

Cyanide in Cassava: A Review

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Abstract

The work presented here is a review of cyanide in cassava. The presence of the two cyanogenic glycosides (linamarin and lotaustralin), in cassava, which on enzymatic hydrolysis leads to the production of hydrogen cyanide, is a major factor limiting cassava use as food or feed. Linamarin accounts for more than 80% of the cassava cyanogenic glycosides. It is a β -glucoside of acetone cyanohydrin and ethyl-methyl-ketone-cyanohydrin. Cassava varieties are often categorized as either sweet or bitter, signifying the absence or presence of toxic levels of cyanogenic glycosides, respectively. The so-called sweet (actually not bitter) cultivars can produce as little as 20 milligrams of Cyanide (CN) per kilogram of fresh roots, whereas bitter ones may produce more than 50 times as much (1 g/kg). Cassava grown during drought are especially high in these toxins. In addition, Cyanide is a chemical defense developed to cope with a huge diversity of unfavorable biotic conditions in the field. These glycosides and cyanogenic glycosides liberate cyanide, which, blocking cytochrome c oxidase (responsible for muscle and brain function) and NIS, is poisonous to parasites and herbivores but does not affect the plant cells. A lot of factors affect the level of cyanogenic glycosides in cassava. Many processing methods that reduce cyanide levels in cassava products have been developed.

Keywords: Cassava; Cyanogenic; Hydroxynitrite; Linamarin

Introduction

Cassava (*Manihot esculenta* Crantz) is an important tropical root crop providing energy to about 500 million people [1,2]. Almost all the cassava produced is used for human consumption and less than 5 percent is used in industries. As a food crop, cassava fits well into the farming systems of the smallholder farmers in Nigeria because it is available all year round, thus providing household food security. Compared to grains, cassava is more tolerant to low soil fertility and more resistant to drought, pests and diseases. Furthermore, its roots store well in the ground for months after maturity. Cassava is important, not just as a food crop but even more so as a major source of cash income for producing households. As a cash crop, cassava generates cash income for the largest number of households, in comparison with other staples, contributing positively to poverty alleviation. The presence of cyanogenic glycosides in cassava which when broken down through enzymatic reaction liberates hydrogen cyanide poses a great concern in cassava utilization as food and as industrial raw material.

With respect to Cyanide levels, cassava varieties are broadly divided into two groups; the sweet cassava known for low cyanide

content and the bitter cassava with its high characteristic content of Cyanogenic Glycosides (CGs) that is highly toxic when consumed [3-5]. Total cyanide in cassava products exists in form of CGs (linamarin and lotaustralin), cyanohydrin and free hydrocyanic acid (HCN). Notwithstanding the CGS, according to FAO, FAO, 2001 [6] 172 million tons of cassava were produced world-wide in 2000 with Africa accounting for 45%, Asia 28% and Latin America and the Caribbean 19%. The five main producing countries are Nigeria, Brazil, Thailand, Congo (DRC) and Indonesia.

The on-going challenge is to ensure that the presence of these cyanogenic glycosides is minimized through proper understanding and possibly control of factors that affect cyanogenic glycoside content of cassava. Roots and leaves contain the highest amount of linamarin [7].

Cyanide in Plants

The cyanogenic glycosides are a group of nitrile-containing plant secondary compounds that yield cyanide (cyanogenesis) following their enzymatic breakdown. The functions of cyanogenic glycosides remain to be determined in many plants; however, in some plants they have been implicated as herbivore deterrents and as transportable forms of reduced nitrogen [8-10]. It is

estimated that between 3,000 and 12,000 plant species produce and sequester cyanogenic glycosides. The major edible plants in which cyanogenic glycosides occur are almonds, sorghum, cassava, lima beans, stone fruits and bamboo shoots [11,12]. In certain sapindaceous seeds, HCN may arise during cyanolipid hydrolysis. More frequently, HCN production in higher plants results from the catabolism of cyanogenic glycosides. The approximately 75 documented cyanogenic glycosides are all O-β-glycosidic derivatives of ahydroxynitriles. Depending on their precursor amino acid, they may be aromatic, aliphatic, or cyclopentenoid in nature. Most are cyanogenic monosaccharides in which the unstable cyanohydrin moiety is stabilized by glycosidic linkage to a single sugar residue. Alternatively, in the cyanogenic disaccharides [e.g. (R)-amygdalin, (R)-vicianin, and linustatin] or trisaccharides (e.g. xeranthin), two or three sugar moieties, respectively, are involved in such stabilization. Sulfated, malonylated, and acylated derivatives of cyanogenic glycosides are also known. Cyanogenesis is not exclusive to those plant species accumulating cyanolipids and cyanogenic glycosides. All higher plants probably form low levels of HCN as a coproduct of ethylene biosynthesis [13]. This might explain why even ‘acyanogenic’ plants contain significant levels of the cyanide detoxifying enzyme β-cyanoalanine synthase. Cyanogenesis is also known in animals, but is restricted to the arthropods, notably to certain centipedes, millipedes, and insects. In fungi and bacteria, HCN may originate via oxidative decarboxylation of glycine.

A cyanogenic food of particular economic importance is cassava (*Manihot esculenta*), which is also known by the names manioc, yuca and tapioca. Cassava is by far the most important cyanogenic food crop for humans and is an important source of dietary energy in tropical regions. The predominant cyanoglycoside in cassava is linamarin. It is present in leaves and tubers, both of which are eaten. Linamarin is also present in beans of the lima or butter type. Amygdalin is the cyanogenic glycoside responsible for the toxicity of the seeds of many species of Rosaceae, such as bitter almonds, peaches and apricots. Sweet almonds are low in amygdalin as a result of breeding processes. Their use in marzipan is common but the preparation procedure should eliminate most of the cyanide. Cyanogen levels can vary widely with cultivar, climatic conditions, plant part and degree of processing. Typical levels for some plant materials consumed by humans are found in (Table 1) below:

Food	Major cyanogenic glycosides present	Cyanogen content (mg HCN/kg)
Cassava (<i>Manihot esculenta</i>) - root	Linamarin	15-1000
Sorghum (<i>Sorghum vulgare</i>) - leaves	Dhurrin	750-790

Flax (<i>Linum usitatissimum</i>) - seed meal	Linamarin, linustatin,	360-390
	Neolinustatin	
Lima beans (<i>Phaseolus lunatus</i>)		2000-3000
Giant taro (<i>Alocasia macrorrhizos</i>) - leaves	Triglochinin	29-32
Bamboo (<i>Bambusa arundinacea</i>) - young	Taxiphyllin	100-8000
Shoots		
Apple (<i>Malus spp.</i>) - Seed	Amygdalin	690-790
Peach (<i>Prunus persica</i>) - Kernel	Amygdalin	710-720
Apricot (<i>Prunus armeniaca</i>) - Kernel	Amygdalin	785-813
		89-2170
		2.2 (juice)
Plum (<i>Prunus spp.</i>) - Kernel	Amygdalin	696-764
Nectarine (<i>Prunus persica var nucipersica</i>) - Kernel	Amygdalin	196-209
Cherry (<i>Prunus spp.</i>)	Amygdalin	4.6 (juice)
Bitter almond (<i>Prunus dulcis</i>)	Amygdalin	4700
Cited from Haque and Bradbury, 2002 [14]; Simeonova and Fishbein, 2004 [15]		

Table 1: Levels of cyanogenic glycoside present in sampled plants.

In areas of the world where cyanogenic plants such as cassava and lima beans comprise the major item of the diet, chronic cyanide poisoning and associated pathological conditions still exist [16]. It is highly desirable that the toxicity of cyanogenic plants to humans and livestock be reduced. This is achievable by: (a) selective breeding to produce low-cyanogen varieties, as was accomplished for almonds, (b) screening of natural populations for low-cyanogen varieties, (c) mutagenesis of protoplasts or cell cultures with subsequent regeneration of plants having desired mutant genotypes, or (d) genetic engineering.

Role of Cyanogenic Glycosides in Plants

A common feature of cyanophoric plants is that cyanogenic glycoside hydrolysis occurs at a significant rate only after their tissues have been disrupted by herbivores, fungal attack, or

mechanical means. Although other explanations are possible, it is generally assumed that the glycosides and their catabolic enzymes are separated in the intact plant by compartmentation at either tissue or subcellular levels [17]. These possibilities have been extensively tested in a single organism, namely the leaves of 6-day old light-grown sorghum seedlings [18]. Somewhat unexpectedly, the authors demonstrated that the substrate and its catabolic enzymes were localized within different tissues. The cyanogenic glycoside dhurrin was sequestered in the vacuoles of epidermal cells, whereas the 3- glycosidase and hydroxynitrile lyase were present almost entirely in the underlying mesophyll cells. These two enzymes were located in the chloroplasts and cytosol, respectively. It therefore seems likely that the large-scale hydrolysis of dhurrin, which probably provides a defense mechanism against herbivores by liberating HCN, occurs only after tissue disruption allows the mixing of contents of different tissues.

Available evidence from other plant species, however, favors compartmentation of components of the ‘cyanide bomb’ at the subcellular level. In cassava, cells throughout the entire root cross-section possess both cyanogens (principally linamarin) and linamarase [19]. As in sorghum, highest glycoside levels are found in outer cell layers, again suggesting the involvement of cyanogens in defense against herbivores or pathogens, but the subcellular localizations of linamarin and linamarase remain unknown. In *Phaseolus lunatus*, the low recoveries of linamarin, linamarase, and hydroxynitrile lyase in leaf mesophyll protoplasts pointed to other tissues, perhaps the epidermis, as the principal site for these components [20]. Although these data cannot unequivocally distinguish between an epidermal or mesophyll location, it seems certain that the *P. lunatus* linamarase is apoplastic. Leaf discs hydrolyzed externally supplied linamarin, and about one-third of the total linamarase activity was extractable by multiple infiltrations of the leaves. The *T. repens* linamarase was detected by immunocytofluorescence in cell walls, especially those of the epidermis, and in the cuticle. More recently, protoplast isolation and tissue filtration experiments with *Hevea* endosperm showed that linamarin and the hydroxynitrile lyase were intracellular but that linamarase occurred both intra- and extracellularly. The apoplastic distribution of most linamarases contrasts with the intracellular location of sorghum dhurrinase, a fact perhaps related to the nonglycoprotein character of the latter [17].

The physiological importance of cyanogenic compounds in plant metabolism is currently receiving renewed interest. As with other secondary products, cyanogenics were originally viewed as excretory substances, but their turnover (seasonal and even diurnal) argues strongly against this hypothesis.

Given the well documented toxicity of HCN, a role in plant protection against herbivores, pathogens, and competitors is appealing. Much evidence, indeed, favors a defence function for cyanogenics against certain animals including insects [21].

Cyanide in Cassava

Of all cyanogenic crops, the most agronomically important, is the tropical root crop, cassava (*Manihot esculenta*, Crantz). More than 153 million tons of cassava is produced annually, and it is the major source of calories for many people living in the tropics, particularly sub-Saharan Africa [22].

Cassava leaves have higher protein content, contain vitamin C and vitamin A and provide some dietary fiber [23]. Much of the protein in the leaves is made up of linamarase, the enzyme that detoxifies the cyanogenic glycosides in cassava [24]. However, each parts of cassava plants (leaves, stem, root) contains high levels of cyanogenic glycosides; linamarin, lotaustralin, and amygdalin [25,26] (Figure 1), with linamarin been the most predominant cyanogen. Linamarin is rapidly hydrolyzed by linamarase to glucose, acetone cyanohydrin, and hydrogen cyanide.

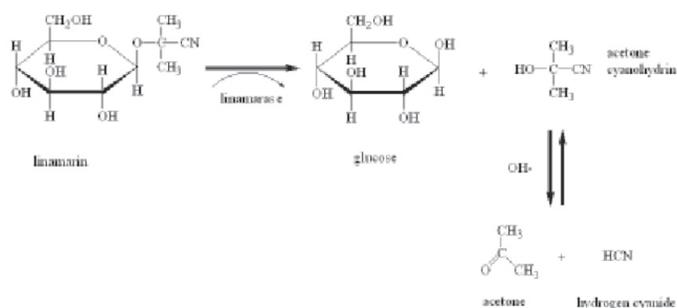


Figure 1: Hydrolysis of Linamarin to produce cyanide (Cited from Lykkesfeldt and Moller, 1994; Bolariwa et al., 2016).

Under neutral conditions, acetone cyanohydrin decomposes to acetone and hydrogen cyanide.

The cyanide level of cassava varies from about 75 to 350 ppm but can be up to 1000 ppm or more depending on the variety, plant age, soil condition, fertilizer application, weather, and other factors [27-29]. Studies have shown that the levels of cyanogenic glycosides in cassava roots are generally lower than that in the leaves and stems [30,31]. Cassava roots have been reported to contain cyanide content of 10-500 mg/kg of dry matter [32] and the leaves were reported to contain 53-1300 cyanide equivalents/kg of dry matter [33].

Cassava cultivars are classified as “bitter” or “sweet” depending on the level of cyanogenic glucoside (hence hydrogen cyanide). Values from 15-400 mg of hydrogen cyanide per kilogram of fresh weight of cassava roots have been reported for bitter varieties. Sweet varieties of cassava (low cyanide content) will typically contain approximately 15-50 mg hydrogen cyanide/kg fresh cassava. Sweet varieties of cassava can be processed adequately by peeling and roasting, baking or boiling, while bitter varieties of cassava (high cyanide content) require more extensive processing such as drying, fermentation etc. Bitter cassava varieties

are more drought resistant and thus more readily available and cheaper. However, owing to food shortage in times of drought, less time is available for the additional processing required for cassava products. Highly toxic hydrocyanic acid (HCN) is released from the cyanogenic glucosides during hydrolysis by the enzyme linamarase (present in the root peel of cassava).

The World Health Organisation (WHO) has set the safe level of cyanogens in cassava flour at 10 ppm or 10 mg HCN /kg, while in Indonesia the acceptable limit is 40 ppm [34-37]. Consumption of cassava and its products that contain large amounts of cyanogens may cause cyanide poisoning with symptoms of vomiting, nausea, dizziness, stomach pains, weakness, headache, exacerbates goitre and diarrhoea and occasionally death [37-47].

Although processing methods can reduce linamarin and cyanide in food, improperly processed cassava products would contain some amount of residual linamarin and hydrogen cyanide. This would result in the potential toxicity of the cassava products. Indeed, cases of cyanide toxicity from the consumption of inadequately processed cassava products have been reported [40].

Factors Affecting Cyanide Content of Cassava

Cultivar

Thousands of cassava cultivars have been developed that are adapted to local conditions and differ in their ability to tolerate pest and diseases, yield, nutritional and cooking qualities of food products. Cassava is propagated clonally from stem cuttings so there is minimal variation between individuals of one cultivar when grown under the same environmental conditions. All cassava cultivars contain cyanogenic glucosides, however, a wide variation in the concentration of cyanogens exists among different cultivars. This can range from 1 to 2,000 mg/kg [37]. Cultivars with 100mg/kg are called bitter [43]. A study in Fiji by [44] on 17 different cultivars grown in the same environmental confirmed the influence of cassava variety on levels of cyanogenic glucosides (and hence hydrogen cyanide) content. The 17 different cultivars had cyanide levels of 14 -121 mg/kg.

Climatic Conditions

Cassava, a perennial shrub thrives in tropical and sub-tropical conditions. In general, the crop requires a warm humid climate. Temperature is important, as all growth stops at about 10°C. Typically, the crop is grown in areas that are frost free the year round. The highest root production can be expected in the tropical lowlands, below 150 m altitude, where temperatures average 25-27°C, but some varieties grow at altitudes of up to 1500 m. The plant produces best when rainfall is fairly abundant, but it can be grown where annual rainfall is as low as 500 mm or where it is as high as 5,000 mm. The plant can stand prolonged periods of drought in which most other food crops would perish. This makes it valuable in regions where annual rainfall is low or

where seasonal distribution is irregular. In tropical climates the dry season has about the same effect on Cassava as low temperature has on deciduous perennials in other parts of the world. The period of dormancy lasts two to three months and growth resumes when the rains begin again. Cassava is drought resistant and grows well in poor soil (Java Cassava, 2007). The problem however is that cyanide content of cassava tends to increase during periods of droughts and or prolonged dry weather due to water stress on the plant [24]. For example, in Mozambique, about 55% of the sweet fresh roots were extremely toxic and the remainder moderately so during drought like conditions. Similar observations were recorded in Democratic Republic of Congo [45], and various citations in Africa. Splittstoesser and Tunya (1992) [46] reported that cassava grown in wet areas contain relatively lower amount of cyanide than those grown in drier areas.

Fertilizer

There is a general consensus that crop yields do increase with application of fertilizer, there is debate however on the relationship between addition of fertilizer and cyanide content of cassava. Studies in the Philippines [47] concluded that application of fertilizer does not significantly affect cyanide content. It further suggested that the amount of nutrient in the soil does not considerably contribute to the cyanogenic character of the cultivar. In Ethiopia, Endris (1977) [48] suggested that the cyanogenic content of cassava roots was significantly reduced by potassium application. In Nigeria, Okwu and Awurum (2001) [49] were able to prove that the value of HCN in the cassava samples decreases as fertilizer levels increases.

Health Implications of Cyanide

The toxicity of cyanogenic glycosides and their derivatives is dependent on the release of hydrogen cyanide. Toxicity may result in acute cyanide poisoning and has also been implicated in the etiology of several chronic diseases (FAO/WHO, 2012 [50]. Dietary exposure to elevated levels of some cyanogenic glycosides in food has the potential to cause acute cyanide poisoning or a debili- tating irreversible neurological condition in the long term.

High and sustained cyanogens intake at sub-lethal concentrations from cassava or cassava flour in combination with a low intake of sulfur amino acids has been reported to cause Konzo in women and children [5]. Konzo is an upper motor neuron disease characterized by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age [42].

Tropical Ataxic Neuropathy (TAN)

It is another health problem associated with continuous consumption of improperly processed cassava products. TAN is used to describe several neurological syndromes attributed to toxic nutritional causes. TAN has occurred mainly in Africa,

particularly Nigeria [52] and is common among people of 40 years and above (FSANZ, 2004). Dietary exposure to cyanide from the monotonous consumption of inadequately processed cassava products over years is responsible for the cause of the disease. Symptoms of TAN include sore tongue, optical atrophy, neuro sensory deafness, and sensory gait ataxia [52].

Goiter and Cretinism

They are common diseases in developing countries due to low intake of iodine (<100 µg/day). The disease is particularly common in Africa because of their over dependent on cassava as a staple food. Continuous exposure to dietary cyanide from insufficiently processed cassava products aggravate the disease [53] by the interferences of thiocyanate (the end products of cyanide detoxification in human system) with dietary iodine, thus leading to iodine deficiency According to reference Rosling, 1987 [53], populations with very low iodine intake and high thiocyanate levels from consumption of cassava, showed severe endemic goiter, which decreases with iodine supplementation. Study has shown that consumption of cyanogenic glycosides even at a very low concentration can also cause iodine deficiency leading to goiter [54].

Growth Retardation

It is a common health problem especially among children in developing countries. Exposure to cyanogenic glycosides has been a contributing factor to this health problem. Growth retardation is particularly a serious problem in populations consuming foods with inadequate proteins especially diets that are low in sulfur containing amino acids (methionine and cysteine). This is because detoxification of cyanide in human body requires sulfur donors from sulfur-containing amino acids. Thus, dietary exposure to cyanide is a contributing factor to growth retardation [55].

Cyanide Poisoning

Cyanide toxicity occurs when cytochrome oxidase a₃ inhibits the terminal enzyme in the respiratory chain and halts electron transport and oxidative phosphorylation (which is essential to the synthesis of Adenosine Triphosphate (ATP) and the continuation of cellular respiration) [56]. Cyanide poisoning occurs as a result of consumption of bitter cassava, almond kernels, or apricot kernels and their products without proper processing. Cases of cyanide poisoning after consumption of drink produced from the blends of apricot kernels and orange juice have been reported [57]. Clinical symptoms of cyanide toxicity are vomiting, nausea, dizziness, stomach pains, weakness, headache, diarrhea, and occasionally death [14,38,58-60].

Effect of Processing Cassava on Cyanide Content

Cyanide in cassava can be found as bound glucosides, cyanohydrins, and free cyanide [61]. Each of the 3 forms has different toxicity

and reacts differently to processing techniques that remove cyanide [62]. Thus, it is important to take into account the proportion of each cyanide form in the processed cassava. Many different processing techniques are used for cassava roots. Depending on the nature and duration of the processing methods, the residual level of cyanogens in cassava products will differ. Processing methods, such as peeling, drying, grinding, soaking, boiling or cooking, soaking and fermentation have been reported by several studies to cause significant reduction in the cyanogenic glycosides of processed foods. These processes can be applied to cassava roots to cause significant reduction in the cyanogen contents of the crops. Food-processing methods generally disintegrate cyanogens contents of plants, and this leads to the production of cyanide. Since cyanide is volatile, further processing techniques, such as roasting and drying, will volatilize the remaining cyanide to low level (Table 2).

PROCESS	RETENTION %	CYANOGEN GLYCOSIDE mg HCN/kg
Fresh Root	100	140
Boiling	55.5	77.6
Steaming	86.5	121
Frying	89.3	125
Baking	87.1	122
Changing size boiling (30 min)		
Fresh Root	100	160
2-g pieces	25.6	41
5-g pieces	50	80
50-g pieces	75	120
Changing water ratio boiling (30 min)		
Fresh root	100	165
Root: water (1: 1)	69.9	115
Root: water (1: 2)	36.7	60.5
Root: water (1: 5)	24.2	40.1
Root: water (1: 10)	22.3	36.8
Cited from Nambisan and Sundaresan (1985) [63]. Expressed as µg cyanide/g fresh weight in reference.		

Table 2: Effects of different processing methods and boiling technique variations on cyanogen glucoside content of cassava roots.

Boiling

Boiling is not an effective method for cyanide removal (50%). The inefficiency of this processing method is due to the high temperatures. At 100°C, linamarase, a heat-labile β-glucosidase, is denatured and linamarin cannot then be hydrolyzed into cyanohydrin. Cooke and Maduagwu (1978) [62] reported that

bound glucosides were reduced to 45% to 50% after 25 min of boiling. Free cyanide and cyanohydrin in boiled cassava roots are found at very low concentrations. Nambisan (1994) [64] reported a cyanohydrin and free cyanide content of 6% of the total cyanogens content in 50 g of boiled cassava roots, and only 3% in small pieces (2 g). Furthermore, Oke (1994) [65] reported that cyanohydrin and free cyanide were volatilized during boiling, which reduced the content in boiled cassava roots.

However, using small-sized cassava pieces or increasing the volume of water in which cassava roots are boiled can increase the efficiency of the boiling method (Table 2). For example by reducing cassava chip size, Nambisan and Sundaresan (1985) [63] demonstrated that boiling 2- and 50-g pieces of cassava root for 30 min resulted in a 75% and 25% reduction in cyanide content, respectively. Similarly, by increasing the volume of water from 1- to 5-fold, cyanogen retention was reduced from 70% to 24%. Oke (1994) [65] reported that the solubilization of cyanogenic glucosides from the small cassava chips into the large volume of water seemed to better explain the cyanogen removal than enzymatic degradation.

Steaming, Baking and Frying

The loss of cyanide resulting from steaming, baking, or frying is small (Table 1) due to processing temperatures of over 100°C and to the stability of linamarin in neutral or weak acid conditions [63,66]. These methods are only suitable for sweet cassava, common in the South Pacific, because they contain low cyanide content [67].

Drying

Two kinds of drying are used for cassava: mechanical drying, such as in an oven, and natural drying by the sun. In the drying process, endogenous linamarase controls the cyanogenic glucoside removal, and thus is responsible for cyanohydrin and free cyanide accumulation in dried cassava.

Generally, drying is not an efficient means of detoxification, especially for cassava varieties with high initial cyanogen glucoside content. In Tanzania, sun-drying whole roots into *makopa* reduced cyanide levels from 751 to 254 mg HCN equivalents/kg DW, that is, 66% of total cyanogens were removed [39]. Cyanogenic glucoside breakdown during sun-drying depends on enzymatic hydrolysis and on gradual root cell disintegration. Thinner cassava pieces dry faster, and at low moisture content levels (13%) linamarase is inactivated, and cyanogen glucoside break down ceases [39]. Cyanohydrin removal is increased with complete sun-drying. A possible explanation would be that dehydration of the roots and moisture losses results in pH changes, which affects cyanohydrin stability [39]. Because drying temperatures are above the boiling point of HCN (26°C) and free cyanide is easily released into the atmosphere, free cyanide can readily be removed [39].

Fermentation

Fermentation by lactic acid bacteria is a processing method commonly used in Africa. Fermentation is done with grated or soaked cassava roots and results in a decrease in pH value. The efficiency of the 2 kinds of fermentation differs due to the mechanisms of cyanogen removal. The microorganisms in the traditional fermentation process of grated roots have been characterized [68]. The fermentation of grated cassava roots is efficient at removing cyanogen glucosides. Westby and Choo (1994) [69] reported that 95% of linamarin was removed within 3 hours of grating. Vasconcelos and others (1990) [70] showed that microorganisms played only a small role in cyanogen reduction and that grating was mainly responsible for linamarin hydrolysis.

The process of roasting after fermentation of grated cassava, which is used for *gari*, is relatively efficient as free HCN and cyanohydrin are steadily removed into the atmosphere leaving little free HCN (3.4 mg/kg DW) and cyanohydrin (2.2 mg/kg DW) [70]. Cyanide content of *gari* further decreases during storage. Indeed, Mahungu and others (1987) [71] showed that a 4-month-old *gari* (2.9 mg HCN equivalents/kg) had a cyanogen content 9 times less than its initial content (26.6 mg HCN equivalents/kg), and after 2 y of storage, *gari* seemed to be a cyanogen-free product, that is, in 57 samples analyzed, no cyanogen could be detected. The fermentation of soaked roots in water is much more effective than that of grated roots in terms of cyanogen reduction. Indeed, more than 90% of total cyanogens were removed after 3 d of fermentation and about one-third of initial linamarin was found in the water. No significant accumulation of cyanohydrin or free cyanide was noted [69]. In this case, microbial growth is essential for removing cyanogens. The cyanogen removal process can be improved by increasing the soaking and fermentation times [65] and by peeling and grating cassava roots between the soaking and fermentation stages.

Other Processing Methods

Steam Distillation

Meuser and Smolnick (1980) [72] showed that steam distillation of fresh cassava pulp resulted in total cyanogen removal for a minimum distillate volume of 100 mL (assumed to be normalized to 1 kg). Steam distillation of fermented pulp slowly removed cyanogens. For a 100-mL distillate volume, only 65% of cyanogens were removed. A distillate volume \geq 550 mL was needed to remove about 90% of cyanogens. The resistance of fermented pulp to cyanogen removal can be explained by cyanohydrin stability at low pH values.

Starch Production

The process of starch extraction results in total cyanogen removal. Starch extraction involves different processing steps.

First, cassava roots are grated or rasped, and then starch is extracted with a large volume of water; residues are removed by sieving. In this way, a complete hydrolysis of cyanogenic glucosides occurs, and cyanohydrins, free cyanide, and the remaining cyanogenic glucosides solubilize in the supernatant water [63].

Combination of Several Processing Methods

To increase the efficiency of cyanogen removal, efficient processing techniques are usually combined with others that are less efficient. Sun-drying cassava roots usually retains about 25% to 33% of total cyanogens. Nambisan (1994) [63] reported that boiling thin pieces of cassava roots in water for 5 to 10 min prior to sun-drying removed only 50% of cyanogenic glucoside content. However, if these pieces are further boiled in water, 50% of the remaining glucoside can be removed. Furthermore, if cassava roots are soaked before sun-drying, cyanogen removal is greater (97.8% to 98.7%) [65]. Soaking fresh cassava roots for 3 days followed by 3 days of drying resulted in the removal of 85.9% of total cyanogens. The flour of *fufu* obtained at the end of the process had 2.2% retention of total cyanogens [65]. Similarly, crushing and then sun-drying cassava roots into flour allowed a total cyanogen removal of 96% to 99% [63]. This last combination of processing steps is effective. Indeed, *chinyanya*, which is obtained after pounding and sun-drying fresh cassava roots for 1 day, retains 22% of total cyanogens, while *makopa*, which is obtained after sun-drying whole cassava roots, retains 33% of total cyanogen [39]. In fact, crushing cassava roots damages the plant cells and, therefore, puts linamarase directly into contact with linamarin. Then, sun-drying reduces cyanohydrin and free cyanide to low levels [73].

The fermentation process can be improved. Soaking, fermenting, and then roasting cassava roots into *gari* or *farina* reduces total cyanogen content to 1.8 to 2.4% in the final product [65]. Soaking and fermenting cassava roots for 3 days allows a reduction of total cyanogen content to 5.7% [69]. Oke (1994) [65] reported that peeling and grating cassava before fermenting, and then sun-drying or oven-drying with moderate heat can result in a cyanogen-free product for any cassava variety that is used. Sun-drying for 6 hours instead of roasting for 10 min before fermenting for 2 days resulted in 22% less cyanogens.

Comparison of the different Processing Techniques

Methods involving grating and crushing are usually very efficient in cyanide removal because they completely rupture plant cells of cassava and allow direct contact between linamarase and linamarin [37,65]. However, sun-drying and heap fermentation are less efficient because peeled roots are usually cut in half longitudinally [37] and most of the plant cells remain intact. Hydrolysis of cyanogenic glucosides is prevented or reduced because linamarin and linamarase are located in different compartments of the plant cell. Heap fermentation retains half the cyanide of sun-drying because of the presence of microflora

that can break down the linamarin during the fermentation process [74]. Boiling, which is relatively inefficient for removing cyanide (50%), is much more efficient than baking, steaming, or frying (15% to 20% of cyanogen removal). Even if linamarase is inactivated at high temperatures (100°C), cyanogens are water-soluble and, therefore, they can be removed during the dewatering process as in (Figure 2) below.

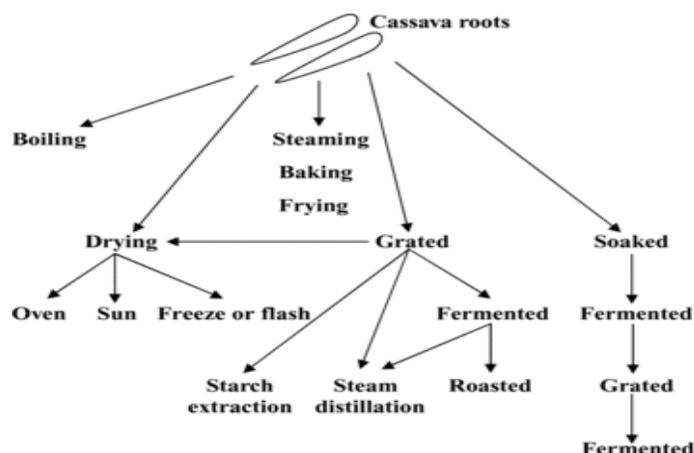


Figure 2: Cassava is processed in many different ways to be used in a variety of snacks and main dishes. Many times, the processing techniques are combined to either improve the end product or further reduce the amount of cyanide and/or phytate.

Processed Cassava Foods Found on the Market

Although efficient processing techniques to remove cyanide have been developed, many processed foods have a final cyanide content well above FAO/WHO's safe recommendation (1991) of 10 ppm. Oke (1994) [65] reported from work by cyanide content values of 0 to 32 ppm in 202 *gari* samples from all over Nigeria (with a mean value of 6 ppm). Similarly, Adindu and others (2003) [75] reported that *fufu*, *gari*, and *tapioca* contained cyanide contents up to 30 ppm on Nigerian markets, which is well above the safe value. These values are lower than the cyanide content of cassava flour in East Africa [37]. Indeed, Mlingi and Bainbridge (1994) reported values for cyanide contents of *makiopa* and *chinyanya* (sun-dried flours) to be above 130 ppm in Tanzania. The values vary by geographic location and are usually related to the amount of cyanide present in the raw product.

Cardoso and others (2005) [37] developed an equation to predict differences in the total cyanide content of cassava root parenchyma and the processed food. Using this equation, they showed that to have cassava root flours with less cyanide than the WHO safe level, the roots should have a maximum content of 12 to 16 ppm for sun-drying or 24 to 32 ppm if roots were to undergo heap fermentation. Sun-drying and heap fermentation remove 67% to 75% and 83.5% to 87.5% of total cyanogens, respectively. Cardoso and others (1998) reported that flour

produced by sun-drying and heap fermentation of cassava roots from markets in 3 areas of Mozambique contained 59 and 32 ppm cyanide, respectively, which is higher than what is recommended. In cassava roots with high cyanogen content, processes sometimes are not sufficient to reduce levels of cyanide to < 10 mg/kg. To produce safe cassava flour by sun-drying or heap fermentation, only sweet cassava roots with cyanide content < 32 ppm should be used [37], which are more common in the South Pacific than Africa (Bradbury and Holloway 1988).

During drought years, total cyanide content of cassava roots increases, exacerbating the situation because traditional processing techniques may not adequately reduce the cyanide content of the cassava. Cardoso and others (2005) reported that the percentage of flour samples exceeding cyanide content of 100 ppm increased in Mozambique from 6% to 43% to 65% during low-rainfall years because of water stress. It is noteworthy that low rainfall is a common feature of the African climate. Therefore, other strategies are needed to reduce daily cyanide intake. One of these strategies could be the introduction of low-cyanide varieties of cassava.

Safety Assessments

Cyanogenic glycosides were assessed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1993 (Speijers, 1993) [76], by Food Standards Australia New Zealand (FSANZ) in 2004 (FSANZ, 2004) [30] and by the International Programme on Chemical Safety (IPCS) in 2004 (Simeonova and Fishbein, 2004) [15]. None of these assessments established a safe level of exposure to cyanogenic glycosides, mainly due a lack of quantitative toxicological and epidemiological information.

Safety and Regulatory Limits

Safety limits are levels of dietary exposure that are without appreciable risk for a lifetime of exposure. Regulatory limits define the maximum amount of a substance that is permitted in a particular food (FSANZ, 2004) (Table 3).

Source	Limit Type	Limit
Safety Limits		
No safety limits have been set for cyanogenic glycosides		
Regulatory Limits		
Maximum Level Total hydrocyanic acid Content **:		
Australia, New Zealand Food Standards Code	Confectionery	25 mg/kg
	Stone fruit drinks	5 mg/kg
	Marzipan	50 mg/kg
	Alcoholic beverages	1 mg/kg per 1% alcohol

Table 3: Cyanide content approved regulatory limits by FSANZ.

Conclusion

Cyanogenic glycosides occur in cassava at variable levels. When cyanogenic glycosides undergo enzymatic hydrolysis hydrogen cyanide is liberated. Consumption of improperly processed cassava can lead to chronic and acute health problems as a result of cyanide poisoning. Understanding the appropriate processing methods for Cassava will help in reducing the problem of unintentional cyanide toxicity. Similarly, to prevent adverse effects of cyanogenic glycoside, consumers should prepare foods properly before consumption. This review presents the origin of hydrogen cyanide and its functions in cassava. Various processing methods and their effect on the levels of cyanide in cassava processed products were also reviewed.

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