# **CSIR-FRI/NRI CASSAVA GMARKET PROJECT**

Work Package 4: Ensuring the safety and quality of processed cassava products in market-oriented production.

Report on the Review of previous experiences and works on cyanogenic glycosides in cassava processing

December, 2012

## Contents

EXECUTIVE SUMMARY ii
1.0 INTRODUCTION
1.1 Objectives1
2.0 GENERAL OVERVIEW OF CASSAVA AND ITS IMPORTANCE
2.1 Nutritional and anti-nutritional properties
3.0 CYANOGENIC GLYCOSIDES
3.1 Toxicity of cyanogens
3.2 Relationship between bitterness and toxicity of cassava
4.0 DETOXIFICATION OF CASSAVA CYANOGENS7
4.1 Biotechnology and conventional breeding
4.2 Processing
4.2.1 Fermentation
4.2.2 Soaking
4.2.3 Cooking
4.2.4 Roasting Drying
4.2.5 Other unit operations
5.0 CONCLUSION
REFERENCES

## **EXECUTIVE SUMMARY**

Cassava is one of the most important root and tuber crops, providing nourishment for more than half a billion people the world over. It derives its importance from the fact that it is a valuable source of less costly calories, widespread and an integral contributor to food security in developing countries. The crop can grow and produce significant harvests even in environmental conditions which are inclement for most crops. World production quantity of the produce increased by more than 30 % over the period between year 2000 and 2010, with more than half of the total amount produced in Africa. Cassava is primarily grown for use as food and has over the years played an inimitable role in providing valuable calories for people of diverse socio-cultural standings. That notwithstanding, the crop has received attention as a raw material for a wide range of industrial applications including the production of bioethanol, adhesives, pharmaceuticals, plastics as well as pelletized animal feed.

One of the drawbacks of the root crop for use as food is its potential toxicity, a phenomenon which stems from the cyanogenic glycoside content of the crop. These compounds, which naturally serve wards off insect and herbivore attack, undergo enzymatic degradation to produce HCN which is lethal at 35 - 150  $\mu$ mol/kg, administered in a single dose. Sub-fatal doses over a long period have been reported to affect the nervous system and thyroid glands. HCN has also accounted for cases of reduced blood pressure, diabetes mellitus and growth retardation in children. Cyanogenic compounds have also been identified as contributing to bitterness in certain cassava varieties. Reduction of cyanogen content reduces the risk of intoxication associated with cassava consumption.

Detoxifying cassava of cyanide presents an avenue for expanding both domestic and industrial applications. Even though contemporary interventions such as genetic engineering and breeding have been applied to generate cyanide-free varieties, traditionally, detoxification is achieved by processing. Methods such as fermentation, cooking, drying and roasting have resulted in significant reduction of cyanide content of cassava. These techniques involve a combination of unit operations that trigger the breakdown of cyanogens by endogenous enzymes into HCN, which is subsequently evaporated (by heating) or dissolved in water (depending on the processing method under consideration). Processing has resulted in markedly lessened potency of cyanogenic glycosides in cassava, even though the reduction in toxicity depends on the starting material, and the method used and the extent of processing.

## **1.0 INTRODUCTION**

This report presents review of previous works on cassava cyanogenic glycosides, their structure, toxicity and its relationship with bitterness as well as interventions and previous attempts made at detoxifying cassava. It begins with a general overview of cassava, its socio-economic and nutritional significance to the cassava producing regions. The write-up focuses on detoxifying these cyanogenic glycosides during processing, the mechanisms and the unit operations involved in these processes. Both the roots and leaves of cassava are covered in this review.

## **1.1 Objectives**

To review various unit operations involved in cassava processing and their effect on the degradation of cyanogenic glycosides.

## 2.0 GENERAL OVERVIEW OF CASSAVA AND ITS IMPORTANCE

Cassava (*Manihotesculenta*Crantz) is arguably the most important staple in most tropical regions of the world. With a somewhat ambiguous origin (Allem, 2002), the plant is widely grown in areas with different geographical conditions. It has been identified as a potentially valuable source of food for addressing food security in developing countries (Montagnac*et al.*, 2009). The crop is hardy and can survive adverse conditions such as infertile soil, drought, pests and diseases (Bokanga, 1999; El-Sharkawy, 2003) and plays several important roles in Africa such as serving as a rural staple food, famine-reserve crop, cash crop for households and as a raw material for feed and industrial manufacturing (Nweke*et al.*, 2002).

Primarily, cassava is cultivated on small-scale farmers on small plots of land. African produces more cassava than the rest of the world put together (FAO, 2012), with production hitting 230 million tonnes in 2010. Although African countries present the lowest yields, Nigeria, DR Congo, Angola and Ghana are among the 10 in the league of world cassava production. In 2010, Angola was highest in terms of production per capita (726 kg/person), followed by Ghana (563 kg/person) and Thailand (314 kg/person) (FAO 2012). While it is used predominantly for food in Africa, in South America, Asia and Europe, the crop mainly serves industries (mostly starch and ethanol) and some used for the production of animal feed.

The leaves and roots are which make up 50% and 6% of the mature plant respectively (Tewe and Lutaladio, 2004) are considered important in terms of its use for food and animal feed. The edible part of the root accounts for 80 - 90 % of the total weight of the root (Alves, 2002; Wheatley and Chuzel, 1993) and is rich in digestible carbohydrate, mainly starch (Charles *et al.*, 2005). It's mineral content is comparable to that of several legumes but is low in fat and protein (of low quality) and should be eaten with other foods that may supplement the deficiency.

Conversely, the leaves are richer in proteins, minerals and vitamins and lower in carbohydrates compared to the roots (Adewusi and Bradbury, 1993). Much of the protein in the leaves is made up of linamarase, the enzyme that detoxifies the cyanogenic glycosides in cassava (Bokanga, 1995).

If the contribution of cassava to the livelihood of producers, processers and traders are to be realized fully, there is the need to counter the three major limitations to its use; i.e., poor shelf-life, low protein content and cyanogenic potential (Westby, 2002). The cyanogenic potential of cassava is by far the single factor that adversely constraints the use of cassava as food and feed for animals. This is as a result of the toxic effect of cyanide on humans and animal who rely on cassava as food.

## 2.1 Nutritional and anti-nutritional properties

Cassava is cultivated primarily in areas with limited soil fertility by farmers with restricted economic resources and used as food and as raw material for certain industrial products. As presented in Table 1, both leaves and roots are nutritionally valuable (Tewe and Lutaladio, 2004). The roots are a rich source of energy, appreciable in mineral content but marginal in vitamins. The leaves, which are also used for food in certain areas, are rich in vitamins, proteins, minerals (Lebot, 2009) and carbohydrate, which is mainly starch (Gil and Buitrago, 2002). A lot of programmes and strategies have been put in place to prop up cassava's zinc, iron, protein and vitamin A content (Montagnac*et al.*, 2009). Generally, however, the leaves present valuable nutritional potential compared to the root which is widely utilized.

	Cassava roots	Cassava leaves	
Proximate composition			
Food energy (kcal)	100 - 149	91	
Moisture (g)	45.9 - 85.3	64.8 - 88.6	
Dry weight g)	29.8 - 39.3	19 - 28.3	
Protein (g)	0.3 - 3.5	1.0 - 10.0	
Lipid (g)	0.03 - 0.5	0.2 - 2.9	
Total carbohydrate (g)	25.3 - 35.7	7 - 18.3	
Dietary fiber(g)	0.1 - 3.7	0.5 - 10.0	
Ash(g)	0.4 - 1.7	0.7 - 4.5	
Vitamins			
Thiamin (mg)	0.03 - 0.28	0.06 - 0.31	
Riboflavin (mg)	0.03 - 0.06	0.21 - 0.74	
Niacin (mg)	0.6 - 1.09	1.3 - 2.8	
Ascorbic acid (mg)	14.9 - 50	60 - 370	
Vitamin A (µg)	5.0 - 35.0	8300 - 11800	
Minerals			
Calcium (mg)	19 - 176	34 - 708	
Phosphorus (mg)	6 - 152	27 - 211	

 Table 1. Nutritional composition of cassava roots and leaves

Iron (mg)	0.3 - 14.0	0.4 - 8.3	
Potassium (%)	0.25 (0.72)	0.35 (1.23)	
Magnesium (%)	0.03 (0.08)	0.12 (0.42)	
Copper (ppm)	2.00 (6.00)	3.00 (12.0)	
Zinc (ppm)	14.00 (41.00)	71.0 (249.0)	
Sodium (ppm)	76.00 (213.00)	51.0 (177.0)	
Manganese (ppm)	3.00 (10.00)	72.0 (252.0)	
· · · · · · · · · · · · · · · · · · ·	· · · · · ·	· · · · · · · · · · · · · · · · · · ·	

Bradbury and Holloway, 1988

In spite of the impressive nutritional value (roots and leaves), cassava contains toxic substances and anti-nutrients which restricts the digestibility and absorption of some nutrients. Phytates and oxalates abound in cassava, with contents of 624 mg/100 g (Marfo*et al.*, 1990) and 1.35 - 2.88 g/100 g (Correa, 2000; Wobeto*et al.*, 2007) respectively. Phytic acid binds calcium, magnesium, iron and zinc (Hambidge, 2008) while oxalate complexes with calcium and magnesium and makes them bio-unavailable (Massey, 2007). They may also complex with protein and inhibit peptic digestion. Other antinutritional factors in cassava including saponins, tannins (Wobeto*et al.*, 2007), trypsin inhibitors in the leaves (Correa *et al.*, 2004)and the cyanogens have also been reported.

## **3.0 CYANOGENIC GLYCOSIDES**

Cyanogenic glycosides are derivatives of  $\alpha$ -hydroxynitriles from aliphatic and aromatic protein amino acids and aliphatic non-protein amino acids, found in plants and some animals belonging to the phylum arthropoda (Zagrobelny*et al.*, 2004). They are secondary metabolites that are widespread in plants and act as defense compounds to fight against herbivore and pathogen attack (Heldt and Piechulla, 2011; Vetter, 2000). Several forms of these compounds abound and have been reported in a number of edible plants.

Bound forms of cyanogenic glycosides occur as Linamarin, Lotaustralin (Acetonehydrin), Amygdalin, and Dhurrin. These compounds are generally stable at neutral pH. Linamarin and Lotaustralin have a wide distribution and have been found in cassava and lima beans (Jorgensen *et al.*, 2011; Vetter, 2000) while Amygdalin has been reported in apples, peaches and cherries and Dhurrin in sorghum leaves (Haque and Bradbury, 2002). Other forms of cyanogenic glycosides have also been reported in other plant species (Bak*et al.*, 2006). Chemical structures of some common cyanogenic glycosides are shown in Fig. 1

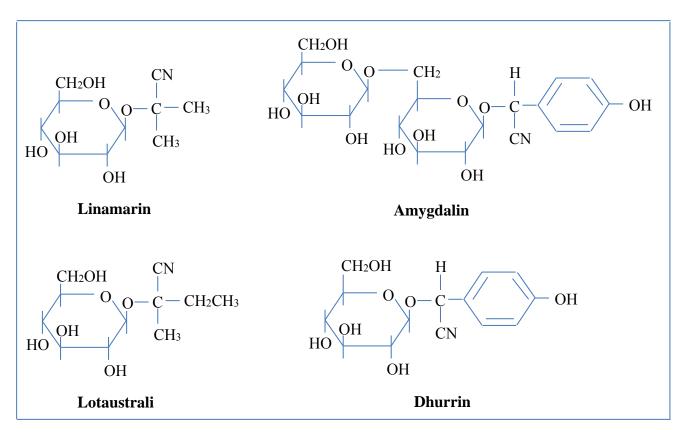


Fig. 1. Chemical Structures of some cyanogenic glycosides

Upon hydrolysis, cyanogenic glycosides breakdown into a sugar and a cyanohydrin which rapidly decomposes to hydrogen cyanide (HCN), a compound that has a long-term damaging effect on the central nervous system and the thyroid glands (Anhwange*et al.*, 2011). The production of HCN from cyanogenic glycosides is an enzymatic process, commonly called cyanogenesis which occurs when a cyanogenic plant tissue is pulped. This may occur during processing of the plant tissue or when it is directly chewed by animals.

In order not to poison themselves, cyanogenic glycosides (which are themselves non-toxic) and the enzyme that catalyzes its hydrolysis are stored in different compartments in plants tissues (Fig 2). Glycosides are stored in the vacuoles while the enzyme, glycosidase is stored in the cytosol (Heldt and Piechulla, 2011) and only come into contact when the partition is broken. When cell wall structures are raptured andthe bound form of the glycosides is brought into contact with the glucosidase, hydrogen cyanide is released through a two-reaction process (Shibamoto and Bjeldanes, 2009). The first reaction involves the breakdown to yield a cyanohydrin and a sugar, while the second one involves the decomposition of the highly unstable cyanohydrin into an aldehyde or ketone and hydrogen cyanide (HCN) and is catalyzed by hydroxynitrilelyase. The degradation of cyanogens to produce HCNby this two-step process is referred to as cyanogenesis (Deshpande, 2002).

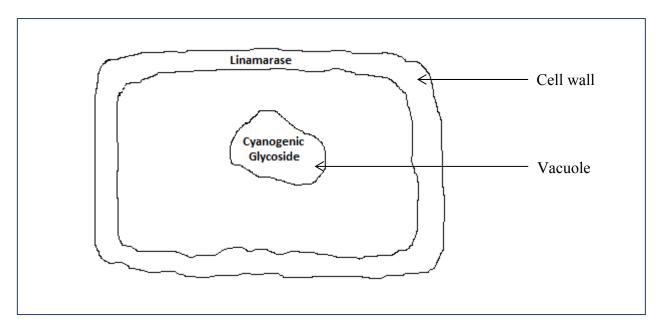


Fig 2. Location of cyanogenic glycoside and linamarase in the plant cell, adapted from Conn (1994)

## 3.1 Toxicity of cyanogens

The toxicity of cyanogenic glycosides results from the production of HCN, resulting in cyanide poisoning. Cyanide is a highly toxic compound with both acute and chronic effects (Shibamoto and Bjeldanes, 2009) stemming from ability to inhibit respiration and the action of some metalloenzymes (Deshpande, 2002). The lethal dose of HCN for humans, according to Deshpande (2002), has been estimated as ranging between 0.5 - 3.5 mg/kg body weight. Indeed, Jansz and Uluwaduge (1997) reported damage to the central nervous system in people who have been exposed to low levels of cyanide through their food over a long period of time. Thyroid glands may also be affected when exposed to sub-lethal doses because at these levels, HCN is converted to goitrogens such as thiocyanate (Deshpande, 2002; Abuyeet al., 1998). Other reports have implicated HCN in cases of neuropathy (Harris and Koomson, 2011; Madhusudananet al., 2008), diabetes mellitus (Morrison et al., 2006) and growth retardation in children (Banea-Mayambuet al., 2000) while consumption of up to 100 mg in adults has resulted in death (Yeoh and Sun, 2001). Detoxification of CN in humans is by conversion to thiocyanate, which is excreted in urine, in a process catalyzed by rhodanese. The process expends methionine and cysteine which are obtained through diets, and as such depletion of these amino acids without replacement may lead to protein malnutrition and stunting (Baneaet al., 2012). A lack of these essential amino to detoxify ingested cyanide, leads to an increase in blood cyanide concentration, an occurrence that manifests in certain neurological disorders (Cardoso et al., 2004; Harris and Koomson, 2011). Certain pancreatic disorders have been reported among cassava consumers who lack the right levels of proteins in their diets.

Many edible plants have been found to contain significant amounts of cyanogens whichplace a restriction on their use to a very large extent. Substantial concentrations have been reported in cassava (Manihotesculenta), a staple food of economic importance in Africa, South America and South Eastern parts of Asia that feeds more than half a billion people (Anhwage*et al.*, 2011; Nhassico*et al.*, 2008; Nweke, 2004; Nweke*et al.*, 2002).All parts of the cassava plant contain cyanogenic glycosides in the form of linamarin and lotaustralin, in a ratio of 97:3 (Lykkesfeldt and Moller, 1994). The concentration of cyanogens in roots and leaves differ from the same plant (Riis *et al.*, 2003) and is known to be more intense in the leaves than the stem and roots(Nambisan, 2011). The leaves and the roots have cyanide contents ranging from 53 - 1300 and 10 - 500 mg cyanide equivalents/kg of dry matter respectively (Siritunga and Sayre, 2003; Wobeto*et al.*, 2007).The cyanogen principles are produced at the apex of the shoot (Andersen *et al.*, 2000) and transported to the roots and leaves.

The use of cassava tuber for food and other industrial products is greatly hampered by its short shelf life (Zidenga*et al.*, 2012) and cyanogenic potential (Falade and Akingbala, 2010), even though it is known to be a good source of energy (Jisha*et al.*, 2010; Montagnac*et al.*, 2009). Many industrial and food products processed from cassava have been found to contain significant levels of degradation products of cyanogenic glycosides. Yeoh and Sun (2001) reported 15 – 61 mg of HCN/kg in various foods containing cassava flour, whileCumbana*et al.*, (2007) reported 8 - 85 mg for cassava flour. Other reports by Adindu*et al.*, (2003), Djazuli and Bradbury (1999) andSopade (2000) showed significantly higher amounts of cyanide containing compoundsthan recommended by FAO/WHO (1991).

Processing plays an effective role in the reduction/removal of cyanogens and their degradation products. This is accomplished by two separate treatments; that is, one that raptures the cellular compartments and brings the degradation enzymes into contact with the bound and inactive forms of the cyanogens and another that destroys the products formed from this reaction and favours the evaporation of HCN (Bainbridge *et al.*, 1998; ).The efficiency of cyanogen removal depends largely on the kinds of unit operations involved in the processing method (Nambisan, 1994) as well as the initial cyanogen load (Cardoso *et al.*, 2005). In order to attain levels within the recommended safe limits set by WHO, initial root cyanide load not exceeding 250ug/g has been proposed for efficient processing(Cardoso *et al.*, 2005).

## 3.2 Relationship between bitterness and toxicity of cassava

Depending on the cyanogenic glycoside content and taste, cassava is categorized into three classes namely; sweet, average toxic and bitter, with <50, 50 - 100 and > 100 ppm of linamarin calculated as mg CN/kg of edible portions (fresh weigh basis) respectively (Jansz and Uluwaduge, 1997; Nhassico*et al.*, 2008).Bitterness in cassava has been associated with linamarin

because this cyanogen is bitter (King and Bradbury, 1995). This relationship, not clear cut, though, may not be a good indicator of toxicity as was previously thought(Jansz and Uluwaduge, 1997) because other compounds in the parenchyma and cortex also impart bitterness.That notwithstanding, local farmers classify cassava as being bitter or not bitter (Chiwona-Karltun, 2004; Kebede*et al.*, 2012) and use this grouping as an indicator of toxicity (Chiwona-Karltun, 2004). Bitter cassava has been observed to be less prone to theft and predation(Chinowa-Kartun*et al.*, 1998).

#### 4.0 DETOXIFICATION OF CASSAVA CYANOGENS

The presence of toxic cyanogenic glycosides in cassava constitutes a critical limiting factor to its use, together with other considerations such as deficiency in some essential nutrients and high deterioration rate. Detoxification through breeding/genetic engineering and processing offers an opening to scaling this debacle that confronts economic and social prospects of the plant. This reduces the exposure to cyanogenic compounds and thus lowers or eliminates the risk of cyanide intoxication (Onabolu*et al.*, 2002). Autolysis of linamarin is extensively relied on in detoxifying cassava (especially during processing) for human consumption. This is triggered by maceration or cell disruption, which results in bringing linamarase into contact with the glycosides and hydrolyses them. The activity of linamarase, however decreases a few days after harvest (Iwatsuki*et al.*, 1984). The reasons responsible for this lowered activity is not certain, but has been related to the formation of enzyme inhibiting compounds such as polyphenols (Essers*et al.*,, 1996).

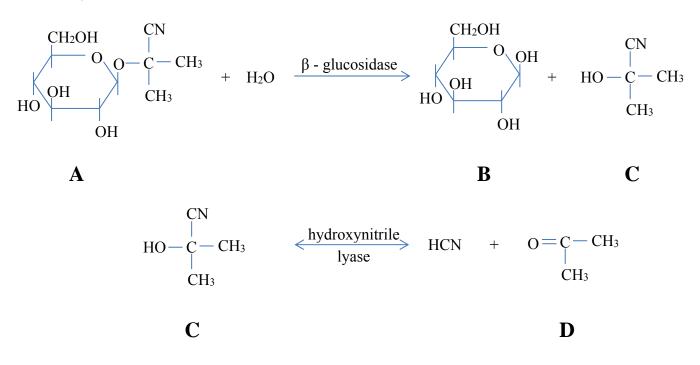


Figure 3: Enzymatic breakdown of Linamarin (McMahon et al.,,, 1995)

Fig. 3 shows the breakdown of linamarin into HCN and acetone. Linamarin (A) hydrolyses into glucose (B) and acetone cyanohydrin (C), and further into hydrogen cyanide and acetone (D). The breakdown of acetone cyanohydrin is influenced by pH and temperature (occurs spontaneously at pH > 4 and temperatures > 30 C) McMahon *et al.*, (1995).

## 4.1 Biotechnology and conventional breeding

The hindrance to attaining optimal use of cassava can best be achieved when cyanide-free strains are obtained from breeding programmes because they do not occur naturally (Bradbury and Holloway, 1988). Cyanide-free strains would make cassava reliably safe, more acceptable and marketable and reduce cyanide effluent from cassava processing plants (Siritunga and Sayre, 2003). Genetic engineering, using antisense technology, has been used to block the synthesis of linamarin, resulting in cyanide-free cassava. Dramatically reduced linamarin content in leaves and roots of wild-types has also been achieved by genetic manipulation (Anderson et al., 2000; Siritunga and Sayre, 2003; Siritunga and Sayre, 2004). The downside to this development, however, is the likelihood of having reduced plant yield as a result of stalling the synthesis of linamarin (Taylor et al., 2004). The resulting transgenic plant could not produce roots because of a lack of ammonia, which is produced by the roots using linamarin as its source. Obstructing the synthesis of linamarin also leaves the plant vulnerable to animal and insect attack since linamarin is used in a defensive mechanism (Vetter, 2000). Besides these technical and research issues, controversy and skepticism surrounding genetically modified organisms (Falkner, 2004) may pose a challenge to the introduction and use of transgenic "strains" in part of the world.Genetic transformation and molecular biology techniques have not made any commercially remarkable impact even though theypresent great potential.

Conventional methods of breeding, which involves selection and crossing varieties to yield desirable traits, have also been applied in a bid to reduce the cyanogen content in cassava. Previous studies by Iglesias *et al.*, (2002) showed reduced cyanogen content in some clones compared to their parental variety. The low vegetative multiplication rate and the fact that several factors affect the quality of planting material (Ceballos*et al.*, 2010), however, complicates and makes this method quite difficult to implement.

## 4.2 Processing

Aside of genetic/breeding interventions embarked upon to obtain significantly reduced cyanogen content in cassava, biological detoxification methods such as enzyme and bacteria action and physical methods such as processing present suitable options to attaining a similar goal. These methods have resulted in tremendous and significant economic gains as far as the use of cassava is concerned.Detoxification essentially involves two separate treatments; first is one that enhances the contact between linamarase and its substrates (cyanohydrins) followed by a second

that volatilizes the HCN produced as a result of contact between the enzyme and its substrates. Processing largely promotes these conditions that are required for adequate detoxification. Cassava processing improves shelf-life, detoxifies the roots, facilitates transport and enhances consumer acceptability (Westby, 2002; Nyirenda*et al.*, 2011). The shortcoming of processing as a detoxification method, conversely, is that a lot of them result in loss of nutrients (Murugan*et al.*, 2012).

Enzymatic removal of cyanogens is commonly accomplished by treating samples with enzymes isolated from bacteria to breakdown cyanogenic compounds into acetone cyanohydrins, which decomposes spontaneously to HCN or by treating with plant cell wall-degrading enzymes such as cellulolytic and pectolytic enzymes to enhance the release of linamarin and allow for more contact time with linamarinase (Yeoh and Sun, 2001). The latter principle has been exploited in the production of cassava starch (Sornyothaet al., 2010). The HCN produced is subsequently dissolves readily in water or is released into the air (Rolle, 1998; Muruganet al., 2012). The enzyme hydrolyses of the cyanogens is sensitive to changes in pH (Cumbanaet al., 2007), with pH > 5 favouring the breakdown. Certain species of Bacillus, pseudomonas and klebsiellaoxytoca have been reported to utilize cyanide as the only source of nitrogen under aerobic and anaerobic conditions thus breaking it down into non-toxic compounds (Kaewkannetraet al., 2009). Bacillus subtilis KM05 isolated from cassava peels has been used to detoxify cassava flour (Muruganet al., 2012) by degrading linamarin into HCN and subsequently releasing ammonia. In another study by Nwokoro and Anya (2011), cassava flour samples treated with linamarinase enzyme isolated from L.delbrueckiiresulted in an 89.5% reduction in cyanide content.

## 4.2.1 Fermentation

Fermentation as a method of processing primarily enhances nutritional properties through biosynthesis of vitamins, essential amino acids and proteins, by improving protein quality and fibre digestibility as well as the enhancement of micronutrient bioavailability and degradation of anti-nutritional factors (Achinewhu*et al.*, 1998; Motarjemi, 2002). Fermentation of cassava, both aerobic and anaerobic, favors the hydrolysis of linamarin into HCN. Even though details of the mechanism involved are unclear (Vasconcelos*et al.*, 1990), fermentation softens the cells of the roots and favours contact of the enzymes with its substrate (Errers, 1995). In the case of submerged fermentation, this process synergiseswith leaching of cyanogen to detoxify the cassava roots (Westby and Choo, 1994).

Fermentation has been applied in the production of gari (Onaboluet al., 2002), akyeke/atioke (Tetchiet al., 2012), bikedi and ntobambodi (Kobawilaet al., 2005), cassava dough (Amoa-Awuaet al., 1997), farinhapuba (Aidoo, 1992) and several other foods customary to cassava production areas worldwide (Westby, 2002). Three major types of fermentation are widely

practiced in different parts of Africa; these are the grated root fermentation, mould fermentation of roots in heaps and fermentation of roots under water (Westby, 2002). Fermentation of cassava roots is largely acidic (pH 3.8) while that for leaves is alkaline (pH 8.5) with lactic acid bacteria dominating the microbiota (Ngaba and Lee, 1979; Oyewole and Odunfa, 1988). Some lactic acid bacteria and yeast possess linamarase activity and are recognized for significantly contributing to cyanogenic glycoside breakdown during fermentation of cassava (Amoa-Awua*et al.*, 1996, Kimaryo*et al.*, 2000; Lei *et al.*, 1999). These microorganisms are capable of utilizing the cyanogens and their degradation products (Akindahunsi*et al.*, 1999) thereby ridding their substrate of these noxious substances and rendering the substrate safe.

Previous reports have shown a remarkable reduction in cyanogenic potential of cassava following fermentation. More than 50 % and 35 % reduction in cyanogen levels has previously been achieved in the production of gari and fermented cassava flour respectively (Kemdirim*et al.*, 1995). Iyayi and Dosel (2000) and Enidiok*et al.*, (2008) have also reported up to 80 % and 41% reduction in cyanide levels respectively during fermentation. Other researchers have also reported varying levels of decline in cyanogen potential after fermentation (Cardoso *et al.*, 2005;Bradbury, 2004; Djoulde*et al.*, 2007;Oyewole and Ogundele, 2001; Zvauya and Muzondo, 1995). Indeed reduction in cyanide level in all cases depends on the initial cyanide levels of the raw material.

## 4.2.2 Soaking

Soaking cassava roots usually precedes fermentation, cooking or drying. Retting, followed by sun drying is exploited as a method of processing cassava roots in some parts of Africa. This technique of long soaking cassava roots in stagnant or slow running ponds and causes the breakdown of tissues and extraction of the starchy mass (Ayernor, 1985). The water softens the cells of the cassava roots, provides a larger medium for fermentation and facilitates leaching of cyanogenic glycosides. The method removes a substantial amount of free cyanide but has little effect on bound cyanide.Soaking peeled or unpeeled cassava roots is practiced in the northern and central regions of Malawi (Nyirenda, 2003) to produce 'waluwa' and 'kanyakaska' which are dried and pounded into flour and used to prepare a local delicacy called 'kodowole'. The cassava roots come out of the process having lost between 31.0% and 49.9% (for unpeeled and peeled roots respectively) of their cyanogenic potential.Other studies have resulted in remarkably significant reduction in cyanogenic glycosides after soaking (Ampe and Brauman, 1995)

## 4.2.3 Cooking

Boiling cassava roots, which is often for direct consumption with accompaniments such as soups and stews, is commonplace in most areas where cassava is produced for culinary purposes.Cooking is Processing cassava roots by this method is preceded by peeling, cutting into chunks/dicing and washing. Disruption of cell membrane during cooking largely occurs between 60 and 70 °C and not long after that linamarase is destroyed, making contact with its substrate inadequate for thorough detoxification. This causes a possible retention of cyanogenic glycoside levels (Jansz and Uluwaduge, 1997). Cyanohydrins from aldehydes, may also exist even after cooking because they are thermo-stable (Onabolu*et al.*, 2002). As a result, boiling is often criticized and an ineffective standalone method of detoxifying cassava roots and hence is preferred as a method of processing sweet cassava(), although the heat favours rapid evaporation of HCN produced (Bokanga, 1994).Indeed, the extent of reduction of cyanogenic glycosides has been related to the cooking time (Hidayat*et al.*, 2002). Jansz and Uluwaduge (1997) have reported cooking to reduce cyanogen potential by 50 -70% in Southern Asia. Fukuba*et al.*, (1982) introduced a soaking and squeezing stage prior to cooking and achieved a remarkable reduction in cyanogenic potential of up to 70%. Boiling/cooking has also been applied to process cassava leaves and resulted in 75 % reduction (Hidayat*et al.*, 2002) and in some cases more than 90% reduction in cyanide level (Ngudi*et al.*, 2003).

## 4.2.4 Roasting Drying

Cassava roots have been processed into a lot of dried products. Drying is widely accepted as an efficient processing method for cassava roots as it results in products that are shelf-stable with relatively reduced cyanide content. In as much as advanced systems of drying exist, sun drying is the most adopted method in cassava processing regions of Africaand as such sun-dried cassava products are the most common (Westby, 2002). Dried cassava pieces can be processed further into other preferred forms. Drying or roasting cassava is usually preceded by peeling, chipping, chunking or grating before spreading out in the sun to dry. Detoxification is achieved by

The drying mechanism in itself does not play any significant role in the detoxification process but the tissue disruption that precedes drying (Essers*et al.*, 1996). The efficiency of cyanide removal during drying is dependent on moisture content of the roots, rate of moisture loss (which relates to drying conditions), and the extent of tissue disruption (Essers*et al.*, 1996; Tivana, 2012). The influence of moisture content on detoxification is crucial, as glucoside degradation has been observed to stop between 13% and 18% moisture. This is because diffusion of linamarin during drying continually decreases and at a point where bulk water for transport is lacking, it becomes immobilized thus preventing its interaction with linamarase in the drying medium(Essers*et al.*, 1996, Mlingi*et al.*, 1995). Extending the period of drying with higher moisture levels have been observed to result in enhanced linamarin breakdown, thus explaining the fact that fast drying rates result in lower detoxification while slower rates result in higher cyanogen removal (Essers*et al.*, 1996). Cyanohydrin levels remain high in the product during drying because of the enzyme hydrolysis that takes place, especially when root pieces are humid. Their levels could be reduced further by thorough drying well below 12 or 13% moisture (Mlingi*et al.*, 1995). HCN levels conversely remain low during drying because it volatilizes as a result of its exposure to heat.

## 4.2.5 Other unit operations

Several other unit operations or a combination of unit operations employed during cassava processing also contribute to the reduction in cyanide potential. These include size reduce operations such as cutting, pulping (grating/chipping and crushing), washing/soaking.Size reduction precedes a lot of processing operations. In cassava processing, cells break open during size reduction and bring endogenous enzymes into contact with their substrates, consequently initiating the hydrolysis of cyanogens into hydrogen cyanide and acetone. Processes that begin with pulping result in the greatest detoxification of the final products (Bokanga, 1999). Other size reduction operations such as mincing and rasping have been reported to result in a loss of more than 70% of cyanogenic glycosides (Jansz and Uluwaduge, 1997).

## **5.0 CONCLUSION**

Cassava is by far the most important tuber crop in the lives of many people the world over and in recent times serves as a less costly source of raw material for industrial applications. Its uses, however, is hampered because of its potential toxicity which is due to the presence of cyanogenic glycosides. Processing successfully detoxifies cassava and reduces the risk of intoxication by consuming cassava. The efficiency of cyanide removal however, depends on the processing technique employed and the extent of processing. Processing operations such as fermentation, boiling/cooking, roasting and drying, applied to process cassava have been able to reduce cyanide content to acceptably safe levels.

## REFERENCES

Abuye, C., Kelbessa, U. and Wolde – Gebriel, S. (1998). Health effects of cassava consumption in south Ethiopia. *East African Medical Journal*, 75: 166 – 170.

Achinewhu, S.C., Barber, L.I. and Ijeoma, I.O. (1998). Physicochemical properties and garification (gari yield) of selected cassava cultivars in River States, Nigeria. *Plant Food for Human Nutrition*, 52: 133 – 140.

Adewusi, S.R.A. and Bradbury, J.H. (1993). Carotenoid in cassava: comparison of open column and HPLC methods of analysis. *Journal of the Science of Food and Agriculture*, 62:375 – 383.

Adindu, M.N., Olayemi, F.F. and Nze-Dike, O.U. (2003). Cyanogenic potential of some cassava products in port Harcourt markets in Nigeria. *Journal of Food Composition and Analysis*, 16: 21 – 24.

Aidoo, K.E. (1992). Lesser-Known Fermented Plant Foods. In: Applications of Biotechnology to Traditional Fermented Foods. Report of an Ad Hoc panel of the board of science and technology for international development, Office of International Affairs, National Research Council. National Academy Press, Washington DC, pp

Akindahunsi, A.A., Ohoh, G., and Oshodi, A.A. (1999). Effect of fermenting cassava with Rhizopusoryzae on the chemical composition of its flour and gari. *Rivista Italiana delle Sostanze Grasse*, 76:437 – 440.

Allem, A.C. (2002). The Origins and Taxonomy of cassava. In: Hillocks, R.J., thresh, J.M. Bellotti, A., (eds) Cassava: Biology, production and utilization. CAB Publishing International. Pp. 1 - 16.

Ampe, F. and Brauman, A. (1995). Origin of enzymes involved in detoxification and root softening during cassava retting. *World Journal of Microbiology and Biotechnology*, 11:178 – 182.

Andersen, M.D., Busk, P.K., Svendsen, I. and Moller, B.L. (2000). Cytochromes P-450 from cassava (ManihotesculentaCrantz) catalyzing the first steps in the biosysthesis of the cyanogenic glycosides linamarin and lotaustralin: cloning, functional expression in *Pichiapastoris* and substrate specificity of the isolated recombinant enzymes. *Journal of Biology and Chemistry*, 275:1966 – 1975.

Anhwange, B.A., Asemave, K., Ikyenge, B.A. and Oklo, D.A. (2011). Hydrogen Cyanide content of Manihortutilissima, colocasia esculenta, dioscore abulbefera and dioscore adomentorum tubers found in Benue state. *International Journal of Chemistry*, 3:69 – 71.

Ayernor, G.S. (1985). Effects of the retting of cassava on product yield and cyanide detoxification. *Journal of Food Technology*, 20:89 – 96.

Bainbridge, Z., Harding, S., French, L., Kapinga, R. and Westby, A. (1998). A study of the role of tissues disruption in the removal of cyanogens during cassava root processing. *Food Chemistry*, 62:291 - 297.

Bak, S., Paquette, M.S., Morant, M., Morant, A.V., Saito, S., Bjarnholt, N., Zagrobelny, M., Jorgensen, K., Osmani, S., Simonsen, H.T., Perez, R.S. van Heeswijck, T.B., Jorgensen, B. and Moller, B.L. (2006). Cyanogenic glycosides: A case study for evolution and application of cytochromes P450. *Phytochemical Reviews*, 5:309 – 329.

Banea, M.J.P., Tylleskar, T., Tylleskar, K., Gebre – Medhin, M. and Rosling, H. (2000).Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo, former zaire.*Annals of Tropical Paedriatrics*, 20: 34 – 40.

Banea, M.J.P., Nahimana, G., Mandombi, C., Bradbury, J.H., Denton, I.C. and Kuwa, N. (2012).Control of konzo in DRC using the wetting method on cassava flour.*Food and Chemical Toxicology*, 50: 1517 – 1523.

Bokanga, M. (1995).Biotechnology and cassava processing in Africa.*Food Technology*, 49:86 – 90.

Bradbury, H. (2004). Processing of cassava to reduce cyanide content. In: Cassava cyanide diseases network.pp3

Bradbury, J.H. and Holloway, W.D. (1988).Cassava, M. esculenta. Chemistry of tropical root crops: significance for nutrition and agriculture in the pacific. Australian center for international agricultural research, Monograph 6, Canberra, Australia, pp 76 - 104.

Cardoso, A.P., Mirione, E., Ernesto, M., Massaza, F., Cliff, J., Haque, R.M. and Bradbury, J.H. (2005).Processing of cassava roots to remove cyanogens.*Journal of Food Composition and Analysis*, 18:451 – 460.

Ceballos, H., Okogbenin, E., Perez, J.C, Lopez-Valle, L.A.B. and Dubouck, D. (2010).Cassava. In: Bradshaw, J.E. (ed). Roots and Tuber Crops, Springer Science and Business media, London, pp 53 – 96. Charles, A.L., Sriroth, K. and huang, T.C. (2005).Proximate composition, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes.*Food Chemistry*, 92: 615 – 620.

Chinowa-Kartun, L., Mkumbira, J. and Saka, J., Bovin, M., Mahungu, N.M. and Rosling, H. (1998). The importance of being bitter – a qualitative study on cassava cultivar preference in malawi. *Ecology of Food and Nutrition*, 37:219–245.

Chiwona-Karltun, L., Brimer, L., Saka, J.D.K., Mhone, A.R., Mkumbira, J., Johansson, L., Bokanga, M., Mahungu, N.M. and Rosling, H. (2004). Bitter taste in cassava roots correlates with cyanogenic glucoside levels. *Journal of the Science of Food and Agriculture*, 84: 581 – 590.

Conn, E.E. (1994). Cyanogenesis – a personal perspective. In, Bokanga, M., Essers, A.J.A., Poulter, N., Rosling, H. and Tewe, O. (eds), Proceedings of the international workshop on cassava safety, March 1-4, 1994, Ibadan, Nigeria, *ActaHorticulturae*, 375, 31 – 43.

Cumbana, A., Mirione, E., Cliff, J. and Bradbury, J.H. (2007).Reduction of cyanide content of cassava flour in Mozambique by the wetting method.*Food Chemistry*, 101: 849 – 897.

Deshpande, S.S. (2002). Handbook of Food Toxicology. Marcel Dekker, Inc, New York pp

Djazuli, M. and Bradbury, J.H. (1999).Cyanogen content of cassava roots and flour in Indonesia.*Food Chemistry*, 65:523 – 525.

Djoulde, D.R., Essia, N.J.J. and Etoa, F.X. (2007). Nutritive value, toxicological and hygienic quality of some cassava based products consumed in Cameroon. *Pakistani Journal of Nutrition*, 6:404 – 408.

El-Sharkawy, M.A. (2003).Cassava biology and physiology.*Plant Molecular Biology*, 53:621 – 641.

Enidiok, S.E., Attah, L.E. and Otuechere, C.A. (2008). Evaluation of moisture, total cyanide and fiber contents of Garri produced from cassava (Manihotutilissima) varieties obtained from Awassa in Southern Ethiopia. *Pakistan Journal of Nutrition*, 7:625 – 629.

Essers, A.J.A. (1995). Removal of cyanogens from cassava roots: studies on domestic sun-drying and solid substrate fermentation in rural Africa. PhD thesis, Wageningen Agricultural University, The Netherlands.

Essers, A.J.A., Van der Grift, R.M. and Voragen, A.G.J. (1996). Cyanogen removal from cassava roots during sun-drying. *Food Chemistry*, 55: 319 – 325.

Falade, K.O. and Akingbala, J.O. (2010).Utilization of cassava for food.*Food Reviews International*, 27:51-83.

Falkner, R. (2004). The first meeting of the parties to the Cartagena protocol on biosafety. *Environmental Politics*, 13:635 – 641.

FAO/WHO.(1991). Joint FAO/WHO food standards programme. Codex alimentarius commission XII, Supplement 4. Rome, FAO.

Fukuba, H., Igarashi, O., Briones, C.M. and Mendoza, E.M.T. (1982).Determination and detoxification of cyanide in cassava and cassava products.*Philippians Journal of Crop Science*, 7:170–175.

Hambidge, K.M., Miller, L.V., Westcott, J.L., Krebs, N.F. (2008). Dietary reference intakes for zinc may require adjustment of phytate intake based upon model predictions. *Journal of Nutrition*, 138: 2363-2366.

Haque, M.R.and Bradbury, J.H. (2004). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry*, 77:107 – 114.

Harris, M.A. and Koomson, C.K. (2011). Moisture-Pressure combination treatments for cyanide reduction in grated cassava. *Journal of Food Science*, 76: T20 – T24.

Heldt, HW and Piechulla, B. (2011) Plant Biochemistry, 4<sup>th</sup> Ed. Academic Press, London pp 399 – 408.

Hidayat, A., Zuraida, N. and Hanarida, I. ((2002) The cyanogenic potential of roots and leaves of ninety nine cassava cultivars. *Indonesian Journal of Agricultural Science*, 3:25 – 32.

Iglesias, C.A., Sanchez, T. and Yeoh, H.H. (2002).Cyanogens and Linamarase activities in storage roots of cassava plants from breeding program.*Journal of Food Composition and Analysis*, 15:379 – 387.

Iwastuki, N., Kojima, M. Data, E.S. and Villegas-Godoy, C.D.V. (1984). Changes in cyanide content and linamarase activity in cassava roots after harvest. In: Uritani, I. and Reyes, E.D. (eds), Tropical root crops – postharvest physiology and processing. Japan Scientific press, Tokyo, pp 151-161

Iyayi, E.A. and Losel, D.M. (2000).Cyanide detoxification in cassava by-products by fungal solid state fermentation.*The Journal of Food Technology in Africa*, 5:48 – 51.

Jansz, E.R. and Uluwaduge, D.I. (1997).Biochemical aspects of cassava (manihotesculentacrantz) with special emphasis on cyanogenic glucosides – A Review.*Journal of the National.Science Council of Sri lanka*, 25:1–24.

Jisha, S., Padmaja, G. and Sajeev, M.S. (2010).Nutritional and textural studies on dietary fiberenriched muffins and biscuits from cassava-based composite flours.*Journal of Food Quality*, 33:79 – 99.

Jorgensen, K., Morant, A.V., Morant, M., Jensen, N.B., Olsen, C.E., Kannangara, R., Motawia, M.S., Moller, B.L. and Bak, S. (2011). Biosynthesis of the Cyanogenic GlucosidesLinamarin and Lotaustralin in cassava: Isolation, Biochemical Characterization, and Expression Pattern of CYP71E7, the Oxime-Metabolizing cytochrome P450 Enzyme. *Plant Physiology*, 155: 282 – 292.

Kaewkannetra, P., Imai, T., Garcia-Garcia, F.J. and Chiu, T.Y. (2009).Cyanide removal from cassava mill wastewater using Azotobactorvinelandii TISTR 1094 with mixed micro-organisms in activated sludge treatment system.*Journal of Hazardous Material*, 172:224 – 228.

Kebede, A., Teshome, B., Wondimu, A., Belay, A., Wodajo, B. and Lakew, A. (2012). Detoxification and consumption of cassava based foods in south west Ethiopia. *Pakistan Journal of Nutrition*, 11: 237 – 242.

Kemdirim, O.C., chukwu, O.A. and Achinewhu, S.C. (1995).Effect of traditional processing of cassava on the cyanide content of gari and cassava flour.*Plant food for Human Nutrition*, 48:335 – 339.

Kimaryo, V.M., Massawe, G.A., Olasupo, N.A. and Holzapfel, W.H. (2000). The use of a starter culture on the fermentation of cassava for the production of kivumde a traditional Tanzanian food product. *International Journal of Food Microbiology*, 56:179 – 190.

King, N.L.R. and Bradbury, J.H. (1995). Bitterness of cassava: identification of a new apiosylglucoside and other compounds that affect its bitter taste. *Journal of the Science of Food and Agriculture*, 68, 223 – 230.

Kobawila, S.C., Louembe, D., Keleke, S., Hounhouigan, J. and Gamba, C. (2005).Reduction of the cyanide content during the fermentation of cassava roots and leaves to produce bikedi and ntobambodi, two food products from Congo.*African Journal of Biotechnology*, 4:689 – 696.

Lebot, V. (2009). Tropical root and tuber crops: cassava, sweet potato, yams, aroids. CAB International, Oxfordshire, UK. pp 2 - 87.

Lykkesfeldt, J. and Moller, B.L. (1994).Cyanogenic glycosides in cassava, ManihotesculentaCrantz.*ActaChemScandA* 48: 178 – 180.

Madhusudanan, M., Menon, M.K., Ummer, K. and Radhakrishnan, K. (2008).Clinical and Etiological Profiling of tropical ataxic neuropathy in Kerala, South India.*European Neurology*, 60: 21 – 26.

Marfo, E.K., Simpson, B.K., Idowu, J.S., Oke, O.L. (1990). Effect of local food processing on phytate levels in cassava, cocoyam, yam, maize, sorghum, rice, cowpea and soybean. *Journal of Agriculture and Food Chemistry*, 38:1580 – 1585.

Massey, L.K. (2007). Food Oxalate: Factors affecting measurement, biological variation and bioavailability. *Journal of American Dietetic Association*, 107: 1191-1194.

McMahon, J.M., White, W.L.B. and Sayre, R.T. (1995).Cyanogenesis in cassava (ManihotesculentaCrantz).*Journal of Experimental Botany*, 46: 731 – 741.

Mlingi, N. L.V., Bainbridge, Z.A., Poulter, N.H. and Rosling, H. (1995). Critical stages in cyanogen removal during cassava processing in southern Tanzania. *Food Chmistry*, 53:29 – 33.

Montagnac, J.A., Davis, C.R. and Tanumihardjo, S.A. (2009).Nutritional value of cassava for use as a staple food and recent advances for improvement.*Comprehensive Review in food Science and Food Safety*, 8:181 – 188.

Morrison, E., Ragoobirsingh, D. and Peter, S. (2006). The Unitarian Hypothesis for the aetiology of diabetes mellitus. *Medical Hypotheses*, 67:1115 – 1120.

Motarjemi, Y. (2002). Impact of small scale fermentation technology on food safety in developing countries. *International Journal of Food Microbiology*, 75:213 – 229.

Murugan, K., Yashotha, Sekar, K. and Al-Sohaibani, S. (2012). Detoxification of cyanides in cassava flour by linamarase of Bacillus subtilis KM05 isolated from cassava peel. *African Journal of Biotechnology*, 11:7232 – 7237.

Nambisan, B. (1994). Evaluation of the effect of various processing techniques on the cyanogens reduction in cassava. *Acta Horticulture*, 375: 193 – 202.

Nambisan, B. (2011). Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. *Food and Chemical Toxicology*, 49:690 – 693.

Ngaba, P.R. and Lee, J.S. (1979).Fermentation of cassava (ManihotesculentaCrantz).*Journal of Food Science*, 44: 1570-1571.

Ngudi, D.D., Kuo, Y.H. and Lambein, F. (2003).Cassava cyanogens and free amino acids in raw and cooked leaves.*Food and Chemical Toxicology*, 41: 1193 – 1197.

Nhassico, D., Muquingue, H., Cliff, J., Cumbana, A. and Bradbury, J.H. (2008).Rising African cassava production, diseases due to high cyanide intake and control measures.*Journal of the Science of Food and Agriculture*, 88: 2043 – 2049.

Nweke, F. (2004). New challenges in the cassava transformation in nigeria and Ghana. EPTD Discussion Paper No. 118. International Food Policy Research Institute, Washington, D.C.

Nweke, F.I., Spencer, D.S.C. and Lynam, J.K. (2002). The cassava transformation. Michigan state university press, East Lansing, MI.

Nwokoro, O. and Anya, F.O. (2011).Linamarinase enzyme from Lactobacillus delbrueckii NRRL B-763: Purification and some properties of a  $\beta$ -Glucosidase. *Journal of Mexican Chemical Society*, 55:246 – 250.

Nyirenda, D.B., Chiwona-Karltun, L., Chitundu, M., Haggblade, S. and Brimer, L. (2011). Chemical safety of cassava products in regions adopting cassava production and processing – Experience form Southern Africa. *Food and Chemical Toxicology*, 49:607–612.

Onabolu, A.O., Oluwole, O.S.A., Rosling, H. and Bokanga, M. (2002). Processing factors affecting the level of residual cyanohydrins in Gari. *Journal of the Science of Food and Agriculture*, 82:966–969.

Oyewole, O.B. and Odunfa, S.A. (1988). Microbiological studies on cassava fermentation for "lafun" production. Food Microbiology, 5:125 - 133.

Oyewole, O.B. and Ogundele, S.L. (2001). Effect of length of fermentation on the functional characteristics of fermented cassava 'fufu'. *The Journal of Food Technology in Africa*, 6:38 – 40.

Riis, L., Bellotti, A.C., Bonierbale, M., and O'Brien, G.M. (2003). Cyanogenic potential in cassava and its influence on a generalist insect herbivore Crytomenusbergi (Hemiptera: Cydnidae). *Journal of Economic Entomology*, 96:1905 – 1914.

Rolle, R.S. (1998). Enzyme application for agro-processing in developing countries: an inventory of current and potential applications. *World Journal of Microbiology and Biotechnology*, 14:611–619.

Shibamoto, T and Bjeldanes, L. (2009).Introduction to food toxicology 2<sup>nd</sup> edition, Academoc press, California, USA pp 124 – 154.

Siritunga, D. and Sayre, R.T. (2003).Generation of cyanogen – free transgenic cassava.*Planta*, 217:367 – 373.

Siritunga, D. and Sayre, R.T. (2004). Engineering cyanogen synthesis and turnover in cassava (Manihotesculenta). *Plant Molecular Biology*, 56:661 – 669.

Sopade, P.A. (2000). The fate of cyanogens during the cooking of cassava in mumu, a traditional oven in Papua New Guinea.*International Journal of Food Science and Technology*, 35:173–182.

Sornyotha, S., Kyu, K.L. and Ratanakhanokchai, K. (2010). An efficient treatment for detoxification process of cassava starch by plant cell wall-degrading enzymes. *Journal of Biosciences and Bioengineering*, 109: 9 - 14.

Taylor, N., Chavarriaga, P., Raemakers, K., Siritunga, D. and Zhang, P. (2004) Development and application of transgenic technologies in cassava. *Plant Molecular Biology*, 56: 671–688.

Tetchi, F.A., Solomen, O.W., Celah, K.A. and Georges, A.N. (2012). Effect of cassava variety and fermentation time on biochemical and microbiological characteristics of raw artisanal starter for attieke production. *Innovative Romanian Food Biotechnology*, 10: 40 – 47.

Tewe, O.O. and Lutaladio, N. (2004). Cassava for livestock feed in sub-sahara Africa. FAO, Rome, Italy.

Tivana, T.D. (2012). Cassava Processing: safety and protein fortification. PhD Thesis submitted to the Department of Food Technology, Engineering and Nutrition, Lund University, Sweden.

Vasconcelos, A.T., Twiddy, D.R., Westby, A. and Reilly, P.J.A. (1990).Detoxification of cassava during gari preparation.*International Journal of Food Science and Technology*, 25:198–203.

Vetter, J. (2000).Plant cyanogenic glycosides. Toxicon, 38:11 – 36.

Westby, A. (2002). Cassava Utilization, storage and small-scale processing. In: Hillocks, R.J., thresh, J.M. Bellotti, A., (eds) Cassava: Biology, production and utilization. CAB Publishing International, pp 281 – 300.

Westby, A. and Choo, B.K. (1994).Cyanogen reduction during the lactic fermentation of cassava.*ActaHorticulturae*, 373: 209 – 215.

Wheatley, C.C. and Chuzel, G. (1993). Cassava: the nature of the tuber and use as raw material. In: Macrae, R., Robinson, R.K. and Sadler, M.J. (eds). Encyclopedia of food science, food technology and Nutrition. Academic Press, San Diego, California, pp 734 – 743.

Wobeto, C., Correa, A.D., de Abreu, C.M.P., dos Santos, C.D. and Pereira, H.V. (2007). Antinutrients in the cassava (ManihotesculentaCrantz) leaf powder at three ages of the plant. *CienceTechnologieAlimentaire*, 27:108 – 112.

Yeoh, H.H. and Sun, F. (2001). Assessing cyanogen content in cassava-based food using the enzyme – dipstick method. *Food and Chemical Toxicology*, 39: 649 – 653.

Zagrobelny, M., Bak, S., Rasmussen, A.V. Jorgensen, B., Naumann, C.M. and Moller, L.B. (2004). Cyanogenic glucosides and plant-insect interactions. *Phytochemistry*, 65:293 – 306.

Zidenga, T., Leyva-Guerrero, E., Moon, H., Siritunga, D. and Sayre, R. (2012).Extending cassava root shelf life via reduction of reactive oxygen species production.*Plant Physiology*, 159: 1396 – 1407.

Zvauya, R. and Muzondo, M.I. (1995). Reduction of cyanide levels in cassava during sequential sun-drying and solid state fermentation. *International Journal of Food Sciences and Nutrition*.46:13 – 16.