Simple Picrate Method for the Determination of Cyanide in Cassava Flour

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Abstract

A simple picrate method was used to quantify the cyanide contents of food samples. The cyanide in the food samples reacted with hot 20% HCl solution to produce hydrogen cyanide vapour which reacted with alkaline picrate test strips to form red colour on the test strips. The red coloured complex on the strips was extracted with 50% ethanol solution and the absorbance of the extract was measured at 510nm using a spectrophotometer. The method was reproducible and cyanide as low as 1 microgram could be determined. Cyanide levels of all the cassava varieties tested were higher than the 10ppm WHO safe level. Recovery of cyanide from acyanogenic foods fortified at levels of 5 and 10mg KCN/10g were 98.6% and 99.1% respectively. The picrate method is simple and useful for routine determination of cyanide content of cassava flour.

Keywords: Picrate method, Cyanide, Cassava flour

Introduction

Cassava is the third most important food source in the tropics after rice and maize and it is a staple food for at least 600 million people (Stupak, *et al.*, 2006). However, cassava consumption both as human food and animal feed is associated with certain health problems (Padmaja, 1995). In cassava, there are two cyanogenic glucosides, linamarin and lotaustralin (McMahon *et al.,* 1995). The breakdown of these cyanogens releases hydrogen cyanide (HCN) which causes chronic cyanide toxicity among populations subsisting on cassava (Howlette *et al.*, 1990, Akintowa *et al.*, 1994; Osuntokun, 1994). The total cyanide content of cassava parenchyma depends on the cultivar, the environment and various other factors. There is a continuous distribution of cyanide content from 1 to 500 ppm (Bokanga, 1994). Although acyanogenic roots were reported, but this has never been confirmed (Bradbury and Egan 1992). Roots containing only 1 – 2 ppm have been reported (Bradbury *et al.,* 1991). There are reports of cyanide levels greater than 1000 ppm in Tanzania (Mlingi *et al.*, 1992). In some tropical countries where cassava is a major staple food product, it is difficult to measure the cyanogens levels of cassava because of lack of facilities to carry out the assay procedure and problems associated with accurate methods of analysis. This paper describes a simple and rapid method for the determination of cyanide in cassava flour which may be useful in tropical developing countries.

Materials and Methods

Sample collection: Five cassava varieties (TMS 98/0510, TMS 98/0505, TMS 98/0581, TMS 97/2205 and TMS 50395) were collected. The cassava samples (2kg per sample) were harvested from a farm in the National Root Crops Research Institute, Umudike, Nigeria after 10 months of planting. The cassava roots were peeled with a knife. Maize (NARZO-16) and groundnut (SAMNUT-3) varieties were obtained from the International Institute of Tropical Agriculture Ibadan, Nigeria. All the samples were dried, milled and sieved with a stainless steel mesh. The maize and groundnut samples were fortified at levels of 5 and 10 mg KCN/10g respectively.

Determination of cyanide: Alkaline picrate reagent was prepared by a modification of the method described by Williams and Edwards (1980) as follows: Test tubes with 2mL of 2% KOH and 1mL of picric acid: Na_2CO_3:H_2O (1:5:200 v/w/v) were prepared. Standard absorbance curves were made with 3 Whatman No 1 papers each with a dimension of 8×1cm. The papers were dipped into the alkaline picrate solution for 15 minutes. The picrate impregnated papers were removed from the solution and used immediately for cyanide determination. Cyanide solutions containing (50-200µgKCN/mL) were each prepared in glass bottles. The cyanide was acidified with 20% HCl solution heated to 80^0C and immediately sealed with 3 picrate impregnated papers. The system was incubated at room temperature (28 ± 2^0C) for 24 h. The red- coloured complex formed was eluted with 50% ethanol solution for 30 minutes. The eluate absorbance was measured at 510nm using a Spectrumlab 23A spectrophotometer (Fig. 1).

Preparation of samples and cyanide analysis: Whatman number 1 filter papers (8 x 1cm) were dipped into the alkaline picrate solution and drained free of excess liquid just before use. The filter paper strips were prepared under identical conditions. The samples (10g per sample) were loaded into glass bottles and acidified with 15mL of hot 20% HCl solution. The bottles were sealed with 3 picrate impregnated strips suspended above the acidified samples as the bottles were sealed. The system was left at room temperature (28 ± 2^0C) for 24h. The red-coloured picrate paper strips were removed from the bottles and rinsed in 5mL of 50% ethanol solution for 30 minutes and the absorbance of the solution measured at 510nm using a Spectrumlab

23A spectrophotometer. Cyanide levels of the samples were extrapolated from the standard curve.

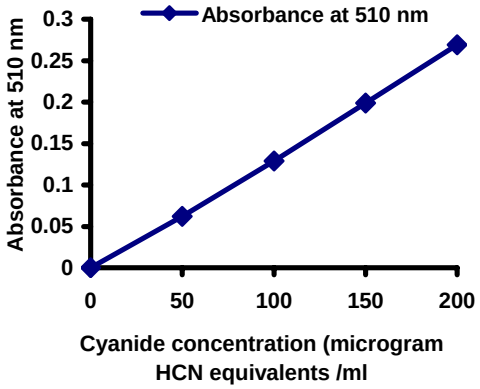

Fig. 1: Calibration curve for cyanide determination by the alkaline picrate method

Results and Discussion

A linear relationship was obtained between cyanide concentrations of 0-200 µg HCN equivalents/mL. The values obtained were reproducible and cyanide as low as 1µg could be detected. The picrate method was used to determine the cyanide content of cassava flour and two acyanogenic food products fortified at levels of 5 and 10mg KCN/10g. In all cases, 10g of sample was sufficient for quantitative analysis.

The cyanide in the test samples evaporated as HCN with the addition of 20% HCL solution heated to 80^0C. Tempest (1959) reported the volatitization of HCN from cyanogenic biological substances with the addition of hot mineral acid. In the presence of sodium carbonate, the quantity of HCN released was sufficient for quantitative determination. The alkaline picrate solution acted as a trapping agent of the liberated HCN (Williams and Edwards, 1980). The HCN liberated slowly changed the colour of the picrate paper strips from orange to red at 28± 2^0C. The colour was fully developed after 20h.

For quantitative determination of cyanide by the suspended strip technique, an important factor is the amount of picrate present on the test strips. In preparing the test strips, efforts were made to prepare the strips under identical conditions so that the picrate contents of the strips were as constant as possible.

The result presented in Table 1 shows the cyanide levels of cassava flour samples. There values were compared with values previously reported for these cassava varieties by Oyekan and Sarumi (2004) who determined the cyanide levels by acid hydrolysis method. The picrate method gave higher cyanide levels than reported except TMS 98/0581 variety which gave a lower cyanide level. The variations in the cyanide content of similar cassava varieties may be as a result of some environmental factors (Bradbury *et al*., 1991) and the method of analysis.

Recovery of cyanide from maize and groundnut samples fortified at levels of 5 and 10mgKCN/10g were 98.6% as 99.1% respectively. Therefore the picrate method gave acceptable result for cyanide quantification. The picrate method is also comparable to the linamarase method for cyanide estimation in cassava flour (Ikediobi *et al*., 1980; Yeoh *et al*., 1997). The picrate method was considered more suitable for cyanide determination of cassava food products in tropical developing countries where methods of linamarase enzyme extraction and storage may be lacking.

Table 1: Cyanide levels of cassava flour samples determined by the suspended strip picrate method.

Cultivar	Cyanide level (mgHCN equivalents /10g sample)
TMS 98/0510	1.58
TMS 98/0505	0.86
TMS 98/0581	1.01
TMS 97/2205	1.26
TMS 50395	0.52

The WHO safe concentration for cassava flour is 10ppm (FAO/WHO, 1991). All the cassava samples analyzed gave cyanide levels greater than 10ppm (Table 1). Consumption of cassava and its products that contain large amounts of cyanide has resulted in several cyanide poisoning with symptoms of vomiting, nausea, dizziness, stomach pains, weakness, headache, diarrhea and occasionally death (Mlingi *et al*., 1992, Akintonwa *et al*., 1994). To minimize further outbreaks of these health disorders in populations who rely on cassava as a staple food, development of this picrate method to assay cyanide levels in cassava food products and to quantify the retention of cyanogens in finished cassava products becomes necessary.

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