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Titrimetric Evaluation of Cyanogens in Parts of Some Nigerian Cassava Species

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Abstract: In this study, Tropical Manihot Selection TMS 30572 fresh cassava samples from Kubwa (in Abuja) and Agbara (in Ogun State) of Nigeria were evaluated for cyanogens using titrimetric method. The variations in the cyanide concentrations from different parts of the plant were significant at $p<0.05$ and of the order leave> peel> tuber (flesh)> stem. The average cyanide contents, respectively for the Abuja and Agbara samples ranged from 32.85±1.55 ppm to 36.17±11.80 ppm for stems; 50.24±0.18 ppm to 74.38±0.41 ppm for tubers; 74.38±0.41 ppm to 84.96±1.70 ppm for peels and 105.21±0.95 ppm to 118.00±13.70 ppm for leaves.

Key words: Cassava parts, cyanogens, Nigeria

INTRODUCTION

Cassava *(Manihot esculenta*) and (*Manihot utillisima phol*) are shrub perennial plants of the *Euphorbia ceae* (spurge family) also known as *yoca*, *manioc* and *mandioca*. They are characterized by palmate lobed leaves, inconspicuous flowers and a large, starchy, tuberous root with a tough papery brown bark and white to yellow flesh (New World Encyclopedia, 2008). It originated from South America and now is extensively cultivated as an annual crop in many tropical and subtropical regions of the world including Africa and Asia, with Africa as its largest center of production (New World Encyclopedia, 2008; RMRDC, 2004). Cassava is a prolific crop that can grow in poor soil and even strive well in dry season. It is one of the most important food plants in the tropics and the third largest source of carbohydrates for human food in the world (New World Encyclopedia, 2008; O'Hair, 1995).

Cassava is a major agro-raw material (RMRDC, 2004) that contains significant amount of iron, phosphorus, calcium and vitamin C (O' Hair, 1995), but is a poor source of protein as reliance on cassava as a staple food is associated with kwashiorkor (New World Encyclopedia, 2008).

The different cultivars or varieties of cassava can be distinguished by such features as size, colour and shape of the leaf, stem and petiole, branching habit, plant height, tuber and amount of the root tuber produced per plant, the nutritive content of the tubers, the resistance to certain diseases and weeds, the climatic and nutrient requirements such as fertilizers for maximum yield of the plants and "sweet" or "bitter", depending on the level of cyanide content (Nweke *et al*., 1999; RMRDC, 2004). In Nigeria research efforts are on going to develop new cassava varieties and some of the best-known improved varieties are Tropical Manihot Selection (TMS) 30572, TMS 30211, NR 8082, TMS 30555 and NR 8083 (NRCRI, 1982-1997; IITA, 1976-1996). These improved varieties yield between 25-40 tonnes/ha, are resistant to pests and diseases and have acceptable culinary and industrial qualities (Breekelbaum *et al*., 1978; NRCRI, 1982-1997; IITA, 1976-1996). In comparison, local varieties yield between 5-10 tonnes/ha and are very susceptible to pests and diseases. A total of 1,200 local cassava varieties have been identified in Africa and with a higher genetic diversity for the sweet than for the bitter varieties (Asadu *et al*., 1999). Also that farmers in Cote D'Ivoire, Ghana and Uganda planted more of the sweet varieties, while bitter varieties were more popular in the Congo, Nigeria and Tanzania. And within each country, the distribution of the area planted varied by agro-ecological zone. Farmers in the Nigerian forest zone planted the bitter varieties because they have the processing technology to convert the roots into garri, which does not require sun-drying (Nweke *et al*., 1999).

The cyanogenic glycosides, linamarin and lotaustralin (occurring in the ratio 93:7) are distributed throughout the cassava plant. The cyanogenic glycosides, which offer a protection against some herbivores, also make the plant toxic to humans, if consumed without prior treatment such as leaching, drying, fermentation, boiling, ensiling and grating (Vasconcelos *et al*., 1990). The levels of the cyanogens and the activity of linamarase vary considerably between tissues (De Bruijn, 1971; Nambisan and Sundaresan, 1991). These cyanogens sometimes produce acute intoxication leading to death, exacerbate goitre and cretinism in iodine-deficient regions, cause konzo and are implicated in the occurrence of Tropical Ataxic Neuropathy (TAN) (Onabolu *et al*., 2002; Oluwole *et al*., 2003) and stunting of children (Stephenson *et al*., 2010). In Nigeria, cassava is a staple

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food and about 84% of the productions are used for food. Endemic ataxic polyneuropathy was first described from some communities in south-western Nigeria in the mid-1950s (Money and Smith, 1955; Money, 1958). Although most Nigerians who consume cassava do not develop the disease because of the way the cassava is prepared (Asadu *et al*., 1999), variation in biological susceptibility among individuals and variation in consumption of other food and supplements, especially animal protein (Nweke *et al*., 1999). Recent epidemiological studies have shown that occurrence of ataxic polyneuropathy persists in the endemic area in south-western Nigeria and may result in high mortality rate (Oluwole *et al*., 2000, 2003; Oluwole and Onabolu, 2004).

A statistical evaluation and comparison of the cyanogen levels in different parts of some cassava species grown in Kubwa in Abuja (North Central Nigeria) and Agbara in Ondo State (South West Nigeria) is the purpose of this research.

MATERIALS AND METHODS

Sample collection and preparation: Eight different fresh samples of TMS 30572 cassava species (locally called "Oko Iyawo" in the Yoruba language) were collected. The plant leaves, stems, tubers and peels were collected from local farmers in Bwazin in Kubwa-Abuja (North Central Nigeria) and Agbara in Ogun State (Southern Nigeria). 40 g each of the leaves, stems, tubers and peels was mashed and weighed for analysis. The prepared samples were stored at -4°C till further use.

Determination of cyanide in cassava: Twenty grams of cassava sample (leave, tuber, stem or peel) was weighed and soaked in 20 ml of distilled water in separate 500 ml round bottom flask. 200 ml of phosphate buffer (pH 6.0) and 10 ml of 2% mercuric chloride solution were added to each sample and left overnight. Samples were prepared in duplicate. After 24 h, 5.0 g of hydrated stannous chloride ($SnCl_2$. $2H_2O$) was added into the soaked mixture just before each flask was fixed to steam distillation apparatus to release the hydrocyanic acid (HCN). Thereafter, the mixtures in the flask were heated by steam distillation and the cyanide released was collected in a conical flask containing 50 ml of 1% alcoholic NaOH solution until the volume of the distillate was 200 ml. The distillate was then titrated against 0.02M $AgNO_3$ and 1 ml of freshly prepared 0.5% w/v dithrozone in ethanol as an indicator to determine the amount of CN^- in the samples (Equation 2). At the end point of titration, the colour of the indicator changed from red to purple (FIIRO, 2000-2004).

Using the relationship 1 ml of 0.02M $AgNO_3$ = 0.52 mg CN^-:

$$\text{Concentration of } CN^- = \frac{\text{Titre value x 0.52 x 1000 (ppm)}}{\text{Weight of sample taken}} \quad (1)$$

Chemical equation of the reaction:

$$HCN(aq) + AgNO_3(aq) \rightarrow HNO_3(aq) + AgCN(aq) \quad (2)$$

Statistical analysis: The data of the fresh cassava species from the two different localities (Abuja and Agbara) were subjected to the non-parametric Kruskal-Wallis analysis. Significance was accepted at 95% probability level. Values were reported as the mean±SD for three determinations.

RESULTS AND DISCUSSION

Table 1 showed that the mean cyanide concentrations in different parts of cassava samples from Abuja in North-Central Nigeria ranged from 32.85±1.55 ppm (for stem) to 105.21±0.95 ppm (for leave). There was a significant difference ($p<0.05$) between the total cyanide concentrations in the different parts of the plant, as the concentration of cyanide in the leaves was three fold higher than in the stem. Similarly, the cyanide concentration in the peels was half of that in leaves. The cyanide concentration in this cassava specie was in the range leave> peel> tuber (flesh)> stem.

The mean cyanide concentration in the different parts of cassava from Agbara in Ogun State of South West of Nigeria is shown in Table 2. In these samples, cyanide concentration was lowest in stems (36.17±11.80 ppm) but highest in leaves (118.04±13.70 ppm). There was a significant difference ($p<0.05$) between the concentrations obtained from the different parts of the plant. Again, the cyanide concentration was of the order stem < tuber (flesh) < peel < leave.

The results of this study (Table 1 and 2) showed higher cyanide concentration in leaves compared to other parts of the cassava samples and agrees with the findings of other researches (Nambisan and Sundaresan, 1985). The cyanide ion (CN^-) inhibits the enzyme cytochrome c oxidase (also known as aa_3) in the fourth complex of electron transport chain and prevents the cells from aerobic production of ATP for energy (Banea-Mayambu *et al*., 1997). In cassava the cyanogenic glucoside (linamarin) is present in large amounts in the leaves and the peel of the roots (900-2000 mg HCN/kg) (Cardoso *et al*., 2005). The leaves also contain the enzyme hydroxynitrile lyase, which catalysis the hydrolysis of acetone cyanohydrin to produce HCN and acetone (Siritunga *et al*., 2004). The peel part of the cassava tuber is the outermost layer part that is mostly exposed to different biochemical nutrient in the soil. As a result, exposure to hyrdocynide in the soil could lead to increase proportion of cyanide in part of the plant. The peels and stem of cassava are not totally exposed to sun which can lead to cyanide depletion. The profile of cyanide concentration in this study showed that tubers are lower cyanide storage part of cassava than peels and leaves and explains the reason for the higher consumption of this part by man and animals and the

Table 1: Cyanide concentration of cassava samples from Kubwa in Abuja (in ppm)

Sample	Stem	Tuber	Peel	Leave
TMS 30572 A_1	33.710	50.370	74.210	104.740
TMS 30572 B_1	30.520	50.220	74.100	106.620
TMS 30572 C_1	33.660	49.990	75.000	104.920
TMS 30572 D_1	33.520	50.370	74.220	104.540
Mean±SD	32.85±1.55	50.24±0.18	74.38±0.41	105.21±0.95

Where A, B, C and D = Farmlands; 1 = Sample location; SD = Standard Deviation

Table 2: Cyanide concentration of cassava samples from Agbara in Ogun State (in ppm)

Sample	Stem	Tuber	Peels	Leaves
TMS 30572 A_2	41.31	74.21	83.05	104.74
TMS 30572 B_2	34.36	74.10	84.32	115.85
TMS 30572 C_2	48.34	75.00	87.06	114.35
TMS 30572 D_2	20.68	74.22	85.41	137.21
Mean±SD	36.17±11.80	74.38±0.41	84.96±1.70	118.04±13.70

Where A, B, C and D = Farmlands; 2 = Sample location; SD = Standard Deviation

Table 3: Comparison of average cyanide content between cassava samples from Kubwa (Abuja) and Agbara (Ogun State) of Nigeria

Sample	Abuja (ppm)	Agbara (ppm)
Stems	32.85±1.55	36.17±11.80
Range	30.52-33.70	20.68-48.34
Tubers	50.24±0.18	74.38±0.41
Range	49.99-50.37	74.21-75.00
Peels	74.38±0.41	84.96±1.70
Range	74.10-75.00	83.05-87.06
Leaves	105.21±0.95	118.00±13.70
Range	104.74-106.62	104.74-137.24
Kruskal-Wallis value ($H_{\chi 1, 0.05}$) = 0.52*		

Values are mean±standard deviation; *Significant

cyanide level is furthermore reduced significantly during the processing, to accepted level of 10 ppm by WHO (Anonymous, 1991) or 2 to 3 mgHCN/100 g by IITA (1989). As one dose of pure cassava cyanogenic glycoside (40 mg) is sufficient to kill a cow (White *et al*., 1994). The cyanide content of processed cassava products from Nigeria have been reported by Okafor (2004) to range from 0.14±0.03 to 0.82±0.32 on dry matter basis. The south-west of Nigeria is one of the highest producers and consumers of processed cassava in the form of "fufu" and "garri". During the various stages of processing, 80 to 95% cyanide loss occurs (Vasconcelos *et al*., 1990; Cardoso *et al*., 2005). The study by Onabolu *et al*. (2002) has shown that there is loss of cyanohydrin and linamarin in garri during short-term storage and when garri is made into eba, which reduces dietary cyanide load in consumers. However, frequent intake of garri could lead to high cyanide accumulation in plasma (Oluwole *et al*., 2002). A comparison of the cyanide concentrations of the same cassava specie from the two different locations showed higher cyanide content in the Agbara samples (Table 3). Statistically ($p<0.05$), the cyanide concentrations of the different parts of cassava samples from Agbara in south-west Nigeria were higher than the Abuja samples and ranged from 20.68-137.24 ppm. Agbara is an industrial city and the high cassava cyanide content from this area may be due to environmental factors or industrialization. Not only does the cyanogenic glucoside content vary within each individual plant, its age, variety, the geographical locations and other factors like soil, fertilization and climate also contribute to the quantities of cyanogenic glucoside in the plants (Nartey, 1977). It has been reported that growth and nutritive values of cassava are reduced when grown in areas with high emission of carbon monoxide that may increase the cyanide content of the plant (Gleadow *et al*., 2009). Addition of potassium to soil has been reported to reduce the HCN content of cassava roots. Other locally available and cheap sources of potassium such as wood ash can alternatively be used by subsistent farmers who usually cultivate the crop (Endris, 2009).

Conclusion: In this titrimetric method of analysis of cyanogens in some cassava samples, the cyanide content of leaves and peels were higher than the other parts of the plant. Furthermore, the cyanide concentrations of the different parts of cassava samples from Agbara in Ogun State were significantly higher than the Abuja samples.

REFERENCES

Anonymous, 1991. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission XII, Supplement 4, FAO, Rome.

Asadu, C.L., A. Felix and I. Nweke, 1999. Soils of Arable Crop Fields in Sub-Saharan Africa: Focus on Cassava Growing Areas. Collaborative Study of Cassava in Africa (COSCA) working Paper No.18. Ibadan, Nigeria: International Institute of Tropical Agriculture.

Banea-Mayambu, J.P., T. Tylleskar, N. Gitebo, N. Matadi, Gebre-Medhin and H. Rosling, 1997. Geographical and regional association between linamarin and cyanide exposure from cassava and the upper motor neuron disease konzo in former Zaire. Trop. Med. Int. Health, 2: 1143-1151.

Breekelbaum, T., A. Bellotti and J.C. Lozano, 1978. Cassava Protection Workshop. CIAT Cali, Columbia.

Cardoso, A.P., E. Mirione, M. Ernesto, F. Massaza, J. Cliff and M.R. Haque, 2005. Processing of cassava roots to remove cyanogens. J. Food Comp. Anal., 18: 451-460.

De Bruijn, G.H., 1971. A study of the cyanogenic character of cassava. Mededelingen Landbouwhoge Wageningen, 71: 1-40.

Endris, S., 2009. Cyanogenic Potential of Cassava Cultivars Grown under Varying Levels of Potassium Nutrition in Southwestern Ethiopia. Ethiopian Institute of Agricultural Research (EIAR), Jimma Center.

Federal Institute for Industrial Research, Oshodi, FIIRO, 2000-2004. Annual Reports. Lagos, Nigeria.

Gleadow, R.M., J.R. Evans, S. McCaffery and T.R. Cavagnaro, 2009. Growth and nutritive value of cassava (*Manihot esculenta* Cranz.) are reduced when grown in elevated CO. J. Plant Biol., 1: 76-82.

International Institute of Tropical Agriculture, IITA, 1976-1996. Annual Reports. Ibadan, Nigeria.

International Institute of Tropical Agriculture, IITA, 1989. Cassava Processing and Utilization. Ibadan, Nigeria, 28: 16-18.

Money, G.L., 1958. Endemic neuropathies in the Epe District of Southern Nigeria. West Afr. Med. J., 7: 58-62.

Money, G.L. and A.S. Smith, 1955. Nutritional spinal ataxia. West Afr. Med. J., 4: 117-123.

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

Nambisan, B. and S. Sundaresan, 1991. Cyanoglycosides in cassava. Tech. Bull. Cental Tuber Crop Res. Inst., 12: 15.

Nartey, F., 1977. *Manihot Esculenta* (Cassava): Cyanogenesis Ultra-Structive and Seed Germination. Ph.D Thesis, University of Copenhagen, Munkshaard, Copenhagen, Demark.

New World Encyclopedia, 2008. Cassava. (http://www.newworldencyclopedia.org/entry/special:cite?page=cassava (Date last accessed October 23, 2010).

National Root Crops Research Institute, NRCRI, 1982-1997. Annual Reports. Umudike, Nigeria.

Nweke, F.I., B.O. Ugwu, A.G.O. Dixon, C.L.A. Asadu and O. Ajodo, 1999. Cassava Production in Nigeria: A function of Farmer Access to Markets and Improved Production and Processing Technologies. COSCA working paper No. 20. IITA, Ibadan.

O'Hair, S.K., 1995. Cassava: Tropical Research and Education Centre. University of Florida.

Okafor, P.N., 2004. Assessment of cyanide overload in cassava consuming populations of Nigeria and the cyanide content of some cassava based foods. Afr. J. Biotech., 3: 358-361.

Oluwole, O.S. and A.O. Onabolu, 2004. High mortality of subjects with endemic ataxic polyneuropathy in Nigeria. Acta Neurological Scandinavicca, 110: 94-99.

Oluwole, O.S.A., A.O. Onabolu, H. Link and H. Rosling, 2000. Persistence of tropical ataxic neuropathy in a Nigerian community. J. Neurol. Neurosurgery Psychiatry, 69: 96-101.

Oluwole, O.S., A.O. Onabolu and A. Sowunmi, 2002. Exposure to cyanide following a meal of cassava food. Toxicol. Lett., 135: 19-23.

Oluwole, O.S.A., A.O. Onabolu, I.A. Cotgreave, H. Rosling, A. Persson and H. Link, 2003. Incidence of ataxic polyneuropathy and its relationship to exposure to cyanide in a Nigerian community. J. Neurol. Neurosurgery Psychiatry, 74: 1412-1416.

Onabolu, A.O., O.S. Oluwole and M. Bokanga, 2002. Loss of residual cyanogens in a cassava food during short-term storage. Int. J. Food Sci. Nutr., 53: 343-349.

Raw Materials Research and Development Council, RMRDC, 2004. Cassava Report on Survey of Agro Raw Materials in Nigeria-Raw Materials Research and Development Council (Maiden Edition), Abuja, pp: 1-2, 18-19.

Siritunga, D., D. Arias-Garzon, W. White and R.T. Sayre, 2004. Over expression of hydroxynitrile lyase in cassava roots accelerates cyanogenesis and detoxification. Plant Biotechnol. J., 2: 37-43.

Stephenson, K., R. Amthor, S. Mallowa, R. Nungo, B. Maziya-Dixon and S. Gichuki, 2010. Consuming cassava as a staple food places children 2-5 years old at risk for inadequate protein intake, an observational study in Kenya and Nigeria. Nutr. J., 26: 1-9.

Vasconcelos, A.T., D.R. Twiddy, A. Westby and P.J.A. Reilly, 1990. Detoxification of cassava during Garri preparation. Int. J. Food Sci. Technol., 25: 198-203.

White, W.L.B., J.M. Mcmahon and R.T. Sayre, 1994. Regulation of cyanogenesis in cassava. Acta Hoticulturae Wagningen, 375: 225-226.