Cyanide levels, starch content and antioxidant composition of three cassava varieties (Benguela, Malawi 1 and Malawi 7)

By

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A dissertation submitted in partial fulfilment of the requirements for the Bachelor of Science in Applied Biological sciences and Biotechnology Honours Degree

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Approval Form

This is to certify that the dissertation entitled “Cyanide levels, starch content and antioxidant composition of three cassava varieties (Benguela, Malawi 1 and Malawi) found in Zimbabwe”, submitted in partial fulfillment of the requirements for Bachelor of Science Honors Degree in Applied Biological Sciences and Biotechnology at Midlands State University, is a record of the original research carried out by Tsitsi Bessie Masawi R141626Q under my supervision and no part of the dissertation has been submitted for any other degree or diploma.

The assistance and the help received during the course of this research have been duly acknowledged. Therefore, I recommend that it be accepted as fulfilling the dissertation requirements.

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ABSTRACT

Cassava (*Manihot esculenta*) is a drought resistant crop that can potentially contribute to food security through diversification of the staple diet in Zimbabwe. A major hindrance to its adoption as a major food item is its perceived toxicity because it contains a cyanogenic glycoside, which can be hydrolyzed into hydrogen cyanide, HCN, and there is also lack of its nutritional benefits. A comparative study of three cassava varieties (Benguela, Malawi 1 and Malawi 7) grown in Zimbabwe, was done to: characterize their nutrient status (i.e. starch content), antioxidant properties, and dry matter content, determine cyanide concentration, and determine the effect of processing (boiling and drying) on cyanide concentration. Cyanide concentration differed significantly among varieties (ANOVA, $p = 0.00$) and between plant parts (ANOVA, $p = 0.00$). Cyanide concentration was highest in Malawi 7 (69 ± 4.00 and 134 ± 6.35 mg $kg^{-1,}$ in leaves and roots, respectively) followed by Benguela with a cyanide concentration of 65 ± 4.21 and 131 ± 3.48 mg $kg^{-1,}$ and Malawi 1 with a concentration of 63 ± 3.30 and 125 ± 4.99 mg kg^{-1}, in roots and leaves, respectively. Boiling reduced cyanide concentration by an average of 66% to 24 ± 3.63 mg kg^{-1} in Benguela, 20 ± 2.64 mg kg^{-1} in Malawi 1 and 23 ± 3.35 mg $kg^{-}1$ in Malawi 7. Drying had a similar effect, reducing cyanide concentration by about 59% to 25 ± 4.21 mg $kg^{-}1$ in Benguela, 29 ± 3.88 mg kg^{-1} in Malawi 1 and 27 ± 4.12 mg kg^{-1} in Malawi 7. The HCN concentrations for all the three varieties before processing were within the WHO recommended level (10 mg kg^{-1} body weight). A 200 g average cassava meal after processing contains 4.5 mg kg^{-1} of HCN, which lies within the acceptable WHO limits and much lower than the body toxic level. Starch content varied significantly among the three varieties (ANOVA, $p = 0.00$), being 19 % in Benguela followed by Malawi 7 with 16% and Malawi 1 with 15%). Dried mass also varied significantly among the three varieties (ANOVA, $p = 0.00$). It was highest in Benguela (44%) followed by Malawi 7 (40%) and Malawi 1 (38%). Cassava leaves of all three varieties contained the following antioxidants: alkaloids, flavones and phenolic flavonoids, meaning that cassava is not only a good source of calories but also antioxidants, which are well sought after by consumers. HCN content in the three varieties, even before processing, was within the WHO recommended safe level therefore the varieties can be safely adopted for consumption. The best variety of choice in terms of HCN content would be Malawi 1 with the least HCN content. Benguela would also be the best choice in terms of producing high yield with higher starch and dry matter. In light of these results, I recommend that consumers prepare their boiling. Further similar studies should be carried out focusing on other varieties in Zimbabwe.

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DEDICATION

This is for my grandparents; I love you both.

ACRONYMS AND ABBREVIATION

CN - Cyanide.

CNP - Cyanogenic Potential.

DM - Dry Matter

FAO - Food and Agriculture Organization

FAOSTAT - Food and Agriculture Organization Statistical Database

FSANZ - Food Standards Australia New Zealand.

FW - Fresh Weight.

HCN - Hydrocyanic acid.

HNL- Hydroxynitrile Lyase.

MDG - Millennium Development Goals.

NSN - Non-Nutritional Substances.

SCN - Thiocyanate.

SDG – Strategic Development Goals

SPSS – Statistical Package for Social Sciences

SSA - sub-Saharan African.

TAN - Tropical ataxic neuropathy.

WHO - World Health Organization.

ZIMVAC - Zimbabwe Vulnerability Assessment Committee.

ZMWF - Zimbabwe Microfinace Wholesale Facility.

ZZHSR - Zimbabwe Zero Hunger Strategic Review.

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CHAPTER: 1 INTRODUCTION

1.1 Background

Cassava (*Manihot esculenta*), locally known as *Mupfarinya,* is the third most important source of dietary energy in the world after rice and maize (Kwok, 2008). Cassava cultivation and processing provides food security, employment and income in communities having access to markets for over 500 million people in Africa, Asia and South America (www.ctahr.hawaii.edu).

Cassava is a potentially high yielding root crop that originated from South America where, for centuries, it has been grown as staple food. It was introduced to Africa by Portuguese traders during the 16th century and to Zimbabwe from Angola, Malawi, Mozambique and Zaire (Carter *et al*., 1992). It is now found in almost all parts of tropical Africa. Today Nigeria and Congo are the biggest producers of cassava after Brazil and Thailand (Cassava Production Guide, 2010).

Cassava production in Zimbabwe takes place on a limited scale but it has been identified as a possible food security crop (Mutenga, 2014). Cassava production in Zimbabwe has been negligible, and has not been a focus on agricultural policy. Zimbabwe ranks 83 in the world, in terms of cassava production, contributing 0.1% to the world share (FAOSTAT 2014). However, because of the economic challenges, declining soil fertility and climate change, cassava is becoming increasingly important in the food basket and as a buffer against drought shocks (Chiredzi Research Station, 2016). Mutenga (2014) called for an increase in the cassava production in Zimbabwe by smallholder farmers to ensure food security by embracing cassava production at commercial level. Cassava nursery trials in Mushumbi pools, headed by Dr Ian Robertson of Agri-Biotech, together with Sunbird, aim to of produce 120 million litres of ethanol per year for the

domestic fuel market and 15 000 tons of starch for the local food market (www.sunbirdbioenergy.com

Cassava roots are tuberous, long and tapered, with flesh enclosed in a removable peel, which is thick, rough and brown on the outside. 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, makes up about 85% of the total weight, consisting of starch containing cells and can either be white or yellowish in color (Wheatley *et al.*, 1993). The tubers are very rich in starch, but have lower nutritional value compared to cereals, legumes, and even some other root and tuber crops (Cassava Production Guide, 2010; Charles *et al.*, 2005). The stem with a milky fluid radiates from the tuber. As the plant grows, the main stem usually divides into three branches, each of which then divides in the same way. The leaves are large and palmate and have five to seven lobes borne on a long, slender petiole. They are dark green above, and light green below, and grow only towards the end of the branches (New World Encyclopedia, 2008).

Cock (1985) reported that the roots and the leaves are the most utilized parts of the plants. The starchy roots are a major source of energy, highly tolerant to low soil fertility, drought conditions as well as most pest and diseases. Cassava's success as a staple food in developing countries lies in its ability to thrive in poor soils as well as low rainfall areas (400mm annual average), and most importantly as a perennial crop with no specific season of harvest (Wilberforce *et al.,* 2016). These characteristics have made cassava tailored into a crop of primary importance, advantageously valued for its role in food security, mainly in insubstantial ecosystems (Bokanga, 1995).

It also plays a part in poverty alleviation and as a source of raw materials for agro-allied industries, with a huge potential for the export market (Egesi *et al.*, 2007), as it provides a good source of alcohol and industrial starch.

Post-harvest activities of cassava include milling and drying, which are not complicated and are inexpensive hence they can be done by anyone on farm or village level. It can be processed into a range of products that can be used by numerous industries. Cassava flour is used in preparation of porridge, bread, biscuits, confectionery, pasta and couscous-like products and in the preparation of adhesives while cassava starch is used in various food products and can also be used for alcohol production, among other agro-based industrial uses (Kenyon *et al.*, 2006).

Recently the aerial part of the plant, which was regarded as a by-product, has been shown to be of great benefit for both human and animal consumption (Corrêa *et al.*, 2004). The leaves are rich in proteins and vitamins A and C (Corrêa *et al.*, 2004), and minerals, especially magnesium, iron, zinc and manganese (Mg, Fe, Zn and Mn respectively) (Wobesto *et al.*, 2006), that can be obtained at a low cost. Use of the leaves can provide an extra income to various producers that survive on the cassava culture. Cassava leaves contain natural substances that can bring benefits, mainly to health. These natural substances such as antioxidants reduce risk of many diseases, such as cancer, cardiovascular diseases, chronic diseases and aging, among others (Simão *et al.*, 2013)

Hydrocyanic acid (HCN) is toxic to man and hence much of the processing of cassava tubers is to remove HCN before consumption by processes such as fermentation (AttahDaniel *et al.*, 2013). Several varieties of cassava have been identified and grouped into bitter and sweet depending on the quantity of linamarin in the tuber (Guédé *et al.*, 2013). Bitter cassava has a high concentration (HCN>100 mg kg^{-1}) of toxic HCN, whereas sweet cassava has a small amount of the substance (HCN<100 mg kg^{-1}), (www.ctahr.hawaii.edu, (FSANZ., 2004), however both bitter

and sweet varieties must be thoroughly processed before consumption (AttahDaniel *et al.*, 2013). A level of 10 mg HCN kg^{-1} of body weight after processing is considered safe for consumption by WHO standards (Bradbury *et al*., 1991), while FSANZ limit permissible on the market is 10 mg HCN kg^{-1} fresh weight (FSANZ., 2004).

1.2 Problem Statement

Climate change may significantly reduce yields of agronomic crops in southern Africa (Makado, 1992). There is therefore need for plants with high water use efficiency for sustainable agriculture (Kamukondiwa, 1996). Cassava could therefore become an important food source in Zimbabwe since it copes well with harsh conditions. With the estimated population of about 13 million (ZIMSTAT, 2013), and the country's reliance on starchy food for carbohydrates, the growing population could strain other food sources causing food shortages. However, adoption of cassava as a staple and food security crop is slow in Zimbabwe, owing to its perceived toxicity due to presence of cyanogenic compounds and little nutritional knowledge of the crop by the majority of the population.

Cyanide concentration in cassava differs in different parts of the plant, and this is determined by variety, location, age, and environmental conditions (Wangari., 2013). Older cassava plants tend to have more cyanide content than young plants, the soil chemistry in location also influences the ability of the cassava plant to take up cyanide from the soil (Mburu F, 2013). Cyanogenic glucosides and their catabolic enzymes in cassava are found in separate parts of the cassava tuber. The enzyme linamarase is in the cell wall, thus grating or injuring the tuber brings linamarase and linamarin into contact, the enzyme degrades linamarin to produce cyanohydrin which precipitously degenerates to yield HCN at about pH 5 (AttahDaniel *et al.*, 2013).

1.3 Justification

Cassava contains a cyanogenic glycoside, which is converted to poisonous hydrogen cyanide as an anti-predatory mechanism. Cyanide is potentially harmful to humans especially if it exceeds concentrations that the body can deal with. The information on cyanide concentration in cassava obtained from this study will be crucial for determining if cassava grown in Zimbabwe is safe for human consumption. Studies on cyanide concentration in cassava have been done elsewhere, in Nigeria and Kenya (Aalberberg *et al.*, 1991), but none have been reported in Zimbabwe. We cannot rely on results obtained elsewhere because cyanide concentration, nutritional status and dry matter content in cassava can vary with geographical location and variety. This study generated information on effective methods for safe processing of cassava.

Additionally, assessment of nutritional status in terms of starch and dry matter content, presence and absence of antioxidants would provide the much needed information for informed decision making.

1.4 Objectives

The main objective of the study was to characterize the nutrient status (starch, dry matter content and antioxidant composition) and cyanide concentration of three cassava varieties found in Zimbabwe and infer on the benefits and risks to consumers.

The specific objectives were:

i. to determine cyanide content in cassava,
ii. to determine effect of processing on cyanide content,
iii. to determine the starch and dry matter of the tubers, and
v. to investigate the presence and absence of antioxidants in cassava leaves.

CHAPTER 2: LITERATURE REVIEW

Owing to recurrent food insecurity and economic difficulties over the past 15 years, Zimbabwe did not achieve the first Millennium Development Goal (MDG), which included halving extreme poverty and hunger. The proportion of people suffering from hunger should have halved between 1990 and 2015, and the proportion of the malnourished children under five should have reduced by two thirds (Zimbabwe Zero Hunger Strategic Review Report, 2015).

Cassava has five important roles that it plays in African food security. It is regarded as a famine reserve crop, rural staple, cash crop for urban consumption, livestock feed and industrial raw materials (Curran *et al.,* 2009), thus it can be a great breakthrough for Zimbabwe to address the gaps in sustainable development. Its production is of significance as a staple crop for sub-Saharan African (SSA) smallholder farmers (Nginya, 2015). The crop continues to be the second most important food crop in Africa (after maize) in terms of the calories consumed (Curran *et al.*, 2009). Even though it has predominantly been grown as a staple crop, it is now intensifying as a cash crop as markets continue to grow in urban areas of Africa and around the world (Nginya. 2015).

The bulkiness and high perishability of the cassava crop limits its trade as a fresh crop, therefore most of the cassava in SSA is used domestically or traded among bordering countries, limiting its international role in foreign exchange and import substitution (Curran *et al.,* 2009). The plant's ability to acclimatize to various climates and delayed harvest for a long time, makes it a very important food reserve during the dry seasons, food shortage or famine (FAO, 2000). This leads to its contribution to solve food insecurity problems and poverty in rural areas in Africa and beyond.

2.1 Cassava production

In the last 100 years there has been an incredible increase in the world's population growth. Africa is not following the small growth patterns of other continents, it is now home of 1.2 billion, from 477 million in 1980. It is expected that by 2050, annual increases will surpass the current 30 million to 42 million per year and the total population will have doubled to 2.4 billion according to United Nations (www.theguardian.com; Essers *et al.*, 2005). There is therefore the need to intensify production of crops that can sustain the highly increasing population growth.

Table 2.1: Uses of cassava by continent (in percentage of production)

Producing region	Food	Feed	Industry	Export	Waste
Africa	88.7	1.4	0.1	0.1	9.5
Asia	55.3	2.9	8.6	26.9	6.3
Americas	42.4	33.4	9.6	0.1	14.0

Source: FAOSTAT, 1997

An increasing trend evolving across Africa is the consumption of cassava as a basic urban food staple and an important cash crop for rural farmers. This therefore entails that high-yielding cultivars and labor-saving technologies are required for efficient cassava production (Essers *et al.*, 2005). Almost all cassava grown in Africa is for human consumption; 30 percent is consumed after peeling, cleaning and boiling, while 70 percent is processed into a wide variety of food products including dry chips and flour, cooked pastes, roasted or steamed granules, beverages, etc, (Cassava production Guide, 2010; Nginya, 2015).

In South Africa, cassava is grown as a secondary crop by smallholders and is utilized for the production of starch (commercial and food grade starch). Currently, 20 000 tons of its starch are produced commercially, (Cassava Production Guide, 2010).

Table 2.2 Average annual export of cassava products to the European Union in the period 1994-1996 (in metric tons)

Producing Region	Fresh	Pellets	Chips	Starch	Total
Africa	159	25	21 031	12	21 227
Americas	3 935	5	33 223	821	37 984
Asia	20 925	1 238	3 766 885	8 447	3 797 696
Total	25 019	1 468	3 821 139	9 281	3 856 907

Source: European Commission for Agriculture, 1997

2.1.1 Cassava Production in Zimbabwe

Cassava production in Zimbabwe has been negligible, and has not been a focus on agricultural policy. Worsening drought situation, cassava is becoming increasingly important. Currently there are cassava pilot projects in Manicaland being run by the Zimbabwe Microfinace Wholesale Facility (ZMWF), same as those being run by Agri-Biotech (Pvt) Ltd. ZMWF recognized the various opportunities that come with commercial cassava production after information on expected frequent drought periods that are expected in the next 50 years emerged (Mutenga, 2014). The country is in the cluster initiative that will contribute to the MDGs together with nine other countries (South Africa, Uganda, Tanzania etc) (www.financialgazette.co.zw, Mutenga, 2017). Below is an image showing cassava production in Zimbabwe in the past 20 years.

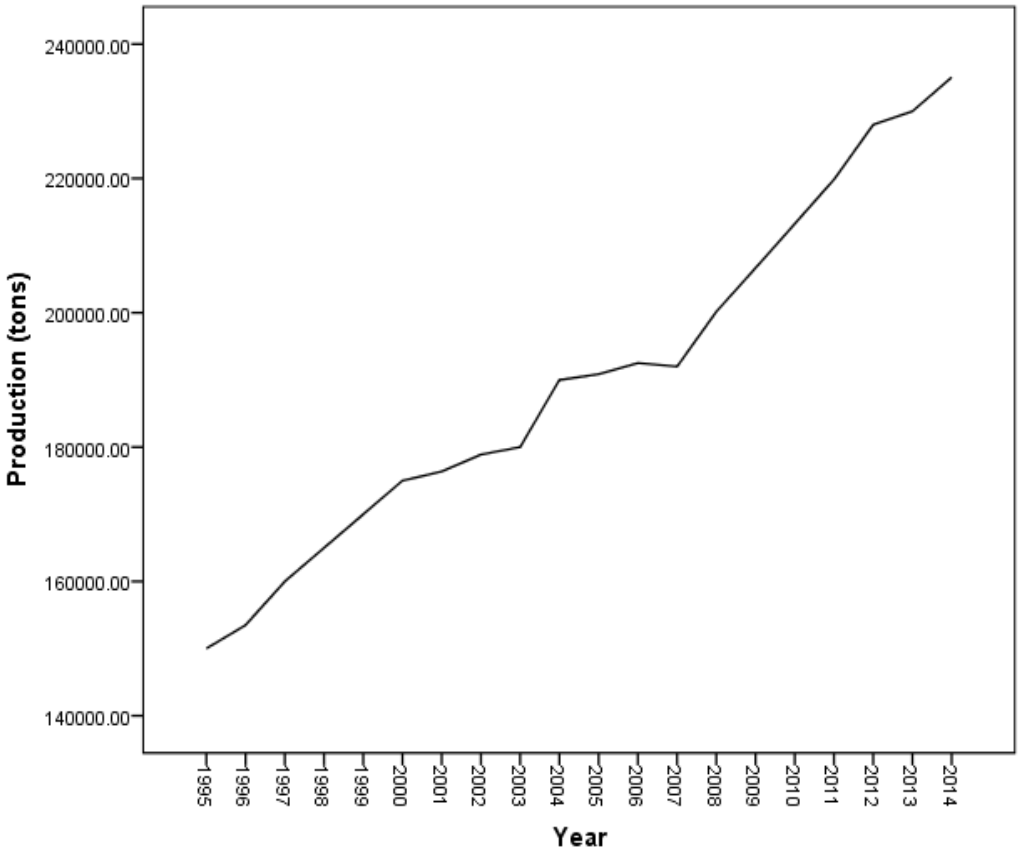

Figure 2.1: Cassava production in Zimbabwe; *Source: FAOSTAT (www.factfish.com)*

Zimbabwe ranks 83 in the world, in terms of cassava production, contributing 0.1% to the world share (FAOSTAT 2014). Mupakati and Tanyanyiwa (2017) assessed the cassava climate exchange adaptation in Chiredzi, pointing out that the root can penetrate greater depths that maize would not reach. However, the issue of cultivating drought tolerant crops is not yet a policy, instead the government has been encouraging the growth of cash crops such as tobacco (Mupakati and Tanyanyiwa 2017). Results from the study carried out by the above mentioned authors stated that 78% of study population did not have much knowledge of the crop, and limited quality seed is a limiting factor on large scale production.

FAO statistics, as reported by Nginya (2015), stated that the worldwide production of cassava roots has nearly doubled in the last 30 years, reaching 213 million tons in 2005. With the

estimated population of 13,061,239 (ZIMSTAT, 2013a), and the country's reliance on starchy food for carbohydrates, it could slowly serve as an ideal alternative source of carbohydrates to maize and other cereals which are more prone to drought and other factors that threaten food security.

The three varieties selected in study have differing genotypes: Benguela came originally from an IITA release, Malawi 1 and Malawi 7 was a selection by Rukuni (Robertson, 2006, *pers.com.*). The three varieties were bred in different regions for possibly different objectives and so have differences in their genome sequences.

2.2 Cassava nutrition

Nutritionally, it is a major source of calories in the form of starch. The nutrient composition depends on the parts of the crop. The roots and leaves are the nutritionally valuable parts of the plant, they constitute 56% of the mature plant with 50% being the root (Montaghac *et al.*, 2009). Cassava roots' nutritional value is important because they are the main part of the plant that is consumed in developing countries, they are a good source of energy while the leaves provide proteins, vitamins and minerals (Montaghac *et al.*, 2009).

Cassava tubers provide about 53% more calories than provided by potatoes. It has been shown that cassava is a good source of energy and that it interferes very little with the digestion of added protein and fat in weaning diets (Morales and Graham, 1987). They are lower in proteins and fats than cereals (Alfred *et al.*, 2014). Cassava is free from gluten, just like many other tuber crops, and hence can be used in preparation of special foods for patients suffering from celiac disease (Mburu, 2013).

The main advantage of cassava tubers is their low cost. Thus, energy requirements can be met at a low cost and a larger proportion of the income can be devoted to other foods and/or needs.

This is important on two counts: first, it makes it easier for poor populations for whom cassava is the main staple to afford other items of their diet; and second, it makes much nutritional sense since meeting one's energy requirements has been recognized as a prerequisite for a good utilization of other elements such as proteins in the diets (FAO/WHO, 1973). A positive effect of cassava on the metabolism of cholesterol has also been reported (Brydon, 1982).

Young cassava leaves are good sources of dietary protein and vitamin K which has a potential role in bone mass building by promoting osteotropic activity in the bones and also has established role in treatment of Alzheimer's disease patients by reducing damage in the brain (Baltha and Cereda, 2006). It's also a moderate source of vitamins such as folates, thiamin, pyridoxine (vitamin B-6), riboflavin and pantothenic acid (Alfred *et al.*, 2014).

The root is a chief source of some important minerals like zinc, magnesium, copper, iron and manganese for many inhabitants in the tropical belt. It also contains a significant amount of potassium, which is an important component of the cell and body fluids that helps regulate heart rates and blood pressure (Baltha and Cereda, 2006).

2.2.1 Antioxidants

Halliwell and Gutteridge, (1995) defined an antioxidant as "any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate". Halliwell gave a more specific definition in 2007, stating that an antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule". Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Free radicals are produced as a result of oxidation reaction and these radicals can start a series of reactions that damage cells. The role of antioxidants is to terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. They

achieve this by being oxidized themselves, so antioxidants are often reducing agents such as ascorbic acid or polyphenols (Shebis *et al.,* 2013)

Low levels of antioxidants in the body, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells resulting into health complications. Sikora *et al.,* (2008) mentioned that, 'Epidemiological and scientific studies show that nutrition plays important role in prevention of the consequences of free radical activity in the organism'. A diet rich in natural antioxidants reduces oxidation by increasing the reactive antioxidant potential of the organism, thereby decreasing the risk of some diseases of free radical origin.

There is a significant increase in the defensive abilities of cells influenced by adequate levels of antioxidants provided in diet which prompts immunological processes (Prior, 2003; Gałek and Targoński, 2003). Scientists and food producers interest has been concentrated on phenolic compounds, belonging to natural non-nutritional substances (NSN), (Sikora *et al.,* 2008). They can be divided to phenolic acids (derivatives of hydroxybenzoic acid and hydroxycinnamic acid) and flavonoids in relation to the basic structure of the carbon frame.

Natural antioxidants are present in plants, and this is why the basic source of these compounds for humans are plant-derived products. Vitamins soluble in lipids and selenium occur also in food derived from animals (milk and fish lipids, eggs), but in smaller amounts, and in dependence on kind of feed consumed (for example, carotenoids content in milk lipids, eggs). That is why, products derived from animals are not significant sources of antioxidants in human diet (Gupta and Sharma, 2006).

2.2.1.1 Effects of Antioxidants

Consistent consumption of foods rich in antioxidants has been linked to reducing the risk of chronic diseases (Gałek and Targoński, 2003). Studies have shown that an antioxidant-rich diet has a very positive health impact in the long run (Sin *et al.*, 2013).

Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease. Although initial studies suggested that antioxidant supplements might promote health, later large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. In addition to these uses of natural antioxidants in medicine, these compounds have many industrial uses, such as preservatives in food and cosmetics and preventing the degradation of rubber and gasoline (Sakora *et al.*, 2008).

The antioxidants travel through the blood vessels to reach damaged cells. Oxidation in cells knocks off electrons in chemical bonds, leaving behind highly reactive free radicals. These free radicals damage healthy molecules such as are found within the DNA structure by stealing electrons from healthy cells' molecules. The damaged healthy cells then literally break down releasing their oxidative stress to surrounding cells. To reverse oxidation, the body requires a reservoir of antioxidant molecules, whose extra electrons neutralizes free radicals, thereby repairing the chemical bonds without the antioxidants being left with free radicals (Gałek and Targoński, 2003).

Oxidation factors include pollution, poor diet, drugs, radiation, stress, injury, aging as well as infection. Disease risks associated with oxidation include cancer, heart disease, autoimmune disease, arthritis, diabetes, nervous system damage, poor skin and accelerated aging among many other diseases (Sikora *et al*., 2008).

2.2.2 Starch

Starch is one of the most abundant substances in nature, and important to man, a renewable and almost unlimited resource. Starch is mostly produced from grain or root crops. It is mainly used as food, but is also readily converted chemically, physically, and biologically into many useful products. To date, starch is used to produce such diverse products as food, paper, textiles, paints, adhesives, beverages, confectionery, pharmaceuticals, and building materials. Cassava starch has many remarkable characteristics, including high paste viscosity, high paste clarity, and high freeze-thaw stability, which are advantageous to many industries. Cassava starch is recommended for use in extruded snacks for improved expansion (*cassavabiz.org*, Fakir *et al*., 2012).

Nutritionally, cassava is a major source of calories in the form of starch. It can contribute to a large proportion of the daily calorific intake, taking up a large portion of the diet. One of the reasons why cassava is being relied upon as a source of food security is because it yields more starch efficiently than any other crop (Thro *et al.*, 1999). Owing to the very low protein content, soluble carbohydrates and fats, extraction and purification of cassava starch is relatively simple. A first-rate quality starch can be obtained from cassava using only water, and this makes the processing of cassava starch and flour particularly suitable for developing countries and rural industries (Ubalua *et al*., 2014). Fakir *et al*. (2012) estimated that 12tons of cassava starch can be produced per hectare, however, age and root quality are critical factors. Starch is one of the most common carbohydrate polymers in nature, the main source of carbon and energy for many organisms in nature because it is highly abundant throughout the plant kingdom (Cock *et al.*, 1979).

Advantages of cassava starch are its high level of purity and its excellent thickening characteristics, a neutral (bland) taste, desirable textural characteristics and a relatively cheap

source of raw tubers containing a high concentration of starch (dry matter basis) that can equal or surpass the properties offered by other starches (maize, wheat, sweet potato and rice).

2.2.3 Dry Matter

The dry matter (DM) (otherwise known as dry weight) is a measurement of the mass of something when completely dried (www.agriculture.vic.gov.au). The cassava DM would be tuber's solids, *i.e.* all its constituents excluding water. DM determination in different genotypes of cassava is important since nutrition and energy calculation is based on magnitude and nature of DM content. Higher DM content naturally would provide greater yield once partition of the same is increased to the economic part. Islam *et al.,* (2008) investigated dry weight of plant parts and observed that leaf, stem and tuber contained 30-60% DM. Its tuberous root contains (fresh weight basis) 34-40% dry matter (DM) and 25-30% starch (Charles *et al.*, 2005). Of the Dry Mass the starch provides energy and the cellulose provides fibre.

Sagrilo *et al*. (2002) assessed the performance of cassava varieties at different harvest times and concluded that, dry matter content is dependent on period of harvest. It is important to put emphasis in selecting the genotypes which had have dry matter content in the storage roots, to obtain better yield. Only a few studies for example those by Richardson (2011), however, explain the performance of cassava varieties regarding their biological production (total dry biomass) and its relationship with the efficiency in the storage root dry matter accumulation at different harvesting times.

2.3 Cyanogenic Compounds in cassava

The roots of cassava contain cyanogenic glycosides that can potentially release cyanide ions (Cliff *et al.,* 2011). Cyanogenic glycosides are a group of chemical compounds which occur naturally in over 2000 plant species (Kwok, 2008). A number of these species are used as food in

some areas of the world. Cassava is especially an important staple food containing cyanogenic glycosides (Rosling, 1987). These cyanide irons are toxic to humans.

The cyanogenic glycosides are amino acid-derived plant constituents (Vetter, 2000) which protects the plants from pests and diseases by immediately providing chemical defense by combination of cyanogenic molecules and hydrolytic enzymes thereby releasing toxic hydrogen cyanide (Moller and Seigler, 1999). The bitter taste of cassava caused by the cyanogenic glucosides also serves a purpose of restricting consumption of the tuber as mentioned by McKey *et al.* (2010).

Cassava produces two cyanogenic glucosides, linamarin (95%) and a small amount of lotaustralin (5%) (AttahDaniel *et al.*, 2013). The glucosides are hydrolysed to release toxic hydrogen cyanide (HCN) when the plant tissues are injured (Guédé *et al.*, 2013). Linamarin is synthesized in the leaves and then transported to the roots, through the phloem (Jorgensen *et al.*, 2005). In cassava plant cells, cyanogenic glucosides are stored inside the vacuoles while enzymes with the capacity of degrading them are located in the cell wall outside the cytoplasm (Cock, 1985). Linamarin and lotaustralin come into contact with linamarase only when the cassava plant is macerated or bruised, disrupting the cellular structures. This occurs when insect or mammalian pests attack.

Therefore, in intact cells the breakdown of cyanogenic glucosides which would otherwise lead to the formation of acetone cyanohydrin and glucose would not occur (Jorgensen *et al.*, 2005). The compartmentalization of linamarin in the vacuole and linamarase in the cell wall prevents the formation of toxic cyanide in undamaged cells. HCN is volatile (boiling temperature 25^{o}C), once it is produced, it will be released in air which will discourage many pests.

Two defense mechanisms are present to detoxify cyanide in the body. The methaemoglobin fraction in the red blood cells can temporarily neutralize cyanide by reversible reaction (Schultz, 1984). 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). Normally about 50mg cyanide can be converted into SCN per day in healthy human tissue (Schultz, 1984), then gradually excreted in the urine.

2.3.1 Cyanogenic Levels in Different Cultivars

A sum of 1,200 native cassava varieties has been identified in Africa, with the sweet cassava varieties dominating with higher genetic varieties compared to the bitter cassava varieties (Asadu *et al.*, 1999). Farmers grow varieties that have preferred qualities such as taste, early maturity as well as easy processing attributes (Salick *et al.*, 1997). They can get these varieties from neighbors, by gathering seedlings of sexually proliferated cassava found in fields left uncultivated for quite a long while (Chiwona-Karltun *et al.*, 1998). The execution of a varieties within local environmental conditions and cultivating systems determines whether it will be encompassed, and continue being cultivated. There is evidence from a few parts of Africa suggesting that few cassava varieties start from rearing projects, most grown nowadays comes from IITA or national breeding programmes (Nweke *et al.*, 1994).

The diverse varieties of cassava can be differentiated by size, shading and state of the leaf, stem and petiole, branching habits, plant stature, tuber and amount of the root tuber per plant, nutritional content of the tubers, resistance to specific sicknesses and weeds, the climatic and supplement requirements, for example, manure for high yield of the plants and either "sweet" or "bitter", contingent upon the level of cyanide content (Nweke *et al.*, 1999).

Certain environmental variables, for example, pest attacks, prolonged dry season and low phosphorus and potassium levels in soils may cause intense bitterness in the roots, and this corresponds with an increase in the levels of cyanogenic glucosides (De Bruijn, 1971). Detailed levels of cyanogenic glucosides in new root parenchyma have been from 10 up to 2000 mg HCN proportionate kg^{-1} dry weight (Coursey, 1973). Linamarin and linamarase levels can differ broadly among cultivars, plants of a similar cultivar, tissues of a similar plant, and even inside the root parenchyma (De Bruijn, 1971; Bourdoux *et al.*, 1980).

2.3.2 Effects of Cyanogenic Compounds

Consumption of 50 to 100 mg of HCN within 24 hours in an adult human, can completely block cellular respiration leading to death, therefore consumption of cassava and its derivative products with high concentrations of cyanogen can lead to illness or death (Rosling, 1994). HCN 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).

When large quantities of HCN are rapidly absorbed and the body's detoxification mechanisms are overwhelmed, acute cyanide poisoning occurs (Salkowski and Penney 1994). Cyanide in the body, directly or indirectly interferes with the functioning of certain organs and enzymes (Rocha-e-Silva *et al.*, 2010). Goiter, cretinism, tropical ataxic neuropathy or spastic paralysis are among the diseases attributed to the toxic effects of HCN diets, dominated by root of cassava non or poorly treated, and of low protein content (Cliff *et al.*, 2011). Therefore, the elimination of HCN is very crucial in cassava processing. Any high HCN content in the food only depends on cooking method, which can expose consumers to CN intoxication (Nambisan, 2011).

Although the situation is rare, the ingestion of large quantities of cassava or prolonged exposure to improperly processed cassava food has been shown to be associated with chronic cyanide toxicity in several areas of Africa (Tylleskar *et al.*, 1992; Mlingi *et al.*, 1992). 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). These observations came at a time of civil war when refugees were desperate for food and a neglected root crop was sometimes the only diet available.

Since the advent of highly bred maize availability of cassava has been neglected, indeed abandoned, in Zimbabwe for sixty years. Available literature gives a clear indication of the need to carry out a research in Zimbabwe specifically in the most used cassava varieties so as to assess the risks associated with their consumption before it can be fully adapted as a secondary source of starch. The research could fill in the gap of food security which is defined by ZIMVAC 2014 as, "A state of existence when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active, healthy life".

This could also necessitate need for extra care when preparing the cassava for either pharmaceutical uses or for food consumption. For antioxidants, cassava leaves provide alternative natural source which is healthier and cheap. The risks of diseases associated with high cyanide levels may be reduced by opting for varieties with little cyanide concentration which can be easily hydrolyzed in the body, thoroughly preparing the cassava tubers to reduce the concentration of the cyanide and to wear protective face masks to avoid inhaling the cyanide gas.

CHAPTER 3: MATERIALS AND METHODS

3.1 Sample Collection

Cassava samples were collected from Agri-Biotech Pvt Ltd, a company located in the northern part of Harare (Figure 3.1). Tubers from three varieties were randomly chosen for the study (Benguela, Malawi 1 and Malawi 7, Fig 3.2). The sampling site was selected because it is a research centre where most of the varieties are found, in a controlled environment with minimum attack from pests and diseases. From each of the three varieties, leaves and tubers were collected from five plants of the same age into distinctly labeled bags, and transported to the laboratory, where they were refrigerated pending analyses.

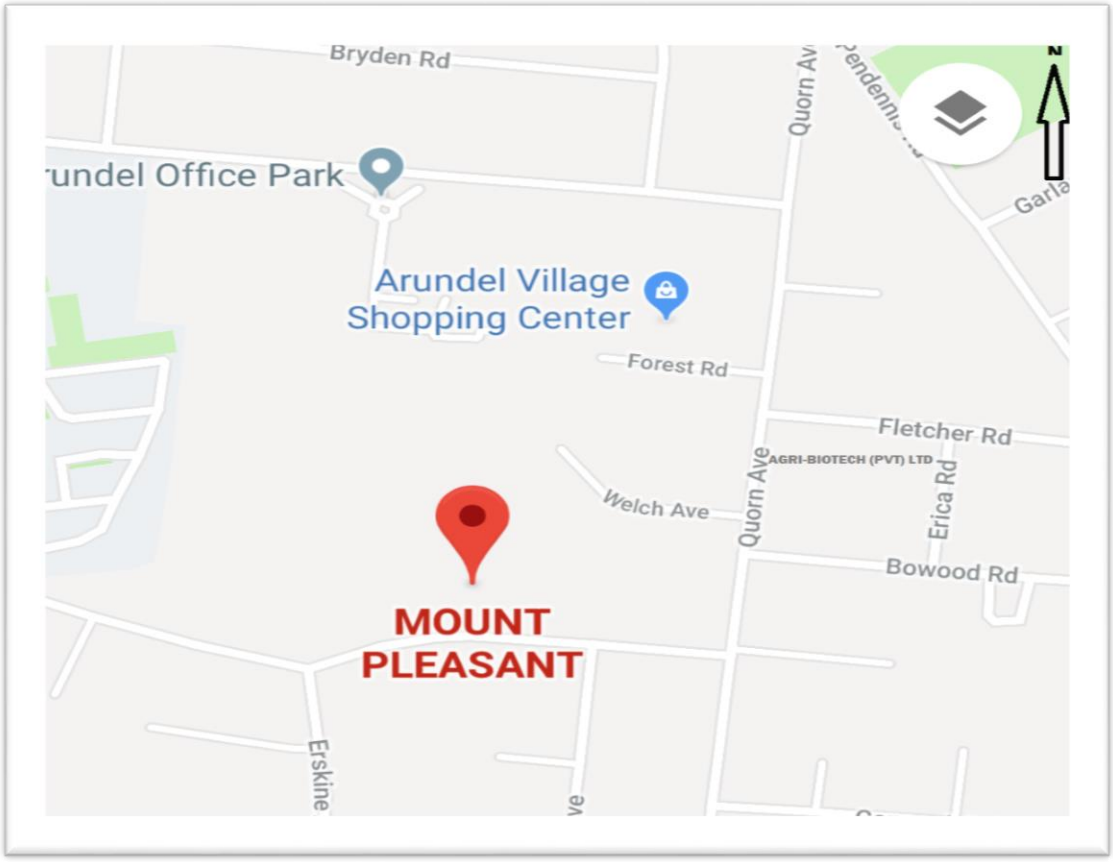

Figure 3.1 Location of Agribiotech in Harare *(Source: Google maps)*

a) Malawi 1 b) Benguela c) Malawi 7

Figure 3.2 Cassava varieties

3.2 Laboratory analyses

3.2.1 Cyanide determination

HCN concentration was measured following AttahDaniel, *et al. (*2013). Fresh cassava tubers and leaves were obtained. The tubers were washed and extracted with 150 ml of cold dilute orthophosphoric acid (0.1M) and 10 ml sulphiric acid (4M). Leaves were dried overnight at 70 ^{o}C before HCN was extracted with 150 ml orthophosphoric acid (0.1M). Following addition of 10 ml sulphuric acid for hydrolysis as in the tubers, the extraction was carried out in a boiling bath for 60 min (for the breakdown of cyanohydrin to yield HCN). Sodium hydroxide (5 ml) was then added, the cyanogenic potential (CNP) was quantified with Silver nitrate, ammonium hydroxide and potassium iodide as the indicator (figure 3.3).

The same procedure was followed to determine concentration on boiled and dried cassava tubers. 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).

Fig 3.3 a) HCN extract before titration; b) HCN extract at end point

3.2.2 Starch content determination

Starch was extracted following the wet method of Benesi *et al*. (2004). Fresh tubers were washed, peeled, weighed, chopped into about 1 cm cubes and then pulverized in a high speed blender for 5 min. The pulp was suspended in ten times its volume of water, stirred for 5 min and filtered using double fold cotton cloth. The filtrate was allowed to stand for 2 h for the starch to settle and the top liquid was decanted and discarded. Water was added to the sediment and the mixture was stirred again for 5 min. Filtration was repeated as before and the starch from the filtrate was allowed to settle. After decanting the top liquid, the sediment (starch) (figure 3.4) was sun dried for 24 h, weighed and stored.

Fig 3.4 a) Starch as a pure white paste before drying; **b)** powder starch after drying

3.2.3 Dry matter content determination

After harvest, tubers were peeled and cut into small cubes (2 cm long and 0.5 cm wide) for dry matter content. The dry matter contents of the different plant parts of cassava were determined following Benesi *et al*. (2004). The samples were sun dried following oven drying (60 °C for 24h) and weighed immediately. The drying and weighing steps were repeated until consecutive constant weights were achieved.

Fig 3.5 Cassava chips in drying oven

3.2.4 Antioxidant determination

Antioxidant determination was done following a protocol by Anbuselvi and Balamurugan (2014). For each variety, 200 g of dried cassava leaves were pulverized using an electric blender. The aqueous extracts of cassava samples were prepared by soaking 100 g in 200 ml of different solvents namely acetone and ethyl acetate. The extraction was performed for a period of 24 h. The crude extract was subjected to centrifugation at 10,000 rpm and supernatant was used to analyze the anti-nutritive content of tuber.

To test for alkaloids, the crude extract (2 ml) was dissolved in 2N Hydrochloric acid (1 ml). The mixture was filtered and the filtrate was mixed with equal amount of Mayer's reagent. A white precipitate was expected. A 2 ml extract was treated with 1.5 ml of 50% methanol solution and heated to test for flavonoids. To this solution magnesium and few drops of concentrated hydrochloric acid were added. An orange color confirmed presence of flavones, red for flavonoids.

Dilute hydrochloric acid (5 ml) was added to 1 ml of filtrate to determine presence of anthocyanin. A pale pink color development confirmed their presence. To test for anthraquinones test, a 0.5 ml extract was dissolved in 5ml of chloroform and shaken well for 5 min. It was then filtered before an equal volume of 10% ammonia solution was added and left to stand for 5 min awaiting a color change. A pink- violet color was expected.

To 1 ml of the filtrate 2 ml of 10% lead acetate was added. A brown color confirms the presence of phenolic flavonoidsTo the 5 ml of the extract, 2ml of chloroform was added. Concentrated sulphuric acid was added along the sides of the test tubes. A reddish-brown ring confirmed a positive result for a tri-terphenoids.

3.3 Data analyses

Data analyses focused on comparing the concentrations of HCN, starch and dry matter among the cassava varieties. Data were subjected to the software SPSS version 2.1 to compare the concentrations of HCN, starch, dry matter and antioxidant properties of cassava using ANOVA. This test was also used to determine the effect of processing on HCN concentration amongst the varieties. The dependent variables were HCN concentration, starch and antioxidants content, the predictor variable was the cassava varieties. Tukey tests for multiple comparisons were done to locate differences among varieties where ANOVA showed significant differences.

CHAPTER: 4 RESULTS

4.1 Cyanide (HCN) concentration

HCN concentration in cassava roots and leaves ranged from Malawi 1 (63 ± 3.30 mg kg^{-1}; 125 ± 4.99 mg kg^{-1}) and Benguela (65 ± 4.21 mg kg^{-1};132 mg kg^{-1}) to Malawi 7 (69 ± 4.00 mg kg^{-1};134 ± 6.35 mg kg^{-1}, Fig. 4.1; Appendix 1a), respectively. HCN concentration differed significantly among varieties (ANOVA, p = 0.00; Appendix 1b). In all varieties, HCN concentration in the leaves was nearly twice higher than that in the roots (Fig 4.1).

Malawi 1 had the least HCN concentration compared to the other two varieties which had no significant differences (Tukey's multiple comparison; Appendix 1b).

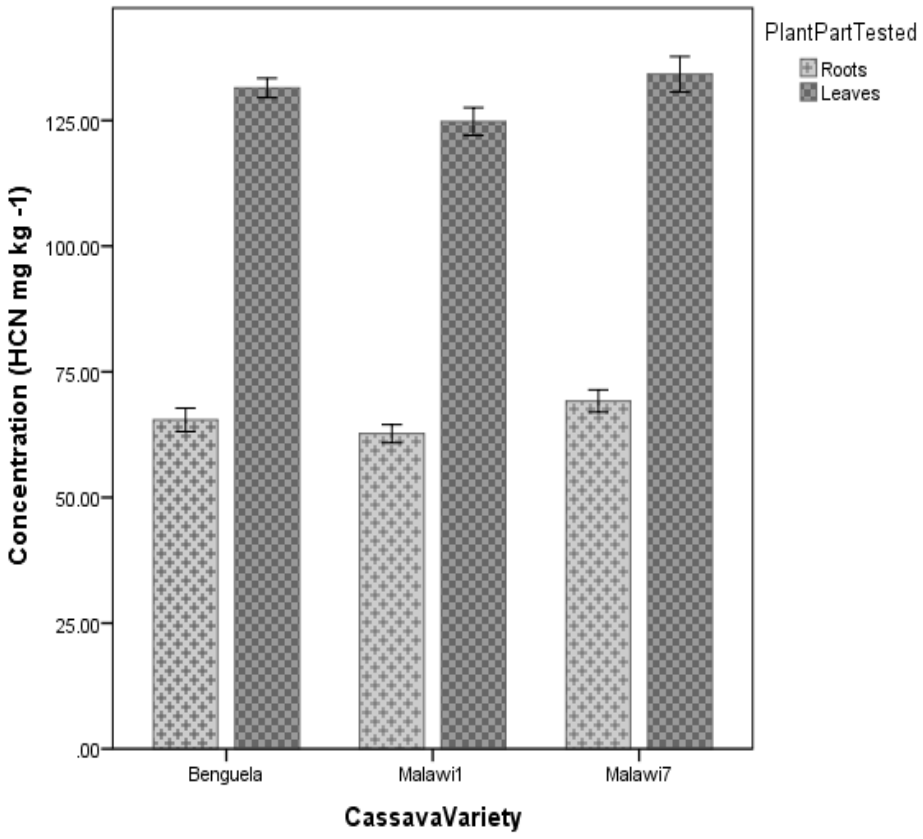

Fig 4.1 Cyanide concentration in roots and leaves of three cassava varieties

4.2 Effect of tuber processing on HCN concentration

Boiling and drying as methods of processing reduced the HCN concentration in the tubers. Boiling reduced HCN concentration by about 66% from about 65 mg kg^{-1} in fresh tubers in all varieties to about 22 mg kg^{-1} whereas drying reduced it by about 59 % to about 27 mg kg^{-1}(Fig.4.2). Boiling and drying had significantly different effects on HCN concentration (ANOVA p = 0.00; Appendix 2b), with boiling being more effective. The processing methods had no significant differences in Benguela as in Malawi 1 and Malawi 7,

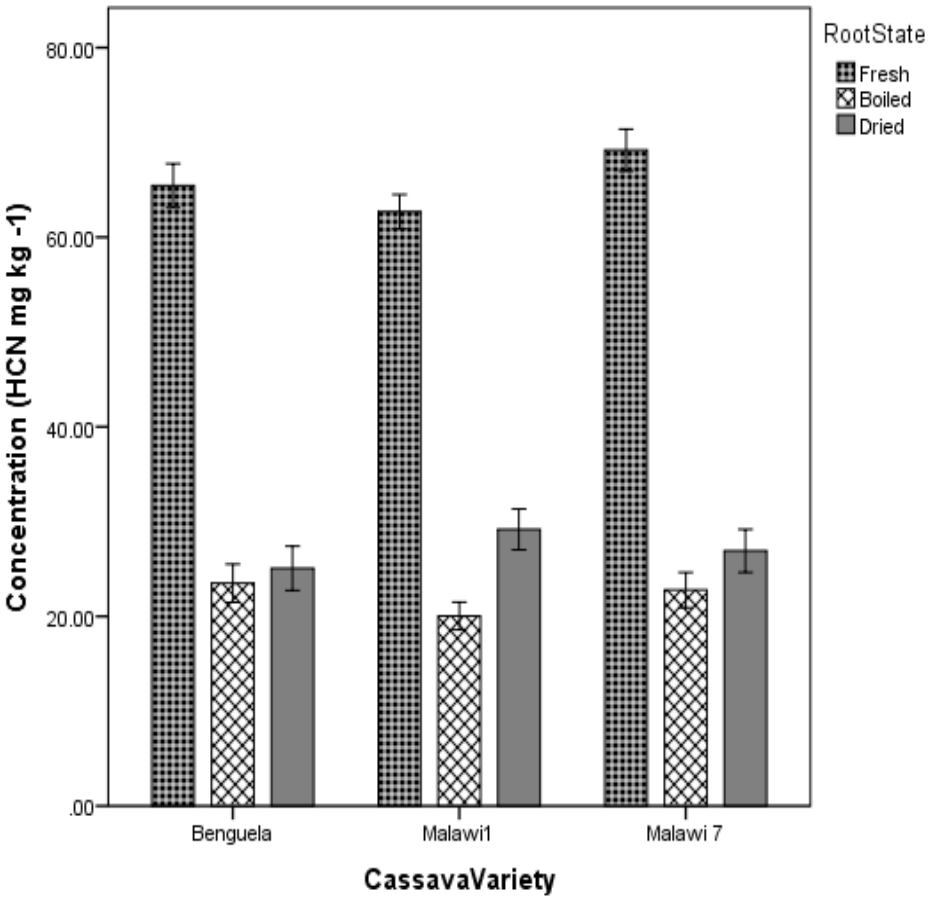

Fig 4.2 Effect of processing on cyanide concentration.

4.3 Starch and dry matter content

The mean percentage starch content ranged from Malawi 1 (15 ± 0.55%), Malawi 7 (16 ± 0.71%) to Benguela (19 ± 0.89%, Fig. 4.4). Dry matter content ranged from Malawi 1(38 ± 3.00%), Malawi 7 (40 ± 2.57%) to Benguela (44 ± 1.25%, Fig. 4.4). Starch and dry matter contents were significantly differently across varieties (ANOVA, p = 0.00). Figure 4.4 shows that there are no interactions between starch and dry matter contents.

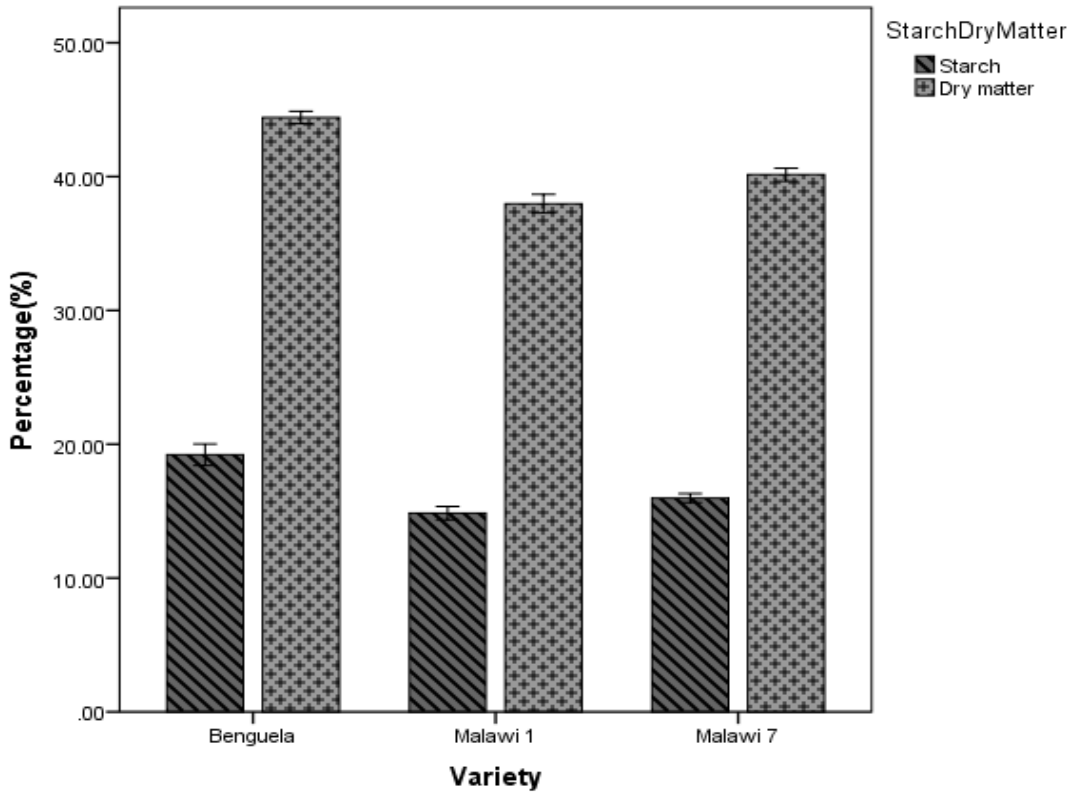

Fig 4.3 Cassava starch and dry matter content as a percentage

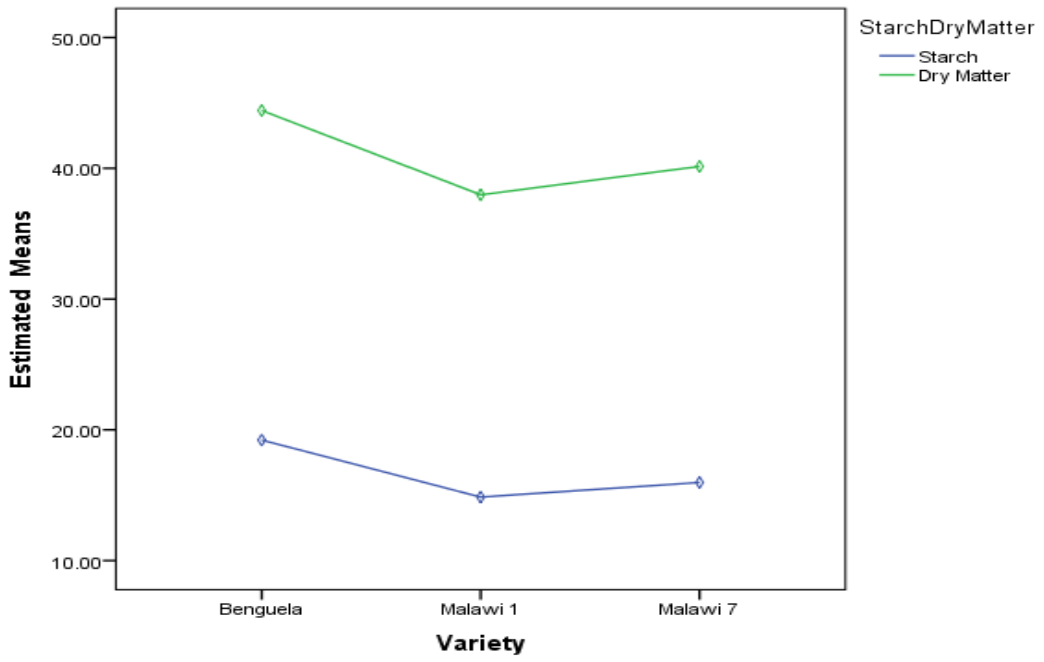

Fig 4.4 Relationship between starch content and dry matter.

4.4 Antioxidants

All three cassava varieties had the same antioxidant properties in that they all contained alkaloids, flavonoids and flavones but none contained terpenoids, anthraquinones, and anthocyanin (Table 4.1).

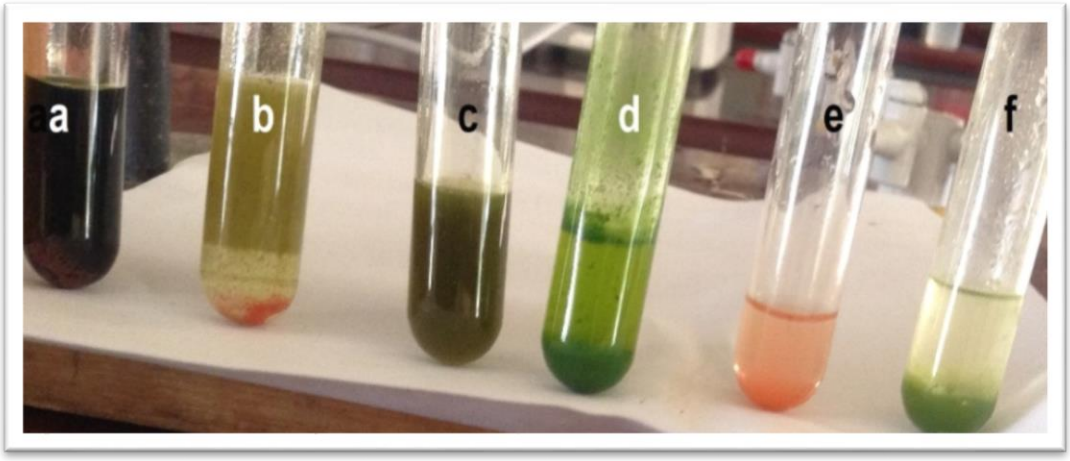

Fig 4.5 Image showing colorimetric antioxidant tests

Key;

a- negative for tri-terpenoids;

b- positive for alkaloids;

c- positive for phenolic flavonoids;

d- negative for anthraquinones;

e- positive for flavones (flavonoids);

f- negative for anthocyanin

Table 4.1 Presence absence of antioxidants in cassava leaves of Benguela, Malawi 1 and Malawi 7.

Variety	Benguela	Malawi 1	Malawi 7
Alkaloids	+	+	+
Anthocyanin	-	-	-
Anthraquinones	-	-	-
Flavonoids	+	+	+
Phenolic Flavonoids	+	+	+
Tri-terpenoids	-	-	-

Key: + present; - absent

In the above table 4.1, alkaloids, flavonoinds and phenolic flavonoids were present in all the three varieties, while anthocyanins, anthraquinones and tri-terpenoids were absent among the varieties.

CHAPTER 5: DISCUSSION

The main objective of the study was to characterize the nutrient status (starch), dry matter content, antioxidant composition and cyanide concentration of three cassava varieties found in Zimbabwe and infer on the benefits and risks to consumers.

5.1 Cyanide Concentration

5.1.1 Cyanide content in Benguela, Malawi 1 and Malawi 7

The differences in HCN concentration among different varieties can be attributed to the genotypic differences introduced in the process of developing the varieties. Differences can arise in response to pressures from pest attacks, age and prevailing phosphorus level (De Bruijn, 1971). The differences in HCN concentration could also be attributed to individual variety response to the soil chemistry (Charles *et al.*, 2005).

The observed concentrations were higher than those obtained by Wilberforce and Ngele (2016) and Ubwa *et al*. (2015) who used picrate solution and picrate paper, respectively for concentration determination. The levels of HCN concentration noted in titration method could be attributed to interferences, for example by nitrates (US EPA, 2004), which may act as masking agents, preventing the formation of silver cyanide. It could also be attributed to inaccuracies in determination of titre volumes during titration, since use of turbidity end point is subject to being arbitrary depending on the analyst (Mburu (2013).

The similarity in HCN concentration between Benguela and Malawi 1 could be explained by the varieties having the same biochemical response to the same environmental conditions for example the same key components like potassium, calcium and magnesium ions adversely affect uptake of cyanide by cassava (Solomon, 2011). This is in line with previous studies by Mburu

(2013) in which concentration of HCN levels of different varieties did not significantly differ. The higher concentration in Malawi 7 could have been caused by the variety's inability to take up phosphorus, low phosphorus levels are closely related to an increase in cyanogenic potential (De Bruijn, 1971). Inter-breeding of cassava to develop best varieties that adapt to local conditions could also cause similarities in HCN concentrations as all breeders usually focus on low cyanide for food consumption and since cassava is propagated clonally from stem cuttings, there is minimal variation between individuals of one cultivar when grown under the same environmental conditions.

Leaves had a higher HCN concentration than the roots. A research carried out by Bokanga (1994) on the cyanogenic potential of leaves proposed that 'cassava leaves with petioles have a cyanogenic potential 5 to 20 times higher than that of the root', however the ratios in the study were smaller compared to Bokanga's findings. The difference in concentrations could be attributed to the difference in tissues and their varying biochemical characteristics. This has an influence on the distribution of key enzymes in HCN metabolism (Mburu, 2013). However, Bokanga's analysis used a less accurate method which has been replaced by more accurate chemistry (Robertson, 2017).

The relationship between gene expression and plant age could explain the variations of HCN content in the different parts of cassava. This explains why the leaves have a higher HCN concentration than the roots, different tissue parts mature at different times, different gene action is expected to come into play at different ages of the plant (Westby, 2002). Another factor contributing to higher HCN concentration in leaves is because linamarin the cyanogenic glucoside, is synthesized in the leaves and then transported to the roots, through the phloem (Jorgensen *et al.*, 2005).

The HCN levels from the study were comparatively within the WHO recommended values of 10 mg of HCN kg^{-1} body weight (Bradbury *et al*., 1991). For instance, an average of 200 g (69±0.965 mg $kg^{-1,}$ raw) cassava meal would contain 13.84 mg HCN kg^{-1} fresh weight, this concentration can be metabolized and excreted from the body since it is lower than 50 mg HCN kg^{-1} (Appendix 2d) that the body can tolerate. The concentration is however according to FSANZ (2004), not permissible on the market, their maximum limit is 10 mg kg^{-1} fresh weight.

However, there is need to effectively process cassava meant for consumption by children since they are at more risk of the accumulative effects. This is supported by Kwok (2008) who reported that children are especially at more risk since they weigh less than adults.

5.1.2 Effect of processing on cyanide concentration

Processing reduced the HCN concentration in the tubers. Boiling was more effective by reducing the HCN by about 66% while drying reduced it by about 59%. The more effectiveness of boiling in reducing HCN is most probably caused by water hydrolysing the linamarin together with linamarase within cassava tubers to release HCN. The decrease in concentration could be explained by the formation of HCN under the action of linamarase on the cyanogenic glycoside especially at the beginning of the cooking when the temperature is still low to deactivate enzymes. Part of the cyanide could also have been lost through evaporation (Bradbury, 1992).

More HCN could have been lost if the cassava was first grated before boiling due to increased surface area (Ubwa *et al.*, 2015). Losses of up to 80% have been recorded by Pacific Islanders (Aprianita *et al.*, 2014; www.respository.usp.ac). A probable reason for the lower effectiveness of drying in lowering HCN concentration would be that there is no water interacting in hydrolyzing linamarin, but only release of volatile HCN by drying.

The HCN concentrations after processing were within the WHO recommended values of 10 mg of HCN kg^{-1} of body weight (Bradbury *et al*., 1991) For instance, an average of 200 g (29±0.965 mg kg^{-1}, dried) cassava meal would expose the body to an average of 5.84 mg HCN which is far less than what an average adult human body can handle. The more weight one has, the more HCN concentration they can tolerate, this is supported by Kwok (2008) who reported that children are especially at more risk since they weigh less than adults. Hence the need to effectively process cassava since they are still developing and at more risk of accumulative effects.

5.2 Starch and dry matter

The starch and dry matter content in cassava varied among the three varieties (Benguela (19%; 44%) > Malawi 7(16%; 40%) > Malawi 1(15%; 38%) starch and dry matter content, respectively). A similar range of percentage starch content was observed by Fakir *et al*. (2012) and Sajeev *et al*. (2003) who recorded a starch content range of 15-25% in cassava. Tuber starch content varies with availability of nutrients, and dependent on how much would have been utilized by the plant canopy (Charles *et al.*, 2005). Cassava starch is healthier than maize starch, it has lower calories, gluten free and has less fat content (www.foodstruct.com). Tonukari *et al.* (2004), mentioned that among the starchy staples, cassava gives a carbohydrate production which is about 40% higher than rice and 25% more than maize, with the result that cassava is the cheapest source of calories for both human nutrition and animal feeding. On fresh weight basis, current cassava starch yield is generally higher than potato and sweet potato (Jannat, 2011). Cassava starch is comparatively higher than that of maize (5.7%) and rice (5.4%) (www.skipthepie.org).

A range of dry matter content of 40-44% was observed in the study. A similar range was observed by Teye *et al.* (2011) who reported a dry matter content of 32-43%. Lower dry matter contents of 17% have been documented (Braima *et al*., 2000). There are various factors that

influence dry matter including soil moisture content, humidity, nutrients, period of harvest and genetic variations among varieties (Oetzel *et al.*, 1993).

Varieties with a high moisture content contain less dry matter, and are more susceptible to pest attacks. Cassava varieties with low moisture content are more suitable for long term storage of their roots than varieties with moisture content (Adejumo, 2012). In general terms, the weight of a specific tuber comes from either the moisture or from the DM portion (www.extension.purdue.edu). DM refers to material remaining after removal of water, and it reflects on the nutritional status of the tuber. Knowing the moisture content is important because the moisture content affects the weight, but does not provide nutrient value. The nutrients in tubers are part of the DM portion of the tubers and they reflect on the chemical potential of crops to produce a high yield (Teye *et al*., 2011). For industrial purposes, the greater the percentage starch the cheaper the purification costs which is why breeders are urged to aim at low water percentages.

Cassava starch was influenced by the dry matter, the higher the dry matter in the variety, the higher the starch content. Varieties with high dry matter content in the storage roots give better yields of starch. This suggests that, all other factors being constant, Benguela and Malawi 7 provide better cassava yields than Malawi 1. However, there are only a few studies that explain the performance of cassava cultivars regarding their biological production (total dry biomass) and its relationship with the efficiency in the storage root dry matter accumulation at different harvesting times (Sagrilo *et al.*, 2008).

5.3 Antioxidants

Out of the six compounds with antioxidant properties tested for, only flavones, alkaloids and phenolic flavonoids were present, while anthocyanins, anthraquinones and tri-terpenoids were absent.

Anbuselvi and Balamurugan (2014) obtained all the six antioxidants in the varieties they studied. Anthocyanins, anthraquinones and tri-terpenoids are known to be prevalent in cassava leaves. Failure to detect them in this study could have been caused by the solvents of extraction (ethyl acetate and acetone) used. Anbuselvi and Balamurugan (2014) found that cassava leaves showed maximum amount of anthrocyanins if methanol is used as a solvent compared to ethyl acetate and acetone. So this could imply that the antioxidants could have been present, but in small amounts that could not be detected.

The presence of flavones, alkaloids and phenolic flavonoids has added benefits on physical wellbeing. Flavones have received increasing attention due to their anti-inflammatory, anti-microbial and anti-cancer, anti-aging, anti-viral activities and vasodilating effects. (Andersen and Jordheim, 2006). In humans, they appear to function as "biological response modifiers" (Percival, 1996), thus they can modify immune responses by either enhancing or suppressing them. Apart from getting calories these cassava varieties, consumers also get flavones with their additional health benefits.

Phenolic compounds also have similar effects as flavones (Balasundram *et al.*, 2006). Alkaloids, which are the largest group of secondary chemical constituents are made largely of ammonia compounds. Even though there are some which are illicit drugs and poisons, majority have diverse medicinal uses. They can be used as anesthetics as well as anti-malarial agents.

Certain alkaloids act as cardiac or respiratory stimulants, treating irregular rhythms of the heartbeat (www.britannica.com).

5.4 Implications of HCN starch yield and antioxidant composition of the three varieties.

The WHO set a standard at 10 mg kg^{-1} as the safest concentration of HCN that the body can take for every serving of a cassava based diet which the body can easily metabolize without the gradual accumulation of residue HCN (FAO and WHO, 1973). The presence of high HCN residues in the body would cause chronic effects that pose danger to health, causing diseases that could be incurable such as symmetrical hyperreflexia of the upper limbs and the lower limbs (Ministry of Health, Mozambique, 1984). These residues would only accumulate when one consumes a high amount of HCN that the body cannot metabolize. This would put a strain on the economy and cause emotional burden on families that cannot afford health care, the more reason why there is need to know the HCN contents of varieties found in the country to minimize these risks.

The study revealed that the HCN content in the three varieties are safe, while others may choose to consume it raw, effective processing methods should be practiced as a lot of people would turn away from cassava as a secondary staple if many deaths are recorded due to cyanide poisoning. Alternative cassava based products which go under thorough screening could be introduced to minimize people's fears of its uptake as a secondary staple.

The reasonably high starch and dry matter contents obtained mean that more yield can be obtained per unit area of any starch bearing crop grown in Zimbabwe. This would increase food for consumption, thereby feeding more people. It could also increase industrial processes that generate income for the country. The high dry matter yield obtained means that all three varieties are not susceptible to pest attacks, minimizes yield loses, and could extend the cassava shelf life

as opposed to varieties with more moisture content. The consumption of these cassava leaves as relish could provide added benefits in the diet by increasing nutrition security through reducing postharvest due to them being thrown away.

5.5 Recommendations

5.5.1 For Producers and Consumers

Producers and consumers should be sensitized on effective methods of cassava processing and encouraged to use them before consumption of cassava in order to reduce the risk of HCN poisoning. Varying the time of boiling or drying will change the levels of CN achieved and informed decisions can be made to render the food safe to eat.

More studies should be done on varieties that are in different regions of Zimbabwe, and the findings should be documented and made available to the public so that consumers would easily opt for varieties with little HCN concentration which can be safely hydrolyzed in the body.

Both consumers and producers need to be informed of the different ways the leaves may be processed, or rather the antioxidants could be extracted and used to add value to other products in the fight against various diseases that come with age. Cassava can be utilized in Zimbabwe where maize, rice and wheat starch are currently used. It may also be used in pharmaceutical industries, where it may serve as a filler material and bonding agent for making tablets. Because of the variation in dry matter among the varieties, there is, therefore, opportunity for selection of improved genotype(s) for higher dry matter and flour yield.

5.5.2 For policy makers

The government should disengage from the reactionary practice where there is major importation of staple food (starch) such as maize and adopt a proactive approach of growing

cassava. Polices that guard the growth of cassava should be implemented. Investments that stimulate the growth of cassava should also be encouraged, and there is need for agricultural transformation strategies that highlight these policies, making them known to the public.

5.6 Conclusion

This study was able to determine the different concentrations of HCN in different varieties and plant parts of cassava, as well as infer on the effect of processing to the HCN content. Cyanide concentration differed significantly among varieties and between plant parts. Cyanide concentration was highest in Malawi 7 followed by Benguela and Malawi 1. Boiling reduced cyanide concentration by an average of 66%, while drying had a similar effect, reducing cyanide concentration by about 59% and therefore leaving minimal cyanide residue. The HCN concentrations for all the three varieties before processing were within the WHO recommended level of 10 mg HCN kg^{-1} body weight. Starch and dry matter contents were significantly different among the three varieties, being highest in Benguela. Cassava leaves of all three varieties contained the following antioxidants: alkaloids, flavones and phenolic flavonoids, therefore they can be utilized as a food source and possible enhancement of the agricultural by-product. Since the study revealed that HCN content in cassava falls within the WHO recommended safe level, the varieties can be safely adopted for consumption. The best variety of choice in terms of HCN content would be Malawi 1 with the least HCN content. Benguela would also be the best choice in terms of producing high yield with higher starch and dry matter. Consumers should be sensitized on the effective methods of cassava processing, both methods should be recommended, and prolong cooking time. More studies should be carried on other varieties in Zimbabwe.

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APPENDICES

Appendix 1: Cyanide concentrations among varieties

a) (Benguela, Malawi 1 and Malawi 7).

Cassava Variety	HCN Mean±SE	Minimum	Maximum
Benguela Roots	65.436±4.21	63.121	67.752
Leaves	131.446±3.38	129.131	133.761
Malawi 1 Roots	62.692±3.30	60.377	65.007
Leaves	124.792±4.99	122.477	127.108
Malawi 7 Roots	69.185±4.01	66.870	71.500
Leaves	134.190±6.35	131.875	136.505

b) SPSS Output HCN concentration among Benguela, Malawi 1 and Malawi 7.

Tests of Between-Subjects Effects

Dependent Variable: Concentration

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	94252.628[a]	5	18850.526	927.130	.000
Intercept	863600.902	1	863600.902	42474.706	.000
CassavaVariety	957.503	2	478.752	23.547	.000
PlantPart	93233.302	1	93233.302	4585.518	.000
CassavaVariety * PlantPart	61.822	2	30.911	1.520	.225
Error	1707.898	84	20.332		
Total	959561.428	90			
Corrected Total	95960.526	89			

c) SPSS output for HCN concentration among Benguela, Malawi 1 and Malawi 7

Pairwise Comparisons

Dependent Variable: Concentration

(I) CassavaVariety	(J) CassavaVariety	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Benguela	Malawi1	.697	.788	.378	-.862	2.257
	Malawi 7	-1.622*	.788	.042	-3.181	-.062
Malawi1	Benguela	-.697	.788	.378	-2.257	.862
	Malawi 7	-2.319*	.788	.004	-3.878	-.760
Malawi 7	Benguela	1.622*	.788	.042	.062	3.181
	Malawi1	2.319*	.788	.004	.760	3.878

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

Appendix 2: Effect of processing on cyanide concentration

a) Cyanide concentration in fresh, boiled and dried roots (mg/kg HCN equivalent) in Benguela, Malawi 1and Malawi 7.

Cassava Variety		HCN Mean±SE	Minimum	Maximum
Benguela	**Fresh**	65.44±4.21	63.527	67.346
	Boiled	23.50±3.63	21.590	25.410
	Dried	25.10±4.21	23.154	26.794
Malawi 1	**Fresh**	62.70±3.30	60.782	64.602
	Boiled	20.04±2.64	18.126	21.945
	Dried	29.18±3.88	27.271	31.090
Malawi 7	**Fresh**	69.19±4.01	67.276	71.095
	Boiled	22.77±3.35	20.864	24.683
	Dried	26.91±4.12	24.997	28.816

b) SPSS output for effect of processing in HCN concentration

Tests of Between-Subjects Effects

Dependent Variable: Concentration

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	52006.861[a]	8	6500.858	465.483	.000
Intercept	198114.388	1	198114.388	14185.645	.000
CassavaVariety	127.408	2	63.704	4.561	.012
RootState	51460.415	2	25730.207	1842.368	.000
CassavaVariety * RootState	419.038	4	104.759	7.501	.000
Error	1759.695	126	13.966		
Total	251880.944	135			
Corrected Total	53766.556	134			

R Squared = .967 (Adjusted R Squared = .965)

c) SPSS output for effect of Processing in HCN concentration

Pairwise Comparisons

Dependent Variable: Concentration

(I) RootState	(J) RootState	Mean Difference (I-J)	Std. Error	Sig.[b]	95% Confidence Interval for Difference[b]	
					Lower Bound	Upper Bound
Fresh	Boiled	43.668*	.788	.000	42.109	45.227
	Dried	38.721*	.788	.000	37.162	40.280
Boiled	Fresh	-43.668*	.788	.000	-45.227	-42.109
	Dried	-4.948*	.788	.000	-6.507	-3.388
Dried	Fresh	-38.721*	.788	.000	-40.280	-37.162
	Boiled	4.948*	.788	.000	3.388	6.507

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. Adjustment for multiple comparisons: Least Significant Difference (equivalent to no adjustments).

d) HCN concentration per 200g meal cassava

Cassava Variety		HCN Mean±SE mg kg^{-1}	200 g meal mg kg^{-1}
Benguela	**Leaves**	131±3.48	26.3
	Fresh	65±4.21	13.1
	Boiled	24±3.63	4.7
	Dried	25±4.12	5.02
Malawi 1	**Leaves**	125±4.99	25
	Fresh	63±3.30	12.54
	Boiled	20±2.64	4.01
	Dried	29±3.88	5.84
Malawi 7	**Leaves**	134±6.35	27
	Fresh	69±4.01	13.84
	Boiled	23±3.35	4.6
	Dried	27±4.12	5.4

Appendix 3: SPSS outputs for starch and dry matter contents.

a)

Multiple Comparisons

Dependent Variable: Starch

	(I) Variety	(J) Variety	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Benguela	Malawi 1	4.35733*	.38168	.000	3.4300	5.2846
		Malawi 7	3.23600*	.38168	.000	2.3087	4.1633
	Malawi 1	Benguela	-4.35733*	.38168	.000	-5.2846	-3.4300
		Malawi 7	-1.12133*	.38168	.014	-2.0486	-.1940
	Malawi 7	Benguela	-3.23600*	.38168	.000	-4.1633	-2.3087
		Malawi 1	1.12133*	.38168	.014	.1940	2.0486

*. The mean difference is significant at the 0.05 level.

b)

Multiple Comparisons

Dependent Variable: DryMatter

	(I) Variety	(J) Variety	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Benguela	Malawi 1	6.45000*	.36931	.000	5.5528	7.3472
		Malawi 7	4.27867*	.36931	.000	3.3814	5.1759
	Malawi 1	Benguela	-6.45000*	.36931	.000	-7.3472	-5.5528
		Malawi 7	-2.17133*	.36931	.000	-3.0686	-1.2741
	Malawi 7	Benguela	-4.27867*	.36931	.000	-5.1759	-3.3814
		Malawi 1	2.17133*	.36931	.000	1.2741	3.0686

*. The mean difference is significant at the 0.05 level.