Variation of Cyanogenic Potential of Selected Cassava Varieties with Age in Zombo District, Uganda

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Abstract:

Cassava is a major staple food in the developing world, providing a basic diet for around 500 million people. In Uganda, it is currently one of the most important food crops and ranks second to bananas in terms of area occupied, total production and per capita consumption. However, cassava contains cyanogenic glycosides such as linamarin and Lotaustralin, which are toxic. Consumption of such toxins in sufficient quantities can cause acute cyanide poisoning and death in humans and animals. Thus, cassava is a possible health risk to the consumers. Awareness about the trend of cyanogenic glycosides content in cassava as it matures is paramount in minimizing the health risks associated with cassava consumption. As such, the most common local cultivars; Bisimwenge, Nyar-anderiano, Nyar-udota, in Zombo district and two improved cultivars: NASE 9 and TME 14, were investigated for variation in their cyanogenic content with age. The concentrations of cyanogens were determined using standard titration method. The results showed that cyanogeniccontent was highest at the ages of 8-10 months and generally decreased after the tenth month. It is therefore safer to harvest cassava after at least ten months when the levels of the hydrogen cyanide have reduced and stabilized.

***Key Words**: Cassava, Cyanogenic potential, Food safety, Linamarin, Lotaustralin*

1. Introduction:

Cassava (*Manihotesculenta*Crantz) is the most important root crop in the world and ranks second among African staple crops(Nweke, Spencer, & Lynam, 2002). It is a major source of calories in the tropics, where its roots are processed into several foods (Agbor-Egbe & Lape Mbome, 2006; Aloys, 2006), and it is sometimes described as the 'bread of the tropics' (Adams, Murrieta, Siqueira, Neves, & Sanches, 2009).According to FAO/WHO report (2009), cassava is the second most vital staple food in Uganda with per capita consumption of 132 kg, accounting for about 13% of the caloric intake. In Zombo district, cassava is the most widely cultivated crop with about 72% of households growing it (Uganda Bureau of Statistics, 2012).

Cassava plays a particularly important role in agriculture in developing countries, especially in sub-Saharan Africa, because it does well on poor soils and under low rainfall. Furthermore, it is a perennial crop that can be harvested as required (ability to be harvested as soon as six months and as late as 3 years after planting) (Nweke, 1994). Its wide harvesting window allows it to act as a famine reserve and is invaluable in managing labour schedules. It also offers flexibility to resource-poor farmers because it serves as either subsistence or a cash crop(Stone, 2002).

Cassava usage is being extended to some regions in Africa and elsewhere in which it was not formerly used, because of the apparent agricultural advantages of growing cassava and increasing demand for food due to the population pressures (Cardoso et al., 2005).

Nevertheless, cassava contains two cyanogenic glycosides, linamarin and a small amount of lotaustralin, which are catalytically hydrolyzed to release toxic hydrogen cyanide (HCN) when the plant tissue is crushed (Balagopalan, Padmaja, Nanda, & Moorthy, 1988; McMahon, White, & Sayre, 1995). The cyanogenic glycosides are produced as a defence mechanism against attack by predators(Bradbury &

Holloway, 1988). The cyanogenic content of cassava cultivars can be as small as less than 10 mg/kg for some cultivars and can be as high as more than 500 mg/kg fresh weight basis (O'Brien, Wheatley, Iglesias, & Poulter, 1994). The improved varieties have lower level of the cyanides than the local varieties (Oloya, Adaku, Ntambi, & Andama, 2017). Yet, some farmers from cassava growing countries often prefer the bitter varieties because they deter pests, animals, and thieves (Linley et al., 2002). This is because the bitter cassava varieties (bitterness largely due to linamarin) contain higher amounts of cyanide than sweet varieties(King & Bradbury, 1995; Sundaresan, Nambisan, & Eswari Amma, 1987).

Consumption of cassava that contain large amounts of cyanogens may cause cyanide poisoning (Akintonwa, Tunwashe, & Onifade, 1994). Hydrogen cyanide (HCN) is so poisonous because it binds to the Fe^{2+} in heamoglobin forming cyanoheamoglobin(Cereda & Mattos, 1996). As the binding affinity of cyanide is much higher than the equivalent binding affinity of oxygen, the respiratory cycle is impeded. High levels of HCN in the bloodstream lead to suffocation of the cells and result in acute poisoning with symptoms such as diarrhoea, stomach ache, and headache and, in some cases, even death (Fuchs, 2008). Thus, HCN and the cyanogenic glycosides are so dangerous.

But usually the erythrocytes containing cyano-hemoglobin just circulate uselessly in the blood stream until they age enough to be broken down. This lowers the oxygen transport capacity of the blood and if it is too low this can result in chronic poisoning causing serious illnesses such as Konzo or Tropical Ataxic Neuropathy (TAN) (Cardoso et al., 2005). Konzo is an irreversible paralysis of the legs of sudden onset, which occurs particularly in children and women of child bearing age(Cliff et al., 1997; Howlett, Brubaker, Mlingi, & Rosling, 1990; Ministry of Health Mozambique, 1984). Tropical ataxic neuropathy (TAN) is a chronic condition of gradual onset that occurs in older people who consume a monotonous cassava diet. It causes loss of vision, ataxia of gait, deafness and weakness (Howlett, 1994; Onabolu, Oluwole, Bokanga, & Rosling, 2001; Osuntokun, 1994). Cyanide also causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism (EPA, 1990).

Individuals with low protein and particularly low cysteine intake in their diets are more susceptible to cyanide poisoning since the detoxification of cyanide to thiocyanate by rhodanese requires cysteine as a substrate (Siritunga & Sayre, 2003). The medical conditions caused by cyanide overload could be prevented by a considerable reduction in the per capita cyanide intake (Cardoso et al., 2005).

Therefore, it was critical to study how the cyanogenic potential of cassava cultivars vary with age so that the safest period for harvesting the tubers, when they have the lowest levels of the toxins, is recommended. Coupled with detoxification, especially fermentation which is the favourite(Andama & Lejju, 2012), this would reduce further the cyanide content to within acceptable limits (safe level) of cyanogens in cassava flour of 10 mg/kg, set by the World Health Organization (WHO) (FAO/WHO, 1991).

2. Materials and Methods:

2.1 Study area:

This study was conducted in Zombo district which lies approximately 27 kilometres (17 miles), by road, west of Nebbi, the nearest large town. This location is approximately 70 kilometres (43 miles), by road, south of Arua, the only Municipality in the sub-region. The coordinates of Zombo town are: 02° 30' 54N, 30° 54' 06E (Latitude: 2.5150; Longitude: 30.9100).

2.2 Materials:

The apparatus used during this research included polythene bags, a kitchen knife, refrigerator, containers (basins), distillation flask, reciprocating shaker, 125 mL Erlenmeyer flasks, filter funnels, filter papers, micro-burette, distillation apparatus, pestle and motor. The main reagents that were used during laboratory analysis were sodium hydroxide, 5 % potassium iodide solution, 0.02N silver nitrate, and distilled water.

2.3 Cultivation of cassava:

A plot of land (10 m x 7 m) was prepared and 5 ridges were made with a spacing of 0.5 metres between them. Stem cuttings of two improved cultivars of cassava (NASE 9 and TME 14) were obtained from National Agricultural Research Organization (NARO) at Abii Farm in Arua District, Uganda. The stem cuttings of three local cassava cultivars (*Nyar-anderiano*, *Nyar-udota* and *Bisimwenge*) were collected from local peasant farmers in Zombo district, Uganda. Each cultivar was planted in a ridge following the order of their names as listed above.

The cuttings from each cultivar, measuring 27 cm in length, were planted at about 45° on the crest of the ridges. Care was taken to ensure that the buds were not inverted during planting, in order to prevent delayed sprouting (Okigbo, 1980). Planting distance was 0.5 m x 0.5 m and weeding was done after every 4 weeks after planting, since the crop was planted as a sole crop (Melinfonwu et al., 2002).

2.4 Harvesting and preparation of cassava samples:

Sample collection and preparation was done monthly (on the fifteenth day of each month) for cassava at the ages ranging from eight months to thirteen months, considering that cassava matures from the age of about 12 months (Ngendahayo & Dixon, 1998; Odigboh, 1976; Oluwole, Onabolu, Mtunda, & Mlingi, 2007; Sriroth et al., 1999).

Samples of the fresh cassava root tubers were got directly from the garden using a hoe. After removing the soil, they were transported in polythene bags to the Government Analytical Laboratory (GE058/07) at Wandegeya in Kampala, Uganda. Each of the peeled and washed sample (40.0 g) was weighed and mashed using a wooden pestle and mortar. The samples were then stored at a temperature of – 4^{o}C awaiting analysis within 24 hours.

2.5 Determination of level of cyanides in cassava:

The cassava samples were analysed by the standard method of FAO (FAO, 2000). In brief, the sample (10 g to 20 g) was put in a distillation flask; distilled water (about 200ml) was added and allowed to stand for three hours, in order to set free all the bound hydrocyanic acid. The mixture was distilled with steam and 200ml of distillate was collected in a solution of 0.625M sodium hydroxide in water (20 ml). The distillate was then diluted with distilled water to a volume of 250ml.

To the distillate (100 ml) was added potassium iodide solution (5%, 8 ml) and titrated with 0.02N silver nitrate (1ml of 0.02N silver nitrate corresponds to 1.08mg of hydrocyanic acid) using a micro-burette. The end point was indicated by a faint but permanent turbidity, which was easily recognized, especially against a black background.

When all the cyanide ions have reacted with the silver ions, any excess silver ions react with the iodide ions giving a precipitate of silver iodide.

$HCN_{(aq)} + AgNO_{3(aq)} \rightarrow HNO_3\ (aq) + AgCN_{(aq)}$ (1)

$Ag^+\ (aq) + I^-_{(aq)} \rightarrow AgI_{(s)}$ (2)

Excess

2.6 Data analysis:

A line graph was generated from the results of laboratory analysis using Microsoft Excel 2007 computer package.

3. Results and discussion

Effect of age of cassava on the levels of hydrogen cyanide:

The level of hydrogen cyanide generally increased from the eighth to the tenth month of planting for varieties (NASE 9, TME 14, *Nyar-anderiano* and *Bisimwenge*) and then started decreasing until the thirteenth month, except for *Bisimwenge*, which showed a slight increase from the twelfth month (154.56 mg/kg) up to the thirteenth month (181.48 mg/kg) (Figure 1). This increase was an anomaly that could not be accounted for, but a further analysis of a sample of *Bisimwenge* variety after 21 months of planting gave 91.11 mg/kg HCN, which still agreed with the general decreasing trend.

For *Nyar-udota*, there was an increase from the eighth month up to the ninth month after which the level of the hydrogen cyanide started decreasing until the thirteenth month. By the thirteenth month, *Bisimwenge* had the highest amount of the hydrogen cyanide (181.48 mg/kg) followed by NASE 9 (109.33 mg/kg), TME 14 (105.60 mg/kg), *Nyar-anderiano* (90.00 mg/kg) and finally *Nyar-udota* (88.50 mg/kg) with the lowest amount of the hydrogen cyanide.

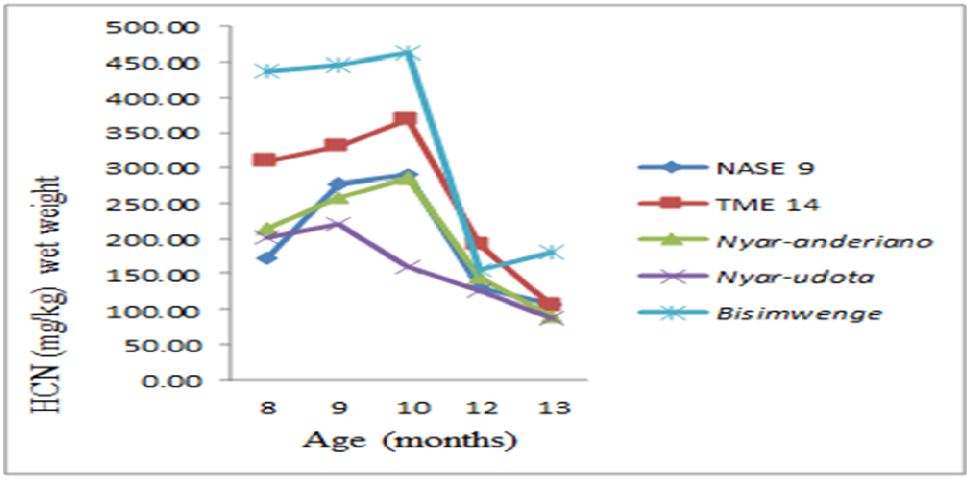

Figure 1: *Variation of levels of hydrogen cyanide (mg/kg) with age in five cassava cultivars (NASE 9, TME 14, Nyar-anderiano, Nyar-udota and Bisimwenge).*

The obtained trends of HCN concentrations in *Nyar-udota* cassava with age (Figure 1) is similar to the variations in cyanide concentration in Nyamkagile cassava landrace planted in Chambezi and Amani sites, Tanzania which decreased with age especially from the 9^{th} to the 15^{th} month after planting (Mtunguja, Laswai, Kanju, Ndunguru, & Muzanila, 2016). Nevertheless, the HCN levels in the other cassava varieties (NASE 9, TME 14, *Nyar-anderiano*, *Bisimwenge*) started decreasing from the 10^{th} month after planting. However, the slight increase in HCN levels in *Bisimwenge* from the twelfth month to the thirteenth month is also concurrent with the findings of Mtunguja et al.(2016), which showed increase in HCN concentrations from the 12^{th} to the 15^{th} month after planting in Kalolo, Kiroba, Msenene landraces of Chambezi and Amani sites in Tanzania.

The decreasing trend of HCN with age could be attributed to the following two opposing factors:

Firstly, cyanogen synthesis which is based on the expression of the gene CYP79D1/D2, takes place in the young shoots. After the synthesis, it is translocated to the roots (Siritunga, Arias-Garzon, White, & Sayre, 2004). This increases the level of cyanogen in the roots.

Secondly, cyanogen re-assimilating based on expression of the gene β-CAS, where they are utilized for amino acid synthesis(Jørgensen, 2005), as well as linamarase and hydroxy nitrile lyase (HNL) genes, both involved in the breakdown of cyanogens upon tissue disruption, based on their expression clustered together.Both cyanogen re-assimilation and the action of linamarase and HNL reduce the level of cyanogen in the roots.

When the action of cyanogen synthesis outweighed the factors that reduce the cyanide levels, there was an increasing trend of the cyanide level (8-10 months). During the tender age of the cassava, cyanogen synthesis is enhanced because of the increased number of young shoots sprouting which are responsible for synthesis of the cyanogens. This led to an overall increase in the cyanogen level.

Meanwhile, when cyanogen re-assimilation and action of linamarase and HNL outweighed cyanogen synthesis there was a decreasing trend (10-13 months) except for *Nyar-udota* where the decrease was from the ninth month (Figure 1). As the cassava matures, the number of young shoots being produced reduces drastically hence leading to a decrease in the amount of cyanogen synthesized, as the rate of cyanogen re-assimilation and action of linamarase and HNL remains fairly constant. Thus, overall, the level is reduced. This seems to agree with investigations by Chotineeranat, et al.(2006) and the report by Bokanga, Essers, Poulter, Rosling, & Tewe, (1994).

4. Conclusions:

The concentration of cyanogenic glycosides in cassava cultivars is highest at the ages of 8-10 months and thereafter, generally, decreases with age.

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