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 Aghara (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 Aghara samples ranged from 32.8±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 utilissima phol) are shrub perennial plants of the Euphorbia eae (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, 2006). 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-rust 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 (Nwke 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, 1978-1998). These improved varieties yield between 25-40 tonnes/ha, are resistant to pests and diseases and have acceptable culinary and industrial qualities (Breukeilaum et al., 1978, NRCRI, 1982-1997; IITA, 1978-1998). 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 (Nwke 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; Oluwolere et al., 2003) and stunting of children (Stephenson et al., 2010). In Nigeria, cassava is a staple...
MATERIALS AND METHODS

Sample collection and methods: 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 kept overnight. Samples were prepared in duplicate. After 24 h, 5.0 g of hydrated stannous chloride (SnCl2·H2O) 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 AgNO3 and 1 ml of freshly prepared 0.5% w/v dithizone 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 (PFIRO, 2000-2004).

Using the relationship 1 ml of 0.02M AgNO3 = 0.52 mg CN-:

\[
\text{Concentration of CN}^- = \frac{\text{Titre value} \times 0.52 \times 1000 \text{ (ppm)}}{\text{Weight of sample taken}} \tag{1}
\]

Chemical equation of the reaction:

\[
\text{HCN(aq) + AgNO}_3(\text{aq}) \rightarrow \text{HNO}_3(\text{aq}) + \text{AgCN(aq)} \tag{2}
\]

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 < leaf.

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 aa3) 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 catalyzes 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 hydrocynide 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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stem</th>
<th>Tuber</th>
<th>Peel</th>
<th>Leave</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS 30572 A&lt;sub&gt;c&lt;/sub&gt;</td>
<td>33.710</td>
<td>50.370</td>
<td>74.210</td>
<td>104.740</td>
</tr>
<tr>
<td>TMS 30572 B&lt;sub&gt;c&lt;/sub&gt;</td>
<td>30.520</td>
<td>50.220</td>
<td>74.100</td>
<td>106.620</td>
</tr>
<tr>
<td>TMS 30572 C&lt;sub&gt;c&lt;/sub&gt;</td>
<td>33.660</td>
<td>49.990</td>
<td>75.000</td>
<td>104.920</td>
</tr>
<tr>
<td>TMS 30572 D&lt;sub&gt;c&lt;/sub&gt;</td>
<td>33.520</td>
<td>50.370</td>
<td>74.220</td>
<td>104.540</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>32.85±1.55</td>
<td>50.24±0.18</td>
<td>74.38±0.41</td>
<td>105.21±0.95</td>
</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Stem</th>
<th>Tuber</th>
<th>Peels</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMS 30572 A&lt;sub&gt;c&lt;/sub&gt;</td>
<td>41.31</td>
<td>74.21</td>
<td>83.05</td>
<td>104.74</td>
</tr>
<tr>
<td>TMS 30572 B&lt;sub&gt;c&lt;/sub&gt;</td>
<td>34.36</td>
<td>74.10</td>
<td>84.32</td>
<td>115.85</td>
</tr>
<tr>
<td>TMS 30572 C&lt;sub&gt;c&lt;/sub&gt;</td>
<td>48.34</td>
<td>75.00</td>
<td>87.06</td>
<td>114.35</td>
</tr>
<tr>
<td>TMS 30572 D&lt;sub&gt;c&lt;/sub&gt;</td>
<td>20.88</td>
<td>74.22</td>
<td>85.41</td>
<td>137.21</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>36.17±11.80</td>
<td>74.38±0.41</td>
<td>84.96±1.70</td>
<td>118.04±13.70</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Abuja (ppm)</th>
<th>Agbara (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stems</td>
<td>32.85±1.55</td>
<td>38.17±11.80</td>
</tr>
<tr>
<td>Range</td>
<td>30.52-33.70</td>
<td>20.68-48.34</td>
</tr>
<tr>
<td>Tubers</td>
<td>50.24±0.18</td>
<td>74.38±0.41</td>
</tr>
<tr>
<td>Range</td>
<td>49.09-50.37</td>
<td>74.21-75.00</td>
</tr>
<tr>
<td>Peels</td>
<td>74.38±0.41</td>
<td>84.96±1.70</td>
</tr>
<tr>
<td>Range</td>
<td>74.10-75.00</td>
<td>83.05-87.06</td>
</tr>
<tr>
<td>Leaves</td>
<td>105.21±0.95</td>
<td>118.00±13.70</td>
</tr>
<tr>
<td>Range</td>
<td>104.74-106.62</td>
<td>104.74-137.24</td>
</tr>
</tbody>
</table>

Kruskal-Wallis value (H<sub>.05</sub>) = 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 mg HCN/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 0.62±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., 1890; 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 species 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 (Gledow 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

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