consume high amounts of cyanide and high amounts of nitrates and nitrites have the risk of developing stomach cancer (Maduagwu and Umoh, 1988). The mixture develops into high amount of thiocyanate in the stomach due to cyanide detoxification by the body, which may catalyze the formation of carcinogenic nitrosamines (Mirvish, 1983; Maduagwu and Umoh, 1988; Onyesom and Okoh, 2006). Since the levels are high in younger plants, there is need for proper preparation to reduce the nitrate levels in the leaves before consumption.

The tannin content ranged between 1604 and 3474 and, 2040 and 3293, 1208 and 2976 mg/100g for Kibanda Meno, Tajirika and Karembo, respectively. The tannin content in all the three varieties at different maturity stages increased as the plant matures. The tannin content was significantly higher ($p<0.05$) (Appendix 3) in all the three varieties at the three maturity stages although higher levels were observed at 9 months. Results from the current study slightly compare with studies by (Wobeto *et al*. 2007) and (Simao *et al*. 2013) who sampled cassava leaves from plants of ages between 10 and 17 months and concluded that phenollic compound (tannins) increase with plant maturity. In comparing cassava leaves with other vegetables, it was established that tannins levels are high in most green vegetables. Muchoki, *et al*., (2010) recorded values of 2783mg/100g in cowpeas leaves while (Onyango *et al*., 2008) reported values in amaranth leaves to have ranged from 505 to 1056 mg/100g. Tannins, particularly the condensed types, are known to lower protein digestibility by forming indigestible tannin-protein complexes and/or by inhibiting enzyme activities (Price & Butler, 1980). Further, tannins are known to bind proteins and digestive enzymes forming compounds that are not easily digestible by human (Makkar, 1991, 1993).

The implication of the results obtained in this study is that the phenollic levels are high and since they bind protein which is one of the important nutrients in the cassava leaves and a means in preventing malnutrition, there is need to determine a way that can reduce phenollic compounds in cassava leaves.

3.6 Conclusion

Different varieties of cassava varieties at different ages have different levels of nutrients and anti-nutrients. Nutrients and anti-nutrients contents also varied with the plant age. Some nutrients like Vitamin C and Calcium increased with plant maturity while β- carotene, 1ron and zinc decreased with plant maturity. Cyanide content increased with plant maturity while Oxalates were almost equal in all the varieties at the different maturity stages.

Nitrates were high in both 3 and 6 months and significantly lower at 9 months showing a decrease in plant maturity. Tannins were high at all ages but slightly increased with plant maturity. Most of the anti- nutrients increased with plant maturity except for the nitrates that exhibited the opposite trend. These results imply that the most appropriate month for harvesting cassava leaves is 6 months. This is because the cassava leaves at 3 months may have high nutrient levels but the plant is small hence harvesting at this age denies the plant growth nutrients. The levels of anti nutrients are high at nine months hence from the three stages the most appropriate harvesting stage is at six months. Although the levels of most anti-nutrients increased with plant age, the cyanide and tannin levels decreased with leaf age. There were no significant differences in the levels of most nutrients and anti-nutrients at different leaf ages.

3.7 Recommendations

This study recommends harvesting of cassava leaves at 6 months due to high nutrient content and lower anti nutrient content.

1. Since there were no significant differences in the different leafages, the leaves should be mixed during preparation to be able to access all the nutrients concentrated in both young and older leaves.
2. From the results obtained Kibanda Meno and Karembo had higher levels of anti-nutrients than Tajirika. Therefore, the varieties that exhibited the favorable characteristics were Tajirika followed by Kibanda Meno.

CHAPTER FOUR

NUTRIENT AND ANTI-NUTRIENT CONTENT OF FERMENTED, SOLAR-DRIED AND POUNDED CASSAVA LEAVES AND UTILIZATION AMONG COASTAL COMMUNITIES IN KENYA.

4.1 Abstract

Cassava leaves are popular vegetables among the Coastal communities of Kenya. They are preferred because of their colour, taste (sweet-bitter) flavour and availability throughout the year. This study was conducted to determine the effect of processing treatments of cassava leaves on its nutrients (Vitamin C, β-carotene, iron, zinc and calcium) and anti-nutrients: nitrates, oxalates and phenols, cyanide and phytates and oxalates) components. The processing treatments included; pounding-boiling, fermenting-boiling and solar drying-boiling the cassava leaves. Three varieties of cassava leaves commonly used for vegetables included; *Kibanda Meno, Tajirika and Sinitie Nazi* were used. Five topmost leaves from the apex were sampled and collected from different farmers at about 6 months old and transported to Food Science Nutrition and Technology at the Chemistry laboratory, University of Nairobi in cooler boxes with ice packs. Some 250 g of the leaves were solar dried using the forced convectional hygienic solar drier at the Food science department. The other 250 g were fermented in 3% salt and 3% sugar in airtight plastic buckets for 16 days while others were pounded in mortar and pestle. All the products were boiled in water at about 100^0C for one hour until all the water dried. The samples were analyzed using proximate and chemical analyses for specific nutrients and anti nutrients. The raw cassava leaves traditional preparation methods of pounding, fermentation and blanching/solar drying significantly ($p<0.05$) lowered nutrient and anti nutrient content. The Cyanide content ranged 170-380 for blanched/solar dried/boiled, 260-410 for fermented/boiled and 150-320 mg/kg for pounded/boiled.

The average losses of the anti-nutrients Cyanide, Tannins, Nitrates, Oxalates and Phytates were: 83, 76, 46, 16 and 88 % through pounding-boiling. The Cyanide, Tannins, Nitrates, Oxalates and Phytates losses were 72, 85, 66, 48 and 54% through solar drying/boiled while The Cyanide, Tannins, Nitrates, Oxalates and Phytates losses were 63, 86, 26, 59 and 23% through fermentation. Average Vitamin C and β carotene retention were 18% and 61% in blanched/solar drying, respectively while retention of Vitamin C and β carotene in pounded leaves were 52 and 63%, respectively. Iron and Calcium levels slightly increased to 109 and 159%, 112 and 114% with fermentation and solar drying, respectively. The best preparation method was pounding which reduced the Cyanide, Tannins and Phytate content followed by solar drying. Therefore, reducing the anti-nutrient toxicity is thus essential to encourage consumption of cassava leaves for their nutritional value.

Key words: Cassava variety, fermentation, blanching, solar drying, anti-nutrients, nutrients

4.2 Introduction

Cassava is a perennial shrub which is grown throughout the lowland Tropics (Udoetok, 2001). It is an important food crop. The plant has wide and palmated leaves that include 5 in 7 lobes which are used as a vegetable because of their high nutritional value (Eggum, 1970). Cassava grows and produces high tuber yields in areas where maize and other crops will not grow or produce well. It can tolerate drought and grows on soils with a low nutrient capacity (FAO, 2001). It responds well to irrigation or higher rainfall and to the use of fertilizers (FAO, 2001). Cassava is highly flexible in its management requirements, and has the potential of high-energy production per unit area of land. The cassava crop has long been considered as a famine reserve and food security crop (Nweke *et al*., 2002).

In Africa, people have always depended on traditional leafy vegetables to meet their nutritional needs. These vegetables represent a cheap but quality nutrition for large segments of both the urban and rural populations (Nweke *et al*., 2002). These vegetables are rich in vitamins, especially vitamin A,B and C and minerals such as iron, zinc, calcium and phosphorus(Mnzava,1997). In Rwanda, the leaves from three species of cassava, *Manihot utilissima*(bitter), *Manihot dulcis*(sweet) and *Manihot glaziovii*(wild) are valued and highly utilized as green vegetables(Umuhozariho *et al*., 2011). In Kenya, cassava is grown virtually in most parts and it is a major source of income to farmers in agro climatically disadvantaged regions and high potential areas of coastal, central and western Kenya (Githunguri *et al*., 2007). People at the coast, use the cassava leaves for vegetables and especially during dry seasons. The leaves are more nutritionally balanced as they are rich in proteins (17-34% dry weight basis), minerals and vitamins (Eggum, 1970; Gomez *et al*., 1985; Ravindran *et al*., 1988) than the roots that have (1.4% proteins, and 0.6% ash) and can help to prevent certain nutrient deficiencies in humans.

Similar observation has been reported by (Achidi *et al.*, (2005); (Ayodeji, (2005); (Mulokozi *et al.*, (2007) and (Akinwale *et al*., (2010) who also reported the potential contribution of cassava leaves in human nutrition, especially in vitamin A and suggested that if properly prepared households can profitly benefit from their nutrients.

However, leafy vegetables including cassava leaves have a number of anti-nutrients like, cyanide, oxalates, phytates, nitrates and phenols. Their consumption in fresh form is a major health concern. It has been suggested that toxicity problems in cassava leaves may be reduced by preparation methods such as drying, fermenting or pounding and long periods of boiling (Lancaster and Brooks, 1983; Lewis and Fenwick, 1987; Aletor and Adeogun, 1995; Fasuyi, 2005; Ajibade *et al*., 2006). Sun-drying for example has been said to be an inexpensive way to preserve micronutrients in foods (Tontisirin *et al*., 2002) but may reduce ascorbic acid content. Blanching and drying reduces the anti-nutrients, cyanide but unluckly accompanied by loss of nutrients losses (Udofia *et al*., 2010; Oguche G., 2011; Anhwange *et al*., 2011). According to (Akinwale *et al*., 2010) and Faber and Van Jaarsveld (2007) preparation techniques if improved, like in optimizing time of thermal treatment, drying process and preliminary preparations can preserve quality of treated food. The extent to which traditional methods used in processing cassava leaves to reduce anti nutrients are not known. The objective of the current study was to determine the means of utilization and the effect of solar drying, fermentation and pounding on nutrient and anti-nutrient contents in leaves of selected cassava varieties in coastal Kenya.

4.3 Materials and Methods

4.3.1 Focused Group Discussions and Key informant Interviews

The respondents of two focused group discussions (FGD) of 10 and 12 farmers, respectively were randomly selected from the list of active farmers in Kilifi and Kwale Sub-Counties provided by the key informants (the chairmen of the farmers' Association). Names of farmers were written on pieces of paper and a blind- folded individual used to select the 10 and 12 out of the list of 50. The checklist of questions was as shown in Appendix 1.

Two Key Informant Interviews (KII) were conducted among leaders of the teams and County Nutritionists using the same prepared checklists (Appendix 2). The FGD and KII were used to collect information on the varieties of cassava used as vegetables by the coastal people, the harvesting ages and the different preparation methods used. In addition, information on why most of them preferred the cassava leaves as a vegetable was also explored based on several attributes and responses recorded.

4.3.2 Collection of Cassava Leaves

Cassava leaves were harvested from farmers at about 6 months of maturity in both Kwale and Kilifi Counties. About 1 kg of the five topmost leaves of the three most popular varieties namely; Kibanda Meno, Tajirika, and Sinitie Nazi were randomly harvested from different farmers in Kwale and Kilifi counties. The fresh cassava leaves were transported in cooler boxes containing frozen ice packs to the Department of Food Science, Nutrition and Technology laboratory, University of Nairobi (UON), for preparation and analysis of moisture, dry matter, protein, ash, iron, calcium, zinc, beta-carotene, vitamin C and Anti-nutrients (cyanide, tannins, nitrates, oxalates and phytates).

4.3.3 Preparation of the cassava Leaves

The fresh leaves were sorted, de-stemmed, washed in clean water and divided into 3 batches of 250 g each. The first batch was pounded in mortar and pestle and boiled in water for 1 hour. The second batch was blanched in hot boiling water with 3% of salt added to maintain color and improve flavor for 4-5 minutes, cooled in running water and then spread on wire meshed trays and solar dried using the forced air convectional hygienic solar drier installed at the department of Food Science Nutrition and Technology for 5days and later boiled on steady heat for 1 hour. The third batch was cut into 2mm thickness and treated with 3% salt and 3% sugar to increase fermentable sugar content as reported by Muchoki *et al*., (2010) and fermented for 16 days. The fermented leaves were further boiled for 1 hour.

4.3.3.1 Fermentation of cassava leaves

Raw cleaned leaves were cut into 2mm thickness and 500 g of each variety weighed into airtight buckets. About 3% of salt and 3% of sugar by weight were added to increase fermentable sugar content as reported by Muchoki *et al*., (2010). The mixture was gently mixed and the buckets were covered with a sheet of polythene paper. The leave pieces in the bucket were kept under liquid by pressure exerted by placing another sheet of paper filled with water. The buckets were kept at ambient temperatures of approximately 22-25°C for 16 days. The exudate generated from the leaves was subjected to titratable acidity test every 3 days, to ascertain the extent of fermentation changes. The Titratable acidity (TA) of fermenting liquor was determined at specific intervals by addition of 10 ml of distilled water to 2 ml of the liquor, followed by boiling to drive off carbon dioxide. 5 drops of 1% phenolphthalein solution were added and sample titrated with 0.1N sodium hydroxide. Percent lactic acid was calculated as;

Percent Lactic acid= ml alkali x alkali Normality x 9/weight of sample in grams.

NB: 1ml is equivalent to 1g.

The leaves that had undergone the three treatments above were then analyzed for nutrient and anti-nutrient levels.

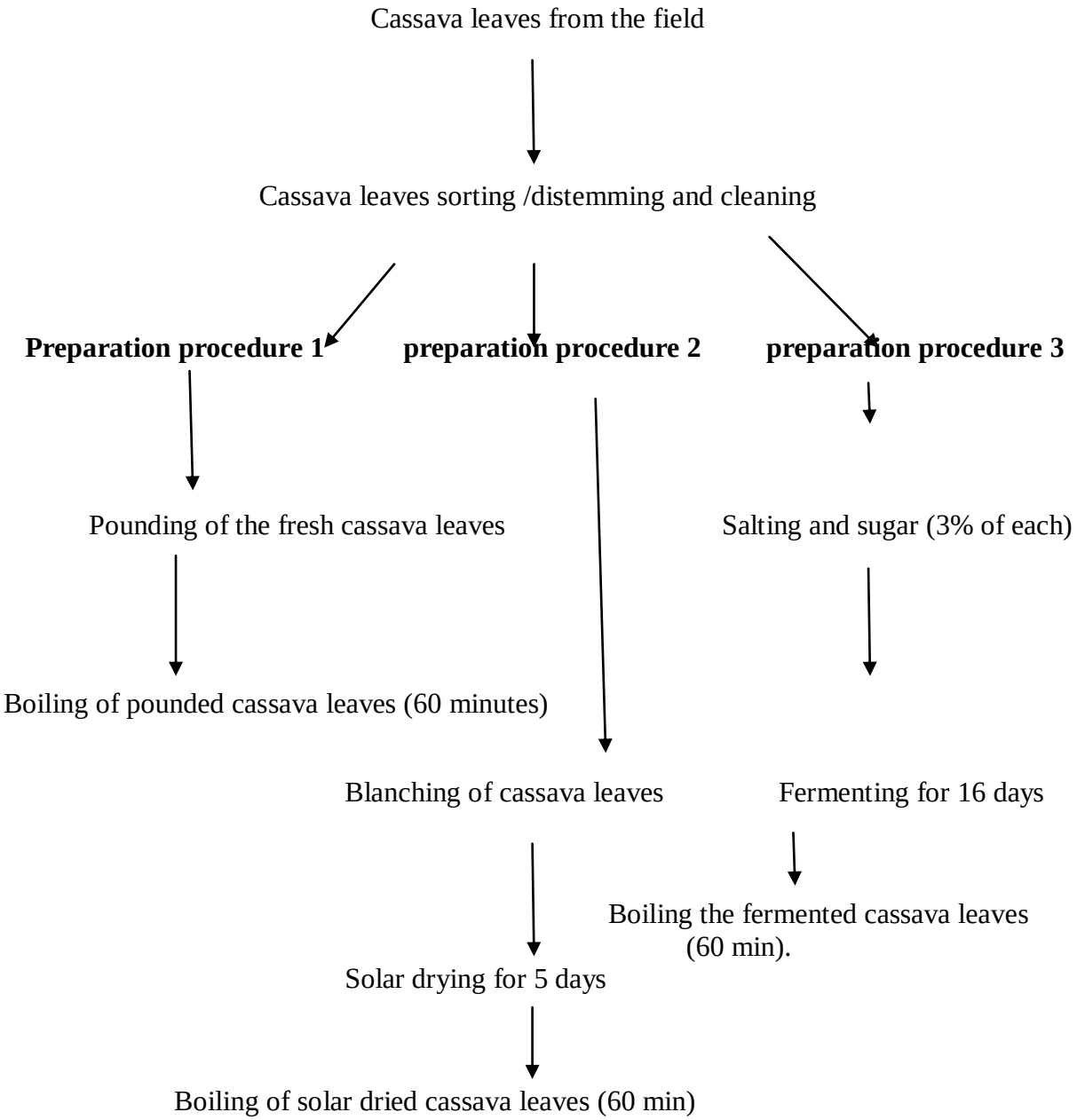

Figure 2: Flow diagram illustrating the Preparation procedures of cassava leaves

4.4 Nutrient and anti-nutrient analysis.

Levels of nutrients and anti-nutrients in raw, pound/boiled, solar dried/boiled, fermented/boiled and cassava leaves were analyzed using the standard analytical procedures outlined in chapter 3. All the analytical analyses were done in duplicates and results were expressed as means ± Standard Error of the Mean (SEM).

4.5 Statistical Data Analysis

Results were subjected to Analysis of Variance (ANOVA) and means were separated by Least Significant Difference (LSD) test calculated at 95% confidence interval using Genstat 15th Edition.

4.6 Results and Discussion

Survey

The Focused group discussions (FGD) established that different varieties of cassava are cultivated and used as vegetables, especially during dry seasons in the two Counties. The most popular cassava varieties include:-*Kibanda Meno, Shibe, Sagalato, Karembo, Gushe, Sinitie Nazi, Kileso, Tajirika, Mnyamwezi and Lisuna.* From the survey conducted, the popular varieties in Kilifi County were *Kibanda Meno and Tajirika* while in Kwale County they are *Kibanda Meno, Sinitie Nazi, Sagalato, Kileso and Gushe* although *Gushe* is also a bitter variety as informed by the key informants. These are grown for both the roots and vegetables. The most popular varieties used for vegetables are, *Kibanda Meno, Tajirika, Sagalato, Sinitie Nazi and Gushe.* These results agree with the study by Mwango'mbe, (2013) who reported that the most common varieties grown in Kilifi and Kaloleni Sub-counties were *Kibanda Meno* at 45.5 % and *Tajirika*. Only 2% of the farmers were grew the variety *Shibe*.

Karembo is being faced out by the farmers and by KALRO because of low yields. In addition, the leaves are bitter and the roots have fibre hence they don't do well on the market. This was also reported in previous studies by (Mwango'mbe, 2013).

The focus groups respondents were asked to vote on the popularity of the local traditional vegetables as utilized by the community and the results were as shown in Figure 3. Cassava leaves were more popular at (35%) followed by Terere (26%) and Mnavu (22%) as utilized during dry seasons.

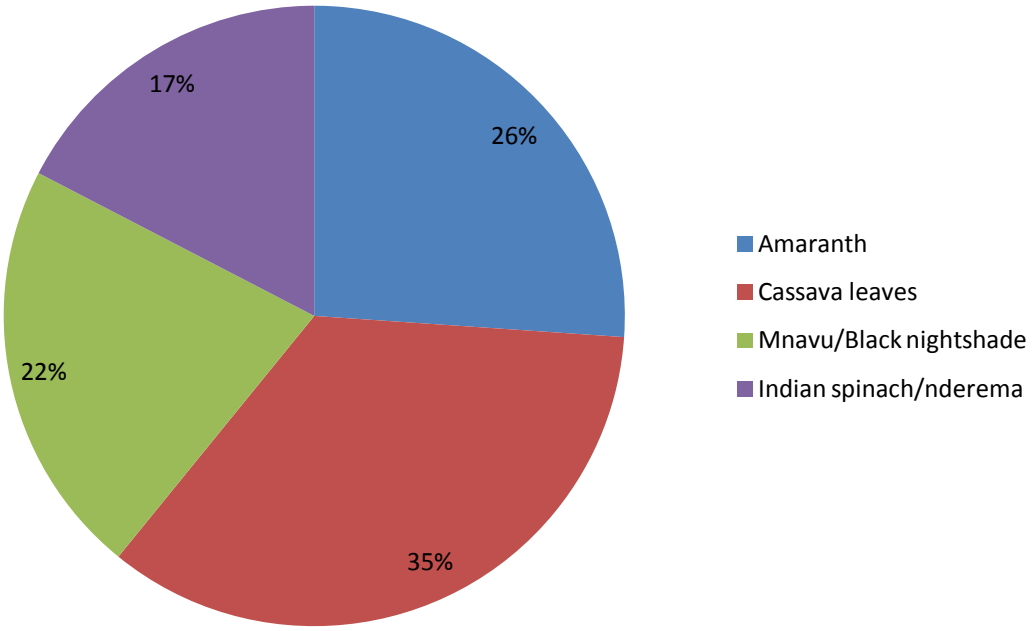

Figure 3: Popularity of the local vegetables as used by the community members

From the Focused Group discussions all the attributes were given the highest score of three which meant that the vegetable was preferred based on the given attribute in Table 9.

Table 8: Scores on attributes of various traditional vegetables compared to cassava leaves

Vegetable	Appearance	Texture	Taste	Flavour	Availability
Cassava Leaves	3	3	3	3	3
Terere/Amaranth	2	3	2	2	3
Cowpea Leaves /mkunde	2	1	2	2	1
Mnavu/Black nightshade	2	2	1	2	1
Nderema/Indian spinach	1	2	1	2	2

3= Good, 2= Fair 1 = Bad/poor

The harvesting of leaves starts after the 5th month of planting because a lower age than that would mean denying nutrients for the plant to grow well. The leaves are continually harvested until the plant maturity is one year and beyond. The leaves harvested are the top most 4 or 5 as long as they are soft. The local preparation method for the cassava leaves as established from the FGD was: the leaves are picked from the farm, sorted, stems removed, washed, pounded in mortar and pestle and boiled in water covering the vegetables for 1 hour or until all the water evaporates out or the leaves become tender with change in taste/flavour. No drying or fermentation of leaves as methods of preparation was reported.

Since cassava leaves had the highest total score, this meant that cassava leaves were preferred based on colour (brownish), smooth texture and good flavour. The cassava leaves are readily availability since the plant is drought resistant and this makes it useful during dry seasons.

The results for percentage Moisture, crude protein and ash levels in raw, fermented-boiled, solar dried-boiled and pounded-boiled samples are as summarized in Table. 10.

Table 9: Percentage crude protein and total ash content for raw, pounded, solar dried and fermented *Kibanda Meno, Tajirika and Sinitie Nazi* Cassava varieties

Variety	Preparation of cassava leaves	Crude Protein (%)	Ash (%)
Kibanda Meno	Raw	38.4±6.7[a]	6.3±0.1[a]
	Solar Dried	32.14±0.9[a]	14.11±0.1[b]
	Fermented	29.73±0.3[a]	16.27±1.1[b]
	Pounded	32.65±0.6[a]	5.44±1.4[a]
Tajirika	Raw	27.4±0.9[a]	5.02±0.02[a]
	Solar Dried	27.29±2.6[a]	10.79±0.09[b]
	Fermented	23.49±0.4[a]	12.98±0.8[b]
	Pounded	22.74±1.4[a]	4.025±0.3[a]
Sinitie Nazi	Raw	25.72±8.4[a]	4.705±0.03[a]
	Solar Dried	24.6±1.8[a]	12.96±0.04[b]
	Fermented	23.99±0.3[a]	15.345±0.9[b]
	Pounded	21.43±1.0[a]	4.325±0.2[a]

*Mean± Standard Deviation (n=12).

**Values within a column followed by the same superscript per variety are not significantly different from each other (p=<0.05)

Percentage crude protein contents for cassava leaves of different cassava varieties' indicated no significant differences (p>0.05) in raw, solar dried, fermented and pounded boiled leaves. The crude protein content ranged from 38.4% to 25.7% for raw leaves, 32.1 to 24.6% for solar dried/boiled leaves, 29.7 to 23.9% for fermented/boiled leaves and 32.6 to 21.4% for pound/boiled leaves for Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. Even though the protein levels were insignificantly different for all the preparation methods under study, there was a slight decrease in the levels that could be attributed to leaching effect during the boiling stage.

Highest losses of 23% were reported in fermented Kibanda Meno while the pounded cassava leaves had 15, 18 and 17% losses for Kibanda meno, Tajirika and Sinitie Nazi varieties, respectively. Gernah and Ajir (2007) also reported a decrease in protein content with the boiling of cassava leaves. The protein levels in raw cassava leaves are within the values of between 14% and 40% reported in other studies (Eggum, 1970). In addition, cassava leaves are still a better and cheap source of protein when compared with protein levels of other foods types like eggs (12.00%), white bread (7.80%), rice (6.50%), milk (3.30%), and potatoes (2.10%) (Gamman and Sherrington, 1990).

Ash content in raw cassava leaves ranged from 6.3% to 4.7% while it ranged from 4.02% to 16.27% with the different cassava leaves preparation methods. Ash content increased from 6.3%, 5.02% and 4.79% in raw to 14.1%, 10.79 % and 12.96% for solar dried and 16.26%, 12.98% and 15.34 % for fermented in Kibanda Meno, Tajirika and Sinitie Nazi, respectively. There were highly significant differences ($p=0.001$) in ash content between the solar dried and fermented cassava leaves compared to pounded and raw cassava leaves. The ash levels were significantly higher in fermented and solar dried ($p<0.05$) with 223.9 214.9 and 275% for solar dried and 258.258 and 326% in fermented samples, respectively.

High ash levels indicate high concentrations of the mineral composition (Udoetok, 2012). These results agree with the study by Adewusi, (1999) who reported that fermentation and blanching of cassava and amaranthus leaves increased the total calcium and iron contents but reduced magnesium level while zinc remained fairly constant in cassava vegetable diets. Adewusi (1999) also stated that fermentation increased the availability of these minerals in both cassava products and simulated cassava-vegetable diets. The ash content in pound/boiled were not significantly different ($p>0.05$) from the raw cassava leaves.

Ash levels in pounded- boiled leaves reduced to 5.44%, 4.02% and 4.32% for Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. Pounding-boiling may have reduced the mineral content through leaching (Bakr and Gawish, 1997). Since fermentation and blanching of cassava leaves play an important role in making the minerals more available than those either from raw or pound preparation methods they should be encouraged to potentially improve the disease states associated with mineral deficiency (Adewusi, 1999).

Beta –carotene, vitamin C, Iron, zinc and calcium levels in raw, solar dried, fermented and pounded/boiled are shown in Table 11.

Table 10: Iron, zinc, vitamin C and Beta- carotene contents in raw-boiled, fermented-boiled, solar dried-boiled and pound -boiled for *Kibanda Meno, Tajirika and Sinitie Nazi* cassava variety' leaves (Expressed in mg/100g edible portion)

Variety	Preparation of cassava leaves	Vitamin C (mg/100g)	β-carotene (mg/100g)	Iron (mg/100g)	Zinc (mg/100g)	Calcium (mg/100g)
Kibanda Meno						
	Raw	96.28 ± 10.2^{d}	15.38 ± 1.9^{b}	58.78 ± 0.1^{b}	31.58 ± 0.01^{c}	1031 ± 0.5^{b}
	Solar Dried	26.64 ± 2.9^{b}	11.42 ± 1.4^{a}	96.47 ± 0.04^{d}	24.05 ± 0.02^{bc}	1157.5 ± 0.5^{d}
	Fermented	14.51 ± 2.9^{a}	10.72 ± 0.1^{a}	60.85 ± 0.3^{c}	17.02 ± 0.01^{a}	1073.1 ± 0.3^{c}
	Pounded	53.47 ± 5.3^{c}	17.32 ± 2.4^{b}	12.45 ± 0.05^{a}	27.44 ± 0.01^{b}	214.4 ± 0.4^{a}
Tajirika	Raw	30.33 ± 1.6^{d}	15.73 ± 1.7^{b}	45.62 ± 0.06^{b}	15.61 ± 0.01^{b}	1590 ± 0.7^{c}
	Solar Dried	16.65 ± 2.3^{b}	10.93 ± 1.5^{a}	81.15 ± 0.5^{d}	13.79 ± 0.09^{b}	1686 ± 0.1^{d}
	Fermented	11.62 ± 2.4^{a}	8.26 ± 0.5^{a}	48.62 ± 0.02^{c}	6.17 ± 0.06^{a}	1294 ± 0.1^{b}
	Pounded	22.65 ± 2.4^{c}	16.69 ± 2.0^{b}	23.71 ± 0.1^{a}	11.51 ± 0.02^{b}	412 ± 0.5^{a}
Sinitie Nazi						
	Raw	63.33 ± 3.6^{d}	18.01 ± 0.3^{b}	30.95 ± 0.01^{b}	19.84 ± 0.08^{cd}	1104 ± 50.1^{b}
	Solar Dried	17.74 ± 2.4^{b}	10.59 ± 1.3^{a}	40.76 ± 0.06^{d}	11.6 ± 0.01^{bc}	1372 ± 15.1^{c}
	Fermented	12.92 ± 2.0^{a}	9.44 ± 0.6^{a}	36.36 ± 0.04^{c}	15.06 ± 0.01^{c}	1675 ± 49.6^{d}
	Pounded	40.93 ± 2.5^{c}	19.26 ± 0.4^{b}	24.52 ± 0.4^{a}	11.24 ± 0.03^{a}	827 ± 5.4^{a}

*Values are mean± Standard Deviation;
**Values within a column followed by the same superscript per variety are not significantly different from each other ($p<0.05$).

Beta carotene levels in raw cassava leaves were 15.38, 15.73, and 28.01 mg/100g in Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. The levels of beta carotene in both raw and pounded/boiled cassava leaves were not significantly different at ($p>0.05$). However, there was a slight increase of beta carotene in pounded/boiled cassava leaves.

These results slightly agrees with a study by USDA, (1998), Ejoh *et al*., (2005) and Rickman *et al*., (2007) that pounding- boiling/cooking moderately is superior to solar drying in improving and preserving beta carotene levels. Rickman *et al*., (2007) further reported that loss of soluble solids and the release of protein-bound β-carotenes that occurred during boiling and may contribute to the observed increase in the provitamin content, but the isomerisation of the naturally predominant all-trans carotenoids to cis conformations could reduce the β-carotene of the vegetables during solar drying. There was no significant difference in β carotene between solar dried-boiled and fermented boiled cassava leaves. However, there was significant difference ($p<0.05$) between raw, solar dried-boiled and fermented-boiled cassava leaves. The average retention of β- carotene levels in the three varieties were 67%, 58% and 108% for solar dried-boiled, fermented-boiled and pound-boiled, respectively. The results indicate that solar drying and pounding retained higher levels of β carotene compared to fermentation. Kibanda Meno had better retentions compared to Tajirika and Sinitie Nazi. The RDA for β- carotene is 300 and 900 µg Retinol equivalents for children and adults, respectively. From the results it can be concluded that pounding and solar drying methods retain β carotene in cassava leaves.

The vitamin C content in raw cassava leaves were 96.28, 30.33 and 63.33mg/100g for Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. The levels were significantly different ($p<0.05$) in all the three cassava leaves preparation methods. The Vitamin C content ranged from 27% to 16.6% in solar dried, 15% to 12.1% in fermented and 75% to 55% in pound/boiled cassava leaves for the three varieties.

Vitamin C levels were better retained in pounded/boiled, solar dried and fermented cassava leaves in decreasing order. The reduction of ascorbic acid (vitamin C) during solar drying agrees with studies by Faber and Van Jaarsveld, (2007) who reported that the reduction in vitamin C may be related to the fact that it is thermo-labile at mild heating and very sensitive to blanching, drying and cooking.

In addition, George, (1999), Ogbadoyi *et al.* (1992), Ejoh *et al.,* (2005) and Rickman *et al*. (2007) also attributed the loss of vitamin C to its thermo-sensitive and hydro soluble nature. Further, Mathooka, and Imungi, (1994) and Rickman, (2007) also reported that the amount of vitamin C decreases with increasing cooking time of cowpeas leaves. Fermentation also resulted in significant loss of the Vitamin C compared to solar drying. These results are in agreement with studies by Doblado *et al*., (2005) and Frias *et al*., (2005) that fermentation caused noticeable reductions in vitamin C content of *Vigna sinensis* and *Lupinus albus.* The loss in ascorbic acid (vitamin C) may be as a result of the increase in the activity of the enzyme ascorbate oxidase that might have been produced by the fermentation microorganism converting ascorbic acid to dehydroascorbic acid (Adetuyi *et al.,* 2008). These results indicate that blanching/ solar drying and fermentation of cassava leaves resulted in significant reduction in levels of vitamin C with *Kibanda Meno and Sinitie Nazi* recording high percentage losses as compared to Tajirika. Such losses in vitamin C have also been reported for other vegetables (Mziray *et al*., 2000). The implication of these results is that the levels of Vitamin C in prepared cassava leaves are low and therefore may require supplementation to be able to meet the RDA of 60-75 mg/day for adults.

Iron levels ranged between 31 and 58mg/100g for raw cassava leaves, 41 and 96 mg/100g for solar dried cassava leaves, 36 and 60mg/100g for fermented cassava leaves and 12 and 24mg/100g for pounded/boiled cassava leaves. There was a significant difference ($p<0.05$) in iron levels in solar dried, fermented and pound/boiled cassava leaves.

Iron contents were significantly higher in blanched/solar dried and fermented cassava leaves, but were significantly lower in pound/boiled cassava leaves. Similar increases in iron content have been reported in other studies. Aduwesi (1999) reported that the increase in total iron content with fermentation is as a result of increase in the number of fermentation microorganisms, which may contribute to the total iron content of cassava leaves. Iron content average retentions were 158%, 93% and 51% for solar dried-boiled, fermented-boiled and pounded-boiled samples. The iron levels were lowest in pounded- boiled cassava leaves, probably as a result of leaching during the pounding and boiling (Bakr and Gawish, 1997). The RDA's for men women and children are 8 mg/day, 18 mg/day and 10 mg/day, respectively (Food and Nutrition Board, 2001).

The zinc content in raw cassava leaves were 31.58, 15.61 and 19.84 mg/100g for Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. The zinc values ranged between 24.05 and 11.6 mg/100g, 13.02 and 6.17 mg/100g and 15.44 and 11.24 mg/100g for solar dried-, fermented- and pound-boiled cassava leaves, respectively. Zinc levels for all the preparation methods were significantly different ($p<0.05$) with highest retention levels of 76% and 88%, 87% and 73% being observed in solar dried and pounded– boiled cassava leaves for Kibanda meno and Tajirika, respectively. Even with the losses during preparation, zinc levels in prepared cassava leaves like for other minerals can still meet the RDA's of 15mg/day and 12mg/day for men and women respectively.

The Calcium content in raw cassava leaves were 1030.48, 1589.41, and 1154.16 mg/100g in *Kibanda Meno, Tajirika and Sinitie Nazi* varieties, respectively. Calcium levels were significantly higher ($p<0.05$) in Raw, solar dried-boiled and fermented-boiled than pounded-boiled cassava leaves. The average increase was 1410.35mg/100g (12.1%) for solar dried and 1364.12mg/100g (8.4%) for fermented cassava leaves.

The implication of the results is that calcium levels slightly increased in solar dried and fermented cassava leaves. These results agree with the studies by Bradbury, (1988) that calcium content (like iron) increased with fermentation time reaching a plateau after 48 hours. Oke, (1966) also reported that the calcium content of fermented cassava roots increased from 0.13 to 0.33% when processed into Kpokpogari. From the results, it can be concluded that solar drying and fermentation methods resulted in higher calcium levels.

The increase could have been as a result of reduction in anti-nutrients like phytates and oxalates through pounding that bind calcium and thus releasing the minerals. The pounded - boiled cassava leaves exhibited low calcium levels probably as a result of leaching during boiling stage (Bakr and Gawish, 1997). Levels of calcium in cassava leaves compare well with other vegetables like raw cowpeas with 1736mg/100g (Muchoki, *et al*., 2010). The RDA's for calcium are 800-1000 mg/day. The implication of these results is that the lower calcium levels can be supplemented with other foods. Adequate processing detoxifies cassava leaves for human consumption with considerable nutrient retention (Mahungu *et.al.,* 1997), (Bokanga, 1994), (Achidi *et al*., 2008). The Cyanide, Oxalate, Nitrate, Phytate and Phenollic content in Raw, fermented, solar dried and pounded cassava leaves are as shown in Table 12.

The cyanide, phytate, nitrates and tannins content in raw cassava leaves were significantly higher ($p<0.05$) than in solar dried-boiled, fermented-boiled and pound-boiled cassava leaves. Cyanide content in raw leaves were 1238.21, 960.31, and 767.28 mg/kg for Kibanda Meno, Tajirika and Sinitie Nazi varieties, respectively. Cyanide content decreased significantly ($p < 0.05$) in all the preparation methods. The cyanide content in solar dried and fermented cassava leaves were not significantly different ($p>0.05$) while the cyanide levels were significantly in the pound-boiled cassava leaves. The average percentage losses were 82 % for pounded-boiled, 70% for solar dried and 44% for fermented-boiled.

Table 11: Cyanide, Oxalate, Nitrate, Phytates and Tannins content in Raw, solar dried, fermented and pounded-boiled for *Kibanda Meno, Tajirika and Sinitie Nazi* cassava leaves

Variety	Preparation of cassava leaves	Cyanide mg/kg	Oxalates g/100g	Nitrates mg/100g	Phytates g/100g	Tannins mg/100g
Kibanda Meno	Raw	123.82 ± 9.3^{c}	78.61 ± 0.7^{b}	47.32 ± 0.9^{c}	509.6 ± 84.9^{c}	4241 ± 449.5^{c}
	Solar Dried	38.93 ± 3.9^{b}	33.56 ± 4.2^{a}	13.97 ± 2.2^{a}	144.5 ± 2.4^{b}	775 ± 35.1^{a}
	Fermented	41.2 ± 1.1^{b}	29.65 ± 0.5^{a}	39.81 ± 0.3^{c}	351.8 ± 10.6^{bc}	507 ± 4.5^{a}
	Pounded	18.95 ± 1.9^{a}	67.97 ± 4.6^{b}	27.68 ± 0.9^{b}	47.8 ± 2.5^{a}	1484 ± 13.3^{b}
Tajirika	Raw	96.03 ± 11.2^{c}	37.27 ± 7.1^{b}	30.38 ± 1.2^{c}	319.2 ± 20.2^{c}	3203 ± 45.5^{c}
	Solar Dried	17.36 ± 0.8^{ab}	23.56 ± 4.2^{a}	15.11 ± 1.5^{a}	220.6 ± 69.4^{b}	699 ± 36.5^{a}
	Fermented	26.36 ± 0.3^{b}	21.17 ± 2.7^{a}	21.83 ± 2.4^{c}	264 ± 1.7^{bc}	471 ± 18.8^{a}
	Pounded	12.1 ± 0.8^{a}	34.76 ± 2.5^{b}	18.89 ± 4.1^{b}	45.4 ± 7.9^{a}	1231 ± 330.3^{b}
Sinitie Nazi	Raw	76.73 ± 8.7^{c}	40.88 ± 5.9^{b}	61.18 ± 23^{c}	287.3 ± 29.3^{c}	4682 ± 177.8^{c}
	Solar Dried	28.88 ± 2.5^{b}	22.77 ± 3.8^{a}	19.97 ± 3.3^{a}	147 ± 21.2^{b}	273 ± 29.4^{a}
	Fermented	39.8 ± 1.3^{b}	12.44 ± 2.1^{a}	41.62 ± 1.0^{b}	224.5 ± 12.1^{bc}	757 ± 143.7^{a}
	Pounded	15.39 ± 0.4^{a}	29.87 ± 0.4^{ab}	25.29 ± 5.9^{b}	41.6 ± 5.45^{a}	1351 ± 36.4^{b}

*Mean± Standard Deviation (n=4)

**values within a column per variety followed by the same superscript are not significantly different from each other ($p<0.05$).

The reductions were significantly high ($p<0.05$) in pounding and solar drying preparation method compared to fermentation with better losses in Kibanda meno and Tajirika varieties. This observation is in agreement with the findings of Aganga and Tshwenyane, (2003) and Richard, (1991) who reported that cyanides are volatile compounds and can degenerate while drying. The reduced levels ranged between 390 and 150 mg/kg on dry matter basis for all the varieties. These levels are still above the recommended cyanide toxicity level of 10 mg HCN/kg for foods. The implication of these results is that consumption of unprocessed cassava leaves as a vegetable is likely to have cyanide toxic effects to the body. However, the residual cyanide in the other preparation methods had lower cyanide content than the fresh cassava leaves.

Phytates were highest anti-nutrient in all the cassava varieties. Values for raw cassava leaves were 509.6, 319.2 and 287.3 mg/100g for Kibanda Meno, Tajirika and Sinitie Nazi, respectively. Phytate content was significantly high ($p<0.05$) in raw leaves than in prepared leaves. The presence of phytin in the cassava leaves agrees with a report by Aletor and Adeogun, (1995) of their widespread occurrence in plants. The average losses were 51%, 23% and 88% for solar dried, fermented and pound cassava leaves. The levels were mostly retained in both solar dried and fermented cassava leaves with better losses in Kibanda Meno and Sinitie Nazi varieties. The implication of these results is that since the anti-nutritional stress factors like of phytin chelates mineral elements, especially Calcium, Magnesium, Iron and Zinc (Forbes and Erdman, 1983) thereby rendering them metabolically unavailable. Therefore, there is need to determine a method that can reduce these Phytates levels since the mentioned minerals are of public health concern. Although solar drying has been found to lower phytates and polyphenols significantly (Yadav, 2002), pounding was the most appropriate method in phytate reduction because of the high losses.

The nitrate content was high in raw cassava leaves 47.32, 30.38 and 61.18 mg/100g for *Kibanda Meno, Tajirika and Sinitie Nazi* varieties, respectively. Nitrate levels were significantly higher ($p<0.05$) in raw cassava leaves as compared to pound and solar dried cassava leaves while the levels were not significantly different ($p>0.05$) in fermented cassava leaves. The average retentions were 37, 74 and 54% in solar dried, fermented and pounded cassava leaves, respectively. Fermentation had high retentions while solar drying and pounding had the least. In comparing the nitrate levels in cassava leaves with other vegetables, the levels of 4335.21mg/kg in amaranthus have been reported (Ogbadoyi *et al*., 2011). The nitrate content in raw and prepared cassava leaves is higher than the acceptable daily intake (ADI) of 3.65 mg/kg for 60kg body weight.

The nitrate content obtained in this study are high for human consumption hence more care is needed for nitrogen fertilization not to exceed the leaf nitrate content over the ADI limit. Further, the processing methods adopted should be able to reduce the nitrate content of the vegetable to the acceptable levels.

The oxalate content in raw cassava leaves were 78.61, 37.27 and 40.88 g/100g in *Kibanda Meno, Tajirika and Sinitie Nazi* varieties, respectively. There were no significant differences ($p>0.05$) in oxalate levels in raw and pounded-boiled cassava leaves. However, the oxalates were greatly lost through fermentation (58%) and solar drying (46%). The high levels of oxalates in raw cassava leaves as compared to pounded cassava leaves agree with studies by Ogbadoyi *et al*., (2006), Antia *et al*., (2006), Adeboye and Babajide (2007) and Ojiako and Igwe (2008) who reported that proper cooking of vegetables before use greatly reduce oxalate levels. The levels of oxalate obtained for raw cassava leaves were comparable with oxalate content of leaves of other vegetables, sweet potato leaves 3730.5mg/100g, (Lwasai 2011) while amaranthus grown in Tanzania were 3383-4333 mg/100g (Mziray 2001) and in Kenya 5830mg/100g (Onyango, 2008). Oxalate levels in cowpeas leaves were 1889mg/100g (Muchoki, *et al*., 2010). However, Some Indian vegetables were found to contain extremely higher oxalate values ranging from 5138 to 12576 mg/100g DWB (Radek, 2008). The levels in cassava leaves in the current study are also very high hence the need for proper preparation/processing before utilization.

Tannins content in raw leaves were 4241, 3203, 4682 mg/100g for *Kibanda Meno, Tajirika and Sinitie Nazi* varieties, respectively. Tannins obtained were significantly higher ($p<0.001$) in raw cassava leaves than those in solar dried, fermented and pounded cassava leaves. The retention levels ranged 34, 15 and 14% in pounded-boiled, solar dried-boiled and fermented-boiled, respectively.

From the results, solar drying and fermentation indicate better preparation methods in the reduction of phenolic compounds with *Kibanda Meno and Sinitie Nazi* indicating better losses than Tajirika variety. It has been reported that phenolic could change in form during food processing (Mbugua *et al*., 1992). However the methods used for their determination in this study could not differentiate these forms.

4.7 Conclusion

In conclusion, the study established that most people at the Coast use cassava leaves as vegetables and especially during dry seasons and all the varieties are used for vegetables except the bitter ones like *Karembo*. The most popular variety is *Kibanda Meno* in addition to *Tajirika, Sagalato, Kileso and Sinitie Nazi.* In addition, the preparation methods are similar across the region.

Current findings indicate that different processing methods (blanching/solar drying, fermentation and pounding- boiling) have effects on nutritional and anti-nutritional contents of cassava leaves. Significant differences exist between raw and processed cassava leaves. Pounding- boiling lost 77% and 86% of cyanide and phytates, respectively. Solar drying-boiling had losses of 85%, 74%, 66%, and 54% for tannins, cyanide, nitrates and phytates, respectively. In addition, blanching and solar drying-boiling of the vegetables retained substantial levels of beta carotene, ascorbic acid while levels of iron and calcium slightly increased.

4.8 Recommendations

1. From the results, this study recommends the use of pounding-boiling as the most appropriate method for reduction of anti nutrients and particularly cyanide and Phytates but with further research on toxicity levels of cyanide.

2. Since pounding-boiling of the cassava leaves retained most minerals and protein, the method can be recommended in preparation of cassava leaves and have them being utilized to fight against micronutrient deficiencies.
3. Solar drying can be used as the second best method for processing the cassava leaves to preserve for future use. However, the storage quality of the solar dried product should be established

5.0 CHAPTER FIVE: GENERAL CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

From the analysis conducted, it was observed that cassava leaves have both nutrients and anti-nutrients and the levels depend on the harvesting stage.

1. Nutrients like, Proteins, β- carotene, iron and zinc are concentrated at 3 months and decrease as the plant matures while Vitamin C and calcium increased with cassava plant maturity.
2. Cyanide, Phenollic compounds and Phytates contents increased with plant maturity while the nitrates reduced and oxalates were almost same at all ages and in all the three varieties.
3. The study also established that most people at the Coast use cassava leaves as vegetables and especially during dry seasons and all the varieties are used for vegetables except the bitter ones like Karembo. The most popular variety is *Kibanda Meno* in addition to *Tajirika, Sagalato, Kileso and Sinitie Nazi*.
4. In addition, the preparation methods are similar across the region where the leaves are five topmost leaves are collected, cleaned, pounded and boiled in water until tender.

5. Results from different preparation methods were significantly different ($p<0.05$) in the retention and loss of both nutrients and anti-nutrients. Pounding- boiling lost 77% and 86% of cyanide and Phytates, respectively. Blanching/solar drying was the second best method that could be alternated with boiling for large scale production. In addition, blanching/ solar drying of the vegetables retained substantial levels of 18% and 61% for beta carotene and ascorbic acid, respectively while levels of Iron and calcium increased slightly. Since the findings indicate that pounding-boiling and solar drying-boiling can reduce the levels of toxic substances and anti-nutrients to tolerable levels without dangerously compromising the micronutrient contents of vegetables, they are recommended with further research.

5.2 Recommendations

Considering the results from this study, the following recommendations are made;

1. Boiling as a method proved to be the best method that greatly reduced levels of most anti-nutrients as well as retaining the nutrients. However, further studies need to be carried out to determine the effects of boiling on nutrients and anti nutrients when the boiling water is discarded.
2. A microbial study should be done to ascertain which specific species of the Lactic acid bacteria are involved in fermentation of cassava leaves to give a uniform product and for large scale production.
3. Keeping quality of solar dried cassava leaves should be established.
4. Toxicity tests to be done to know whether the anti nutrients and particularly cyanide are poisonous on continuous consumption.
5. Further research on the bioavailability of cassava leaves micronutrients in vitro should be done.

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APPENDICES

APPENDIX 1: CHECKLIST FOR FOCUSED GROUP DISCUSSION

County _____Sub-county_____Division_____

Location_____Village_____

Question	Tick	Responses
1. Which part of the cassava plant do you consume?		
2. What do you utilize cassava leaves for? If utilized skip question 3		
3. What reasons do you have for not utilizing the leaves as vegetables?		
4. Do you pick the leaves from any or specific varieties?		
5. If from specific list the varieties you use in order of preference		
6. Why do you prefer the specific varieties?		
7. At what age of the plant do you start picking the leaves? b. Why do you start at that particular age?		
8. Do you pick any leaf or specific leaves? If specific, give position of the leaf on the plant and why?		
9. Heard about any problem in the use of the leaves?		
10. Describe the stages of leaf handling from collection to preparation? (Wilting, sorting, cutting, pounding)		
11. How are the leaves prepared? (Drying, boiling, fermentation, frying) show combination		
12. How long does the stated preparation method take?		

Acceptability and preference attributes for local vegetables rated to a scale of 1- 3

Vegetable	Appearance	Texture	Taste	Flavour	Availability
Cassava Leaves					
Terere/Amaranth					
Cowpea Leaves /mkunde					
Managu/night black shade					
Indian spinach-Nderema					

Appendix 2: Interview questions

Question	Response
Which varieties of cassava are grown in the county?	
What are the common uses of the cassava grown?	
What varieties are the most popular and why are they preferred?	
Which varieties are used for vegetables?	
When do they begin picking the leaves for vegetables?	
Which specific leaves are picked for vegetables and why?	
List the varieties in order of popularity	

Appendix 3: ANOVA for different cassava varieties at different maturity stages

Variate: % Moisture content

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	139.9168	69.9584	2.55	0.097
Month	2	179.6646	89.8323	3.27	0.054
Variety.Month	4	198.2140	49.5535	1.80	0.157
Residual	27	741.9900	27.4811	52.97	
leafage.*Units* stratum	36	18.6784	0.5188		
Total 71	1278.4638				

Variate: % Crude protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	345.3960	172.6980	11.15	<.001
Month	2	247.5910	123.7955	7.99	0.002
Variety.Month	4	181.3015	45.3254	2.93	0.039
Residual	27	418.2359	15.4902	33.59	
leafage.*Units* stratum	36	16.6026	0.4612		
Total	71	1209.1270			

Variate: % Total Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	11.71667	5.85833	11.44	<.001
Month	2	36.34974	18.17487	35.49	<.001
Variety.Month	4	9.43886	2.35971	4.61	0.006
Residual	27	13.82768	0.51214	23.53	
leafage.*Units* stratum	36	0.78350	0.02176		
Total	71	72.11644			

Variate: Vitamin C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	1.120E+06	5.602E+05	13.70	<.001
Month	2	6.716E+06	3.358E+06	82.11	<.001
Variety.Month	4	1.812E+06	4.530E+05	11.08	<.001
Residual	27	1.104E+06	4.090E+04	533.92	
leafage.*Units* stratum	36	2.758E+03	7.660E+01		
Total	71	1.076E+07			

Variate: β_carotene

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	419.0161	209.5081	9.32	<.001
Month	2	103.3826	51.6913	2.30	0.120
Variety.Month	4	880.4582	220.1146	9.80	<.001
Residual	27	606.7336	22.4716	49.27	
leafage.*Units* stratum	36	16.4188	0.4561		
Total	71	2026.0093			

Variate: Calcium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	8510850.3	4255425.1	17.88	<.001
Month	2	7498466.2	3749233.1	15.76	<.001
Variety.Month	4	3786597.3	946649.3	3.98	0.012
Residual	27	6424773.2	237954.6	736.10	
leafage.*Units* stratum	36	11637.5	323.3		
Total	71	26232324.5			

Variate: Iron

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	102393.087	51196.543	20.53	<.001
Month	2	29066.728	14533.364	5.83	0.008
Variety.Month	4	26270.343	6567.586	2.63	0.056
Residual	27	67318.934	2493.294	743.83	
leafage.*Units* stratum	36	120.671	3.352		
Total	71	225169.763			

Variate: Zinc

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	71340.952	35670.476	46.31	<.001
Month	2	60850.245	30425.122	39.50	<.001
Variety.Month	4	64761.963	16190.491	21.02	<.001
Residual	27	20796.470	770.240	569.00	
leafage.*Units* stratum	36	48.732	1.354		
Total	71	217798.362			

Variate: Oxalates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	2703.3587	1351.6794	3.64	0.040
Month	2	1889.0370	944.5185	2.54	0.098
Variety.Month	4	2613.9644	653.4911	1.76	0.167
Residual	27	10038.3566	371.7910	522.32	
leafage.*Units* stratum	36	25.6252	0.7118		
Total	71	17270.3420			

Variate: Cyanide

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	84807.441	42403.721	23.65	<.001
Month	2	57103.191	28551.596	15.93	<.001
Variety.Month	4	81834.099	20458.525	11.41	<.001
Residual	27	48400.273	1792.603	800.82	
leafage.*Units* stratum	36	80.584	2.238		
Total	71	272225.589			

Variate: Nitrates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	4639.5443	2319.7721	5.23	0.012
Month	2	2396.4251	1198.2126	2.70	0.085
Variety.Month	4	2153.5727	538.3932	1.21	0.328
Residual	27	11976.5229	443.5749	582.45	
leafage.*Units* stratum	36	27.4167	0.7616		
Total	71	21193.4817			

Variate: Phenolic_compounds

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
leafage stratum					
Variety	2	4885841.	2442921.	4.28	0.024
Month	2	6718415.	3359208.	5.89	0.008
Variety.Month	4	1089342.	272335.	0.48	0.752
Residual	27	15411758.	570806.	320.12	
leafage.*Units* stratum	36	64191.	1783.		
Total	71	28169548.			

Appendix4: Summary of the analysis for all the variables for different varieties at different leafage and plant maturity

Measure: MEASURE_1

Variety	Month	Leafage	Dependent Variable
1	1	1	V1M1L1
		2	V1M1L2
		3	V1M1L3
		4	V1M1L4
	2	1	V1M2L1
		2	V1M2L2
		3	V1M2L3
		4	V1M2L4
	3	1	V1M3L1
		2	V1M3L2
		3	V1M3L3
		4	V1M3L4
2	1	1	V2M1L1
		2	V2M1L2
		3	V2M1L3
		4	V2M1L4
	2	1	V2M2L1
		2	V2M2L2
		3	V2M2L3
		4	V2M2L4
	3	1	V2M3L1
		2	V2M3L2
		3	V2M3L3
		4	V2M3L4
3	1	1	V3M1L1
		2	V3M1L2
		3	V3M1L3
		4	V3M1L4
	2	1	V3M2L1
		2	V3M2L2
		3	V3M2L3
		4	V3M2L4
	3	1	V3M3L1
		2	V3M3L2
		3	V3M3L3
		4	V3M3L4

Tests the null hypothesis that the error covariance matrix of the orthonormalized transformed Dependent variables are proportional to an identity matrix.

a. Design: Intercept

Within Subjects Design: variety + month + leafage + variety * month + variety * leafage + month * leafage + variety * month * leafage

b. May be used to adjust the degrees of freedom for the averaged tests of significance. Corrected tests are displayed in the Tests of Within-Subjects Effects table.

Appendix 5: ANOVA for Raw, pounded, solar dried and fermented cassava leaves of *Kibanda Meno, Tajirika and Sinitie Nazi* varieties

ANOVA FOR PREPARATION METHODS

Variate: Moisture

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	725.5451	65.9586	140.69	<.001
Residual	11	5.1571	0.4688		
Total	22	730.7023			

Variate: Crude_Protein

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	613.69	55.79	3.57	0.023
Residual	11	172.01	15.64		
Total	22	785.70			

Variate: Ash

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
preparation_procedures	11	462.1358	42.0123	71.80	<.001
Residual	11	6.4361	0.5851		
Total	22	468.5720			

Variate: BC

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	636.504	57.864	16.16	<.001
Residual	11	39.391	3.581		
Total	22	675.895			

Variate: AA

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	44166.98	4015.18	234.05	<.001
Residual	11	188.71	17.16		
Total	22	44355.69			

Variate: Calcium

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	4.840E+06	4.400E+05	7.186E+06	<.001
Residual	11	6.736E-01	6.124E-02		
Total	22	4.840E+06			

Variate: Iron

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	12552.0326	1141.0939	10910.72	<.001
Residual	11	1.1504	0.1046		
Total	22	12553.1830			

Variate: Cyanide

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	21272.68	1933.88	46.01	<.001
Residual	11	462.33	42.03		
Total	22	21735.01			

Variate: Nitrates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	4093.3	372.1	2.63	0.062
Residual	11	1555.5	141.4		
Total	22	5648.8			

Variate: Oxalates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	5538.84	503.53	15.76	<.001
Residual	11	351.47	31.95		
Total	22	5890.31			

Variate: Phytates

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	306521.	27866.	22.19	<.001
Residual	11	13811.	1256.		
Total	22	320333.			

Variate: Phenollics

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Preparation_procedures	11	42029903.	3820900.	124.53	<.001

Residual	11	337503.	30682.
Total	22	42367406.	