

Full Length Research Paper

# The use of methemoglobin complex in estimating cyanogen potential of cassava and cassava products

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**An improved method of analysis of cyanide in cassava and cassava products using methemoglobin complex is described. The cyanogen content of cassava mash, pre-fufu mash, fufu and Gari was determined spectrophotometrically. Optimal conditions were determined to be 24 h at 37°C in phosphate buffer (0.05 M; pH 5.6). The average cyanogen content obtained were 14.68 ± 0.66, 13.49 ± 0.02, 11.94 ± 0.02 and 9.87 ± 0.64 mg/kg for cassava mash, pre-fufu, fufu and gari, respectively. The values estimated for cassava mash and pre-fufu are greater than the values obtained for the processed fufu and gari, and also greater than the WHO safe value for cassava flour (10 mg/kg). The fact that the results of this work gave lower values of cyanide in processed cassava products compared with the value of unprocessed cassava suggests the validity of this method.**

**Key words:**Cassava, cyanide, cyanogen glucosides, methemoglobin, linamarin.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is widely used throughout Africa as an important food source (FAO 1987; Nestel 1973). Its importance as livestock feed and in the starch, alcohol, textile and pharmaceutical industries cannot be over emphasized but its major disadvantage is that it contains cyanogenic glucosides which liberate hydrogen cyanide (Coursey, 1973; Abiona et al., 2005). Cyanogen glucosides are a group of widely occurring natural substances that on hydrolysis yield a ketone or aldehyde, a sugar and highly toxic cyanide ion (McMahon et al., 1995). The toxicity of cyanide depends primarily upon its potency as a respiratory poison, its site of action being the cytochrome oxidase of aerobic organisms with which it forms a highly stable complex. This deactivates the enzyme and breaks the electron transport chain, meaning that the cell can no longer use the oxygen which is available to it (Boundoux et al., 1980). However, cyanide is more strongly drawn to methemoglobin than to cytochrome oxidase of the cells, effectively pulling the cyanide of the cells onto the methemoglobin. Once bound to the cyanide, the meth-

moglobin becomes cyanomethemoglobin, (Akanji et al., 1990).

Various communities using cassava as food in different forms have always used various processing methods to obtain cassava seemingly free of harmful amount of cyanogenic glucosides. A number of processing methods including chopping, heating and grinding have been developed which effectively remove the major proportion of cyanogenic glucosides and their degradation products from cassava food products (Charles et al., 2001). Studies have shown that food items prepared by these traditional methods still contain varying amount of residual cyanogen glucosides or HCN (Oke, 1966; Wood, 1965).

Unfortunately, most processors and especially Nigerians do not know the deleterious effects these have on their health and safety. Many modern methods have been developed for the determination of cyanide in cassava. These include acid hydrolysis method, enzymatic assay method, pyridinepyrazole method, alkaline picrate method and so on (Cooke 1978; Benjamin and Emmanuel, 1984; Bradbury et al., 1991; Muhamad and Bradbury 1999; Muzanila et al., 2000). In this study, a method has been developed to access the extent to which cyanomethemoglobin complex formation could be used

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for monitoring cyanogen content of cassava and cassava products (gari and fufu).

## MATERIALS AND METHODS

Analytical grade chemicals were used. All the chemicals used were purchased from British Drug Houses (BDH) except sodium dihydrogen phosphate which is a product of Merck E. (Darmstadt FRG). Partially purified cassava linamarase was prepared from the peel of freshly harvested cassava tuber according to the method of Ikediobi and Onyike (1982). Hemoglobin and methemoglobin were prepared according to normal laboratory procedure (Beetlestone and Irvine, 1964).

The methemoglobin concentration was  $4.54 \times 10^{-3}$  M per heme. The phosphate buffer of 5.6, ionic strength 0.05 M was also used in this study. The absorbance values were measured with Unicam Aurora Helios UV-VIS spectrophotometer. Fresh cassava tubers were obtained from Bowen University (Nigeria) agricultural farm. The cassava roots were peeled and grated with mechanical grater.

### Cassava mash

This is a grated cassava root after being peeled.

### Gari

Gari is a traditional food preparation of peeled tubers which has been grated, fermented, lightly fried and sometimes with the addition of a little palm oil to produce the yellow type.

### Fufu mash

This is a sieved hydrolysed and fermented cassava mash used in the preparation of a traditional food called fufu.

### Fufu

Peeled cassava tuber is soaked and fermented in large quantity of water and then reduced to pulp which is called fufu. It is usually edible after it has been cooked, turned or pounded to change the state of the pulp to a white thick traditional food.

## Experimental procedure

A total of 32 samples were analysed for cyanogen content. 5 g of each sample (cassava mash, pre-fufu, mash, fufu and gari) was taken and pounded into fine mash by the use of mortar and pestle. 10 ml of linamarase solution was added to 1 g of cassava mash and incubated for 24 h at 37°C. The same procedure was repeated for pre-fufu mash, Gari and Fufu before the samples were taken for analysis. The absorbance of each sample was taken spectrophotometrically at 420 nm with an extinction coefficient  $11.5 \times 10^4$  M<sup>-1</sup>cm<sup>-1</sup>.

## Determination of cyanogen content

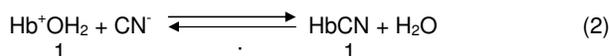
0.1 ml of aliquot of cassava was added to 1.5 ml of methemoglobin. 0.1 ml of this solution was added to 10 ml of distilled water. For quantitative measurement, the absorbance of the diluted cyanomethemoglobin solution was taken at 420 nm, the absorption maxima of cyanide and at  $11.5 \times 10^4$  M<sup>-1</sup>cm<sup>-1</sup>, the extinction coefficient at

that wavelength. The concentration was determined in terms of moles per heme, using the equation below (Beetlestone and Irvine 1964).

$$C_{HbCN} = \frac{A_{420}}{11.5 \times 10^4} = \frac{V + v_i}{L \times v_i} \quad (1)$$

Where  $C_{HbCN}$  = concentration of cyanomethemoglobin in mole of heme per litre,  $A_{420}$  = absorbance maxima of cyanide,  $V$  = volume of distilled water/buffer used,  $v_i$  = volume of cyanomethemoglobin,  $L$  = path length of cuvette, and  $11.5 \times 10^4$  = extinction coefficient at that wavelength.

The stoichiometry of the reaction is:



It shows from equation (2) that one mole of cyanide ligand reacts with one mole of the heme iron. This relationship was used to calculate the concentration of cyanide in mg/kg. The positive charge on the species  $\text{Hb}^+\text{OH}_2$  refers to the net positive charge which the iron atom carries in methemoglobin.

## RESULT AND DISCUSSION

Processing methods play an important role in effective removal of cyanogen glucosides and their degradation products. In this study, cyanomethemoglobin complex formation has been employed to evaluate the cyanogen content of cassava and processed cassava products. Some bound cyanide was removed during 24 h fermentation through the action of the enzyme linamarase contained in the cassava mash. The cyanogen content calculated for the cassava mash, pre-fufu, fufu and gari using equations 1 and 2 is presented in Table 1.

The amount of residual cyanide calculated on the average for cassava mash is  $14.68 \pm 0.66$  mg/kg. There was a drop in residual cyanide from an average of  $14.68 \pm 0.66$  to  $9.87 \pm 0.64$  mg/kg for gari as a result of the action of linamarase during fermentation. This result is in agreement with the work of McMahon et al. (1995) in which they concluded that processing procedures that include incubation at elevated pH (5.0) or a final heat treatment following linamarin hydrolysis facilitate the decomposition of acetone cyanohydrin and thereby reduce the toxicity of the food product.

The amounts of cyanide calculated on the average are  $11.94 \pm 0.02$  mg/kg and  $13.49 \pm 0.02$  mg/kg for fufu and pre-fufu, respectively. These values confirmed the reduction in the level of cyanide during processing of fufu. When the cassava roots are grated, the cyanogenic glucoside (linamarin) will come into contact with its hydrolysis and subsequent removal of breakdown products. This reduction in cyanogen content is based on the observation that the linamarin in root could be hydrolysed by overnight soaking with linamarase. The use of linamarase to facilitate the process of detoxification has also been reported by other workers (Ikediobi and Onyike

**Table 1.** Cyanogen content mg/kg of cassava and cassava products.

Sample	Cassava mash	Sample	Pre-Fufu	Sample	Fufu	Sample	Gari
A1	14.39 ± 0.10	B1	13.70 ± 0.41	C1	12.10 ± 0.52	D1	9.36 ± 0.38
A2	15.30 ± 0.91	B2	13.70 ± 0.22	C2	11.87 ± 0.11	D2	10.50 ± 0.16
A3	14.39 ± 0.44	B3	13.93 ± 0.14	C3	12.10 ± 0.23	D3	9.13 ± 0.61
A4	14.84 ± 0.31	B4	12.47 ± 0.80	C4	11.41 ± 0.33	D4	10.73 ± 0.35
A5	15.61 ± 1.21	B5	13.78 ± 0.50	C5	11.64 ± 0.61	D5	9.81 ± 0.42
A6	15.30 ± 0.63	B6	12.24 ± 0.31	C6	12.33 ± 0.23	D6	10.27 ± 0.28
A7	15.07 ± 1.32	B7	14.13 ± 0.11	C7	11.64 ± 0.22	D7	9.13 ± 0.14
A8	15.53 ± 1.55	B8	13.93 ± 0.63	C8	12.43 ± 0.42	D8	10.06 ± 0.52

A total of 32 samples, 8 each for cassava mash (A1 to A8), pre-fufu (B1 to B8), fufu (C1 to C8) and gari (D1 to D8), were analysed. The results are expressed as the mean ± S.E. of 4 determinations.

1980; Muzanila et al., 2000; Iglesias et al., 2002; Abiona et al., 2005).

The mean percentage difference between the results of 32 samples is 19.85% which agrees with a value of 20% obtained in an earlier study (Egan et al., 1998). The analysis of this work also shows a loss in the cyanogen content of pre-fufu mash and fufu during processing as presented in Table 1. This may be expected since the cyanogen content of cassava mash reduces with time (Benjamin and Emmanuel, 1984).

It has further been shown in the present work that treatment of cassava mash with linamarase solution could enhance the liberation of trapped linamarin and thus result in its hydrolysis and removal. This could pave the way for modifying existing food processing procedures to make cassava consumption safer. The  $9.87 \pm 0.64$  mg/kg of residual cyanide in gari samples analysed still falls within the 10 mg/kg value recommended by WHO and thus tolerable for consumption. It is therefore not unreasonable to conclude that cyanomethemoglobin complex formation method is useful for estimating and monitoring the cyanogen content of raw and cassava products.

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