POTENTIAL TOXIC LEVELS OF CYANIDE IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ) GROWN IN SOME PARTS OF KENYA

BY

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DECLARATION

I hereby declare that this Thesis is my original work and has not been presented for the award of a degree in any other university.

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DEDICATION

This Thesis is dedicated to God Almighty, my parents, Mr Simon Mburu and Felister Mburu, my brother Robert and my sisters Njeri, Susan and Mumbi.

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ABBREVIATIONS

ADP/ATP	Adenosine Diphosphate /Adenosine Triphosphate
ATSDR	Agency for Toxic Substance and Disease Registry
FAO	Food Agricultural Organization
FSANZ	Food Standards Australia New Zealand
ha	Hectare
HCN	Hydrogen Cyanide
IITA	International Institute of Tropical Agriculture
IPCS	International Programed on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KARI	Kenya Agricultural Research Institute
MT	Metric Tonne
PPM	Parts Per Million
RMRDC	Raw Materials Research and Development Council
SNK	Student Newman Keuls
TLC	Thin Layer Chromatography
UV	Ultra Violet
WHO	World Health Organization
HNL	Hydroxyl Nitrile Lyase

ABSTRACT

Cassava (*Manihot esculenta* Crantz) is a cyanogenic plant which is toxic when consumed without sufficient processing. Cassava is characterized by presence of linamarin a cyanogenic glycoside and when acted upon by an enzyme linamarase is hydrolysed into cyanohydrin which is further hydrolyzed to give hydrogen cyanide (HCN) which is toxic. This study aimed at determination of the levels of cyanide in the sweet cassava variety grown in five different geographical regions of Kenya namely Kisii, Kakamega, Thika, Nairobi and Kitui counties. The picrate paper method whose accuracy was higher compared to picrate solution and titration methods was used. The study also reports on variation of cyanide concentration with cultivar type and its concentration in different parts of cassava root. The concentration of cyanide varied significantly ($p<0.05$), ($n=324$) with the geographical location. The concentration of cyanide in cassava from Kakamega was highest (80.79±4.55 mg/kg), while cassava from Kisii had the lowest cyanide concentration (43.27 ± 3.75 mg/kg). The cyanide concentration in cassava from Kitui was 70.46 ± 2.21 mg/kg, while Nairobi and Thika had cyanide concentration of 66.00 ± 2.12 mg/kg and 54.84 ± 0.65 mg/kg respectively. All the eight cultivars (990183, 990014, 990005, 990249, 990006, mm96/5280, ex manakari and 196/0067) analyzed for cyanide showed significant variation ($p<0.05$) in the cyanide content; 55.04±0.02, 44.27±0.07, 47.76±0.07, 48.68±0.07, 49.60±0.07, 45.88±0.07, 46.76±0.00 and 49.14±0.07 in mg/kg respectively. Three parts of cassava root (Pith, Parechyma and Cortex) contained significantly different concentrations of cyanide; 59.24±3.62, 78.35±2.13, 91.03±2.94 mg/kg respectively. The concentrations of cyanide in cassava from all the five regions were higher than the recommended level by WHO (10mg of HCN/kg body weight). This study provides critical information on the potential toxicity of cyanide in cassava from the five different regions.

CHAPTER ONE
INTRODUCTION

1.1 Background information

Cassava (*Manihot esculenta* Cranzt) is a perennial crop native to tropical America (Allen, 1994; Olsen and Schaal, 2001). It originated from Brazil and was introduced to Africa by Portuguese traders in the 16th century (O' Hair, 1995). The plant parts used are the storage root (tuber) and leaves (Cock, 1985). It belongs to the dicotyledon family, Euphorbiaceae. The *Manihot* genus is reported to have about 100 species, among which the only commercially cultivated one is *Manihot esculenta* Crantz. There are two distinct types of cassava plant: erect, with or without branching at the top type and the spreading type (Alves, 2002).

Cassava is a starchy staple whose roots are very rich in carbohydrates, a major source of energy. The cassava plant is the highest producer of carbohydrates among crop plants with perhaps the exception of sugarcane (New World Encyclopedia, 2008). The plant is characterized by palmate lobed leaves, inconspicuous flowers and a large, starchy, tuberous root with a tough papery brown bark and white to yellow flesh (New World Encyclopedia, 2008). It is one of the most perishable tuber crops with a high postharvest loss (Diasolua *et al*., 2003). Anatomically cassava root is not a tuberous root, but a true root, which cannot be used for vegetative propagation. The mature cassava storage root has three distinct tissues: bark (periderm), peel (cortex) and parenchyma. The parenchyma, which is the edible portion of the fresh root, comprises approximately 85% of the total weight, consisting of the xylem vessels radially distributed in a matrix of starch containing cells (Wheatley and Chuzel, 1993). The cyanide concentration in cassava varies in different parts of the plant, according to variety, location, age, and environmental conditions. Consequently, cassava is of lower

nutritional value than cereals, legumes, and even some other root and tuber crops such as yams (Charles *et al*., 2005).

Cassava root contains significant amount of iron, phosphorus, calcium and vitamin C, but is a poor source of proteins. The root contains carbohydrates, 64 to 72 % of which is made up of starch, mainly in the form of amylose and amylopectin. About 17 % sucrose is found in sweet varieties, and small quantities of fructose and dextrose have been reported (Hendershot, 1972). The lipid content of cassava is only 0.5 %, Proteins content is about (1 to 2 %), and the amino acid profile of the cassava root is very low in some essential amino acids, particularly lysine, methionine, and tryptophan. Cassava is reasonably rich in calcium and vitamin C, but the thiamine, riboflavin, and niacin contents are not high (O' Hair, 1995; RMRDC, 2004; New World Encyclopedia, 2008).

Cassava leaves are much richer in proteins than the roots (Chijindu and Boateng, 2008). Although the leaves contain far less methionine than the roots, the levels of all other essential amino acids exceed the FAO's recommended reference protein intake. Cassava-leaf protein is claimed to be superior to soybean protein (Adegbola, 1977). Large proportions of these nutrients have been reported to be lost during processing. Supplementation of Cassava products such as leaf-meal with methionine or any other of the nutrients it lacks serves to improve its biological value significantly and has been widely practiced in industry for the processing of food for human consumption and animal feeds (Adegbola, 1977).

Cassava is grown widely in areas below 1500m above sea levels (Acland, 1985). The crop's starchy root is widely used worldwide, though the fresh foliage is also consumed in several regions of the world. The use of cassava as a food source is increasing, because it yields well even in poor soil without fertilizer, and is drought resistant (El-Sharkawy, 2004). The root

can be left in the ground for up to 3 years as a reserve source of food. In a drought, the leaves drop off, and the plant is kept alive by its large roots, and when the rains come the leaves sprout again (Bradbury and Holloway, 1988).

There are a number of varieties of cassava ‘sweet cassava’ and ‘bitter cassava’. The term "bitter" cassava, as opposed to "sweet" cassava, refers to the taste of the root parenchyma. Bitterness is associated with higher levels of cyanogenic glucosides (Cock, 1985). Certain ecological stress factors, such as pest attacks, prolonged drought and low phosphorus and potassium levels in the soil may cause roots to acquire bitterness, and this coincides with an increase in the levels of cyanogenic glucosides (Ayanru and Sharma, 1984el-). Bitter cassava varieties are more drought resistant and thus more readily available and cheaper (Akintonwa and Tunwashe, 1992).

In Africa, cassava was the crop with the highest total production with 118 million metric tonnes of productions across the continent in 2010, contributing significant energy input to the population, with an average of 196 kcal/capita/day in 2008 (FAO, 2010). It is the staple food of more than 500 million people in the tropics many of whom are very poor (Cock, 1985). The crop offers the advantage of a flexible harvesting time, allowing farmers to keep the roots in the ground until needed (Iglesias *et al*., 1997). In addition, the crop produces reasonably well under growth limiting conditions. In Africa, it is recognized as a famine reserve crop due to its tolerance to drought or infertile soils, and its ability to recover from disease and pest attacks. The area of cassava under unfavorable environments has been continuously increasing (El-Sharkawy, 1993). World production of cassava root was estimated to be 184 million tonnes in 2002, rising to 230 million tonnes in 2008 (Frederick *et al.*, 2008). The majority of production in 2002 was in Africa where 99.1 million tonnes were grown, 51.5 million tonnes were grown in Asia and 33.2 million tonnes in Latin America and

the Caribbean (FAO, 2005). Nigeria is the world's largest producer of cassava. Thailand is the largest exporting country of dried cassava, with a total of 77% of world export in 2005 (Katz and Weaver, 2003). The second-largest exporting country is Vietnam, with 13.6%, followed by Indonesia (5.8%) and Costa Rica (2.1%) (FAO, 2005).

In Kenya, cassava is grown in Western, Eastern, Central and Coastal regions. It is second only to maize in importance in western and coastal regions of Kenya (Njeru and Munga, 2003). In 1986, FAO estimated that the land area in Kenya under cassava cultivation was 30,000 ha producing 380,000 tons (Nweke, 1996). In 2007, about 54,673 ha were planted with cassava in all areas of Kenya except North Eastern Province (Khaemba, 1983). This is shown in Table 1.1.

Table 1.1: Cassava production in Kenya in 2007

Regions	**Nyanza**	**Coast**	**Eastern**	**Western**	**R.Valley**	**Central/ Nairobi**	**Total**
Area (ha)	18010	10745	8101	17144	662	11	54673
Production (MT)	339214	143614	57555	194646	15740	195	750164
Yields (MT/ha)	19	13	7	11	24	18	92
% of Area	32.8	19.7	14.8	31.4	1.2	0.1	100
% of Output	45.2	19.1	7.6	30	2.1	0.02	100

Source: Ministry of Agriculture Kenya (2008)

The Western region of Kenya grows and consumes 60% of national cassava production. In many districts of Western region of Kenya, cassava is the staple food crop, mainly grown in intercrops with beans, maize and bananas (Anon, 1998). The crop provides 9% of the total calories in the diet of Kenyans **(**Anon, 1990**).** Studies show that famine rarely occurs in areas where cassava is widely grown (Nweke, 1996). However its production in Kenya is constrained by Cassava Mosaic Disease (CMD) and high levels of cyanogenic glycosides in

some clones (Lusweti *et al*., 1997). The presence of hydrocyanic acid has been reported to lower the quality of cassava roots and hence the major reason for rejection of cassava cultivars in eastern Kenya and other 'boil and eat' societies like in the coastal region (Githunguri, 1995; Nweke, 1996). In Nyanza and Western provinces of Kenya, roots are also peeled, chopped into small pieces, dried and milled into flour for "Ugali". It is normally milled in combination with maize or sorghum. In the Coast province cassava leaves are used as vegetable (Khaemba, 1983). Cassava in Eastern Kenya is consumed either raw or boiled (Githunguri, 1995).

1.2 Cyanide

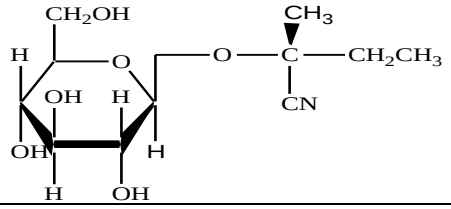
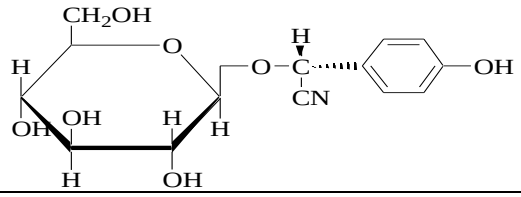
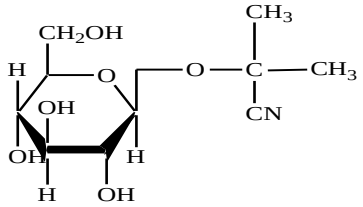
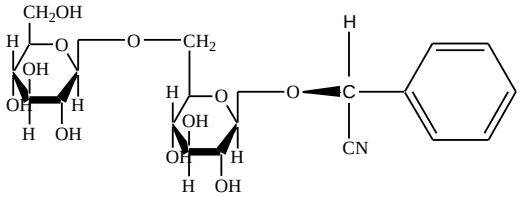
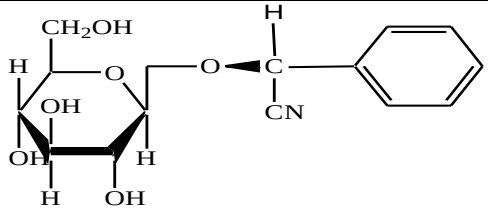
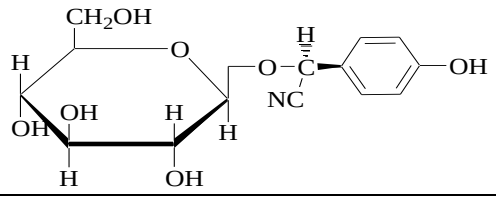
Cyanide is any chemical compound, inorganic or organic in nature that contains the cyano group (C≡N), and is capable of releasing cyanide. (Greenwood and Earnshaw, 1997). Free cyanide is defined as the sum of the cyanide present as either HCN or CN^- (Gosselin *et al*., 1984). Cyanide and chemically related compounds in nature are formed, excreted and degraded by hundreds of species of bacteria, algae, fungi, plants and insects (Knowles, 1976; US Fish and Wildlife Service, 1991). As a result, low levels of cyanide can appear in naturally occurring surface or groundwater, which normally would not be expected to contain it (Duffey, 1981).

Anthropogenic sources of cyanide may include synthetic catalytic process involving reaction of ammonia and natural gas with or without air. Hydrogen cyanide may be obtained as a by-product in the production of acrylonitrile (Carotii and Kaiser, 1972). Other cyanides like sodium and potassium cyanides are principally prepared by direct reaction of hydrogen cyanide released to air as a result of chemical manufacturing and processing industries, volatilization from cyanide wastes deposits in landfills and waste ponds (Carotii and Kaiser, 1972). Airborne sources of cyanide arise from cigarette smoke and smoke generated when

certain synthetic materials such as plastics are burned (Argonne National Laboratory, 2005). Laetrile (an anti-cancer preparation made from apricot kernels) and sodium nitroprusside (a drug used to reduce high blood pressure), release cyanide upon metabolism (ATSDR, 1995).

There are at least 2650 species of plants that contain cyanoglycosides and usually also a corresponding hydrolytic enzyme (β-glycosidase), which are brought together when the cell structure of the plant is disrupted by a predator, with subsequent breakdown to a sugar and a cyanohydrin, that rapidly decomposes to hydrogen cyanide and an aldehyde or a ketone (Haque and Bradbury, 2002). In kernels, this reaction is catalysed by the enzyme emulsin when the seeds are crushed and moistened (Lasch and El Shawa, 1981). Amygdalin (which is also present in bitter almonds and peach stones) is converted to glucose, benzaldehyde, and hydrogen cyanide (IPCS, 1992). There are approximately twenty five (25) cyanogenic glycosides known with the major cyanogenic glycosides found in the edible parts of plants: almonds, sorghum, cassava, stone fruit and bamboo shoots (WHO, 1993). The cyanogenic glycosides in these edible plants are shown in table 1.2.

Table 1.2: Cyanogenic glycosides in major edible plants

Types of cyanogenic glycosides	Structure	Plant Found
Lotaustralin		Cassava and lima beans
Dhurrin		Sorghum
Linamarin		Cassava
Amygdalin		Almonds
Prunasin		Stone fruits like apples, peaches and cherries
Taxiphyllin		Bamboo Shoots

Source: (JECFA, 1993)

In human, hydrogen cyanide is a well-known poison with potential acute and chronic metabolic effects (Kakes, 1990). Cyanide exerts its toxic effects by binding to the ferric ion

of cytochrome oxidase, an enzyme that accounts for about 90 percent of the total oxygen uptake in most cells via the electronic transport chain (Baskin *et al*., 2004). Humans get exposed to cyanide mainly by breathing air, drinking water, eating food or touching soils. Symptoms of acute poisoning include mental confusion, muscular paralysis, and respiratory distress (Kakes, 1990; Essers *et al*., 1992). Chronic exposure may lead to paralysis of legs, a disease called Konzo and even death (Banea *et al*., 1992).

1.3 Cyanide in cassava

Cyanides are produced by certain bacteria, fungi, and algae and are found in a number of foods and plants. In plants, cyanides are usually bound to sugar molecules in the form of cyanogenic glycosides and defend the plant against herbivores (Jones, 1998; Vetter, 2000). They are found, although in small amounts, in certain seeds and stone for example, those of apple, mango, peach, and bitter almonds (Agency for Toxic Substances and Disease Registry, 2004).

All cassava parts except the seed contain cyanogenic glycosides. In cassava plant the cyanogenic glucoside (linamarin) is synthesized in the leaves and transported to the tuber (Wheatley and Chuzel, 1993). Cyanogenic glycoside is present in large amounts in the leaves and the peel of the roots (900-2000 mg HCN/Kg) (Cardoso *et al*., 2005). The leaves contain the enzyme HNL, which catalysis the hydrolysis of acetone cyanohydrin to produce HCN and acetone (Siritunga and Sayre, 2004). The tuber has approximately 20-fold lower linamarin levels compared to the leaves. The root tubers have substantially reduced levels (< 6%) of HNL relative to leaves. There are cultivar- dependent differences in root cyanogen levels. Total tuber linamarin levels range between 100 and 500 mg linamarin/kg fresh weight for low- and high-cyanogenic cultivars, respectively. No cassava cultivars, however, lack cyanogenic glycosides (White *et al*., 1998). The peel (cortex) part of the cassava tuber is the

outermost layer part that is mostly exposed to different biochemical nutrient in the soil. As a result, exposure to hydrogen cyanide in the soil could lead to increase proportion of cyanide in this part of the tuber. The profile of cyanide concentration shows that parenchyma are lower cyanide storage part of cassava than peels and leaves thus the higher consumption of this part by man and animals (Anonymous, 1991). Cyanide level are also reduced significantly during the processing, to accepted level of 10 ppm by WHO or 2 to 3 mgHCN/100 g by IITA (International Institute of tropical agriculture,1989; Anonymous, 1991).

However, consumption of insufficiently processed bitter root tuber with varying levels of glucosides and cyanohydrins can result in dietary cyanide exposure that has been implicated as a cause of acute poisoning and some other toxico-nutritioned disorders (Mlingi *et al*., 1992). The human body detoxifies the main part of cyanide by enzymatic conversion to the less toxic compound thiocyanate (SCN) that is excreted in the urine (Tylleskar *et al*., 1992). HCN whether ingested directly or released from cyanogens in the body is readily absorbed into the blood by binding to iron in hemoglobin and quickly distributed to organs such as liver, kidney, brain and the blood tissue (Barret *et al*, 1977; Carlsson *et al.*, 1999). Acute intoxication leads to death, exacerbate goitre and cretinism in iodine-deficient regions it causes konzo and are implicated in the occurrence of Tropical Ataxic Neuropathy (TAN) and stunting of children (Oluwole *et al*., 2003; Stephenson *et al*., 2010).

1.4 Statement of the problem and justification

Cassava is grown in Kenya as a subsistence crop. It's utilization as food varies from region to region. It is a source of food security, not only because it can be grown on less productive land, but because it is a source of income for urban and rural populations. People in many parts of Kenya rely largely on starchy foods for their carbohydrates intake. Cassava being a

major source of carbohydrates is becoming a major source of food. The crop's agronomic advantages, such as high productivity, ease and flexibility of cultivation, tolerance to drought, and its ability to grow well on relatively poor soils has made it rapidly and extensively adaptable. The status of cassava as a food security crop to most subsistence farmers is, however, threatened by pests, diseases and weeds and the potential toxicity in cassava.

The presence of the cyanogenic glycoside is potential for production of poisonous hydrogen cyanide. Linamarin, a cyanogenic glycoside in cassava when hydrolyzed produces hydrogen cyanide and acetone. Studies on cyanide concentration in cassava have been done, but none has been done on cassava from Kenyan markets. The present work reports on variations in cyanide concentration in cassava growing in different geographical regions using the picrate paper method. The study also compares the concentration of cyanide in different parts of the cassava (pith, cortex and parenchyma).

1.5 Hypothesis

Cassava root contains cyanogenic glycosides which when hydrolyzed produce potentially poisonous HCN which can be assessed.

1.6 Objectives

1.6.1 General objective

To determine the concentration of cyanide in cassava root grown in different regions in Kenya.

1.6.2 Specific objectives

i. To establish the best and reliable method for the determination of cyanide levels.

ii. To determine the concentration of cyanide in cassava root from some parts of Kenya.

iii. To determine the concentration of cyanide in different cassava (parenchyma) cultivars in a farm.

iv. To compare the concentration of cyanide in peel, cortex and parenchyma of the cassava root.

1.7 Significance of the study

The information obtained in this study on, concentration of cyanide in cassava will be crucial for awareness campaigns to its users. Further, such information will be important for extension services geared towards training of farmers and consumers by agricultural officers and other regulatory bodies, on effective methods for processing cassava.

1.8 Scope and limitations

i. The covers specific counties in the country.

ii. Only selected markets in these counties will be sampled for this study.

iii. The sweet variety cassava will be the only one to be considered in this study.

CHAPTER TWO

LITERATURE REVIEW

2.1 Cyanogenic glycosides

Cyanogenic glycosides are phytotoxins which occur in at least 2000 plant species, of which a number of species are used as food in some areas of the world (Rosling, 1987). Cassava and sorghum are especially important staple foods containing cyanogenic glycosides (Rosling, 1987). The cyanogenic glycosides are α-hydroxynitrile secondary metabolites from plants. They are amino acid-derived plant constituents (Vetter, 2000). The biosynthetic precursors of the cyanogenic glycosides are L-amino acids, which are hydroxylated, then the N-hydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to α-hydroxynitriles and then glycosylated to cyanogenic glycosides (Vetter, 2000). This is shown in figure 2.1 below.

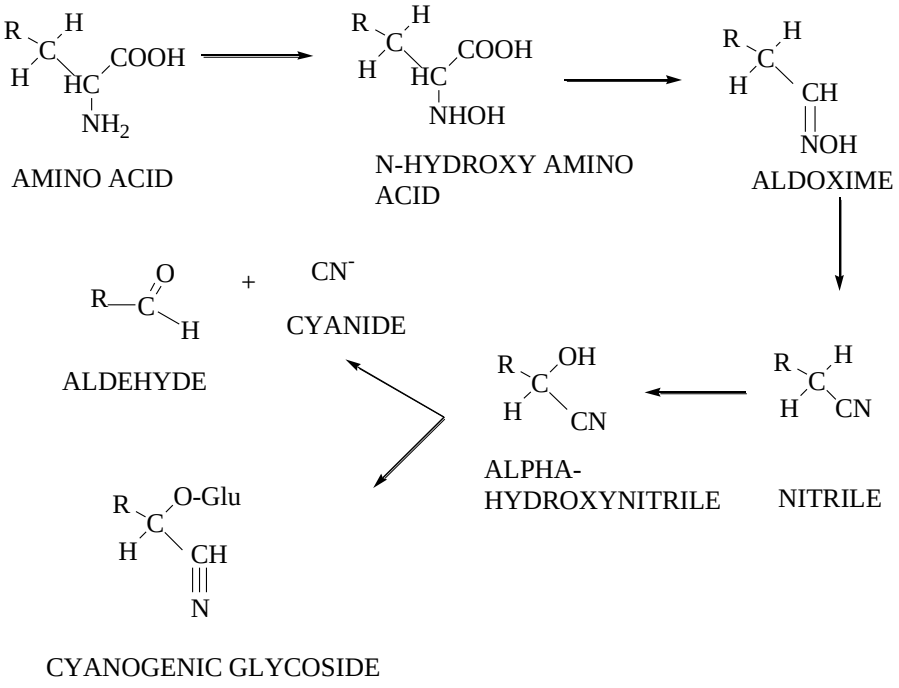


Figure 2.1: Biosynthesis of cyanogenic glycosides (Conn, 1979)

All known cyanogenic glycosides are β-linked, mostly with D-glucose. The glycosides, cyanohydrins and hydrogen cyanide are collectively known as cyanogens. This combination

of cyanogenic and hydrolytic enzyme is the means by which cyanogenic plants are protected against predators (Moller and Seigler, 1999).

Linamarin, a cyanogenic glycoside accounts for 95% of the total cyanogenic glycosides present in intact tissues of cassava (Balagopalan *et al*., 1988). The production of cyanogenic glycosides is thought to provide an immediately chemical defence for the plant against herbivore and pathogen attack through the release of toxic cyanide (Moller, 2010). Cyanogenic glycosides also have a bitter taste that may function as a feeding deterrent (McKey *et al*., 2010). The concentration of cyanogenic glycosides varies within individual plants, among cultivars and with environmental conditions (Bokanga *et al*., 1994; Vetter, 2000). The highest concentrations are in the young leaves, newly germinated seedlings and the outer layers of the tuber (Jorgensen *et al*, 2005).

2.2 Cyanogen in cassava

Cassava, due to the presence of cyanogenic glucosides, is potentially toxic. With the exception of seeds, the cyanogenic glucosides linamarin (95%) and lotaustralin (5%) are present in all parts of the mature cassava plant (Conn, 1994). Lotaustralin (ethyl linamarin) is located in the plant cell vacuole, and the enzyme linamarase is located in the cell wall (Mkpong *et al*., 1990). Linamarin is synthesised in the leaves and then transported to the roots, probably in the phloem, and some additional synthesis occurs in the periderm of the tubers (Jorgensen *et al*, 2005). The cyanogenic glycosides and corresponding hydrolytic enzyme (beta-glycosidase), are brought together when the cell structure of the plant is disrupted by a predator, leading to subsequent breakdown to sugar and a cyanohydrin, the unstable cyanohydrin under neutral condition rapidly decomposes to hydrogen cyanide (HCN) and an aldehyde or a ketone (Moller and Seigler, 1999). The hydrolysis of linamarin is as shown in the Figure 2.2 below.

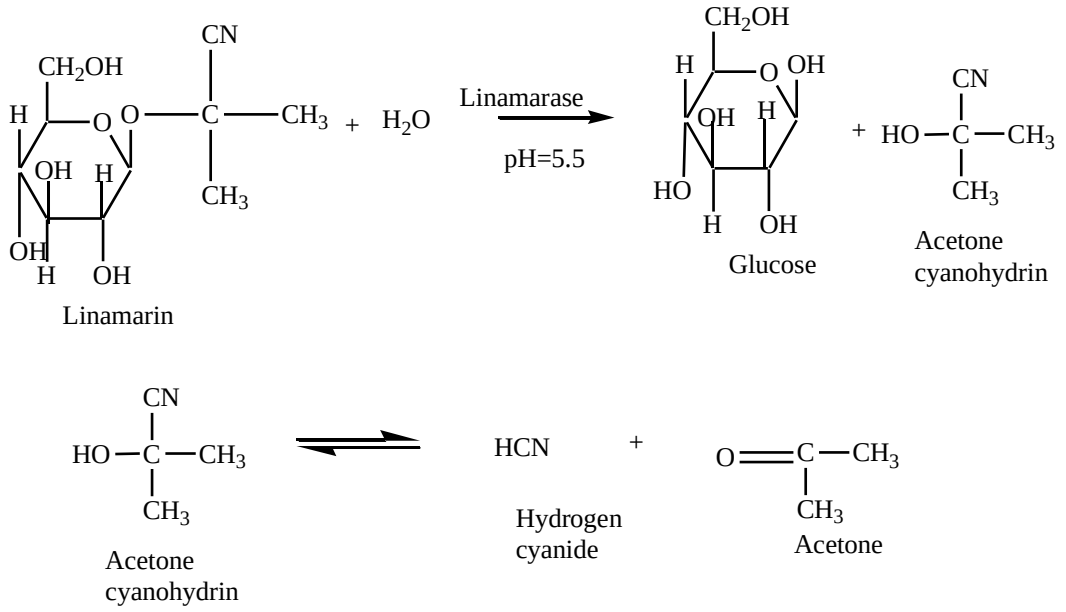


Figure 2.2: Hydrolysis of linamarin (Cooke, 1978).

The compartmentalization of linamarin in the vacuole and linamarase and HNL in the cell wall prevents the formation of toxic cyanide in undamaged cells. The breakdown of the physical barriers between substrates and the enzymes, following tissue damage, initiates cyanogenesis (Poulton, 1990). The potential toxicity of cassava is related to the capacity of all parts of the plant to release hydrogen cyanide from stored cyanogenic glucosides. This ability is known as cyanogenesis (Ermans *et al*., 1980; Nahrstedt, 1993).

The residual cyanogens, linamarin and acetone cyanohydrin, are the apparent source of cyanide toxicity to animals and human, when converted to cyanide inside the body. For an adult human, consumption of 50 to 100 mg or 2 mmol of HCN within 24 hours can completely block cellular respiration leading to death (Rosling, 1994). Consumption of cassava products with high concentrations of cyanogens can lead to illness or even death (Tylleskär *et al*., 1991). While many food crops are cyanogenic, unlike in cassava, the toxic element is generally either in parts of the plant that are not consumed (like apple seeds and wheat leaves or are eaten in small amounts (almonds) (Jones, 1998).

The risk of cyanide toxicity from cassava is increased because not only is the part that is consumed highly cyanogenic but it also frequently forms a large proportion of the overall diet to many countries in Africa Asia and Latin America (Cliff *et al*., 1999).

2.3 Cyanide levels in cassava cultivars

A total of 1,200 local cassava varieties have been identified in Africa and with a higher genetic diversity for the sweet than for the bitter varieties (Asadu *et al*., 1999). Most small-scale cassava farmers grow a number of cultivars, each with locally preferred qualities such as good taste, early maturation or good processing characteristics (Salick *et al*., 1997; Chiwona- Karltun *et al*., 2000). African small-scale farmers mainly acquire new cultivars from their neighbours, during travels or by collecting seedlings of sexually propagated cassava found in fields left in fallow for several years (Chiwona-Karltun *et al*., 1998). The performance of a cultivar within the local environment and farming system determines whether it will be adopted, and continue to be cultivated. There is evidence from several parts of Africa that few cassava cultivars originate from breeding programmes (Nweke *et al*., 1994; Chiwona-Karltun *et al*., 1998).

The different cultivars or varieties of cassava can be distinguished by such features as; size, colour and shape of the leaf, stem and petiole, branching habit, plant height, tuber and amount of the root tuber produced per plant, the nutritive content of the tubers, the resistance to certain diseases and weeds, the climatic and nutrient requirements such as fertilizers for maximum yield of the plants and “sweet” or “bitter”, depending on the level of cyanide content (Nweke *et al*., 1999; RMRDC, 2004). The pulp of most common cultivars varies from white to light yellow (Booth *et al*., 1976).

The term "bitter" cassava, as opposed to "sweet" cassava, refers to the taste of the root parenchyma. Bitterness is associated with higher levels of cyanogenic glucosides (Miya *et al*., 1975; Cock, 1985). Cultivars that usually give bitter roots are called bitter cultivars. However, certain ecological stress factors, such as pest attacks, prolonged drought and low phosphorus and potassium levels in the soil may cause roots to acquire bitterness, and this coincides with an increase in the levels of cyanogenic glucosides (De Bruijn, 1971; Ayanru and Sharma, 1984). The peel of the "bitter" cassava variety contains on average 650 ppm and the parenchyma contains 310 ppm total cyanide; the corresponding values for "sweet" varieties were 200 ppm and 38 ppm total cyanide respectively (Anom, 2005). Reported levels of cyanogenic glucosides in fresh root parenchyma have been from 10 up to 2000 mg HCN equivalent per kg dry weight (Coursey, 1973).

Cassava tubers vary widely in their cyanogenic glycoside content, although most varieties contain 15 – 400 mg cyanide/kg fresh weight. Occasionally varieties of cassava tubers contain 1300 – 2000 mg cyanide/kg fresh weight (Padmaja, 1995). A marked radial gradient in linamarin content exists from the outer peel to the inner parenchyma tissue. It has been shown that the outer peel can contain between 7- and 16-times the linamarin of parenchyma tissue of the same variety (Bradbury and Egan, 1992). Linamarin and linamarase levels vary widely between cassava cultivars, between plants of the same cultivar, between different tissues of the same plant, between roots of the same plant and even within the root parenchyma (De Bruijn, 1971; Bourdoux *et al*., 1980).

2.4 Effects of cyanide on human body

The potential toxicity of cyanogenic plant depends primarily on production of HCN which is toxic to human. The residual cyanogens, linamarin and acetone cyanohydrin in cassava, are

the apparent source of cyanide toxicity to human when converted to cyanide inside the body (Rosling, 1994). Normally, small quantities of cyanide are detoxified by cellular enzymes and thiosulfates in many tissues to form relatively harmless thiocyanate, which is excreted in the urine (Salkowski and Penney, 1994).

For an adult, consumption of 50 to 100 mg or 2 mmol of HCN within 24 hours can completely block cellular respiration leading to death (Rosling, 1994). Upon consumption of unprocessed cassava, β-glycosidase will be released during digestion and remain active until deactivated by the low pH of the stomach. This enzyme will hydrolyse the cyanogenic glycoside and release at least part of the potential hydrogen cyanide content of the plant (WHO, 1993). The ingestion of large quantities of cassava or prolonged exposure to improperly processed cassava food has been associated with chronic cyanide toxicity in several areas of Africa (Tylleskar *et al*., 1992; Mlingi *et al*., 1992). Consumption of unprocessed raw and whole cooked cassava led to food poisoning and fatal cases in Eastern Province, Kenya. The boiled cassava had lower cyanide content (56.0 mg/kg) than the uncooked cassava (73.2 mg/kg). This was attributed to the fact that boiling of cassava reduced the cyanide content to a certain extent (Njue *et al*., 2011).

2.4.1 Acute Toxicity

Cyanide is a chemical asphyxiant that can cause death soon after exposure (Rorison and McPherson, 1992). It inhibits cytochrome oxidase preventing oxygen utilization leading to cytotoxic anoxia. Acute effects depend on the degree of histotoxic hypoxia. Death results from CNS depression (U.S. EPA, 1978). Signs of acute intoxication by cyanide include rapid breathing, gasping, headache, salivation, nausea, anxiety, vertigo, cardiac arrhythmias, tremors, hypotension, respiratory failure, convulsions and death. Venous blood remains oxygenated and victim may appear pink (Timbrell, 1994). The mean lethal dose by mouth of

cyanide in human adults is thought to be in the range of 50 to 200 mg and death is rarely delayed more than one hour (Gosselin *et al*., 1976).

2.4.1.1 Mechanism of Acute Cyanide Poisoning

When large quantities of cyanide are rapidly absorbed and the body's detoxification mechanisms are overwhelmed, acute cyanide poisoning occurs (Salkowski and Penney 1994). The cyanide ion is readily absorbed from the intestinal and respiratory tracts and has a strong affinity for binding with trivalent iron of the cytochrome oxidase molecule (Way, 1984). In acute cyanide poisoning the characteristic cherry red venous blood, is seen due to the failure of the oxygen-saturated hemoglobin, to release its oxygen at the tissues because the enzyme cytochrome oxidase is inhibited by the cyanide (Salkowski and Penney 1994). This causes a decrease in the utilization of oxygen in the tissues. It also causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism (WHO, 1993).

2.4.2 Chronic Toxicity

Exposure to low levels of cyanide over a prolonged period produces symptoms which differ from acute exposures. Several conditions have been observed in cassava eating populations which have been attributed to chronic cyanide intake. These include malnutrition, diabetes, congenital malformations, neurological disorders and myelopathy (FSANZ, 2004). Goiter (swelling of the thyroid glands) is thought to have occurred where cyanogenic glycosides are present in the diet at levels greater than 10 – 50 mg/kg food (FSANZ, 2004).

2.4.3 Neurological Effects

Chronic human exposure to cyanide has been studied in regions of Africa with populations which consume large amounts of cyanide-containing cassava root. Neurological findings include symmetrical hyperreflexia of the upper limbs, symmetrical spastic paraparesis of the lower limbs, spastic dysarthria, diminished visual acuity, peripheral neuropathy, cerebellar signs and deafness (Ministry of Health, Mozambique, 1984). Cyanide intake from cassava-dominated diet is a contributing factor in two forms of nutritional neuropathies, tropical ataxic neuropathy described from Nigeria and epidemic spastic paraparesis described from Mozambique, Tanzania and Zaire (Osuntokun, 1981; Ministry of Health, Mozambique, 1984).

2.4.3.1 Tropical Ataxic Neuropathy (TAN)

Tropical ataxic neuropathy (TAN) describes several neurological symptoms effecting the mouth, eyesight, hearing or gait of mostly older males and females. TAN is attributed to cyanide exposure from the chronic consumption of foods derived from cassava (FSANZ, 2004). In West Africa particularly Nigeria, Tanzania, Uganda, Kenya, the West Indies and tropical Asia it is reported that TAN generally occurs in older people who have consumed a monotonous cassava diet over years. The TAN is progressive and causes unsteady walking, produces loss of sensation in hands, vision, deafness and weakness (Osuntokun, 1981; Osuntokun, 1994).

2.4.3.2 Konzo

In Eastern, Southern and Central Africa there is a disease called ‘konzo’, a tropical spastic paralysis which causes irreversible paralysis of the legs, particularly in children and in women of child-bearing age (Ministry of Health Mozambique, 1984). It is an upper motor neurone disease of sudden onset (Howlett, 1994). It has been reported in drought affected

rural populations in the north east part of Nampula province, Mozambique and in two parts of Tanzania (Howlett *et al*., 1990; Mlingi *et al*., 1992). In all reports of epidemics, konzo has been associated with high and sustained cyanogens intake at sub-lethal concentrations from cassava or cassava flour. The epidemics in East Africa coincided with food shortage and several weeks of exclusive consumption of insufficiently processed bitter cassava roots resulting in high dietary cyanide exposure, as verified by high levels of thiocyanate in serum and urine (Ministry of Health Mozambique, 1984).

2.4.4 Biochemical effect

Hydrogen cyanide after oral ingestion or administration is readily absorbed and rapidly distributed in the body through the blood. It is known to combine with iron in both methaemoglobin and haemoglobin present in erythrocytes (Dreisenbach and Robertson, 1987; Makene and Wilson, 1972)**.** The cyanide level in different human tissues in a fatal case of HCN poisoning has been reported in mg/100g as follows; gastric content (0.03), blood (0.5), liver (0.03), kidney (0.11), brain (0.07), and urine (0.2). Cyanide acts through the inhibition of cytochrome c oxidase in the respiratory electron transport chain of the mitochondria, impairing both oxidative metabolism and the associated process of oxidative phosphorylation. (Dreisenbach and Robertson, 1987; Makene and Wilson, 1972).

In human, cyanide is detoxified by enzymic conversion by rhodanese to thiocyanate using sulphur originating from dietary sulphur amino acids (Cliff *et al*., 1985). Two defense mechanism are present. The methaemoglobin fraction in the red blood cells can temporarily neutralize cyanide by reversible reaction (Lundquist *et al*., 1985). The other major pathway is the conversion of cyanide to a less toxic thiocyanate (SCN). Cyanide is catalysed by the enzyme rhodanese present in most tissues, by a reaction with sulphur (Rosling, 1994), as shown in Figure 3. Normally about 50mg cyanide is converted into SCN per day in healthy

human tissue (Schultz, 1984). The SCN is then gradually excreted in the urine. The sulphur is derived from two amino acids, cysteine and methionine (Diasolua *et al*., 2003). A high intake of cyanide in combination with a low intake of sulphur amino acids has been advanced as the main aetiology, but other dietary deficiencies and predisposing factors may also contribute to it (Cliff *et al*., 1985).

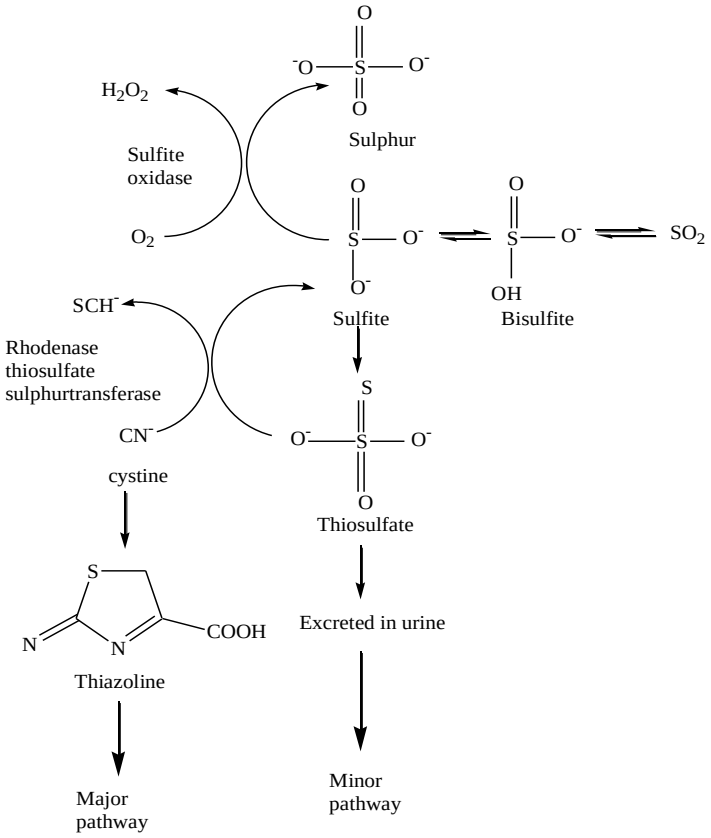


Figure 2.3: Cyanide metabolism in the body (Omaye, 2004).

2.5 Methods of analysis of cassava

There are many methods for cyanide analysis described in literature all of them share three commonalities, the Method of extraction, hydrolysis of cyanogenic glycosides and analytical determination of the free cyanide (Brito *et al*., 2009). Considering the great diversity of toxicity that cyanide species exhibit, cyanide pollutants have been officially classified into three groups. These groups are free cyanide (HCN, alkaline and alkaline earth cyanides), weak acid dissociable cyanide (WAD) – (a collective term for free cyanide and metal-cyanide complexes ($Ag(CN)_2^-$, $Cu(CN)_4^{3-}$, $Cd(CN)_4^{2-}$, $Zn(CN)_4^{2-}$, $Hg(CN)_4^{2-}$, $Ni(CN)_4^{2-}$) which easily

release HCN under slightly acidic environmental conditions) and total cyanide each being potential source of HCN regardless of its origin (U.S EPA,1992).

Cyanide in aqueous matrices is usually measured by colorimetric, titrimetric, or electrochemical methods after pretreatment to produce hydrogen cyanide and absorption in sodium hydroxide solution (US EPA, 1983; US EPA, 1993a). A chromatographic technique with fluorescence detection is used to detect trace amounts of cyanide in blood cells (Chinaka *et al*., 1998). Cyanide in biological tissue and fluids can be measured spectrophotometrically after reaction with methaemoglobin (ATSDR, 1989). Many cyanides are unstable and emit volatile hydrogen cyanide gas. Sampling, storage, and analysis must be done with caution, preferably immediately upon collection. The three commonly used techniques (colorimetric, titrimetric, and electrochemical) may all suffer from interference problems, unless proper precautions are taken (ATSDR, 1989).

The methodology for linamarin and their metabolic analysis require a three-step; extraction of cyanogenic compounds from plants; hydrolysis of cyanogenic compounds in plants and analytical determination of the released cyanide (Cagnon *et al*., 2002). The extraction of cyanogenic compounds from the plant is performed by using an acid diluted solution, since linamarase is inactive at low pH (Bradbury *et al*., 1994). Linamarin is then removed and submitted to a series of hydrolyses until cyanide becomes free cyanide (Rao,1984). Among the methods for the third step, one can cite titration of cyanide with $AgNO_3$ (AOAC, 1990) and reaction with alkaline picrate (Egan *et al*., 1998). The most widely used coloring method is based on the König reaction, in which free cyanide (CN^-) is oxidized into cyanogen halide by chloramines T or N-chloro succinimide (Essers *et al*., 1993). Another method uses specific electrode for cyanide and voltmeter to measure the potential difference. Autolysis method, in which reagents pyridine and pyrazolone or pyridine and barbiturates are used, has very good

specificity to cyanide, and reasonable accuracy (Cooke 1978; Bradbury 1994). The enzymatic method, require an addition of linamarase and use of pyridine and pyrazolone as indicators.

In this study two commonly used techniques of determining cyanide were compared; colorimetric (picrate paper and picric in solution methods) and titrimetric technique. The most reliable, sensitive method, easy to use, and one that had readily available chemicals and equipment was used in determination of cyanide concentration in cassava samples.

2.5.1 Picrate paper method

Bradbury *et al* (1999) developed a picrate paper kit to determine the total cyanogens in cassava root, a linear Beer's law relation between absorbance and cyanogens was obtained over the range 0 – 800mg HCN equivalent kg^{-1} cassava. The picrate paper method has been used in a case study on two incidents, on fatal cassava poisoning in Kitui district in Kenya, where two children died. Levels of cyanide in raw and cooked cassava were determine. The results showed high levels of 46.0 mg/Kg CN and 52.3mg/Kg CN for the raw and cooked respectively (Njue *et al*., 2011). Oliver and Hernaez (2006) also used it in determining level of cyanide in cassava in the Philippine and according to them, the method proved to be effective. This method is simple and requires readily available chemicals and equipments.

The method measures weak acid dissociable cyanide. It's based on reduction of sodium picrate by cyanide to form a colored product which can be measured colorimetrically. This reduction of picric acid is affected by free cyanide only**.** The picrate methods are based on the picrate reaction which has been developed by several authors including Brimer (1994) and Bradbury *et al*. (1999). The method involves impregnating sheets of absorbent paper (or TLC sheets) with picric acid solution, followed by sodium carbonate solution (or picric acid in a solution of sodium carbonate (Brimer *et al*., 1983). Picrate reacts with cyanide in the

presence of sodium carbonate to produce isopurpurin (and similar compounds) which are colored and can be measured spectrophotometrically.

2.5.2 Picric acid in solution method

Baltha and Cereda (2006) used this method for determining the free and potential cyanide based on autolysis of cassava roots in which endogenous linamarase enzyme was used to hydrolyze linamarin and release CN^- (Bradbury, 1994). Free cyanide was determined by adapting the method of Smith described by Winton and Winton (1958), which is based on colorimetric reaction of picrate solution. The authors established that a 15-minute reaction provided the highest values of cyanide, thus indicating an asymptotic curve of color development. Based on the results obtained in a study by Brito *et al.* (2010) it is possible to state that the qualitative and quantitative methods allow the determination of free and potential cyanide contents in different parts of the cassava plant. The method has good sensitivity, good specificity to cyanide, and reasonable accuracy.

After hydrolysis of linamarin the HCN produced reacts with picric acid to form a charge transfer complex that is brown in colour. These complexes are characterised as hybrid of two resonance structures as shown in figure 2.4.

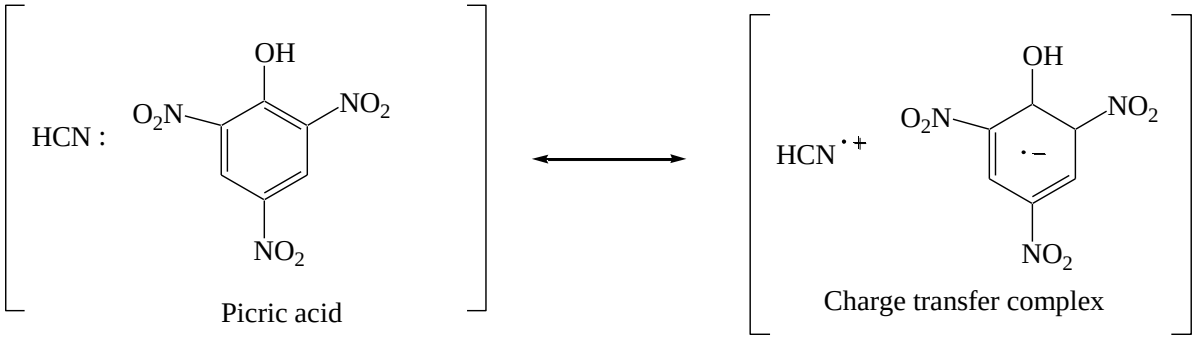


Figure 2.4: Resonance structure of charge transfer complex between picric acid and HCN

Source: Andrew, 1985

2.5.3 Alkaline Titration method

The method was previously used by Onyesom *et al.* (2008) in determining the levels of cyanide in cassava fermented with lemon grass (*cymbopogon citratus*). The cyanide content of each sample was determined using the alkaline titration method (AOAC, 1990). 5% potassium Iodide was used as the indicator and the end point of the titration was reached when the solution changed from a clear solution to a faint turbid solution. The amount of cyanide in the sample was determined from the relation: $1cm^3$ 0.02M $AgNO_3$ = 1.08 mg HCN.

This is one of the most popular cyanide measured methods used in gold extraction industries. It measures free cyanide when the dominant ion is CN^- more appropriately termed as "titratable cyanide" rather than free cyanide (Heath *et al*., 1999). The method involves of addition of silver ions to the solution to complex with the "free cyanide". When all the free cyanide is consumed as silver cyanide complex, the excess silver ions indicate the end point of the titration. The end point is indicated by either color or potential. The easiest possibility is to use an indicator such as potassium Iodide or p-dimethyl amino-benzal-rhodanine that changes color upon appearance of free silver ions. The colored observed is due to formation of diammine Silver (II) complex (Courtney, 2008).

The three methods from the two techniques, colorimetric (picrate paper and picric in solution methods) and titration are the most commonly used in determination of cyanide. The methods were compared to determine the most effective, reliable and easy to use. The most effective, reliable and easy to use method was used to determine the concentration of cyanide in cassava in this study.

CHAPTER THREE

EXPERIMENTAL

3.1 Research design

The research was done in two parts. The first part entailed determination of the most effective method of the three methods: picrate paper method, picric in solution method and alkaline titration method. The method of choice for determination of cyanide concentration in cassava was established by comparing the coefficient relation. The method of choice was used in the second part. The second part involved collection of samples from the different parts of Kenya and experimental laboratory analysis.

3.2 Study area

The study regions were chosen based on two factors. Kakamega, Kitui and Kisii were selected because of the importance of the crop in the areas, while Thika and Nairobi were selected due to easy accessibility to the markets. Nairobi county is located within the coordinate's 1°17′S 36°49′E / 1.283°S 36.817°E cover an area of 700 km^2 with a population of over 3.13 million. Thika district located within the coordinates 01°03′S 37°05′E / 1.050°S 37.083°E covers an area of 1960 km^2 with a population of 646,000, Kakamega county located within the coordinates 0°17′N 34°45′E covers an area of 1395 km^2 with a population 1.66 million (KNBS, 2009). Kitui county located within the coordinates 1°22′S 38°01′E / 1.367°S 38.017°E , covers an area of 20,402 km^2 with a population 1.01 million (KNBS, 2009). Kisii county is located within the coordinates 0°41′ S 34°46′ E / 0.683° S 34.767° E with a population of 1.15 million (KNBS, 2009).

3.3 Sample size, sampling sites and sample procedure

A purposive sampling strategy was used in selecting the counties to be studied. This was done mainly to areas according to literature where cassava is commonly used. Cultivation is

mainly in western Kenya comprising of Nyanza and Western (60%), Eastern (10%) and Coast (30%). Samples were randomly picked form Kakamega, Kisii, Kitui, Thika and Nairobi county. Samples were also collected from Kenya Agricultural Research Institute (KARI) in Kiboko for the different varieties. The sample size was calculated using the formula below reported by Daniel (1999).

$$n = \frac{Z^2 P (1 - P)}{d^2} \quad \text{.....Eq 1}$$

Where n = Sample size

Z = Statistic for a level of confidence (for the level of confidence of 95%, which is convection, Z value is 1.96)

P = Expected prevalence or proportion (in proportion of one; if 80%, P = 0.8)

d = Precision (in proportion of one; if 16%, d = 0.16

$$n = 1.96^2 \frac{\left[0.8 (1 - 0.8)\right]}{0.16^2} \quad \text{.....Eq 2}$$

$$n = 24.01$$

From each of the five regions of study, 3 markets were selected giving a total of nine markets used for study. In each of these markets a sample size of 30 was randomly picked and labeled. A sample size of 90 was collected from each region to give a representative sample size. These sample size included 8 samples from Kari in Kiboko that were to give the values of cyanide concentration in known cassava cultivars. A total of 126 samples were excluded from the study some, due to transportation went bad, and others were found to be diseased thus unfit for study.

3.4 Laboratory procedures

3.4.1 Equipments chemicals and reagents

A UV-visible spectrometer, Cecil CE 2041 2000 series (Cambridge, England, United Kingdom) was used for analysis and the electronic analytical balance (Shimandzu C307

000632 ACX224, Tokyo Japan) AAA model from Britain was used for weighing the samples. Distillation equipment from Great Britain of the model WSB/4 was used for distillation. Picric acid, sodium carbonate, potassium cyanide, sulphuric acid, ammonium solution, potassium iodide, silver nitrate, dihydrogen phosphate and disodium hydrogen phosphate were of analytical grade from Loba Chemie (Mumbai, India). A picrate paper kit used for determination of cyanide was provided to us by Prof Bradbury (Australian National University).

3.4.2 Preparation of the samples

The labeled Cassavas samples obtained from the different regions were cleaned using tap water and refrigerated at -4^{o}C awaiting analysis**.**

3.4.3 Cleaning of glassware and sample containers

All glassware was cleaned in warm soapy water mixed with a detergent (Laser clean from laser Chemicals International). They were then rinsed with distilled water before drying in the oven at 105^{0}C. The dry apparatus were safely stored in clean drawers awaiting usage.

3.4.4 Preparation of reagents

3.4.4.1 Picrate papers

1.4g of moist picric acid was weighed and 100 ml of sodium carbonate solution, made by dissolving 2.5 g of sodium carbonate in 100 ml of water was added. Using whatman 3MM filter paper sheet supplied in the Bradbury kit, 10 cm x 10 cm square of paper were cut and placed in the yellow picrate solution in a dish for about 20 seconds then left to dry. Unevenly colored sections of the paper were cut off and the paper cut into 30 mm x 10 mm rectangular pieces. Each piece was then glued to plastic strip (10 mm x 50 mm) using vinyl acetate glue. The picrate papers were stored in a deep freezer at -4^{0}c to ensure stability.

3.4.4.2 Phosphate buffer

Two one molar solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate were prepared. Acidic sodium dihydrogen phosphate solution was carefully added to disodium hydrogen phosphate to give a solution of pH decreased to 6.0. To make 0.1 M phosphate buffer, 100 ml of 1.0 M phosphate buffer was mixed with 900 ml water.

3.4.4.3 Standard potassium cyanide stock solution (picrate paper method)

2.505 g of Potassium cyanide were accurately weighed, transferred to 1000 ml volumetric flask and diluted up to the mark with 0.01 M NaOH. Working solutions were prepared by serial dilution of the stock solution and checked for consistency of absorption using the UV-spectrophotometer before taking the reading. The absorbance readings were used to draw the calibration curve.

3.4.4.4 Preparation of alkaline picrate solution

Equal volumes of picric acid obtained by diluting 2.56 g of picric acid in 100 ml of distilled water and 5% calcium carbonate solution obtained from 5 g of sodium carbonate dissolved in 100 ml of distilled water were mixed to have alkaline picrate for the analysis.

3.4.4.5 Standard potassium cyanide stock solution (picrate in solution)

6.5 g of potassium cyanide were diluted in distilled water and completed to 1000 ml with 0.01 M H_2SO_4. This stock solution containing 2.6 g of CN^- per ml was obtained with the purity of KCN (see appendix II, table A1). The obtained stock solutions were diluted with a 0.01 M H_2SO_4 to yield eight dilute solutions which were further diluted serially with alkaline picrate solution in distilled water to give eight solutions (see appendix II, table A.2), whose absorbance at 535nm was used to draw a calibration curve (see appendix II, figure A.2).

3.4.5 Determination of cyanide using picrate paper

A sample root (parenchyma) was obtained by cutting a 1 – 2 mm thick section across the root about halfway along its length. After removal of the peel 100 mg sector was cut from this disc. A round paper disc containing buffer at pH 6 was placed in flat bottomed plastic bottle and the weighed sector of cassava root placed on top of it. 0.5 ml of distilled water was added using the plastic pipette provided in the kit. Immediately the picrate paper attached to a plastic strip was placed on the plastic bottle and the bottle closed with a screw capped lid. It was allowed to stand for 16 - 24 hour at room temperature. The bottles were then opened and the color of the picrate papers matched against the shades of color in the color chart provided in Bradbury picrate paper kit (see appendix III). The total cyanide content in mg/kg in the cassava root was determined. This procedure was repeated for blank (same procedure used but no cassava was put in the bottle) and the standard solutions. This gave an estimate of HCN contents in parts per million (mg/kg).

To obtain the actual concentration of HCN equivalent in the sample, a linear calibration curve was drawn. Picrate solutions were made by placing the picrate papers (taken through the procedure above) in 5 ml of distilled water. The absorbance of the picrate solution was then obtained at 510 mn using UV-visible spectrometer Cecil CE 2041 2000 series (Bradbury, 1999). The linear calibration curve ($r^2_=$ 0.9996) for cyanide (see appendix II, figure A3) was used to determine HCN content in cassava samples. Adequate quality control was ensured by analysing the blank samples using the above procedure after every ten samples analysed and carrying out triplicate analyses.

3.4.6 Determination of cyanide using picrate in solution

The cyanide determination was performed using extracts of cassava tissues from the parenchyma. 3.0 g of cassava root (parenchyma) was weighed and placed in 50 ml of distilled

water with a mixer for 60 seconds. The extract was filtered using a filter paper. 0.04 ml of the extract was taken and treated as shown in table in appendix II, table A2. The test tubes containing the samples and color reagents were incubated for 15 minutes in water bath at 37^{o}C. Before reading 15 µL of sulfuric acid (H_2SO_4) were added to stop the reaction and increase the stability of reading. The UV absorption readings of the distillate obtained were used to prepare the standard curve (see appendix II, figure A4).

3.4.7 Determination of cyanide by titration

20 g of the cassava root (parenchyma) was grounded using a blender. It was then transferred into a distillation flask and left to stand for 3 hrs. It was then distilled until 150 cm^3 of the distillate was obtained. 20 cm^3 of 0.02 M sodium hydroxide was added to the distillate and the volume completed to 250 cm^3 in a volumetric flask using distilled water. Three aliquots, two of 100 ml each and one of 50 ml were obtained. 8 cm^3 of 6 M ammonium solution and 2 cm^3 of 5% potassium iodide were added to the 100 ml aliquots, 4 cm^3 and 1 cm^3 were added to the 50 ml aliquot. This was titrated using 0.02 M silver nitrate, using the 50 ml aliquot as the trial. The readings were taken. The equation below was used to get HCN in mg.

1 ml of 0.02 M silver nitrate = 1.08 mg HCN according to AOAC (1990).

3.4.8 Determination of cyanide concentration in the eight cassava cultivars

Eight cassava root (parenchyma) cultivars (all coded differently) were analysed using the picrate paper method. Same procedure as the one mentioned above (picrate paper method) was used.

3.4.9 Determination of cyanide concentration in the three parts of the cassava root

Three cassava root samples were randomly picked. The root was cut and separated according to the different parts (peel, parenchyma and cortex). Picrate paper method was used to analyse cyanide concentration in each of this parts.

3. 5 Data Analysis

Test of significance of cyanide levels was determined by ANOVA. All calculations were done using statistical SPSS program (11.5). The significance level was set at $P = 0.05$.

CHAPTER FOUR

RESULT AND DISCUSSION

4.1 Determination of cyanide concentration in cassava using three different analytical methods

In order to determine the most effective method of cyanide concentration, the three methods were used to determine the concentration of cyanide. Two standard solutions of 40 mg/L and 60 mg/L were considered. The results of analysis are summarized in Table 4.1.

Table 4.1: Concentration of cyanide in standard solutions as determined by three different methods

		Concentration (mg/L)		
	n	**Standard**	**Determined±SE**	**t_{cal}**
Picrate paper	4	40	36.28±3.28	1.134
Picrate solution	4	40	65.15±3.31	7.598
Titration	4	40	184.95±10.03	14.451

		Concentration (mg/L)		
	n	**Standard**	**Determined±SE**	**t_{cal}**
Picrate paper	4	60	57.87±2.14	0.995
Picrate solution	4	60	80.89±4.13	5.058
Titration	4	60	228.6±10.12	16.66

$t_{critical\ (95\%,3\ degrees\ of\ freedom)} = 3.182$

In both picrate in solution and titration methods the standard and determined HCN equivalent (mg/L) differ significantly ($t_{calculated} > t_{critical}$ at 95% confidence level). Picrate solution and titration methods reported values higher than the standard concentrations. This results show that the picrate paper method is reliable and has high reproducibility. The levels of cyanide concentration noted in titration method could be attributed to interferences such as nitrates and /or nitrites (US EPA, 2004). It could also be attributed to bias in determination of titer volumes during titration, since use of turbidity end point is subject to being arbitrary depending on the analyst. The use of picrate paper instead of picrate solution also gives a

possibility of reducing the interferences that may be detected in the solution. The pictrate paper method compared to picrate solution reported less concentration than the standard. This could be attributed to the fact that in picrate paper there is less concentration of interfering anions (US EPA, 2004). The picrate paper method detects cyanide concentration over the range of 0-800 mg HCN equivalent ⁻kg cassava (Bradbury *et al*., 1999). This explains the replacement of this method with modern electrochemical and potentiometric methods in which end point determination is accurate (Courtney, 2008).

4.2 Determination of cyanide concentration in cassava from Kisii, Kakamega, Kitui, Thika and Nairobi using picrate paper

Cyanide levels for three hundred and twenty four (n = 324) cassava root samples were determined using the picrate paper method. The samples had been randomly sampled from five regions. The mean results of the individual analysis are given in table 1 A, appendix I. Analysis of the level of cyanide in cassava samples collected from Kisii, Kakamega, Kitui, Thika and Nairobi using the picrate paper method revealed significant variation ($P < 0.005$) between cyanide concentration and geographical location of the cassava plant (Table 4.2).

Table 4.2: Concentration in cyanide (mg/Kg HCN equivalent) in cassava samples collected from five different regions of Kenya

county	N	Mean±SE (mg/Kg HCN equivalent)	Minimum	Maximum
Kisii	72	43.27 [a] ±3.75	39.29	98.17
Nairobi	54	66.00 [b] ±2.12	45.48	114.04
Thika	90	54.84 [a] ±0.65	44.92	68.73
Kakamega	27	80.79 [c] ±4.55	56.65	127.03
Kitui	81	70.46 [bc] ±2.21	48.49	127.95

Means with different letters are significantly different, n is the number of samples, cyanide levels measured in mg HCN equivalents/ kg fresh cassava weight

From the results there is a significant difference (with a $p<0.05$) between geographical location of the plant and cyanide concentration as revealed by the four different Student Newman Keuls (SNK) groups of means. The concentration of cyanide in cassava from

Kakamega was highest (80.79±4.55mg HCN equivalent/kg), while from Kisii and Thika had the lowest cyanide concentration (43.27 ± 3.75 and 54.84 ±0.65 mg/Kg HCN equivalent respectively). The concentration of cyanide in cassava from Thika and Kisii counties were not significantly different with same SNK group mean (a). The cyanide concentration of cassava from Kitui was 70.46 ± 2.21 mg/Kg HCN equivalents, while Nairobi had its concentration of cyanide at 66.00 ± 2.12 mg/Kg HCN equivalent.

Looking at all the areas, the difference in concentration (HCN equivalent) can be attributed to the fact that, the chemical composition of cassava varies according to variety, location, age, method of analysis, and environmental conditions (Githunguri *et al*., 1998; Githunguri, 2002). Cassava cyanogenic potential changes with change in agro-ecological zone (Githunguri *et al.*, 1998, 2002). This is in line with previous studies in which concentration of cyanide levels has been shown to depend on environmental conditions in which the cassava plant grows (Charles *et al*., 2005). Besides the five regions being ecologically different, the differences in levels of cyanide can be attributed to the different soil chemistry of the regions (Charles *et al*., 2005). This is because variation in key components of soil like potassium, calcium and magnesium ions adversely affect uptake of cyanide by cassava. It has been reported that uptake of cyanide by cassava growing in soil with potassium content of 250 kg/ha was 50.65 ppm compared to 108.37 ppm of cyanide in cassava growing on soil with no potassium (control) (Solomon, 2011).

The concentration of cyanide in Kakamega (80.79±4.55 mg/kg), can be related to the level of potassium and other cations in the soil in Kakamega. Being Ferralsols and acrisols, soils in Kakamega have low available cations like potassium and magnesium, a factor associated with low uptake of cyanide by cassava (Wegulo *et al.,* 2010). The soil in Kitui is characterized with alfisols, ultisols, oxisols, and lithic soils with generally of low fertility, and

many are highly erodible leading to low levels of basic cations a factor that increases the cyanide uptake by cassava (Barber *et al*., 1981). This could explain why the levels of cyanide in cassava from the two regions are comparable. Kisii area whose cassava had the lowest (43.27±3.7 mg/Kg HCN equivalent) concentration of cyanide is characterized with nitisols and phaeozeron, soils which are naturally characterized with high levels cations, potassium and magnesium. Since the rate of uptake of cyanide by cassava reduces with increased cation concentration, this may explain the low levels of cyanide in cassava from Kisii (Mogaka, 2011).

Cassava from Nairobi had a cyanide concentration of 66.00 ± 2.12 mg/Kg HCN equivalent. Compared to other regions, most agricultural products in Nairobi are cultivated at Sasumua area (Kimigo *et al*., 2008). A study carried out in 2008 reveal that there is a general decline in exchangeable calcium and magnesium of the soil collected in this area. This can be related to the relatively high (66.00 ± 2.12 mg/Kg HCN) of cyanide in cassava collected from Nairobi markets.

Cassava from Thika had a cyanide concentration of 54.84±.65 mg/Kg HCN equivalent. Compared to other regions in the study, soils in Thika are formed from tertiary volcanic tuffs in a gentle undulating upland (Muchena *et al*., 1978). The soil is deeply weathered and leached especially in well drained agricultural parts. Due to this, there is heavy application of fertilizer to improve soil fertility a factor that has increased soil pH to a range of 5.0 to 5.5 with a base saturation of 50% (Muthuri, 2004). The different soil chemistry of soils at Thika could explain the difference in cyanide concentration in cassava collected from Thika and other regions.

4.3 Determination of level of cyanide in eight cassava cultivars collected from KARI (Kaboko)

Eight different cultivars of the sweet varieties (990183, 990014, 990005, 990249, 990006, mm96/5280, Ex manakari, 196/0067) of cassava growing on the same soil at the Kenya Agricultural Research Institute (KARI) in Kaboko, Machakos county, Kenya, were collected and analyzed for cyanide composition using the picrate paper method. The eight cultivars considered in this study were developed at KARI by cross breeding of local Kenyan land races with improved International lines from the International Institute of Tropical Agriculture (IITA) and genetic modifications induced during cross-breeding. Results in observed differences in their cyanide content are shown in table below. There was significant variation ($P < 0.05$) in the concentration of cyanide in all the cultivars studied (Table 4.3).

Table 4.3: Concentration (mg/Kg HCN equivalent) of cyanide in eight different cassava cultivars

Cultivars	n	Mean±SE (mg/Kg HCN equivalent)	Minimum	Maximum
990183	3	55.04 ± 0.02^{h}	54.81	55.16
990014	3	44.27 ± 0.07^{a}	44.23	44.35
990005	3	47.76 ± 0.07^{d}	47.68	47.8
990249	3	48.68 ± 0.07^{e}	48.6	48.72
990006	3	49.60 ± 0.07^{g}	49.52	49.64
mm96/5280	3	45.88 ± 0.07^{b}	45.84	45.96
ex manakari	3	46.76 ± 0.00^{c}	46.76	46.76
196/0067	3	49.14 ± 0.07^{f}	49.06	49.18

Means with different letters are significantly different

Cultivar 99183 had the highest concentration (55.04 ± 0.02 mg/Kg HCN equivalent) of cyanide whereas 990014 had the lowest (44.27±0.07 mg/Kg HCN equivalent) and cultivar 990006 had concentration of 49.60±0.07 mg/Kg HCN equivalent. The variations in cyanide concentration among different cultivars can be attributed to the genotypic variations of the cultivars either naturally present or introduced in the process of developing the cultivars.

It is interesting to note that although the cultivars were growing in the same ecological niche, their levels of cyanide are significantly different pointing to the fact that there is a close relationship between cultivar type and cyanide concentration. The reported levels of cyanide are comparatively higher than the WHO recommended values of 10 mg of HCN/kg body weight (Bradbury *et al*, 1991; FAO, 2007). This means these cassavas could be toxic to the consumer, if eaten unprocessed.

In studies involving cyanide composition of cassava as a phenotypic characteristic, several genes have been linked to this characteristic (Sukhuman *et al*., 2011). Cyanide composition of cassava depends on the cyanogenesis pathway in which linamarin and lotaustralin are produced when glycosyl transfarase catalyses the production of L-valine and L- isoleucine (Conn, 1994). Two scaffold genes scaffold 09743 and scaffold 01206 code for synthesis of linamarase and hydroxyl nitrile lyase are key enzymes involved in cyanogenesis (Conn, 1994). Alterations in these two genes in the course of cultivar's development have been linked to the variations in cyanide content of different cultivars (Conn, 1994).

Due to the close relationship between genotype and cyanide content genetic engineering has been adopted as one of the three strategic approaches to reduce Post-harvest Processing Deterioration (PPD) of cassava (Westby, 2002). Expression of the cyanide-insensitive mitochondrial enzyme alternative oxidase (AOX) from Arabidopsis in cassava roots resulted in the production of transgenic cassava plants exhibiting delayed of PPD, approximately three weeks (Sayre *et al*., 2011).

4.4 Concentration of cyanide in different parts of cassava root (cortex, pith and parenchyma)

Concentration of cyanide in different parts of cassava (cortex, pith and parenchyma) of the same type (sweet variety) all collected from Kakamega, was determined using the picrate

paper method. There was significant variation ($P < 0.05$) in the concentration of cyanide in the three parts of the cassava root (Table 4.4).

Table 4.4: Comparison of concentration of cyanide (mg/Kg HCN equivalent) in parts of cassava root

Parts	N	Mean±SE	Minimum	Maximum
Pith	27	59.24±3.62[a]	44.23	96.79
Cortex	27	91.03±2.94[c]	70.45	115.65
Parenchyma	27	78.35±2.13[b]	51.59	91.38

*Mean values followed by different letters are significantly different at $\alpha = 0.05$ ($p < 0.05$, SNK test)

The study revealed that the cortex had the highest concentration of cyanide (91.03±2.94 mg/kg) followed by the parenchyma (78.35 ±2.13 mg/kg), while pith had the lowest concentration (59.24±3.62 mg/kg). These are different tissues with varying biochemical characteristics and this has a bearing to the distribution of key enzymes in cyanide metabolism.

Linamarase a key enzyme in cyanide metabolism has been previously reported to reduce from the cortex towards the Pith. This factor has a direct influence on accumulation of cyanogenic glycosides in the three different parts of the cassava (Westby, 2002). This is consistent with the trend of cyanide concentration in the three parts (Table 6). There is a gradual reduction of cyanide content from the peel (91.03±2.94 mg/kg) through the pith (59.24±3.62 mg/kg).

The relationship between gene expression and plant age could explain the variations of cyanide content in the different parts of cassava. Since different tissue part matures at different times, different gene action is expected to come into play at different ages of the plant (Westby, 2002).

4.5 Comparison of cyanide content from color chart and UV- visible spectrophotometer.

In the picrate paper method, color chart (visual method) and direct spectrophotometric methods were compared. The relationship between the levels of the two measurements was analysed using correlation coefficient. A regression line was plotted where cyanide concentration obtained through the spectrophotometry method was plotted against that obtained by the color chart see appendix I table A3.

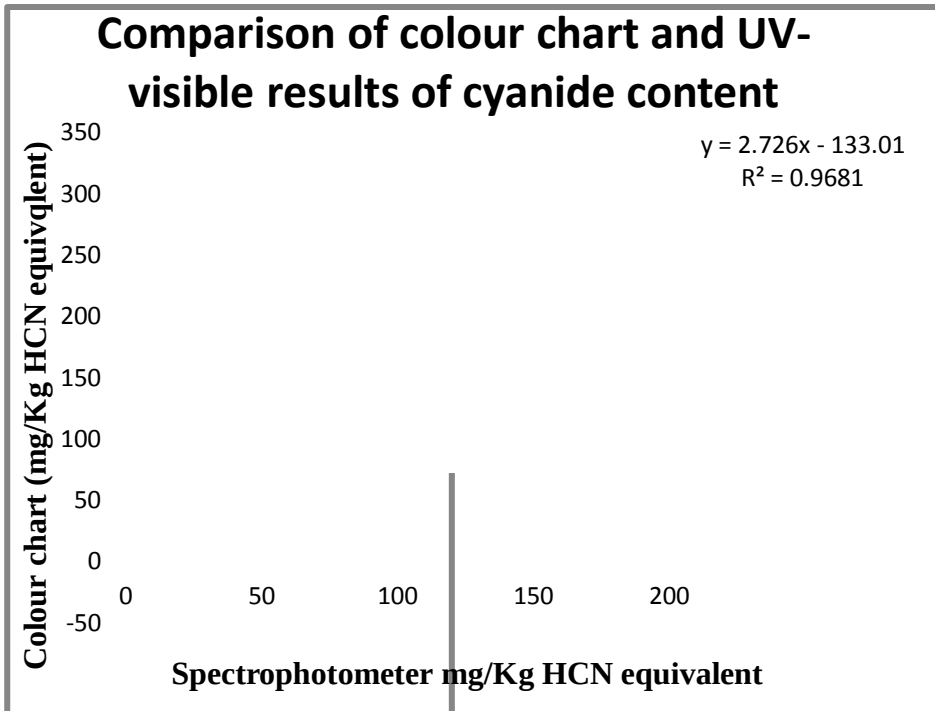


Figure 4.1: A plot of HCN content from color chart and spectrophotometer measurements

There was a good correlation between the results from both procedures with $r^2 = 0.9681$. The results agreed with Bradbury *et al,* (1991) that the color chart can be used for quick analysis where spectrophotometer is not available.

4.6 Conclusions

The following conclusions can be made with respect to the results obtained from the study:-

i. Picrate paper was the most effective and reliable method for determining cyanide concentration in cassava from the three methods considered.

ii. Cassava root from the five different regions was found to have a significant different cyanide concentration.

iii. The different cassava root cultivars, all growing in the same geographical area in the same climatic condition were found to have a significant different cyanide concentration.

iv. Concentration of cyanide was found to have a significant difference in the three parts of cassava root (pith, cortex, and parenchyma).

4.7 Recommendations

Cyanide concentration in cassava root indicates a significant concentration of cyanide in cassava from different markets in different regions. The following recommendations were made from this study:

i. Consumers should be sensitized on effective methods of cassava processing and encouraged to use them before consumption of cassava.

ii. Studies on cyanide content in cassava based products like flours, cassava crisps should be undertaken.

iii. Public awareness campaigns should be carried out to sensitize the public about high levels of HCN equivalent/kg of cassava compared to the recommended standard WHO reference value.

iv. Studies should be carried out on the HCN equivalent/kg of protein rich cassava leaves used as vegetables.

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APPENDIX I

Table A1: Individual raw data of mean mg/Kg HCN equivalent cassava from the five regions

Kisii		Thika		Kisii		Thika	
Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent)	Sample	mg/kg HCN equivalent
Ki1a	81.03	TG1a	62.4			TG13b	52.28
Ki1b	82.295	TG1b	62.4	Ki13c	69.07	TG13c	52.395
Ki1c	82.18	TG1c	62.4	Ki14a	79.765	TG14a	57.8
Ki2a	67.92	TG2a	59.985	Ki14b	79.765	TG14b	57.915
Ki2b	67.92	TG2b	59.87	Ki14c	79.765	TG14c	57.915
Ki2c	68.035	TG2c	59.87	Ki15a	98.05	TG15a	62.4
Ki3a	81.26	TG3a	52.97	Ki15b	98.165	TG15b	62.4
Ki3b	81.26	TG3b	52.97	Ki15c	98.165	TG15c	62.4
Ki3c	81.145	TG3c	52.97	Ki16a	74.245	TR16a	59.41
Ki4a	46.645	TG4a	48.485	Ki16b	74.13	TR16b	59.41
Ki4b	46.645	TG4b	48.485	Ki16c	74.13	TR16c	59.41
Ki4c	46.645	TG4c	48.485	Ki17a	87.24	TR17a	58.26
Ki5a	77.58	TG5a	56.65	Ki17b	87.125	TR17b	58.26
Ki5b	77.58	TG5b	56.65	Ki17c	87.125	TR17c	58.26
Ki5c	77.58	TG5c	56.65	Ki18a	42.85	TR18a	57.685
Ki6a	72.75	TG6a	51.82	Ki18b	42.735	TR18b	57.685
Ki6b	72.865	TG6b	51.82	Ki18c	42.735	TR18c	57.685
Ki6c	72.865	TG6c	51.82	Ki19a	47.45	TR19a	68.725
Ki7a	77.465	TG7a	49.75	Ki19b	47.565	TR19b	68.725
Ki7b	77.35	TG7b	49.75	Ki19c	47.565	TR19c	68.725
Ki7c	77.35	TG7c	49.75	Ki20a	44.805	TR20a	56.765
Ki8a	49.175	TG8a	55.845	Ki20b	44.69	TR20b	56.765
Ki8b	49.175	TG8b	55.845	Ki20c	44.69	TR20c	56.765
Ki8c	49.06	TG8c	55.845	Ki21a	52.28	TR21a	49.29
Ki9a	46.53	TG9a	46.99	Ki21b	52.28	TR21b	49.29
Ki9b	46.53	TG9b	46.99	Ki21c	52.28	TR21c	49.29
Ki9c	46.53	TG9c	46.99	Ki22a	52.74	TR22a	68.035
Ki10a	39.285	TG10a	44.92	Ki22b	52.74	TR22b	68.035
Ki10b	39.285	TG10b	44.92	Ki22c	52.74	TR22c	68.035
Ki10c	39.285	TG10c	44.92	Ki23a	71.6	TR23a	58.49
Ki11a	44.115	TG11a	50.785	Ki23b	71.715	TR23b	58.49
Ki11b	44.115	TG11b	50.785	Ki23c	71.715	TR23c	58.49
Ki11c	44.115	TG11c	50.785	Ki24a	49.175	TR24a	48.485
Ki12a	88.505	TG12a	47.91	Ki24b	49.175	TR24b	48.485
Ki12b	88.62	TG12b	47.91	Ki24c	49.29	TR24c	48.485
Ki12c	88.62	TG12c	48.025			TR25a	54.35
Ki13a	69.07	TG13a	52.395			TR25b	54.35

Sample	mg/kg HCN equivalent)	Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent)	Sample	mg/kg HCN equivalent
Ki13b	68.955					TR25c	54.35
						TR26a	57.11
						TR26b	57.11
						TR26c	57.11
						TR27a	48.37
						TR27b	48.37
						TR27c	48.37
						TR28a	47.565
						TR28b	47.68
						TR28c	47.68
						TR29a	61.25
						TR29b	61.02
						TR29c	61.02
						TR30a	50.325
						TR30b	50.325
						TR30c	50.325

Nairobi		Kakamega		Nairobi		Kakamega	
Sample	mg/kg HCN equivalent)	Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent)
NM1a	45.4835	K1a	127.03	NG10b	63.09	K10b	51.705
NM1b	58.835	K1b	126.915	NG10c	63.09	K10c	51.705
NM1c	58.95	K1c	127.03	NG11a	57.455	K12a	75.51
NM2a	61.71	K2a	67.575	NG11b	57.34	K12b	75.625
NM2b	61.71	K2b	67.575	NG11c	57.455	K12c	75.625
NM2c	61.595	K2c	67.46	NG12a	51.015	K13a	79.075
NM3a	70.68	K3a	67	NG12b	51.245	K13b	78.96
NM3b	70.105	K3b	66.885	NG12c	51.015	K13c	78.96
NM3c	70.22	K3c	67	NG12a	62.17	K14a	87.125
NM4a	58.03	K4a	57.11	NG13b	62.17	K14b	87.125
NM4b	58.145	K4b	57.225	NG13c	62.285	K14c	87.125
NM4c	58.03	K4c	57.225	NG13a	59.64	K15a	72.75
NM5a	71.6	K5a	105.755	NG113b	59.755	K15b	72.865
NM5b	71.485	K5b	105.87	NG13c	59.64	K15c	72.865
NM5c	71.485	K5c	105.87	NG14a	75.855	K16a	85.515
NM6a	70.795	K6a	63.09	NG14b	75.97	K16b	85.63
NM6b	70.22	K6b	62.745	NG14c	75.855	K16c	85.63
NM6c	70.335	K6c	62.745	NG15a	53.66	K17a	81.03
NM7a	56.075	K7a	56.65	NG15b	53.545	K17b	80.915
NM7b	56.305	K7b	56.765	NG15b	53.66	K17c	80.915
NM7c	56.075	K7c	56.65	NG16a	54.12	K18a	81.03
NM8a	93.68	K8a	95.29	NG16b	54.005	K18b	81.145

NM8b	93.68	K8b	95.52	NG16c	54.235	K18c	81.145
NM8c	93.795	K8c	95.52	NG17a	60.56	K19a	91.38
Nairobi		**Kakamega**		**Nairobi**		**Kakamega**	
Sample	**mg/kg HCN equivalent)**	**Sample**	**mg/kg HCN equivalent**	**Sample**	**mg/kg HCN equivalent**	**Sample**	**mg/kg HCN equivalent)**
NM9a	114.035	K9a	87.585	NG17b	60.675	K19b	91.265
NM9b	114.035	K9b	87.7	NG17c	60.675	K19c	91.265

Kitu1		**Kitui**		**Kitui**		**Kitui**	
Sample	**mg/kg HCN equivalent)**	**Sample**	**mg/kg HCN equivalent**	**Sample**	**mg/kg HCN equivalent**	**Sample**	**mg/kg HCN equivalent)**
KM1b	48.485	KM8a	51.475	K5a	80.455	Kk3b	74.82
KM1c	48.485	KM8b	51.475	K5b	80.455	Kk3c	74.82
KM2a	67.345	KM8c	51.36	K5c	80.455	Kk4a	60.1
KM2b	67.345	KM9a	48.945	K6a	66.425	Kk4b	60.1
KM2c	67.23	KM9b	48.945	K6b	66.425	Kk4c	60.1
KM3a	54.235	KM9c	48.945	K6c	66.54	Kk5a	53.545
KM3b	54.235	K1a	52.97	K7a	88.62	Kk5b	53.43
KM3c	54.235	K1b	52.97	K7b	88.62	Kk5c	53.43
KM4a	58.26	K1c	52.97	K7c	88.735	Kk6a	84.135
KM4b	58.145	K2a	50.44	K8a	64.93	Kk7a	50.9
KM4c	58.145	K2b	50.325	K8b	64.93	Kk7b	50.785
KM5a	52.855	K2c	50.44	K8c	64.93	Kk7c	50.785
KM5b	52.855	K3a	63.665	Kk1b	61.94	Kk8a	70.795
KM5c	52.855	K3b	63.78	Kk1c	61.825	Kk8b	70.91
KM6a	74.015	K3c	63.665	Kk2a	82.065	Kk8c	70.91
KM6b	73.9	K4a	59.87	Kk2b	82.065	Kk9	112.425
KM6c	74.015	K4b	59.87	Kk2c	82.065	Kk10	67.92
KM7a	57.225	K4c	59.87	Kk3a	74.935	Kk11	127.95
KM7b	57.34						
KM7c	57.34						

A 2: Individual Raw data for level of cyanide in different parts of cassava

Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent	Sample	mg/kg HCN equivalent
KPr1a	51.59	KP1a	49.175	KC1a	102.88
KPr1b	51.705	KP1b	49.06	KC1b	102.765
KPr1c	51.705	KP1c	49.06	KC1c	102.765
KPr2a	75.51	KP2a	50.095	KC2a	102.075
KPr2b	75.625	KP2b	50.21	KC2b	102.19
KPr2c	75.625	KP2c	50.095	KC2c	102.19
KPr3a	79.075	KP3a	46.3	KC3a	75.28
KPr3b	78.96	KP3b	46.185	KC3b	75.395
KPr3ac	78.96	KP3c	46.185	KC3c	75.28
KPr4a	87.125	KP4a	46.415	KC4a	91.38
KPr4b	87.125	KP4b	46.415	KC4b	91.38
KPr4c	87.125	KP4c	46.415	KC4c	91.265
KPr5a	72.75	KP5a	96.785	KC5a	72.865
KPr5b	72.865	KP5b	96.67	KC5b	7.98
KPr5c	72.865	KP5c	96.67	KC5c	72.98
KPr6a	85.515	KP6a	44.46	KC6a	70.45
KPr6b	85.63	KP6b	44.46	KC6b	70.45
KPr6c	85.63	KP6c	44.575	KC6c	70.565
KPr7a	81.03	KP7a	78.385	KC7a	85.975
KPr7b	80.915	KP7b	78.5	KC7b	86.09
KPr7c	80.915	KP7c	78.5	KC7c	86.09
KPr8a	81.03	KP8a	44.345	KC8a	102.65
KPr8b	81.145	KP8b	44.23	KC8b	102.535
KPr8c	81.145	KP8c	44.23	KC8c	102.535
KPr9a	91.38	KP9a	77.35	KC9a	115.53
KPr9b	91.265	KP9b	77.235	KC9b	115.645
KPr9c	91.265	KP9c	77.35	KC9c	115.645

Table A3: Individual raw data for comparison of colour chart and UV-visible results of cyanide content in cassava

Spectrometer HCN equivalent mg/kg results	Colour Chart mg/kg results
44	0
45.61	0
57.685	30
70.795	50
99.2	100
124.27	200
149.455	300

APPENDIX II

Flasks	Stock solutions	H_2SO_4	HCN^- Concentration
	Ml	ml	mg HCN^-ml^- Equivalent
0	0	100	0
1	1	99	0.026
2	2	98	0.054
3	5	95	0.135
4	10	90	0.27
5	15	85	0.405
6	25	75	0.675
7	50	50	1.35

Table A.3 Stock solution of HCN equivalent in acid conditions (H_2SO_4)

Test tubes					
	Stock solution	Alkaline Picric	Distilled water	mg CN	Absorbance
0	0	2	2	0	0.011
1	0.04	2	1.96	0.001104	0.005
2	0.04	2	1.96	0.00208	0.018
3	0.04	2	1.96	0.0052	0.052
4	0.04	2	1.96	0.0104	0.11
5	0.04	2	1.96	0.0156	0.045
6	0.04	2	1.96	0.026	0.44
7	0.04	2	1.96	0.052	0.847

Table A.4 Standards preparation for picric in solution method

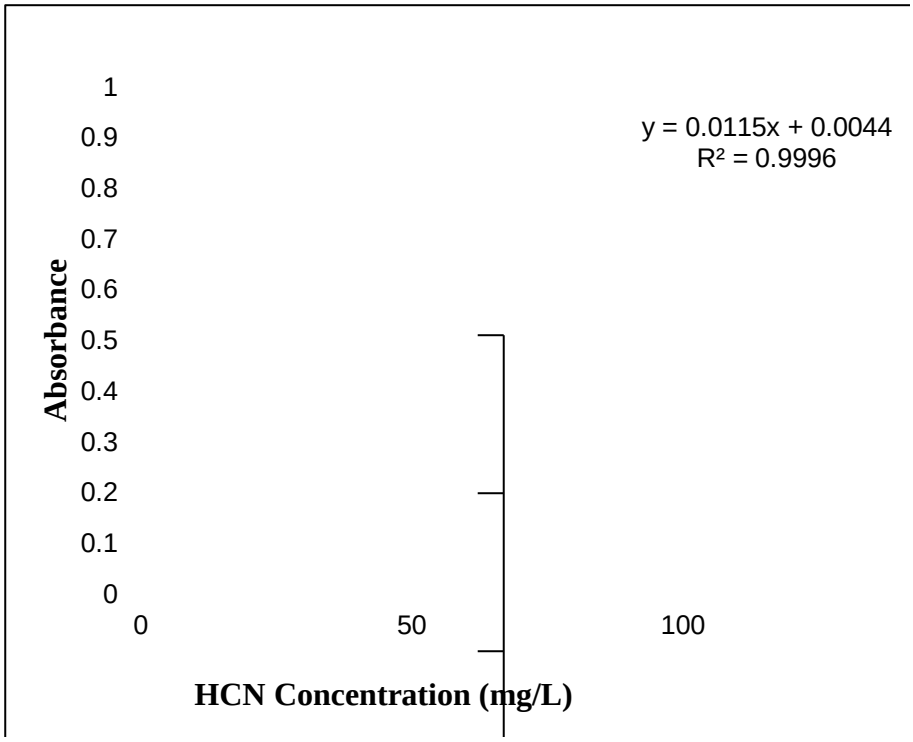

Figure A.1 HCN UV- visible calibration curve for picrate paper method

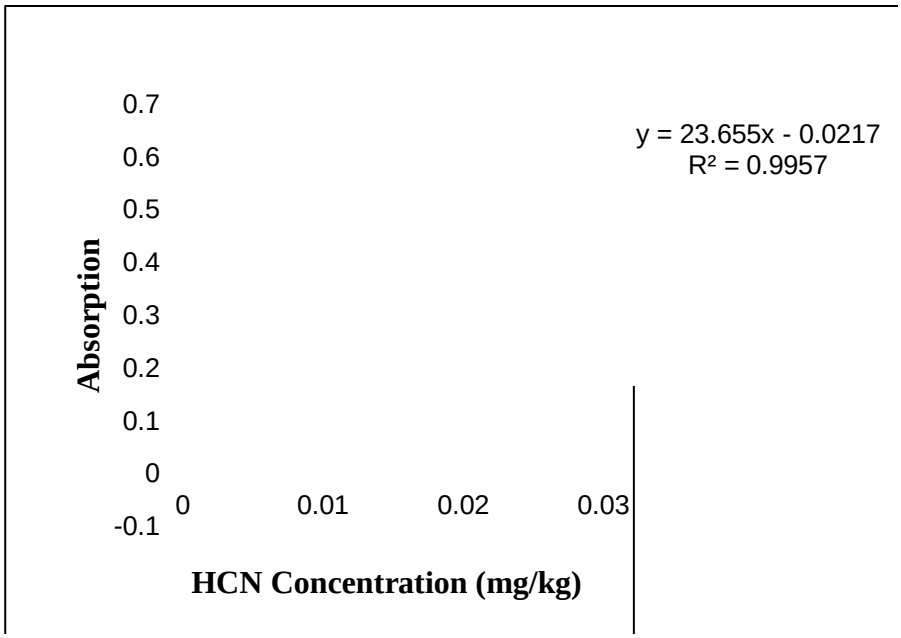

Figure A.2 HCN UV- visible Calibration curve for picrate solution method.

APPENDIX III

COLOUR CHART