International Journal of Innovation in Science and Mathematics
Volume 3, Issue 4, ISSN (Online): 2347–9051

Cyanogenic Glycoside in Food Plants

Onojah, P.K., and Odin, E. M.*
Department of Pure and Industrial Chemistry of Pure and Industrial Chemistry,
Kogi State University, Anyigba, Nigeria.
Correspondence Author's Email: odinem2005@yahoo.com; Phone: 08138637104

Abstract **– Cyanogenic glucoside contents in some edible nuts and seeds (Sesamum indicum, Anacardium occidentale, Garsinia Kola, Sorghum bicolor,Vigna Unguilata) were all analysed to establishe their proximate compositions. The study showed that four (4) out of the six samples studied (Cashew nut, Bitter kola, Corn seeds and Cowpea seeds) contained hydrogen cyanide in the form of cyanogenic glycoside in quantities varying from 29.16mg/100g of dried samples for corn seeds to 56.70mg/100g for bitter kola, while two (2) of the samples sesame seed and sorghum seed tested negative showing the absences of hydrogen cyanide. The toxicological implications of the results have been discussed.**

Keywords **– Cyanogenic Glycoside, Edibe Nuts, Toxins, Glycosylation.**

I. INTRODUCTION

Natural plant toxins may be present inherently in plants such as fruits and vegetables which are common food sources. They are usually metabolites produced by plants to defend themselves against various threats such as bacteria, fungi, insects and predators (Wink, 1988). Natural toxins may also be present in food plants as a result of natural selection and breeding methods that enhance these protective mechanisms.

Cyanogenic glycosides or cyanoglycosides account for approximately 90% of the wider group of plant toxins known as cyanogens. The key characteristic of these toxins is cyanogenesis, the formation of free hydrogen cyanide, and is associated with cyanohydrins that have been stabilized by glycosylation (attachment of sugars) to form the cyanogenic glycosides (Davis, 1991). Examples of Cyanogenic glycosides in plants is usually reported as the level of releasable hydrogen cyanide.

A cyanogenic food of particular economic importance is cassava (*Manihot esculenta*), which is also known by the names Manioc, Yula 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. Amydalin 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.

Potential toxicity of cyanoglycosides arises from enzymatic degradation to produce hydrogen cyanide, resulting in acute cyanide poisoning. The enzyme responsible (β – glucosidase) may arise from the plant material or from gut microflora. Clinical symptoms of acute cyanide poisoning include rapid respiration, drop in blood pressure, rapid pulse, headache, dizziness, vomiting, diarrhea, mental confusion, stupor, blue discolouration of the skin due to lack of oxygen (cyanosis), twitching and convulsions. (Shragg, et al, 1982). Cyanide can be lethal to humans and the acute dose is in the region of 1mg/kg body weight. Cases of acute poisoning have been associated with misuse, particularly of preparations from apricot pits, bitter almonds and cyanide rich apple seeds. Cyanide is detoxified in the body, by the enzyme rhodanese in the presence of sulphur containing amino acids, to produce thiocyanate. Goitre and cretinism due to iodine deficiency can be exacerbated by chronic consumption of insufficiently processed cassava. The detoxification product of cyanide, thiocyanate, is a similar size to the iodine molecule and interferes with iodine uptake by the thyroid, effectively increasing the dietary requirement of iodine. The effect is only seen in iodine deficient population and can be reversed by iodine supplementation (Atkinson, 2006).

Konzo or spastic paraparesis is a motor neuron disease characterized by irreversible weakness in the legs. In severe cases, patients are not able to walk. Speech and arms may be affected. Konzo particularly affects children and women of childbearing age in East Africa in times of food shortage and is associated with a high and sustained intake of cassava (*Manihot esculenta*) in combination with a low intake of protein (Davis, 1991; FSANZ, 2004).

Tropical ataxic neuropatty (TAN) describes several neurological symptoms affecting the mouth, eyesight, hearing or gait of mostly older males and females. TAN is attributed to cyanide exposure from the chronic consumption of foods derived from cassava (FSANZ, 2004). Strong associations have been observed between chronic cassava consumption and these diseases, the observations are confounded by diverse nutritional deficiencies and a causal relationship has not been conclusively established (Davis, 1991; FSANZ, 2004; Speijers, 1993).

Toxicity of cyanogenic glycoside-containing plant is due to the cyanide produced on injestion. The plant species that produce cyanogenic glycosides usually also has a corresponding hydrolytic enzyme (β – glucosidase). In the presence of water, the non-toxic cyanogenic glycosides are hydrolysed by the enzyme producing cyanohydrins which quickly decompose to the toxic hydrogen cyanide. In this way, cyanogenic plants are protected against predators. Cyanogenic glycosides, cyanohydrins and hydrogen cyanide are collectively known as cyanogens.

In human, the clinical signs of acute cyanide intoxication can include: rapid respiration, drop in blood

pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhea, mental confusion, stupor, cyanosis with twitching and convulsion followed by terminal conia (WHO, 1993). Death due to cyanide poisoning can occur when the cyanide level exceeds the limit an individual is able to detoxify. The likelihood of cyanide intoxication from consumption of cyanide – containing food is dependent on body weight. For example, it is possible that a child or person of smaller body weight would not be able to detoxify the cyanide resultant from a meal of inadequately prepared cassava or bamboo shoots. The acute lethal dose of hydrogen cyanide for humans is reported to be 0.5 – 3.5 mg/kg bw. Approximately 50 – 60mg of free cyanide constitutes a lethal dose for an adult man. (WHO, 1993).

It is uncommon for dietary cyanide intake to cause chronic diseases. Adverse effects may, however, accompany individuals who have underlying dietary deficiency such as inadequate protein and/or iodine intake. For example, individuals already suffering from goiter and cretinism due to iodine deficiency may sometimes experience exacerbation of the condition following continuous dietary cyanide exposure.

In view of the poisonous nature of hydrogen cyanide (HCN) and its prevalence in many edible plant seeds and nuts in form of cyanogenic glycoside, this paper estimates the levels of this class of secondary metabolite in order to advise on the average quality to be consumed at a given time.

Table 1: Levels of cyanogenic glycosides for some plants materials consumed by humans

Food	**Major Cyanogenic Glycosides Present**	**Cyanogenic Content (Mg HCN/kg)**
Cassava (*Manihot esculent*) – root	Linamarin	15 – 1000
Sorghum (*Sorghum vulgare*) - leaves	Dhurrin	750 – 790
Flax (*Linum usitatissimum*) – seed meal	Linamarin, linustatin neolinustatin	360 – 390
Lima beans (*Phaseolus innatus*)	-	2000 – 3000
Giant taro (*Alocasia macrorrhizos*) – leaves	Triglochinin	29 – 32
Bamboo (*Bambusa arundinacea*) – young short	Taxiphylin	100 – 8000
Apple (*Malus spp*) – seed	Amygdalin	690 – 790
Peach (*Prunus Perisca*) – kernel	Amygdalin	710 – 720
Apricot (*Prunus armeniace*) – kernel	Amygdalin	-785 – 813 -89 – 2710 -2.2 (juice)
Plum (*Prunus spp*) –kernel	Amygdalin	696 – 764
Nectarine (*Prunus spp*) – kernel	Amygdalin	196 – 209
Cherry (*Prunus spp*)	Amygdalin	4.6 (juice)
Bitter almond (*Prunus dulcis*)	Amygdalin	4700

(Haque and Bradburg, 2002)
(Simeonova and Fishbein, 2004)
(Shragg et al, 1982).

II. Material and Methods

Collection and Treatment of Samples

Dried seeds of the six plants were collected from Anyigba market in Kogi State, Nigeria and Kogi State University Cashew Processing Plant. The samples were sorted and cleaned to remove rotten seeds and debris. The samples were sun dried and were ground into fine powder and dried in the oven at 40^{o}C, then passed through a 40 mesh sieve and stored in a refrigerator at 5^{o}C.

Analysis of the Samples

The qualitative analysis of HCN was done by method described by Nkafamiya and Manji (2006). The quantitative analysis of cyanogenic glycoside was done according to the method described by the Association of Official Analytical Chemist (AOAC, 2005); samples (1g each) dissolved in 200ml water was distilled for 2hrs and 150ml distillate was collected. To the distillate was added 20ml of a 2.5% NaOH and made up to the 250ml with distilled water. To the diluted distillate, 8.0ml of 6M NH_4OH solution and 2.0ml of 5% KI was added. The resulting mixture was then titrated against 0.02M $AgN0_3$ Solution using a 10cm^3 microburette. The end point was noted as a permanent turbicity against a black background. Tire values were obtained and cyanogenic glycoside contents calculated using the formula.

$$\text{Cyanogenic glycoside (mg/100g)} = \frac{Tv \times 1.08 \times Ev}{Sm \times Al} \times 100$$

Where TV = Tire value (ml)
EV = Extract volume (ml)
SM = Sample mass (g)
AL = Aliquot used (ml)

1ml of 0.02M Ag NO_3 = 1.08$_{mg}$ HCN.

III. Results and Discussion

Results

Table 2: Qualitative Determination of Cyanogenic glucoside

Samples	Observation
Cashew Nut (*Anacarduim accidentale*)	+
Cowpea (*Vigna unguiculata*)	+
Bitter Cola (*Garcinia kola*)	+
Sorghum Seed (*Sorghum bicolar*)	–
Corn Seed (*Zea mays*)	+
Sesame Seed (*Sesamum indicum*)	–

Table 2 presents a qualitative determination of cyanogenic glycoside in different samples. All the samples contain cyanogenic glycoside except for sorghum seed and sesame seed indicated by negative (-ve) sign:

Table 3: Quantitative Determination of Cyanogenic Glycoside

Samples	Concentration of HCN in Mg/100g of Dried Sample
Cashew Nut	32.40 ± 0.05
Cowpea	35.64 ± 0.03
Bitter Cola	56.70 ± 0.05
Sorghum Seed	-
Corn Seed	29.16 ± 0.03
Sesame Seed	-

*Values are Mean ± Standard Deviation (SD) of the triplicate analysis.

The concentration of HCN in various samples in mg per 100g of dried sample are also listed in Table 3. Bitter Cola has the highest concentration of 56.70 ± 0.05mg and Corn Seed 29.16 ± 0.03mg being the least. Sorghum seed and sesame seed had no cyanogenic glycoside content according to the result. The result indicate presence of the HCN in the form of cyanogenic glycoside which is below the threshold level of 60m per day in adult (Monago and Akhidue, 2002).

Discussion

The results presented above indicates the presence of cyanogenic glycoside in the form of hydrogen cyanide (HCN) which occurs as a result of hydrolysis and thus making it toxic at certain concentrations. Cyanogenic glycoside is also a known inhibitor of the respiratory chain, inhibiting metallo-enzymes such as cytochrome oxidase (Montgomery, 1980). This makes oxygen unavailable to tissues and might result in death. From the values recorded above, it is observed that the concentrations of the hydrogen cyanide found in these samples were below the threshold level of 60mg per day in adult man (Oyenuga & Amazigo, 1997). The major defence of the organisms to counter toxic effect of cyanide is its conversion to thiocyanate, mediated by the enzyme rhodanese (sulphur transferase) located in the Mitochondria. The enzymatic detoxification requires sulphur donors, which are mostly provided from the dietary sulphur amino acid, cysteine and methionine. Rhodanese catalyses in vitro the formation of thiocynate and sulphite from cyanide and thiosulfate or other suitable sulphur donor, while in vivo, the enzyme is multifunctional.

$$\underset{\text{Hydrogen cyanide}}{HCN} + \underset{\text{thiosulphate}}{S_2O_3^{2-}} \rightarrow \underset{\text{Sulfite}}{SO_3^{2-}} + \underset{\text{thiocyanate}}{SCN}$$

The enzyme (*rhodanese*) contains an active disulphide group which react with the thiosulfate and cyanide. The thiocyanate product is readily excreted in the urine.

In man, cyanide poisoning can be treated by producing a high concentration of methemoglobin (Hb – Fe^{3+}) by administration of nitrite (Louis, 1995).

$Hb – Fe^{2+} + Na\ NO_2 \rightarrow Hb – Fe^{3+}$

Methemoglobin competes with cytochrome oxidant (cyt – Fe^{3+}) for cyanide ion. The concentration gradient favours methemoglobin. Cyanomethemoglobin (Fe – FeCN) is formed and cytochrome oxidase is restored (Lawrence, 1973).

$Hb – Fe^{3+} + cyt – FeCN \rightarrow Hb – FeCN + cyt – Fe^{3+}$.

In animals, the treatment is achieved through intravenous infection of a mixture of sodium nitrite and sodium thiosulfate (Sharman, 1989). The dose rate are 3g of sodium nitrite and 15g of sodium thiosulfate in 200g of water for cattle; 1g of sodium nitrate and 2.5g of sodium thiosulfate in 50g of water for sheep. Treatment may have to be repeated because of further liberation of HCN.

IV. Conclusion

The analysis for the presence of cyanogenic glycoside and its quantification in the selected common edible nuts and seeds indicated that hydrogen cyanide (HCN) in the form of cyanogenic glycoside is present in four out of the six samples studied with their estimated concentrations also given. Having confirmed its presence and concentrations, precautions to ensure detoxifications is therefore imperative.

Acknowledgements

The authors wish to acknowledge with thanks the assistance rendered by the technologists, all of the Department of Pure and Industrial Chemistry, Kogi State University, Anyigba, Kogi State, Nigeria.

References

[1] AOAC (2005). Official method of analysis 18th ed. Association of Analytical Chemists Washington, D.C. 106.

[2] Atkinson, K. (2006). Apricot kernels cavy risk of cyanide poisoning. Accessed at: http://www.nzherald.co.nz/organization/ story.cfm? O id = 324 & objected = 10379786.

[3] Davis, R.H. (1991). Cyanogens. In: D' Mello, J.P.F., Duffus, C.M., Duffus, J.H. (eds.). Toxic substances in crop plants. The Royal Society of Chemistry.

[4] FSANZ (2004). Final assessment report proposal P257. Advice on the preparation of cassava and bamboo shoots. Report Number 2 – 04. Canberra: FSANZ.

[5] Haque, M.R., Brandbury, J.H. (2002). Total cynade determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry*; 77 (1): 107 – 114.

[6] Lawrence, D.R. (1993). Toxicology of young shoot of common baobab. Indian Med. J. 5: 20 – 21.
[7] Louis, S.G. (1995). The pharmacological basis of therapeutics. Macmillan, New York. Pp. 140 – 146.
[8] Monago, C.C., and Akhidue, V. (2002). Cyanide poisoning. J. Appl. Sci. Environ. Manage. 6(1): 22 – 25.
[9] Montgomery, R.D. (1980). Cyanogens in toxic constituents of food stuffs (2nd ed). Acad. Press. New York.
[10] Nkafamiya, II., Manji, A.J. (2006). A study of the cyanogenic glycoside contents of some edible nuts and seeds. J. Chem. Soc. Niger. 31 (1 & 2): 12 – 14.
[11] Oyenuga, V.A. and Amazigo, E.O. (1997). A note of hydrocyanic acid content of cassava. W. Afri. J. – Biol. App Chem. 1; 39 – 43.
[12] Sharman, L.R. (1989). Treatment of cyanide poisoning in animals, N.Z. Vet. J. 15: 19.
[13] Shragg, T.A., Albertson, T.E. and Fisher Jr., C.J. (1982). Cyanide poisoning after bitter almond injestion. Western Journal of Medicine; 136 (1): 65 – 69.
[14] Simeonova, F.P., Fishbein, L. (2004). Hydrogen cyanide and cyanides: Human health aspects. Concise International Chemical Assessment Document 61. Geneva: World Health Organization.
[15] Speijers, G. (1993). Cyanogenic glycosides. Food additive series. No. 30. Geneva: JECFA.
[16] Wink, M. (1988). Plant breeding importance of secondary metabolites for production against pathogens and herbivores. Theor. Appl. Genet. 75: 225 – 233.
[17] World Health Organisation (WHO) (1993). Cyanogenic glycosides. Toxicological evaluation of certain food additives and naturally occurring toxicants. WHO food additives series 30. Geneva. Available from URL: http://www.inchem.org/ documents/jecta/jecmono/r30je18.htm.