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Determination of cyanogenic glucosides in cassava products sold in Okada, Edo State, Nigeria

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Cyanogenic glucosides in some cassava products (cassava flour, fufu and garri) obtained from the market in Okada town, Edo State, Nigeria were determined. The cyanide content of the samples determined ranged from 5 to 10 ppm and falls within the acceptable limit of 10 mg HCN equivalent/kg dry weight recommended by FAO. Fufu product has the highest cyanide concentration of 10 ppm while garri has the lowest value of 5 ppm. The result shows that consumption of cassava products does not pose any health risk to the local consumers although epidemiological studies have shown that exposure to small doses given over a long period of time can produce histological changes in the central nervous system.

Key words: *Manihot exculenta*, cyanogenic glucosides, toxicity.

INTRODUCTION

Cassava is the staple food of more than 500 million people in the tropics, many of whom are very poor (Katz and Weaver, 2003; FAO, 2007). The leaves and roots of the plant contain the cyanogenic glucosides (Linamarin and a small amount of lotaustralin) (Bradbury and Denton, 2010, Mburu et al., 2012). The Linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of the enzyme Linamarase, which is also produced by the plant (Katz and Weaver, 2003, Jansz and Uluwaduge, 2012). The acetone cyanohydrin decomposes rapidly in neutral or alkaline conditions liberating hydrogen cyanide and acetone (Scheme 1).

The sum of the amounts (HCN equivalent) of Linamarin, acetone cyanohydrin, hydrogen cyanides and cyanide ion equals the cyanogenic potential of the cassava sample (Bradbury, 2006; Jansz and Uluwaduge, 2012)

The cyanogenic potential of cassava roots and leaves range from 2 to > 1000 PPM HCN, fresh weight (Odoemelan, 2005; Komolafe and Arawande, 2011). It is generally considered that cassava roots that contains > 100 ppm HCN equivalent should be processed to reduce the cyanogenic potential, before use for human consumption (ANU News, 2007). The traditional methods of peeling and grating, dewatering and fermentation for 72 h reduce the cyanogens in cassava roots to a considerably safe level (Bradbury, 2006; Nambisian, 2011).

There are several heath disorders which have been associated with regular intake of sub-lethal quantities of cyanogens, some of which have resulted into outright poisoning and death due to cyanide intake from consumption of poorly processed cassava products (Adindu and Aproku, 2006; Nhassico et al., 2008; Dufour, 2011). These include the exacerbation of goitre, cretinism and cardiovascular diseases such as encelopathy and neuropathy. Severe cyanide poisoning can lead to heart, brain

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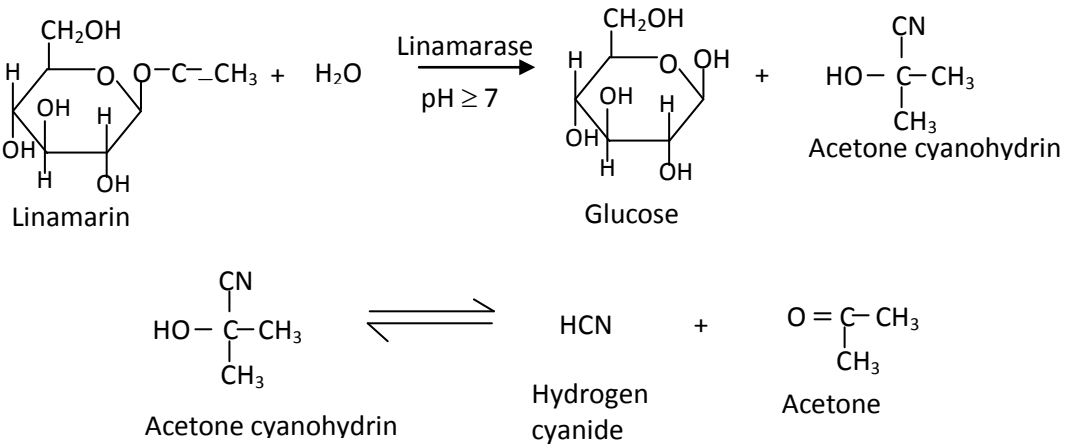

Scheme 1. Enzymatic hydrolysis of linamarin.

and optic nerve degeneration (Soto-Blanko et al., 2002).

In Nigeria, there have been occasional cases of sudden death of a whole family after taking a cassava meal containing lethal dose of cyanide due to poor processing (Adindu and Aproku, 2006; Dufour, 2011). Of recent, there are disturbing incidents of deaths resulting from eating some cassava-based meals reported in Nigerian newspapers (Thisday Live November 14, 2012; Daily Post Newspapers August 2, 2013). Communities around Okada and its environs, are mostly peasant farmers whose major occupation is subsistence farming including cassava processing. Hence there is need to ensure best practices in cassava processing and that the residual cyanide in cassava products from these area are within acceptable limits.

In this present research work, effort have been made to determine the cyanide levels in some local commercial cassava products obtained from the local market in Okada town, Edo State, Nigeria. This is with a view to determining the health risk that consumption of cassava products may pose to the rural dwellers.

MATERIALS AND METHODS

Reagents and test samples

The reagents used are mostly products from BDH and May and Baker, England. They are analytical grades and were used directly without further purification. They include picric acid, potassium cyanide, ethanol, copper (II) sulphate, hydrochloric acid, sodium carbonate, potassium hydroxide and distilled Water. The test samples include cassava flour, garri and fufu.

Sampling area

Okada is the head quarter of Ovia North-East LGA of Edo State, Nigeria. A total of thirty six samples, twelve each of garri, fufu and cassava flour respectively were randomly selected from Okada local market for analysis of cyanide potential. A map of the sampling area is shown in Figure 1

Apparatus

Spectronic 21D Spectrophotometer (Milton Ray Company), water bath (Genlab Ltd, England), Whatman No.1 Filter paper, volumetric flask, measuring cylinder, funnel, conical flask, McCartney bottle).

Methods

Cyanogenic potentials of the test samples were determined by a modified version of the alkaline Picrate impregnated filter paper strips method by Nwokoro et al. (2010). The method involves the preparation of varying concentrations (1 to 10 PPM) of standard KCN solution from a stock solution of KCN (0.2 g %). The KCN solutions in glass bottles were subsequently acidified with 20% HCl acid solution in ratio 1:1 to release the free cyanide as HCN and immediately sealed with 3 Picrate impregnated filter paper strips. The set-up was maintained at 80°C in a water bath for 10 min and was subsequently removed from bath and kept on the laboratory at room temperature for 24 h. The red coloured Picrate paper strips from each glass bottle were removed and rinsed in 5 ml 50% ethanol solution and kept for 30 min. Thereafter, the absorbance of the solution was measured using a spectrophotometer at 510 nm wavelength against a similarly prepared blank developed without KCN solution. A standard calibration curve of absorbance against cyanide concentration in PPM HCN equivalent was plotted (Figure 2). The plot was subsequently used for evaluation of cyanide concentration in the test samples.

Preparation of test samples for analysis of free cyanide (as HCN equivalent)

10 g of each sample (namely, cassava flour, fufu and garri) was mixed with 50 ml of water in a corked conical flask and allowed to stand for 24 h to extract the residual cyanoglucosides in the samples. The mixture was subsequently filtered to obtain the soluble extract containing cyanoglucosides. The same procedure as with standard KCN solutions was followed to determine the free cyanide concentration (as HCN equivalent) in the sample filtrate. The absorbance of the sample solution was equally measured at

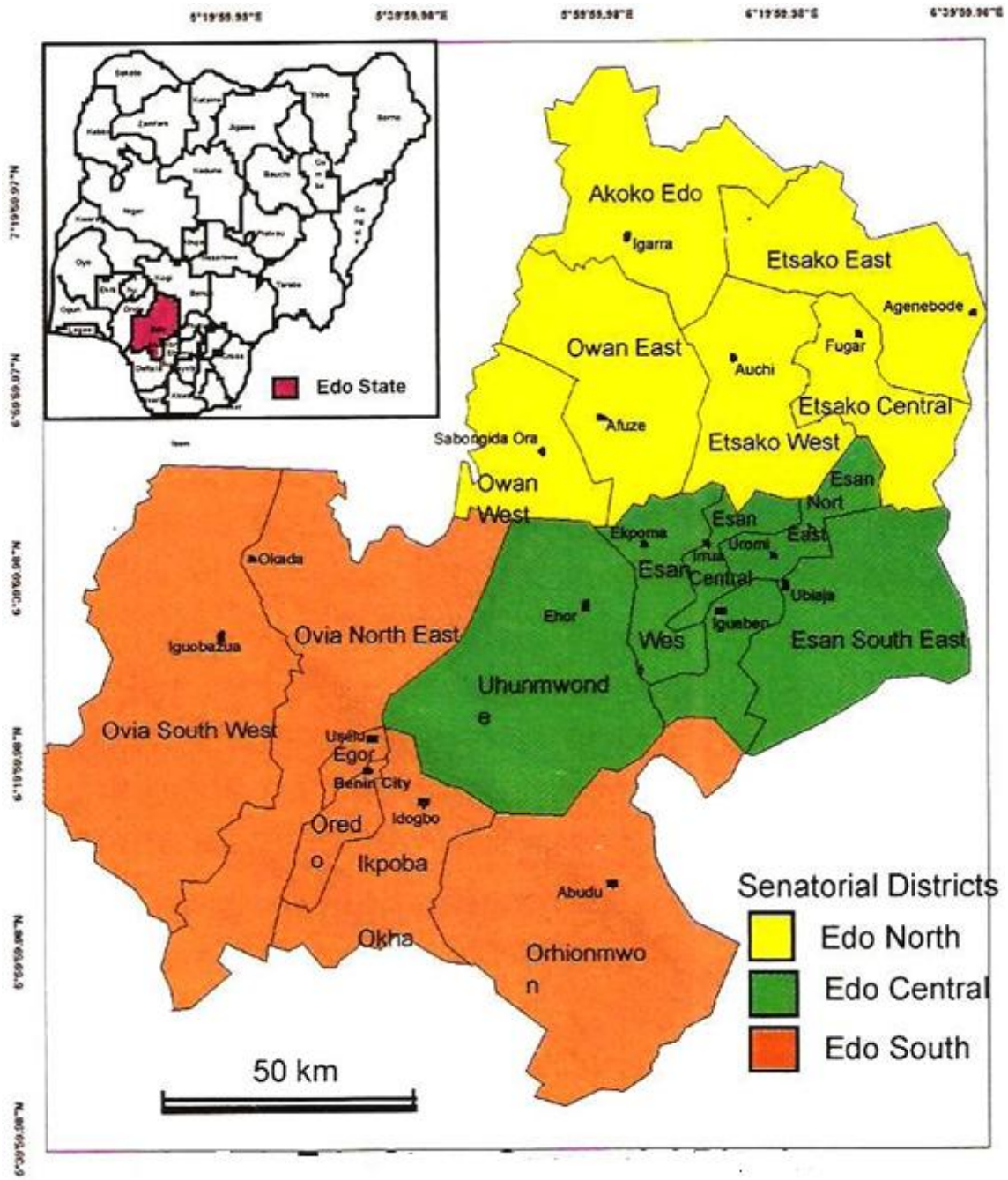

Figure 1. Edo State Senatorial Zones including Ovia North-East LGA with Okada as Head quarter.

510 nm wavelength against a blank devoid of KCN solution. Cyanide levels of the test samples were evaluated from the standard calibration curve by extrapolation.

Data analysis

Data obtained was presented in mean ± standard deviation and analysed in ppm. Data collected were statistically analysed for differences by use of student's t-test. Significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

The standard calibration curve of absorbance against KCN concentration (as HCN equivalent) is shown in Figure 2. Twelve samples each, of the cassava products were randomly selected from the local market in Okada town, Edo State, Nigeria and analyzed for their cyanide content using the modified version of the alkaline Picrate method by Nwokoro et al. (2010). The mean value of the

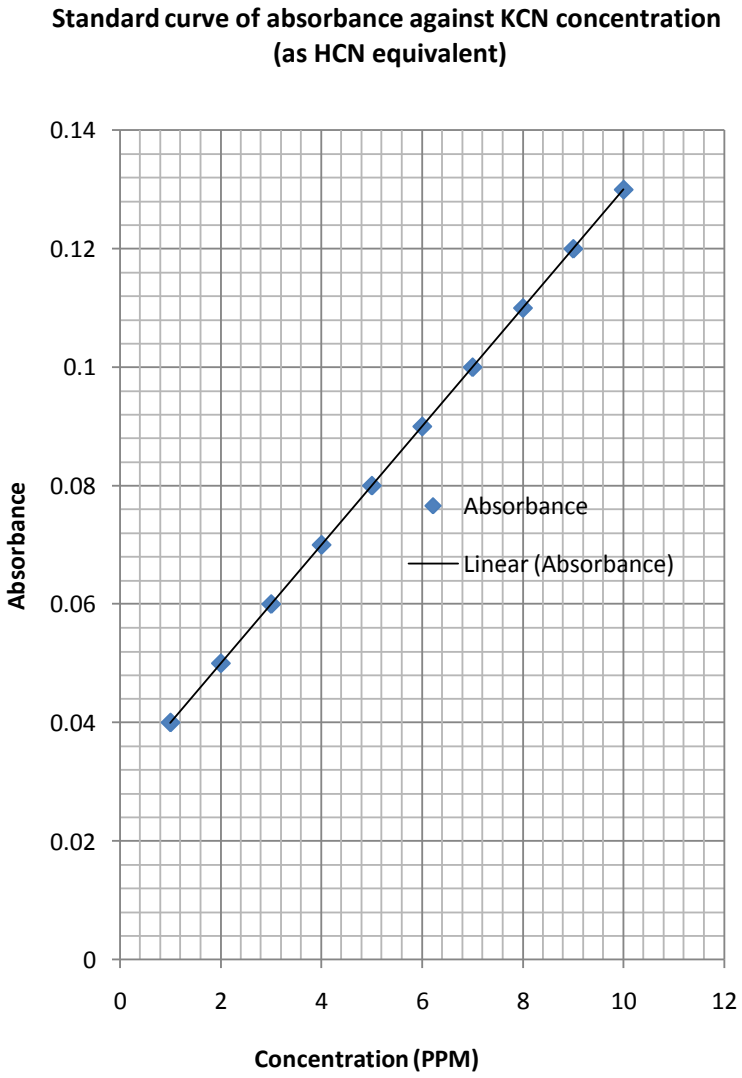

Figure 2. Absorbance against concentration (PPM).

Table 1. Cyanide content of test samples of cassava products sold in Okada market.

Test sample	Botanical name	Cyanide content (PPM)
Cassava flour	*Manihot esculenta* sp.	6 ± 0.12
Fufu	*Manihot esculenta* sp.	10 ± 0.13
Garri	*Manihot esculenta* sp.	5 ± 0.10

Result represents mean value of twelve samples of each cassava product taken in triplicate determinations in PPM equivalent of HCN (mg/Kg sample). 1 PPM = 1 mg/Kg.

cyanide content of the test samples evaluated from the curve is presented in Table 1. The cyanide content of the test samples ranged from 5 to 10 ppm. The result shows that fufu has the highest cyanide level of 10 ppm while garri has the least value of 5 ppm. The cyanide content is due to the presence of a significantly high amount of the cyanogenic glucosides, linamarin contained in cassava tuber which is the source of the cassava products (Lambri et al., 2013). The cyanide level of 5 to 10 PPM in the cassava products falls within the acceptable limits of

10 mg HCN equivalent/Kg dry weight recommended by FAO in 1988 for safe cassava products. The implication here is that the local cassava processors within Okada Community employ best practices in the processing of their cassava products and that the local consumers here are not at risk of consumption of the cassava products. The cyanide level is significantly lower than the lethal dose of cyanide intoxication of human which has been reported as 200 to 300 mg/kg (Akiyama et al., 2006) and the oral toxicity standard of 50 to 90 mg HCN equivalent/Kg body weight (WHO, 2004). Also, the cyanide level reported here is lower than that from the eastern and western parts of Nigeria where the cyanide level is far above the acceptable limit and there have been reported cases of occasional deaths from cassava meal (Odoemelam, 2005; Adindu and Aprioku, 2006; Komolafe and Arawande, 2011).

Although the cyanide levels of the test samples employed in this work are within the acceptable level recommended by FAO, but epidemiological studies have shown that exposure to small doses given over a long period of time can result in increased blood cyanide levels with the following symptoms: dizziness, headache, nausea and vomiting, rapid breathing, restlessness, weakness and even in severe cases paralysis, nerve lesions, hypothyroidism and miscarage (Soto-Blanko et al., 2002; Rachinger et al., 2002). The relatively high amount of cyanide content in fufu (10 ppm) compared to garri (5ppm) may be attributable to the peculiar processing technique employed for each cassava product. Conscious effort should therefore be made by the relevant agencies of the government in Nigeria such as National Agency for Food and Drug Administration Control (NAFDAC) to educate these peasant cassava processors on the modern techniques of cassava processing and the health risk posed to the consumers of adopting short-cut processing techniques. Also, there should be regular routine monitoring of cyanide level in cassava products among millers to avoid sharp practices.

Conclusion

The cyanogenic glucosides levels in the three cassava products studied ranged from 5 to 10 ppm which is relatively very safe and within the acceptable limit of 10 mg HCN equivalent/Kg body weight recommended by FAO. Among the three samples tested for cyanide, fufu has the highest cyanide concentration of 10 ppm, cassava flour has 6 ppm, and garri has the lowest of 5 ppm. The variation in cyanide levels for the three products may probably be a factor of differences in processing techniques for each product. Relevant Government agencies should mount aggressive campaign to sensitize and educate the peasant farmers and cassava millers on the dangers of taking poorly processed cassava meals and the diseases associated with intake of high cyanide concentrated cassava foods.

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