Cyanogenic Potential in Food Crops and its Implication in Cassava (Manihot esculenta Crantz) Production.

Charles N. Fokunang, 1Paul T. Tomkins, 2Alfred G.O. Dixon, 3Estella A. Tembe, 1B. Salwa, 2E. N. Nukenine, 1Ian. Horan
1BioServ, Toxicology Unit, Athlone Institute of Technology, Athlone, County Westmeath, Republic of Ireland
2International Institute of Tropical Agriculture (IITA), Ibadan, PMB 5320, Ibadan, Nigeria
3Elan Pharmaceutical Technologies, Athlone, County Westmeath, Republic of Ireland

Abstract: Cyanide is a by-product from cyanogenic glycosides and is toxic to humans and most living organisms due to its ability of binding to metals such as iron, zinc and copper functional groups of the ligands of most bio enzymes. The cyanide inhibits the reduction of oxygen in the respiratory electron transfer system, the inhibition of plastocyanin reduction in photosynthesis and catalase activity. The magnitude of cyanide metabolism varies greatly between different plant species. Although most plant species produce small amounts of cyanide associated with ethylene production, between 3-12000 plant species produce sufficient amounts of cyanogenic compounds that they may function as translocatable forms of reduced nitrogen or as chemical defense molecules against pests and diseases. This paper discusses the cyanogenic potential (ability to produce hydrogen cyanide), in food crops, the importance of cassava as a cyanogenic food crop, cyanide toxicity, metabolism, the enzyme activities of linamarases (β-glucosidase), hydroxyl nitrile lyase, and cyanide detoxification processes.

Key words: Cyanogenic glycosides, cyanide toxicity, cassava, enzymatic activities, cyanide detoxification.

Introduction
Cyanogenic glycosides are groups of widely occurring natural substances that on hydrolysis yield a ketone or aldehyde, a sugar, and the highly toxic cyanide ion (Millner and Conn, 1980). The major food sources of cyanogenic glycosides include bitter almonds, cassava root, sorghum and lima beans (Shibamoto and Bjeldanes, 1993). Toxicity of cyanogenic glycosides is due to the liberation of hydrogen cyanide for the major food and feed sources (Table 1). Cyanide release from cyanogenic glycosides occurs readily in the laboratory by acid or base hydrolysis. Hydrogen cyanide is released from cyanogenic glycosides in chewed or chopped plants or following ingestion by an enzymatic process involving two enzymes (Bokanga et al., 1984).

Table 1: Food sources of Cyanogenic glycosides and amount of hydrogen cyanide (HCN) produced.

<table>
<thead>
<tr>
<th>Plant type</th>
<th>Amount of HCN</th>
<th>Glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bitter almonds</td>
<td>260</td>
<td>Amygdalin</td>
</tr>
<tr>
<td>Cassava root</td>
<td>52</td>
<td>Linamarin</td>
</tr>
<tr>
<td>Sorghum (whole plant)</td>
<td>260</td>
<td>Dihydrin</td>
</tr>
<tr>
<td>Lima beans</td>
<td>10-312</td>
<td>Linamarin</td>
</tr>
</tbody>
</table>

Source: Shibamoto and Bjeldanes (1993).

The first step in hydrogen cyanide release is the cleavage of sugar catalyzed by β-glucosidase, which yields a cyanohydrin and a sugar (Fig. 1). Most cyanohydrins are relatively unstable and spontaneously decompose to the corresponding ketones or aldehydes and hydrogen cyanide (Kakes, 1980). This decomposition is accelerated by the action of the enzyme, hydroxynitrile lyase. The cyanogenic glycosides and the enzymes necessary for release of hydrogen cyanide are all present separately in the plant (Fomumyam et al., 1984; Mc Mahon et al., 1996).

Cyanogenic Glucoside in Cassava (Manihot esculenta Crantz): Cassava is a widely grown root crop in most countries of the tropical regions of Africa, Latin America and Asia (Cock, 1986). It provides the daily food for more than 200 million Africans, nearly half of the continents population (Cock, 1986; CIAT, 1991). Cassava roots are an important insurance crop for subsistence of farmers throughout the tropics. They can remain in the soil for up to three years before harvesting and the presence of cyanogens protects them from herbivores and theft by vandals (Bellotti and Arias, 1993). Linamarin, the predominant cyanogenic glucoside in cassava, can accumulate to concentrations as high as 500-mg/kg fresh weight in roots (Sundaresan et al., 1987; White et al., 1994). The presence of cyanogens in poorly processed cassava food products can cause health problems for people that subsist on cassava-based diets (Tyllieskar et al., 1992). Research on the cyanogens of cassava has been focused largely on the biochemistry and physiology of linamarin synthesis and metabolism as linamarin accounts for 95% of the total cyanogenic glucosides present in intact tissues (Balagopalan et al., 1983). Linamarin is synthesized in young leaves and petioles and is accumulated in the vacuoles of the leaf cells. In addition, cyanogens can be exported from the leaves via phloem into roots (Fig. 2). Before transport, linamarin is glucosylated within the cells to linustatin, which is then transported via apoplasm (Selmar et al., 1988). In contrast to linamarin, this diglucoside is protected against cleavage by apoplastic β-glucosidases, and can pass the apoplasm via phloem loading without being hydrolyzed. Linustatin at the storage cells of the root is hydrolyzed by diglucosidases back to linamarin, which is then stored in the vacuoles in the storage cells in cassava roots.

Linamarase activity: The first step in the conversion of linamarin to cyanide is the deglycosylation of hydrolysis of linamarin by linamarase to form acetone cyanohydrin and glucose (Mc Mahon et al., 1996). As cyanohydrin may spontaneously or enzymatically decompose to cyanide and acetone, it has generally been assumed that linamarase activity is the rate-limiting step in cyanogenesis (Cooke et al., 1978), achieved a 350-fold increase in linamarase specific activity following fractionation of whole leaf extracts, but
Table 2: Kinetic properties of linamarase from different species.

<table>
<thead>
<tr>
<th>Plant Species</th>
<th>( V_{max} ) (nmol mg(^{-1}) protein h(^{-1}))</th>
<th>( K_m ) (mM)</th>
<th>Temperature Optimum °C</th>
<th>pH Optimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manihot esculenta</td>
<td>0.09</td>
<td>1.45</td>
<td>ND</td>
<td>6</td>
</tr>
<tr>
<td>(a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>3.02</td>
<td>0.97</td>
<td>ND</td>
<td>7.0</td>
</tr>
<tr>
<td>(Pl 4.3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Pl 3.3)</td>
<td>0.71</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(Pl 2.9)</td>
<td>0.39</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>Leaf 2.08*</td>
<td>2.08</td>
<td>SS</td>
<td>6.7-7.3</td>
</tr>
<tr>
<td>(b)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root peel</td>
<td>0.19</td>
<td>2.34</td>
<td>SS</td>
<td>6.7-7.3</td>
</tr>
<tr>
<td>Root cortex</td>
<td>0.16</td>
<td>3.93</td>
<td>SS</td>
<td>6.7-7.3</td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>(b) 1.2</td>
<td>1.2</td>
<td>SS</td>
<td>7.0</td>
</tr>
<tr>
<td>(d)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>(e) 7.6</td>
<td>7.6</td>
<td>SS</td>
<td>5.6</td>
</tr>
<tr>
<td>(f)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>(d) 5.55</td>
<td>5.55</td>
<td>ND</td>
<td>5.1-8</td>
</tr>
</tbody>
</table>

References for experimental details: (a) = Cooke et al., 1987 (partially purified); b = Eksitakil and Chulavatnako, 1986 (measured at 56°C); (c) = Yeah, 1988; (d) = Mloko et al., 1990; (a) = Selmar et al., 1995; (f) = Itoh-Nashida et al., 1997. ND = Not determined.

Fig. 1: Cyanogenesis from linamarin in cassava (Manihot esculenta Crantz). Source: McMahon et al. (1995).

Hydroxynitrile lyase activity: The final step in cyanogenesis of linamarin is the breakdown of acetone cyanohydrin to cyanide and acetone. This reaction can occur spontaneously at temperatures greater than 35°C or at pHs higher than 4.0. Acetone cyanohydrin can also be converted to cyanide and acetone by hydroxynitrile lyase (HNL) (White et al., 1984). Both the spontaneous and the enzyme-catalyzed decomposition of acetone cyanohydrin affect the cyanogens.

Fig. 2: Translocation of cyanogenic glucoside in cassava (Selmar et al., 1989).
Cyanide Toxicity: Hydrogen cyanide is a well-known poison with potential acute and chronic metabolic effects in humans (Kakes, 1990). Symptoms of acute poisoning include mental confusion, muscular paralysis, and respiratory distress (Kakes, 1990; Essers et al., 1992). The minimal lethal dose of hydrogen cyanide is estimated to be 0.5-3.5 mg/kg body weight (Shibamoto and Bjeldanes, 1993). Cyanide exerts its toxic effects by binding to the ferric ion of cytochrome oxidase, an enzyme that accounts for about 90% of the total oxygen uptake in most cells via the electron transport chain (Friedman, 1980). Inhibition of cytochrome oxidase thus virtually completely disrupts cellular oxygen utilization resulting in cytotoxic hypoxia, dysfunctioning and death (Friedman 1980; Abubakre et al., 1988; Grunnett et al., 1994). Cyanide poisoning also leads to reduction in photosynthesis and inhibition of catalase activity (Kakes, 1990; Mc Mahon et al., 1995). The overall effect is cessation of cellular respiration. Plants produce cyanide as a by-product of ethylene synthesis. Cyanide could also regulate development or seed germination in some plants and affect the alternate respiratory pathway (Essah et al., 1991). In addition, cyanide can be assimilated by the ubiquitous plant enzymes, ß-cyanoalanine synthase and rhodanase.

Consumption of cassava in certain parts of Africa and South America is associated with at least two disorders that do not
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seem to occur in areas where consumption is low or in individuals who consume cassava free of cyanide. A disorder known as tropical ataxic neuropathy (TAN) and characterized by optic atrophy, ataxia, and mental disorder is found in areas of West Africa where cassava is a staple of the diet (Akani et al., 1990; Osuntokun, 1981). Individuals with the disorder have very low concentrations of sulphur amino acids in the blood and elevated levels of plasma thioyanate (Bennett et al., 1987).

Neurological disease and more recently, some tropical variants of diabetes mellitus have also been attributed to cyanide exposure from cassava (Abubakare et al., 1986; Akani et al., 1980). Osuntokun (1980) demonstrated that cassava ingestion, together with iodine deficiency, is a factor in the etiology of endemic goitre and cretinism in Central Africa.

Other new severe human diseases called konzo (a paralytic disease) caused by cyanide toxicity has been identified in rural areas of East and Central Africa (Osuntokun, 1981; Howlett et al., 1990; Tyteskar et al., 1992). The clinical features of konzo are characterized by an abrupt onset of a permanent, symmetrical but non-progressive, spastic paraparesis (paralysis of both legs) in a previously healthy person. The disease usually attains epidemic levels in the dry season among very poor rural populations whose diets for weeks and months prior to onset consist almost exclusively of roots of bitter cassava (Howlett et al., 1990; Essers et al., 1992).

Cyanide Metabolism: The principal excretion product of cyanide is thiocyanate, the production of which is catalyzed by rhodanase, an enzyme that is widely occurring in most mammalian tissues (Casadei et al., 1980; Shimamoto and Bjaelde, 1993). Minor metabolic routes of cyanide involve reaction with cysteine to produce a thiosolane and an oxidant pathway leading to carbon dioxide and formate (Fig. 3). An additional minor metabolic pathway for cyanide is complexation with hydroxycobalamin. The magnitude of cyanogens and cyanide metabolism varies greatly between different plant species. Between 3:1:2000 plant species produce sufficient quantities of cyanogenic compounds that may function as translocatable forms of reduced nitrogen or as chemical defense molecules against herbivores (Kakes, 1990; Poultou, 1980).

Generally a survey of most cyanogenic plants have been shown to contain β-cyanolalanine synthase, presumably to metabolize the free cyanide (Mahangu et al., 1987). It has been demonstrated that all tissues have capability to metabolize the cyanide. This conclusion is based on the observation that all tissues have high levels of β-cyanolalanine synthase activity and incorporate free cyanide into β compounds (presumably by β-cyanolalanine synthase), and have rhodanase activity which catalyzes the formation of thiocyanate from CN⁻ and S2O3²⁻ (Poultou, 1980; Koch et al., 1982).

Cyanide Detoxification: A number of processing methods including chopping and grinding have been developed which effectively remove the major proportion of cyanogenic glucosides and their degradation products from cassava food products. In a stable society, the processing method constitutes an important part of the cultural heritage (Bokanga et al., 1993).

In practice, cassava root tubers (Fig. 4) are most often chopped and ground in running water (hydrolysis), a process that can remove both cyanogenic glucosides and any released hydrogen cyanide (Fomunyam et al., 1984). Dehydration by air drying at low temperature of 40°C (Meuser and Smidt, 1980), boiling, (Nambisan and Sundaresan, 1986), fermentation (Nvachukwvu and Edwards, 1987), and storage (Mahangu et al., 1987) have shown some significant decrease in cyanide content in cassava (Table 3). Cassava cultivars with a lower content of cyanogenic glucosides have been obtained through traditional plant breeding (Dixon et al., 1984). However, the breeding efforts have not yet resulted in a cassava variety in which the content of cyanogenic glucosides is so low that processing can be omitted. The major constraint so far to screen a large number of breeding lines for low cyanogenic potential has been the lack of simple, rapid and reliable analytical technique. A complimentary approach in the attempts to control and optimize the contents of cyanogenic glycosides in cassava is the use of molecular biology (Bokanga et al., 1992). Such an approach could be based on specific genes or their products, on the analysis of isoenzyme systems or on the use of DNA probes and restriction fragment length polymorphism (RFLP) markers. These techniques also provide the option to learn how to control and optimize the expression of gene product in different parts of a plant and to identify and modify the genetic elements, which are responsible for a varied production of cyanogenic glycosides independent of growth conditions (McMahon et al., 1986).

Genotype-Environmental interaction for low cyanogenic potential in cassava: Cassava is a widely adapted crop but individual genotypes have a narrow adaptation because of their high sensitivity to genotype-environmental (GxE) interaction (TRIP, 1993). Environmental factors during growing season contribute significantly to the variation in cyanogenic potential among genotypes, with a genotype, and in various parts of the plant (Dixon et al., 1984). Water stress effect on cyanogenic potential of tuberous roots have been reported (CIAT, 1981). The ability of cassava genotypes to maintain low cyanogenic potential under stress is important, especially in areas where fresh cassava is used for human consumption with a minimum processing such as boiling or sun-drying. The growth stage at which water stress occurs appears to have an effect on the building up of cyanogenic potential in plant; low levels of tuberous root cyanogenic potential coincide with the active root-bulking phase (Bokanga et al., 1994). Due to this differential sensitivity, manipulation of planting date appears to be useful in maintaining low cyanogenic potential.

Movement of cassava genotype from one location to another could alter the cyanogenic potential levels in tuberous roots because of differences in climate and soil characteristic (TRIP, 1993). Bokanga et al. (1994) showed a genotypic variation in cyanogenic potential at different planting locations in Nigeria (Table 4). Treatment of acute cyanide poisoning by administration of nitrite or nitrite esters such as amyl nitrite, which converts hemoglobin (Fe2⁺) to methemoglobin (Fe3⁺) has been reported (Akani et al., 1990). Increased circulating levels of methemoglobin will draw cyanide away from cytochrome oxidase, thus allowing cellular respiration to proceed. Final detoxification of the cyanide is facilitated by administration of thiosulphate required for formation of thiocyanate.

In conclusion, cyanogenesis is an important aspect of crop research that needs continuous evaluation, especially with the production of new hybrid cultivars by breeders, of cyanogenic crops. Studies on cyanogenesis and improvement of cyanogenic crops has contributed in the enhancement of sustainable food crop production in the Tropics, where over 200 million people living under subsistnet condition depend on cyanogenic crops like cassava, lima beans, for survival.

References

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