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

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Abstract: Cyanide a by-product from cyanogenic glucosides 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 amount 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 glucosides, cyanide toxicity, cassava, enzymatic activities, cyanide detoxification.

Introduction

Cyanogenic glucosides are groups of widely occurring natural substances that on hydrolysis yield a ketone or aldehyde, a sugar, and the highly toxic cyanide ion (Miller and Conn, 1980). The major food sources of cyanogenic glucoside include bitter almonds, cassava root, sorghum and lima beans (Shibamoto and Bjeldanes, 1993). Toxicity of cyanogenic glucosides is due to the liberation of hydrogen cyanide for the major food and feed sources (Table 1). Cyanide release from cyanogenic glucosides occurs readily in the laboratory by acid or base hydrolysis. Hydrogen cyanide is released from cyanogenic glucosides in chewed or chopped plants or following ingestion by an enzymatic process involving two enzymes (Bokanga *et al.*, 1994).

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

Plant type	Amount of HCN (mg/100g)	Glucoside
Bitter almonds	250	Amygdalin
Cassava root	53	Linamarin
Sorghum (whole plant)	250	Dhurrin
Lima beans	10-312	Linamarin

Source: Shibamoto and Bjeldanes (1993).

The first step in hydrogen cyanide release is the cleavage of sugar catalyzed by ß-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, 1990). This decomposition is accelerated by the action of the enzyme, hydroxynitrile lyase. The cyanogenic glucosides and the enzymes necessary for release of hydrogen cyanide are all present separately in the plant (Fomunyam *et al.*, 1984; Mc Mahon *et al.*, 1995).

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, 1985). It provides the daily food for more than 200 million Africans, nearly half of the continents population (Cock, 1985; 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 (Tyllesskar *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 glycosides present in intact tissues (Balagopalan *et al.*, 1988). 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 apoplast (Selmar *et al.*, 1988). In contrast to linamarin, this diglucoside is protected against cleavage by apoplastic β-glucosidases, and can pass the apoplasm during 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 of 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.*, 1995). 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.

Plant Species	V_{max} (mmol mg^{-1} protein h^{-1})	Km (mM)	Temperature Optimum °C	pH optimum
Manihot esculenta (a)	0.09	1.45	ND	6
Manihot esculenta (b)				
PI = 4.3	3.02	0.57	ND	7.0
PI = 3.3	0.71	ND	ND	ND
PI = 2.9	0.39	ND	ND	ND
Manihot esculenta©				
Leaf	0.54[a]	2.08	55	6-7.3
Root peel	0.18 [a]	2.34	55	6-7.3
Root cortex	0.01 [a]	3.93	55	6-7.3
Manihot esculenta (d)	29.4 [b]	1.9	55	7.0
Manihot esculenta (e)	11.6	7.6	62	5.6
Manihot esculenta (f)	14.6	5.56	ND	5.1-6

References for experimental details: (a) = Cooke *et al.*, 1987 (partially purified), b = Eksittikul and Chulavanatol, 1988 (measured at 55°C); (c) = Yeoh, 1988, (d) = Mkpong *et al.*, 1990; (e) = Selmar *et al.*, 1988; (f) = Itoh-Nashida *et al.*, 1987. ND = Not determined

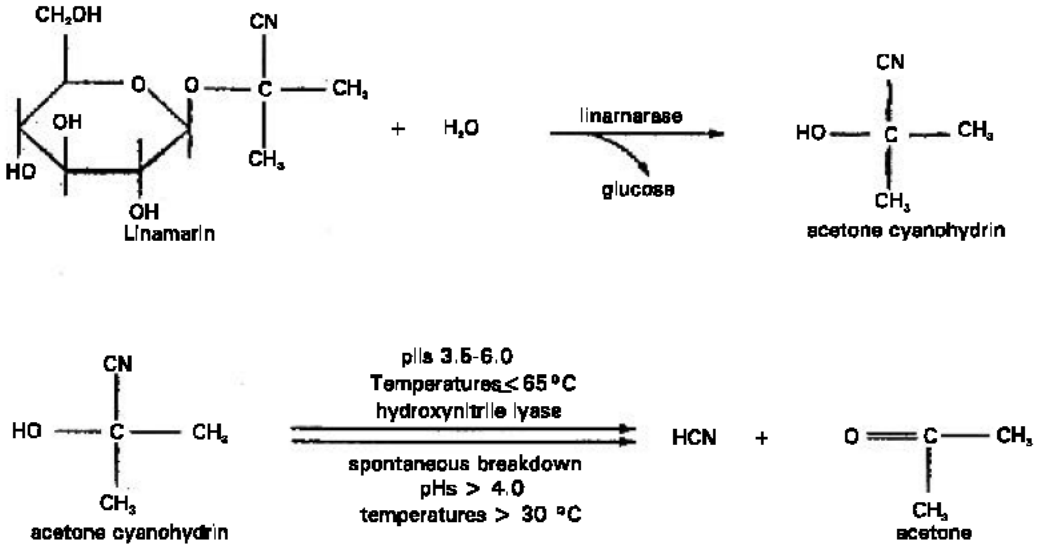

Fig. 1: Cyanogenesis from linamarin in cassava (*Manihot esculenta* Crantz). Source: McMahon *et al.* (1995).

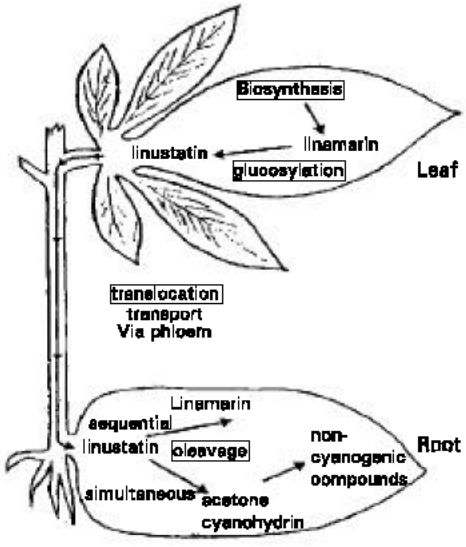

Fig. 2: Translocation of cyanogenic glucoside in cassava (Selmar *et al.*, 1988)

apparently did not achieve a homogenous preparation. Eksittikul and Chulavatnatol (1988), purified cassava linamarase to apparent homogeneity using crude extracts from various plant organs. Three different isoforms (63 kDa) of the enzyme, having isoelectric points (pIs) of 2.9, 3.3 and 4.3, were identified. (Table 2) and differ in their tissue specific localization. The pI = 4.3 isoform was the most abundant in petioles and stems, but equivalent amounts of all three forms were present in root tissue. The pH (6-7) and temperature (55°C) are optima for cassava linamarase activity. The high temperature optimum for cassava linamarase is similar to that of *Hevea* (62°C), which also belongs to *Euphorbiaceae*. Because of high temperature optimum, cassava linamarase has been shown to be extremely stable (McMahon *et al.*, 1995). This unusual stability and kinetic properties of cassava linamarase may be associated with post-translational modifications of proteins (Hughes *et al.*, 1994).

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.*, 1994). Both the spontaneous and the enzyme catalyzed decomposition of acetone cyanohydrin affect the cyanogens

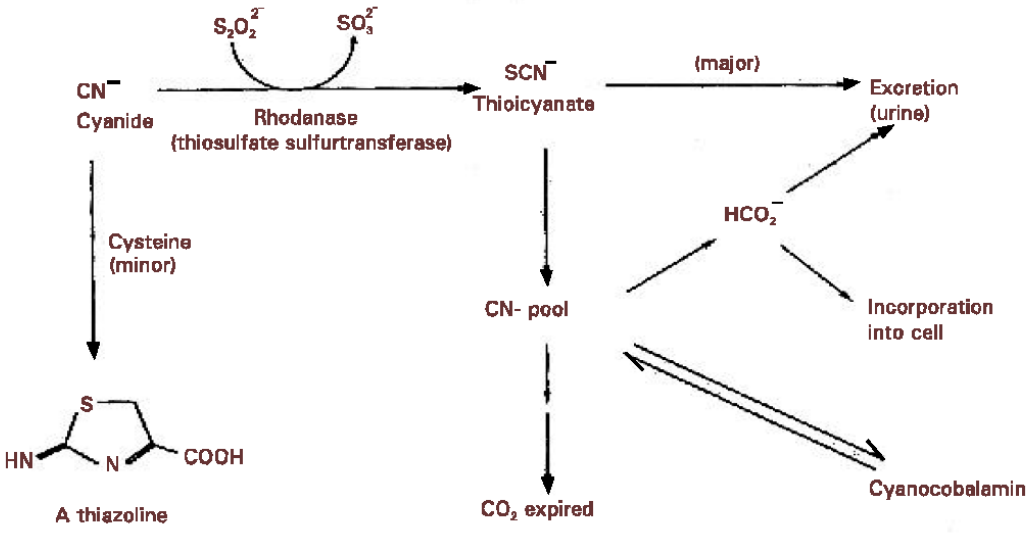

Fig. 3: Normal metabolism of cyanide (Shibamoto and Bjeldanes, 1993)

Fig. 4: Harvested cassava root tubers (a storage tissue for cyanogens)

content of cassava food products. Processing procedures that include incubation at elevated pHs (≥5.0) or a final heat treatment following linamarin hydrolysis facilitate the decomposition of acetone cyanohydrin and thereby reduce the toxicity of the food product (Mc Mahon *et al.*, 1995).

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

Table 3: Effect of different processing methods on cyanogens retention in cassava

Process	Cyanogen content (mg HCN equivalent kg^{-1})		
	Variety H-165	Variety H-2304	Variety H-1687
Fresh	140.0 ± +4.2	82.5 ±1.3	58.2±1.2
Boiling	77.6±1.6(55.5)	43.5±0.8(52.7)	30.7±0.5 (52.7
Baking	122.0±2.8(87.1)	70.1±1.3(85.0)	49.6±1.1 (85.2)
Frying	125.0±2.5(89.3)	75.2±1.3(91.2)	49.8±1.4 (85.3)
Steaming	121.0±2.5(86.5)	70.0±1.5(84.8)	47.5±1.6 (81.6)
Drying	99.2±1.8(70.8)	60.5±1.4(72.2)	43.5± (75.0)

Values are means of five-sample ± SEM; percentage retention of cyanogens is given in parentheses. Source: (Nambisan and Sundaresan, 1985).

Table 4: Mean cyanogenic potential of one cassava genotype, TME1, in 8 different locations in the 1990/91 and 1991/92 planting seasons.

Location	Cyanogenic potential (mg HCN equivalent kg^{-1})	
	1990/91	1991/92
Onne	15.7	55.2
Uyo	20.2	-
Mokwa	53.3	175.7
Ilorin	85.1	55.6
Ubiaja	80.4	98.4
Ibadan	-	50.2
Umudike	-	128.0
Zaria	-	88.1
Mean	50.9	93.0

Source: Bokanga *et al.*, 1994.

virtually completely disrupts cellular oxygen utilization resulting in cytotoxic hypoxia, disfunctioning and death (Friedman 1980; Abubakare *et al.*, 1986; Gruhnert *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 (Esashi *et al.*, 1981). 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

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 (Akanji *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 thiocyanate (Bennett *et al.*, 1987).

Neurological disease and more recently, some tropical variants of diabetes mellitus have also been attributed to cyanide exposure from cassava (Abu-Bakare *et al.*, 1986; Akanji *et al.*, 1990). 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; Tylleskar *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.*, 1990; Shibamoto and Bjeldanes, 1993). Minor metabolic routes of cyanide involve reaction with cysteine to produce a thiazoline and an oxidative pathway leading to carbon dioxide and formate (Fig. 3). An additional minor metabolic pathway for cyanide is complication with hydroxycobalamin. The magnitude of cyanogens and cyanide metabolism varies greatly between different plant species. Between 3-12000 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; Poulton, 1990).

Generally a survey of most cyanogenic plants have been shown to contain β-cyanoalanine synthase activity, presumably to metabolize the free cyanide (Mahungu *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-cyanoalanine synthase activity and incorporate free cyanide into C_4 compounds (presumably by β-cyanoalanine synthase), and have rhodanase activity which catalyzes the formation of thiocyanate from CN^- and $S_2O_3^{-2}$ (Poulton, 1990; Koch *et al.*, 1992).

Cyanide Detoxification: A number of processing methods including chopping and grindling 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 Smolnik, 1980), Boiling, (Nambisan and Sundaresan, 1985), fermentation (Nwachukwu and Edwards, 1987) and storage (Mahungu *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.*, 1994). 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 options 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.*, 1995).

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.*, 1994). Water stress effect on cyanogenic potential of tuberous roots have been reported (CIAT, 1991). 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 (Fe^{2+}) to methemoglobin (Fe^{3+}) has been reported (Akanji *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 subsistent condition depend on cyanogenic crops like cassava, lima beans, for survival.

References

Abu-Bakare, A., R. Taylor and K.G.M. Alberti, 1986. Tropical or malnutrition-related diabetes: a real syndrome Lancet, I: 1135-1138.

Akanji, A.O., I. Adeyefa, M. Charles-Davies and B.O. Osotimehin, 1990. Plasma glucose and thiocyanate response to different mixed cassava meals in non-diabetic Nigerians. Euro. J. Clinic. Nut., 44:71-77.

Bellotti, A.C. and B. Arias, 1993. The possible role of HCN on the biology and feeding behaviour of cassava burrowing bug (Cyrtomenus bergi Froeschhner). In: Roca WM, Thro AM, eds. Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network, Cartegena, Colombia, 25-28 August 1992. Cali, Colombia: Centro Int. Agric. Trop., 406-9.

Bennett, F., O. Morgan, M.H. Golden and M. Stennett-Dawkins, 1987. Plasma thiocyanate and free radicals in Jamaica neuropathy. West Ind. Med. J., 36: 163-165.

Bokanga, M., 1992. Constraints in Food and Nutrition Research. In. G, Thottappilly., L.M, Monti., D.R, Mohan., A,W, Raj., eds. Biotechnology: Enhancing Research on Tropical Crops in Africa. IITA p: 33-38.

Bokanga, M., I.J. Ekanayake, A.G.O. Dixon, 1994. Genotype-Environment Interactions for Cyanogenic potential in Cassava. Acta Horticulturae Cassava Safety 375.

Bokanga, M., B. Halkier and B. Moller, 1993. Studies on the biosynthesis of cyanogenic glucosides in cassava. In: Roca, W.M., Thro, A.M., Eds. Proceedings of the First Scientific Meeting of the Cassava Biotechnology Network, Cartegena, Colombia, 25-28 August 1992. Cali, Colombia: Centro Int. Agric. Trop. (CIAT), 418-23.

Casadei, E., J. Cliff and J. Neves, 1990. Surveillance of urinary thiocyanate concentration after epidemic spastic paraparesis in Mozambique. J. Trop. Med. Hyg., 93: 257-261.

CIAT., 1991. CIAT Cassava Program Annual Report 1987-1991. CIAT, Cali, Colombia.

Cock, J.H., 1985. Cassava: New Potential for a neglected crop. London: Westfield Press. pp: 191.

Cooke, R.D., G.B. Blake and J.M. Battershill, 1987. Purification of cassava linamarase. Phytochem., 17: 381-383.

Dixon, A.G.O., R. Asiedu and M. Bokanga, 1994. Breeding of cassava for low cyanogenic potential: Problems, Progress and Prospect. Acta Horticulturae 375, 1994. Cassava Safety.

Eksittikul, T. and M. Chulavatnatol, 1988. Characterization of cyanogenic ß-glucosidase (linamarase) from cassava (*Manihot esculenta* Crantz). Archives of Biochem. Biophy., 266: 263- 269.

Esashi, Y., Y. Sakai and R.Ushizawa, 1981. Cyanide-sensitive and cyanide resistant respiration in the germination of cocklebur seeds. Pl. physiol., 84: 1296-1300.

Essers, A.J., P. Alsen and H. Rosling, 1992. Insufficient processing of cassava induced acute intoxications and the paralytic disease, konzo in a rural area of Mozambique. Ecol. of Food and Nut., 27: 17-27.

Fomunyam, R.T., A.A. Adegbola and O.L. Oke, 1984. Hydrolysis of linamarin by intestinal bacteria. Can. J. Microbiol., 30: 1530-1535.

Friedman, P.A., 1980. Common poisons. In Harrison's Principles of Internal Medicine, 9th edition. Ed. Isselbacher, K.J., Adams, R.D., Braunwald, B., Petersdorf, R.G. and Wilson, J.D. McGraw-Hill International Student Edition, London, pp: 953-965.

Gruhnert, C.H., B. Bichl and D. Selmar, 1994. Compartmentalization of cyanogenic glucosides and their degrading enzymes. Planta, 195-36-42.

Howlett, W.P., G.R. Brubaker, N. Mlingi and H. Rosling, 1990. Konzo an upper motor neurone disease studied in Tanzania. Brain, 113: 223-235.

Hughes, J., F.J.P.Carvalho, C. De and M.A. Hughes, 1994. Purification, characterization, and cloning of xhydroxylnitrile lyase from cassava (Manihot esculenta Crantz). Archives of Biochem. Biophy., 311, 496-502.

Itoh-Nashida, T., M. Hiraiwa and Y. Uda, 1987. Purification and properties of B-D-glucosidase (linamarase) from the butter bean, Phaseolus lunata. J. Biochem., 101: 847-54.

Kakes, P., 1990. Properties and functions of cyanogenic system in higher plants. Euphytica, 48, 25-43.

Koch, B., V.S. Nielsen, B.A. Halkier, C.E. Olsen and B.L. Moller, 1992. The biosynthesis of cyanogenic glycosides in seedlings of cassava (Manihot esculenta Crantz). Archives of Biochem. Biophy., 292: 141-150.

Mahungu, N.M., Y. Yamaguchi, A.M. Alamazan and S.K. Hahn, 1987. Production of cyanide during processing of cassava into some traditional African foods. J. Food and Agric., 1:11-15.

McMahon, J.M., L.B.W. Wanda and R.T. Sayre, 1995. Cyanogenesis in Cassava (*Manihot esculenta* Crantz). J. Exp. Bot., 46: 731-741.

Meuser, F. and H.D. Smolnik, 1980. Processing of cassava to gari and other foodstuffs. Starch/Starke, 32: 116-122.

Miller, J.M. and E.E. Conn, 1980. Metabolism of hydrogen cyanide by higher plants. Pl. Physiol., 65: 1199-1202.

Mpong, O.E., H. Yan, G. Chism and R.T. Sayre, 1990. Purification, characterization, and localization of linamarase in cassava. Pl. Physiol., 93: 176-81.

Nambisan, B. and S. Sundaresan, 1985. Effect of processing on the cyanoglucoside content of cassava. J. Sci. of Food and Agric., 36: 1197-1203.

Nwachukwu, S.U., A.W.A. Edwards, 1987. Microorganisms associated with cassava fermentation for lafun. J. Food Agric., 1:39-42.

Osuntokun, B.O., 1981. Cassava diet, chronic cyanide intoxication and neuropathy in Nigerian Africans. World Review of and Diebetics, 36: 141-173.

Poulton, J.E., 1990. Cyanogenesis in plants. Pl. Physiol., 94: 401-405.

Rao, P.V. and S.K. Hahn, 1984. An automated enzymatic assay for determinating the cyanide content of cassava (*Manihot esculenta* Crantz) and cassava products. J. Sci. Food and Agric., 35: 426-436.

Selmar, D., R. Lieberei, B. Biehl and J. Voigt, 1988. Hevea linamarase a non-specific β-glycosidase. Pl. Physiol., 86: 711-16.

Shibamoto, S. and L.F. Bjeldanes, 1993. Introduction to Food Toxicology Academic Press Inc, New York, London, Toronto, pp: 213.

Sundaresan, S., B. Nambisan and C.S.E. Amma, 1987. Bitterness in cassava in relation to cyanogenic glucoside content. Ind. J. Agric. Sci., 57: 37-40.

TRIP., 1993. Root and Tuber Crops Improvement Program. Archival Report (1989-1993). Part 1. Cassava breeding, cytogenetics and histology. Volume 2. Germplasm enhancement. IITA, Ibadan, Nigeria.

Tylleskar, T., R.D. Cooke, M. Banea, N.H. Poulter, N. Bikangi and H. Rosling, 1992. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. Lancet, 339: 208-11.

White, W.L.B., J.M. McMahon and R.T. Sayre, 1994. Regulation of cyanogenesis in cassava. Acta Horticulturae, 375: 69-78.

Yeoh, H.H., 1988. Kinetic properties of ß-glucosidase from cassava. Phytochem., 28: 271-4.