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Comparison of an Alkaline Picrate and a Pyridine-pyrazolone Method for the Determination of Hydrogen Cyanide in Cassava and in its Products

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A determination of hydrogen cyanide in fresh cassava tissues and in processed cassava products using an alkaline picrate procedure and a pyridine-pyrazolone method which uses Conway vessels has been made. Results obtained for the fresh cassava tissues by the two methods were similar. Statistically significant higher values for processed cassava products were obtained by the alkaline picrate method which measured both glycosidic and non-glycosidic cyanide. The suggestion is reaffirmed that hydrogen cyanide in processed cassava products may exist in two forms: glycosidic and non-glycosidic (entrapped) cyanide.

Keywords: Cyanide; cassava; alkaline picrate; pyridine-pyrazolone.

1. Introduction

Cassava (*Manihot esculenta Crantz*) is widely used throughout Africa as an important energy source.[1] It is gaining tremendous importance as livestock feed and in the starch, textile and alcohol industries,[2] but it has the major disadvantage that it contains cyanogenic glycosides capable of liberating hydrogen cyanide.[3] Cyanide inhibits many metalloenzymes by forming stable complexes with them.[4] The toxicity of cyanide depends primarily upon its potency as a respiratory poison, its site of action being the cytochrome oxidase system of aerobic organisms with which it forms a highly stable complex, thereby producing death by cellular anoxia.[5] However, various communities using cassava as food in different forms have always used various processing methods to obtain cassava seemingly free of harmful amounts of cyanogenic glycosides.[2,3] Studies,[6,7] have shown that food items prepared by these traditional methods still contain varying amounts of residual cyanogenic glycosides, or HCN.

A pyridine-pyrazolone method for the determination of cyanide in aqueous solutions has been described.[8,9] Subsequent modification by Mao *et al.*[10] which employs Conway vessels have been used for the determination of cyanide in plant tissue extracts. Preliminary experiments in this laboratory using this method gave very low values of cyanide in processed cassava products and the present work was carried out to investigate the technique in more detail and compare it with the suggested alkaline picrate procedure of Ikediobi *et al.*[11]

2. Experimental

2.1. Cassava and cassava products

2.1.1. Cassava parenchymal tissue or flesh

Fresh cassava tubers were obtained from a local cassava plant grown on the university campus. After the removal of the peel, a transverse section was cut from the tuber. Using three cork borers of different diameters, a central disc of the peeled parenchymal tissue was divided into

three radial zones; two annuli and a residual circle referred to as outer, middle, and inner. Separate portions of these were taken and quickly transferred to 0.1M HCl to inactivate the endogeneous linamarase.

2.1.2. Gari

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

2.1.3. 'Akpu'

Peeled cassava tuber is fermented in a large quantity of water and then reduced to pulp which is known as 'akpu'. 'Akpu' is usually wet when freshly prepared.

2.1.4. Powdered cassava or 'Lafun'

This is prepared in a similar way to 'Akpu' but with drying. The final product is a white powder.

Samples of the above gari, 'akpu' and 'lafun' were purchased from the New Benin Market, Bendel State, Nigeria where they were being sold in open basins.

2.2. Reagents

Analytical grade chemicals were used. Linamarin was a product of Calbiochem Ltd, San Diego, USA. Chloramine T, sodium dihydrogen phosphate, acetone, petroleum ether, chloroform and potassium cyanide were products of Merck, E (Darmstadt, FRG). All the other chemicals used were purchased from British Drug Houses (BDH) Chemicals Ltd, Poole, Dorset. Partially purified cassava linamarase was prepared from the peel of freshly harvested cassava tuber according to the method of Ikediobi *et al.*[11]

2.3. Apparatus

A Pye-Unicam SP6-200 spectrophotometer in a fume chamber was used to read the blue colour obtained in the pyridine-pyrazolone procedure while a Pye-Unicam SP-1800 spectrophotometer was used to read the deep orange colour obtained in the alkaline picrate method.

2.4. Methods

2.4.1. Pyridine-pyrazolone procedure

Details of the extraction of cyanogenic glycosides from cassava and cassava products were as previously described.[11] The hydrogen cyanide content of the extracts was determined by the method according to Mao *et al.*[10] except that partially purified cassava linamarase was used for hydrolysis instead of sweet almond emulsin and a 24 h period was allowed for hydrolysis/diffusion time.

2.4.2. Alkaline picrate method

This was carried out as described previously.[11]

2.5. Statistical analysis

Data were analysed using Student's t-test.[12]

Table 1. Comparison of precision of the two methods

	Alkaline picrate method		Pyridine-pyrazolone method	
Sample	mg HCN kg^{-1} freshwt	Coefficient of variation (%)	mg HCN kg^{-1} freshwt	Coefficient of variation (%)
Fresh cassava tuber	222.3±1.2	2	208.3±2.1	3

Results are expressed as the mean ±s.e. (mean) of 10 determinations.

3. Results

Table 2. Glycoside content (mg HCN kg $^{-1}$ freshwt) in different parts of the cassava tuber using the two methods

Sample	mg HCN kg^{-1} freshwt	
	Alkaline picrate method	Pyridine-pyrazolone method
Peel	372.0±14.4	341.3±17.4 (n.s.)
Outer	244.0±18.0	223.7±6.7 (n.s.)
Middle	182.3±5.4	161.7±4.6 (n.s.)
Inner	153.7±3.5	136.7±1.5 (n.s.)

Results are expressed as mean±s.e. (mean) of four determinations.
n.s., not statistically significant compared to the values obtained by the alkaline picrate method ($P>0.2$).

Table 3. Comparison of the results of HCN content of cassava products (A–N) using the two methods

Sample	Description	Moisture content (%)	Mg HCN kg $^{-1}$ dry wt	
			Alk. picrate method	Pyridine-pyrazolone method
A	Gari	17	84±7.6	4.3±0.1
B		16	151±4.7	3.3±0.2
C		16	179±10.1	3.2±0.1
D		17	130±10.1	5.1±0.3
E		16	78±8.7	3.5±0.2
F		16	95±6.7	4.7±0.3
G	'Lafun' (cassava flour)	4	60±2.9	5.5±0.2
H		3	71±3.1	4.2±0.2
I		5	89±15.5	4.2±0.7
J		44	74±4.1	4.5±0.4
K	'Akpu'	54	101±8.2	1.6±0.4
L		51	63±5.3	1.6±0.2
M		52	97±1.8	1.6±0.4
N		55	78±5.2	3.5±0.5

The results are expressed as the mean±s.e. (mean) of four estimations.
The differences in values obtained by the two methods are statistically significant in all cases, $P<0.001$.

4. Discussion

The data in Table 1 demonstrate that the two methods have essentially the same precision as indicated by the coefficient of variation.

In all the cassava products analysed (Table 3) the modified alkaline picrate procedure of Ikediobi *et al.*[11] produces statistically significant higher values for HCN in the processed cassava product compared to the values obtained for the same materials using the pyridine-pyrazolone method in which Conway vessels are used. On the other hand, there was a good agreement between the results for fresh cassava tissues using the two methods (Table 2). Moreover, when for the same cassava products, an alkaline picrate estimation procedure is carried out on an aliquot of the alkaline-cyanide solution in the centre well of the Conway vessel, the results obtained were similar to those obtained by the pyridine-pyrazolone method.

In the alkaline picrate procedure, the colour reaction for cyanide is carried out in the tube containing the reaction mixture while in the pyridine-pyrazolone method, cyanide liberated by

linamarase catalysis is allowed to diffuse out of the reaction mixture and is absorbed by the alkaline solution in the centre well of a Conway vessel before colour reaction is carried out on the alkaline-cyanide solution in a tube (without reaction mixture). This indicates that cyanide in processed cassava products may exist in two forms; (a) cyanogenic glycosides (linamarin and lotaustralin) hydrolysable by linamarase and (b) non-glycosidic cyanide. Butler *et al.* [13] suggested that once cyanogenic glycosides in cassava are hydrolysed to give HCN, the gas is entrapped by reacting with traces of metal ions in cassava[6] and/or reacts with the carbonyl of hexoses in cassava[14] to form cyanohydrins. It is therefore not unreasonable to conclude that the non-glycosidic cyanide in the processed cassava products are these cyanohydrins and the metal cyanides which together with the cyanogenic glycosides are measured by the alkaline picrate procedure of Ikediobi *et al.*[11] The pyridine-pyrazolone method on the other hand only measures the HCN released by enzyme hydrolysis in the intact residual cyanogenic glycoside in the cassava product. This limitation should, therefore, be borne in mind in the choice of a method for the estimation of total cyanide in cassava and cassava products.

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