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Simple Picrate Paper Kit for Determination of the Cyanogenic Potential of Cassava Flour

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Abstract: A simple picrate paper kit method was developed for the semi-quantitative determination of the cyanogenic potential for cassava flour. The method involved the addition of linamarase and pH 8 phosphate buffer (absorbed in a filter paper disc) to 100 mg of flour +0·5 ml water placed in a small vial. A rectangle of yellow picrate paper attached to a plastic strip was added and the vial capped and left overnight. The yellow paper changed colour towards brown and its colour was compared with that of a standard colour card with 10 shades of colour which corresponded with cyanogenic potentials of 0–800 mg HCN equivalents kg^{-1} flour (ppm). To obtain a more accurate measure of cyanogenic potential (±20%) the paper was eluted with water and the absorbance measured at 510 nm. The cyanogenic potential in ppm was determined from a calibration graph. The methodology is available in kit form. The simple method may be used in the field by a relatively unskilled person. The more accurate method requires a spectrophotometer and is suitable for use in simple laboratories in developing countries. © 1998 SCI.

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Key words: cassava flour; cassava roots; cyanogenic potential; cyanide; picrate paper; simple kit method; semiquantitative method

INTRODUCTION

Cassava (*Manihot esculenta*) is the staple food of more than 500 million people in the tropics many of whom are very poor (Cock 1985). The leaves and roots of the plant contain a cyanogenic glucoside (linamarin) and a small amount of lotaustralin (methyl linamarin). The linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of the enzyme linamarase, which is also produced by the plant. The acetone cyanohydrin decomposes rapidly in neutral or alkaline conditions liberating hydrogen cyanide and cyanide ion. The sum of the amounts (HCN equivalents) of linamarin, acetone cyanohydrin, hydrogen cyanide and cyanide ion equals the cyanogenic potential of the cassava sample.

The cyanogenic potential of cassava roots and leaves range from 2 to >1000 ppm HCN (mg HCN equivalents kg^{-1}, fresh weight), (Cooke and de la Cruz 1982; Bradbury *et al* 1991; Bokanga 1994a, b). It is generally considered that cassava roots that contain >100 ppm HCN equivalents should be processed to reduce the cyanogenic potential, before use for human consumption. Some traditional processing methods developed in South America and West Africa remove nearly all the cyanogens from cassava products, but other methods such as those used for cassava flour production in East Africa and Indonesia reduce, but do not eliminate the cyanogens present (Nambisan and Sundaresan 1985; Bainbridge *et al* 1994; Mlingi 1995).

Whilst there are few reports of poisoning and death due to cyanide intake from cassava consumption (Mlingi *et al* 1992; Akintonwa *et al* 1994; Cliff 1994), there are several disorders which have been associated with regular intakes of sub-lethal quantities of cyanogens. These include (1) goitre and cretinism which are due to iodine deficiency, but may be exacerbated by intake of cyanide (Delange and Ahluwalia 1983;

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Delange *et al* 1994), (2) tropical ataxic neuropathy (Osuntokun 1994), and (3) konzo, formerly called epidemic spastic paraparesis, an irreversible spastic paralysis of both legs which affects children and women (Howlett *et al* 1990; Banea *et al* 1992; Cliff 1994; Howlett 1994). Konzo has occurred in Tanzania, Zaire (Banea 1993) and Mozambique usually during periods of famine and drought, when food is scarce and time is not taken to process cassava properly to remove cyanogens.

In order to develop strategies to deal with and hopefully prevent further outbreaks of these crippling disorders, it is important to have available good methods for determination of the cyanogenic potential of cassava roots and cassava products such as flour and gari. Many quantitative methods of analysis have been developed, some based on direct determination of linamarin (Brimer *et al* 1983; Yeoh and Truong 1993; Yeoh and Tan 1994), but most have been based on the three steps of (1) extraction of cyanogens from the substrate using acid, which inactivates linamarase and prevents its catalytic breakdown of linamarin, (2) hydrolysis of cyanogens to hydrogen cyanide and (3) determination of cyanide colorimetrically (Cooke 1978; Bradbury *et al* 1991; Essers *et al* 1993; Brimer 1994; reviewed by Bradbury *et al* 1994). There are also a number of simple methods available (Sadik *et al* 1974; Cooke *et al* 1978; Bradbury and Egan 1992; O'Brien *et al* 1993, 1994).

Many of the quantitative and simple methods rely on the addition of exogenous linamarase to catalyse hydrolysis of linamarin. This presents a problem in developing countries because of lack of equipment for preparing linamarase using existing procedures and the high cost of commercial linamarase. One solution to this problem was the development of a quantitative method of analysis based on acid hydrolysis of linamarin (Bradbury *et al* 1991). A second possible solution is the use of endogenous linamarase (where it is present, as in cassava tubers and leaves) to achieve quantitative hydrolysis of linamarin (Aalbersberg 1996). A third solution has been the recent development of a simple method for isolating linamarase from cassava leaf, that does not require laboratory equipment such as a centrifuge and should therefore be useful in developing countries (Yeoh *et al* 1997).

The problem remains that nearly all of these quantitative and semiquantitative methods can only be carried out properly by skilled personnel in a reasonably well-stocked laboratory. To be able to monitor levels of cyanogenic potential of cassava flour samples in the field, a simple method is needed that can be used by unskilled personnel. This objective has been achieved in the present work. At the same time it has also proved possible to produce a semiquantitative method for use in the laboratory, based on elution of the picrate paper and spectrophotometry (Indira and Sinha 1969; Ikediobi *et al* 1980; Williams and Edwards 1980).

MATERIALS AND METHODS

Materials

Potassium cyanide, AR (98% minimum) was obtained from Prolabo, Paris, France, acetone cyanohydrin (99%) from Aldrich Chemical Co. Milwaukee, WI, USA and linamarin (95% minimum) from Sigma Chemical Co, St Louis, MO, USA. Tetra base (4,4′-methylenebis-*N*,*N*-dimethylaniline) and copper (II) ethyl acetoacetate were from Eastman Kodak, Rochester, NY, USA. Picric acid and linamarase were obtained from BDH Ltd, Poole, UK and linamarase was also prepared by Yeoh (1989) from cassava leaves. Samples of cassava flour were sent from Mozambique and Indonesia by Drs J Cliff and M Djazuli, respectively. Some samples were also obtained from Mr Suharno of the University of Melbourne. Fresh cassava roots were obtained from the Plant Culture Facility at the Australian National University.

Quantitative determination of cyanogens

The determination of linamarin, acetone cyanohydrin and free cyanide ($HCN + CN^-$) was carried out using the acid hydrolysis method (Bradbury *et al* 1991) as modified by Bradbury *et al* (1994).

Determination of cyanogenic potential using tetra base papers

The method followed that of Bradbury and Egan (1992) with replacement of cupric acetate by cupric ethylacetoacetate, which produced a more stable tetra base-treated filter paper.

General method of determination of cyanogenic potential using picrate papers

A weighed amount of cyanogenic sample was placed in a small flat-bottomed glass or clear plastic vial (about 25 mm diam., 50 mm high), followed by a known amount of buffer solution and a known amount of linamarase. A yellow strip of filter paper [previously prepared by dipping filter paper in a solution of moist picric acid (0·5%, w/v) in 2·5% (w/v) sodium carbonate, allowing the paper to dry in air and then cutting to the required size] was suspended above the cyanogenic sample and the vial immediately tightly stoppered. The vials were normally placed in an oven at 30°C overnight and the change in colour from yellow to orange to brown was observed. The shade of colour was compared with that of a colour chart (see below) to obtain the amount of cyanogen present.

The paper was immersed in water for about 30 min and the absorbance measured at 510 nm against a simi-

larly prepared blank developed in the absence of cyanogen. By means of a standard curve (see below) of absorbance vs HCN content (μg) it was possible to obtain a semiquantitative measure of the amount of cyanogen present.

RESULTS AND DISCUSSION

Comparison of simple tetra base and picrate methods for determination of cyanogens

These two methods were compared using a solution of KCN which gave a range from 1 to 10 μg HCN. A solution of KCN was added to the vial, then excess sulphuric acid, followed immediately by suspension of the tetra base or picrate paper and capping of the vial. The colour development of the papers was followed in experiments at room temperature and also at 30°C for periods up to a maximum of 5 days. In the tetra base method the blue colour started developing almost immediately, peaked after 1-3 h (dependent on the amount of HCN and temperature) and then slowly dropped off over 24 h. The orange–brown colour of the picrate paper developed more slowly. It was fully developed after 5 h at 30°C and 22 h at 20°C and was stable for 48 h. We found that the detection limit of the two methods was approximately equal at about 0·5 μg HCN, whereas in our previous work we had reported that the tetra base method was more sensitive (Bradbury and Egan 1992). We believe that the apparent discrepancy was probably due to the more rapid development of the blue colour and its easier visualisation in the tetra base method. However, O'Brien *et al* (1994) found that tetra base performed more reliably with low cyanogen samples. Table 1 gives a comparison between the two methods. The tetra base method is more rapid but the blue colour is not as stable over time as the yellow–orange–brown of the picrate method. The latter has the great advantage that the colour may be quantitatively eluted off the paper and its intensity determined spectrophotometrically, which is not possible with the blue colour. Furthermore, picrate papers are much more stable to storage in the dark and in light than tetra base papers. Finally, the tetra base chemical is carcinogenic and hence more hazardous than picric acid, which is stable in alkaline solution and in filter paper. Thus, picrate papers have four important advantages over tetra base papers and all subsequent work has therefore been done using picrate papers.

The optimum pH for breakdown of cyanogens to HCN

Because of the different pH requirements for decomposition of the various cyanogens it was important to obtain the optimum pH for the process. Thus, the breakdown of linamarin to acetone cyanohydrin is catalysed by linamarase that has maximum activity at about pH 6 (Yeoh 1989), the acetone cyanohydrin produced is reasonably stable in acid (White *et al* 1994) but

TABLE 1
Comparison of picrate and tetra base methods for cyanogens in cassava flour

Property	*Picrate method*	*Tetra base method*
Detection limit (μg HCN)	About 0·5	About 0·5
Ease of observation	Yellow to orange to brown more difficult to see	White to blue easier to see
Speed of test	5 h at 30°C, overnight at 20°C	1–3 h
Stability of colour over time	Stable up to 48 h	Stable for 3 h and then drops off
Elution of colour from paper and spectrometric determination at 510 nm	Very satisfactory	Not possible
Stability of papers on		
(1) Storage in dark at room temperature	Stable for 1 month with no darkening[a] slight darkening after 6 months[a]	Lose sensitivity in 2 months
(2) Storage in dark at 37°C	Slight darkening after 1 month	Lose sensitivity in 1 week
(3) Storage on bench in lab.	Slight darkening after 1 month	Lose sensitivity in 1 week
(4) Exposure to sunlight	—	Green after 20 min and loses sensitivity
Hazards of chemicals	Picric acid explosive when dry, stable in filter paper or in alkaline solution[b]	Tetra base is carcinogen

[a] Studied quantitatively, see Fig 2.
[b] Picrate solution stored for 14 months and papers made from it compared with papers made from fresh picrate solution. No change was observed over 14 months (see text).

decomposes rapidly to cyanide in alkaline solution and the cyanide produced may be retained in solution as CN^- in alkaline solution.

In order to determine the optimum pH, semi-quantitative picrate paper experiments were made in buffer solutions of pH 3, 4, 5, 6, 7 and 8 using equal molar amounts (0·37 μmoles) of the cyanogens (1) KCN, (2) acetone cyanohydrin and (3) linamarin. The absorbances obtained from the KCN solutions were constant over the pH range 3–8. The other standards gave low absorbances at low pH, which increased with increase of pH and became equal to the KCN standard at pH 8. This result shows that at pH 8, equal molar amounts of the three cyanogens break down completely to HCN and produce the same absorbances using the picrate paper method.

Effect of amount of picrate in paper on colour development and calibration curve

We have found that the graph of absorbance of the solutions from the reacted picrate papers as a function of their cyanogen content (μg HCN) is dependent on the amount of picrate in the paper. This confirms the findings of Williams and Edwards (1980) who obtained different graphs using one, two or three picrate paper strips. The effect is shown in Fig 1, in which a comparison is made between picrate papers of about the same size using Whatman No 1 and Whatman No 3MM paper, which is about twice as thick. One notes that there is no difference between the results up to 10 μg HCN, because there is excess picrate present to react with the HCN. However, with larger amounts of HCN, the 3MM paper gives linear behaviour up to 40 μg HCN and then flattens off due to the approaching 'saturation' of the picrate paper with HCN. This effect occurs above 15 μg HCN with the No 1 paper, because it has only about one half of the picrate capacity of the No 3MM paper. The flattening of the curve at higher amounts of HCN reduces the accuracy of the determination in the high range. Since we are interested in working in the range of 0·5–80 μg HCN, we chose to use Whatman No 3MM paper rather than the more readily available No 1 paper.

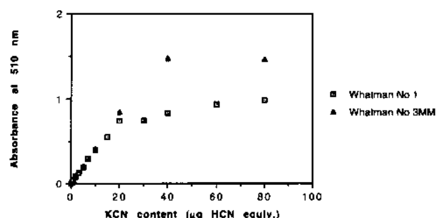

Fig 1. Graph of absorbance (A) at 510 nm vs KCN content (expressed as μg HCN) using 10 mm × 30 mm picrate paper strips of (a) Whatman No 1 paper, (b) Whatman No 3MM paper, which is approximately twice the thickness of the No 1 paper.

Effect of temperature of experiment on colour development

To 100 mg samples of flour in plastic vials was added 0·5 ml 0·2 M phosphate buffer at pH 8·0 and 100 μl linamarase solution (0·055 U). A 30 mm × 10 mm 3MM picrate paper glued to a plastic strip (see below) was placed in the vial which was sealed by a screw cap and maintained at 19, 30 or 37°C for ~18 h. The tube was opened, the colours were compared visually and the picrate paper was removed from the plastic strip. The paper was eluted in 6·0 ml distilled water for 30 min and the absorbance measured at 510 nm. Visual observation of the picrate papers showed that for each sample of flour the colour intensity increased from 19 to 30°C and there was a slight further increase from 30 to 37°C. These increases were much less than would amount to a change of one level on the colour chart (see below). The absorbance results in Table 2 showed that there was an increase in absorbance with increasing temperature, but that exposure over 3 days caused some increase in the results at 19°C but no change in those at 30°C and 37°C. Thus, at 19°C after 18 h the reaction appears to be about 90% complete, whereas at 30° and 37°C the reaction is complete in 18 h.

In practice, we have used 30°C as the temperature for further work, which would approximate to conditions in the tropics. It is noted that there would be no change in the assessment using the simple colour matching method over this range of temperatures. A comparison of the picrate results with those obtained using acid hydrolysis shows good agreement in the low range (sample 12), but the picrate results appear higher than the acid hydrolysis result in the high range (sample 29). However, this difference is not found in the results of samples 18 and 19 given in Table 3.

Stabilities of picrate papers and alkaline picrate solution with time

Picrate papers (Whatman No 1) were prepared, wrapped in gladwrap and stored in the dark at room temperature. Samples (40 mm × 10 mm) were taken at time intervals up to 11 months, eluted with 3·0 ml of distilled water and the absorbance at 510 nm measured against distilled water as a blank. There is a linear increase in absorbance with time due to a very gradual darkening of the paper (see Fig 2). The extent of darkening of the paper after 1 month is not visible to the eye (Table 1) and thus picrate papers may be used for at

TABLE 2
Effect of experimental temperature on colour development and flour analysis

Sample of flour (Table 3)	*Temperature of experiment (°C)*	*A at 510 nm*	*Total cyanide (ppm) by*	
			Picrate method[a]	*Acid hydrolysis*
12	19	0·021	9·5	14
12	30	0·029	13	
12	37	0·032	15	
27	19	0·066	30	30
27	30	0·086	39	
27	37	0·092	42	
29	19	0·193[b]	87	79
29	30	0·226[b]	102	
29	37	0·234[b]	106	

[a] Each result was the average of three experiments.
[b] In these cases there were 3 experiments at each temperature and samples were removed for analysis after about 18 h, 42 h and 66 h. At 19°C the absorbances were 0·193, 0·195 and 0·213 respectively, whereas at 30° and 37°C the absorbances were essentially constant over time.

least one month after preparation, providing that they are wrapped up and stored in the dark.

The response of Whatman No 1 picrate papers of various ages to HCN liberated from standard KCN solutions is shown in Fig 3. It is evident that in the low, linear range there is little difference in response between the three papers, but above 10 μg HCN there is a progressive decrease in response with increasing age of the paper. Clearly, the use of picrate papers stored for more than about one month, will give reduced absorbances (in the high range) and hence low results.

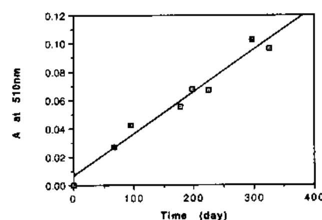

Fig 2. Slow ageing of Whatman No 1 picrate papers. Absorbances measured against water at 510 nm of water eluates of picrate papers stored in the dark at room temperature for long periods.

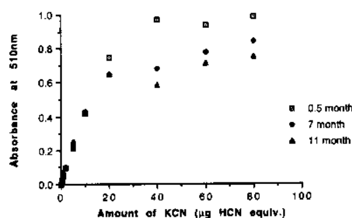

Fig 3. Absorbances at 510 nm of water eluates of Whatman No 1 picrate papers of various ages from 0·5 to 11 m, plotted against the amount of KCN, expressed as μg HCN.

Solutions of 0·5% picric acid in 2·5% sodium carbonate stored in stoppered bottles at room temperature for various times up to 14 months, were used to prepare picrate papers. These papers gave the same response with HCN liberated from KCN, as picrate papers prepared from fresh picrate solution. This shows that it is possible to use picrate solutions up to at least one year old to produce picrate papers.

Development of a standard curve using different cyanogen standards

The results are shown in Fig 4 of duplicate analyses of the three cyanogen standards KCN, acetone cyanohydrin and linamarin using 30 mm × 10 mm picrate Whatman 3MM papers and eluting the colour from the paper using 6·0 ml water.

It is found that the KCN and acetone cyanohydrin results agree within about $\pm 2\%$ over the whole range of concentrations, but that the linamarin results are, on average, about 15% higher than the others over the whole range of concentrations. Each cyanogen standard gives a linear graph up to 40 μg HCN equivalent, from which the gradients of the linear graphs are readily calculated. These values are, respectively, for KCN, acetone cyanohydrin and linamarin 0·0203 (0·0261, 0·0226), 0·0200 (0·0276, 0·0233) and 0·0238 (0·0247, 0·0208), where the two sets of results in brackets refer to

TABLE 3
Summary of cyanogens and linamarase present in cassava flours

Sample	Cyanogenic potential (ppm) by			Content of cyanogens (ppm) by acid hydrolysis			Linamarase[b] (U per 100 g)
	Simple picrate[a]	Semiquantitative picrate	Acid hydrolysis	Linamarase	Acetone cyanohydrin	Free (CN^-, HCN)	
Mozambique							
1	10	11	8·6	—	—	—	—
2	20	15	14	—	—	—	—
3	5	1·0	2·7	—	—	—	—
4	10	9·5	8·4	—	—		—
5	5	2·2	2·8	—	—	—	—
6	5	6·0	8·3	—	—	—	—
7	5	3·5	5·4	—	—	—	—
8 (gari)	20	20	16	—	—	—	0
9 (gari)	15	13	13	—	—	—	0
10	0	1·5	3·9	3·0	0	0·9	3·2
11	5	6·2	8·0	6·5	0·7	0·7	5·0
12	7	11	14	13	0·2	0·5	25
13	5	5·0	5·9	3·6	1·6	0·6	1·5
14	10	14	15	14	0·5	0·7	1·9
15	0	1·0	2·9	2·0	0·1	0·8	1·9
16	5	4·0	5·0	4·4	0·1	0·5	2·2
17	7	4·4	5·8	3·6	1·5	0·7	14
18	100	86	94	90	2·0	1·7	—
19	95	82	95	91	2·1	1·6	—
20	30	23	36	34	0	2·2	—
21	0	0·5[c]	2·1	1·6	0	0·5	—
22	40	26	57	54	1·2	1·7	—
23	85	67	71	62	7·6	1·6	—
24	35	28	39	28	7·9	3·4	—
25	4	7·8[c]	14	12	0	2·3	—
Indonesia							
26	50	53	47	46	0	0·5	0
27	30	30	30	29	0	0·8	0·13
28	70	75	70	69	0	0·7	0·08
29	90	86	80	79	0·1	0·4	0·18
Australia							
30	12	18	13	8·5	3·5	0·9	0·48
31	10	10	7·9	2·9	4·2	0·8	0·82
32	10	12	8·3	0·9	6·7	0·8	0·74
33	5	4·5	4·7	1·5	2·4	0·8	0·14

[a] Samples 18–25 determined in triplicate using a colour chart (see below), prepared from fresh KCN standards. The other samples were 'scored' by an older method using a scale 0, 1, 2, 3, 4, 5 similar to that used for tetra base papers by Bradbury and Egan (1992). The scores were converted into the absorbance (determined by the semiquantitative method) and thence into the cyanogenic potential (ppm) by means of two graphs.
[b] Determined by method of Bradbury and Egan (1994).
[c] These results were low and the flour was very coarse due to the preparation procedure. Both samples were ground and analysed again. Sample 25 increased from 7·8 to 10·5 but sample 21 was unchanged.

the corresponding gradients determined with the same cyanogen standards by Essers *et al* (1993), using their new method and another method formerly used by O'Brien *et al* (1991).

The close similarity of the gradients obtained by Essers *et al* (1993) and ourselves is extraordinary, but is actually fortuitous, because the observed gradients depend on the detailed experimental procedure. Thus, for example, in the picrate method, the papers were eluted in 6·0 ml of water, but if the volume were reduced to 3·0 ml, then the absorbances and hence the gradients would double. However, since the gradients are similar, it shows that the picrate method is at least as sensitive as the Konig method.

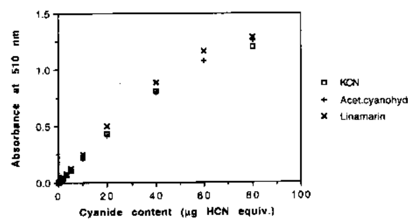

Fig 4. Development of a standard curve of absorbance vs cyanide content (µg HCN equivalents) using Whatman 3MM picrate papers and three different cyanogen standards KCN, acetone cyanohydrin and linamarin.

In principle, one might expect that, within each method, the gradients obtained with each of the cyanogen standards would be the same. However in the picrate method the linamarin gradient is higher by about 18%, than those obtained with KCN and acetone cyanohydrin, which are nearly the same. In both methods of Essers *et al* (1993), the acetone cyanohydrin gradient is about 12% higher than the linamarin gradient with the KCN gradient in between. Since the actual samples of cyanogen standards used in this work and by Essers *et al* (1993) were different, a major source of the difference is undoubtedly the presence of impurities in the 'standards'. Indeed, they are not claimed to be of high purity (see experimental). Another cause of the differences may be the different methodologies used in the three methods used, particularly the sequential steps from linamarin to acetone cyanohydrin to cyanide that require manipulations in the Cooke (1978) methodology but not in the picrate method, where the reagents are all contained within an airtight bottle. An explanation of the source(s) of the variabilities of the gradients across the three cyanogen standards could be resolved by a careful study, which would include preparation of pure cyanogen standards. Until this matter is resolved, there would not appear to be any real advantage in using the particular cyanogen standard that corresponds with the form of the cyanogen compound present in the substrate being studied, as was proposed by Essers *et al* (1993).

Cyanogen and linamarase content of cassava flour determined by different methods

The results obtained for the analyses by different methods of 33 samples of flour from Mozambique, Indonesia and Melbourne, Australia are given in Table 3. It is found that there are wide variations in the cyanogenic potential of the different samples from 2 to 100 ppm HCN equivalents, compared with 10 ppm, which is the safe level recommended by the Codex Alimentarius Committee of the FAO/WHO (1991). About half the samples analysed were below the safe level. The amount of acetone cyanohydrin present in these samples is usually a small fraction of the total cyanogenic potential, which is good because of its acute toxic effects in humans (Mlingi *et al* 1992). However, in four cases of flour produced in laboratory studies in Melbourne, a relatively high percentage of the total cyanogenic potential arose from acetone cyanohydrin. In all cases the free cyanide was a small fraction of the cyanogenic potential. The amount of linamarase present in the flour was very variable from 0 to 25 U per 100 g flour, which showed the necessity for the addition of exogenous linamarase in analysis of flour samples using the picrate method.

Comparison of the analytical methods shows that the cyanogenic potential results obtained by the semiquantitative picrate method show an average deviation of 20% from those obtained by the acid hydrolysis method, providing one excludes results below 5 ppm on the grounds that errors are much larger near the limit of sensitivity of the method. The simple picrate method is seen in Table 3 to be generally less reliable than the semiquantitative method at values below about 10 ppm. Also, where the flour is very coarse (samples 21 and 25), the picrate method tends to give lower results, probably because of incomplete extraction of the linamarin from the coarse particles. In one case a higher result was obtained after grinding. Surprisingly, the coarse and hard gari samples (8 and 9, Table 3) gave results by the picrate method that were about the same as obtained by acid hydrolysis. Nevertheless, it is suggested that with coarse and/or hard samples grinding or pounding before analysis would be useful.

A plot of the results shown in Table 3 for the simple picrate method compared with the acid hydrolysis method gave a good straight line with a correlation coefficient $r = 0.98$ ($r^2 = 0.96$). This means that the simple method gives an acceptable result in 96% of cases, which is much better than that previously obtained using the tetra base method on cassava tubers for which $r = 0.77$ ($r^2 = 0.59$) (Bradbury and Egan 1992). Good straight lines and similar correlation coefficients were also obtained by plotting the results for the semiquantitative picrate vs acid hydrolysis and simple picrate vs semiquantitative picrate.

Preparation of a colour chart for simple picrate kit

A picrate colour chart was prepared by exposing freshly prepared Whatman 3 MM, 30 mm × 10 mm picrate papers to known amounts of HCN liberated from standard KCN solutions, using the methodology described above. On the following day, the picrate papers were aligned on a chart and the colours matched using computer technology. In this way a good match of the colours was possible. The colour chart is used with

100 mg samples of flour (see protocol below) and contains 10 shades of colour from yellow to brown, which correspond to amounts of HCN of 0, 5, 10, 20, 30, 50, 100, 200, 400, 800, mg HCN equivalents kg^{-1} fresh flour (ppm).

Stability of linamarin standard for use in simple picrate kit

In order to check out the methodology, it would be useful to have available a known amount of a standard cyanogen in the simple picrate kit. Filter paper (Whatman 17CHR) 10 mm × 10 mm squares were treated with known amounts of a solution of linamarin in water and allowed to air dry. The stability of the linamarin in the filter paper was studied over three months at room temperature. The absorbance results (Fig 5) do not show a consistent trend with time after preparation of the paper. Thus, incorporation of linamarin-impregnated filter paper into the simple kit, should allow a check to be made on the effective operation of the kit in practice.

Development of a simple picrate kit to determine the cyanogenic potential of cassava flour

The method involved weighing out 100 mg of cassava flour using a small portable balance (SD of 8 weighings = 4%). The flour was placed on top of a 21 mm diam. Whatman 3MM filter paper in a flat-bottomed, clear plastic vial (25 mm id, 50 mm high). This filter paper contained both linamarase and buffer and was prepared beforehand by the following procedure. Phosphate buffer (100 μl), 1 M at pH 8 was added to a 21 mm disc of Whatman 3MM paper and the paper allowed to dry overnight. A 60 μl aliquot of the enzyme mixture (activity of linamarase 0·2 U), which retained 60% of this activity in presence of 1% (w/v) gelatin and 5% (w/v) polyvinylpyrrolidone-10 (Yeoh *et al* 1996), was then applied to the filter paper disc. The paper was again dried at room temperature.

Water (0·5 ml) was added to the flour plus filter paper disc and the yellow picrate paper immediately inserted in the vial. This picrate paper was prepared beforehand by the following procedure. A yellow Whatman 3MM paper (30 mm × 10 mm) was glued, using one small drop of PVA hobby glue, to a plastic strip (10 mm × 52 mm) cut from overhead transparency plastic sheet, so that the upper end of the paper was 5 mm from the upper end of the strip. The vial was immediately sealed with a screw cap lid and allowed to stand at room temperature (25–37°C) for 16–24 h. There was a slow colour change from yellow towards brown. The colour was compared with that of a colour chart containing 10 colours and the cyanogenic potential (mg HCN equivalents kg^{-1}) of the cassava was read off from the chart.

To obtain a semiquantitative estimate of the cyanogenic potential, the plastic backing sheet was taken off the picrate paper, which was immersed in 6·0 ml of water for about 30 min. The net absorbance (after correction for the absorbance of a blank) of the solution was measured at 510 nm and the cyanogenic potential (mg HCN equivalents kg^{-1}) read off the calibration graph (Fig 4).

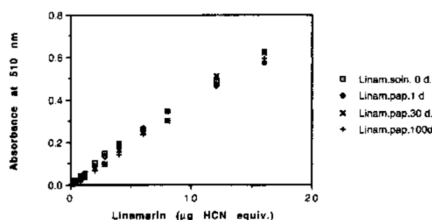

Fig 5. Graphs of absorbances at 510 nm vs linamarin content (μg HCN equivalents) for linamarin-impregnated filter papers as a function of time.

Components of kit for cyanogenic potential determination

A kit composed of the following components, was prepared for the determination of the cyanogenic potential of cassava flour.

(1) Balance, small and portable for weighing 100 mg of flour.
(2) Vials, clear plastic, flat-bottomed, with screw lids (25 mm id., 50 mm high).
(3) Filter paper discs, 21 mm, which contain buffer at pH 8 and linamarase.
(4) Picrate solution (0·5% picric acid in 2·5% sodium carbonate)
(5) Squares of filter paper (Whatman 3 MM) and of clear plastic overhead transparency sheets.
(6) Colour chart containing 10 entries from yellow to brown which correspond to amounts of HCN of 0–800 mg HCN equivalents kg^{-1} flour (ppm).
(7) Graph of absorbance A vs cyanogenic potential (mg HCN equivalents kg^{-1} flour, ppm).
(8) Filter paper squares containing linamarin standard.
(9) A simple step-wise protocol which describes the preparation of picrate papers and their use for the determination of cyanogenic potential of cassava flour.

CONCLUSION

In this paper we compared the well known picrate and tetra base paper methods for determination of cyanide

and found the former to be superior. We then developed a simple picrate paper method for the determination of the cyanogenic potential of cassava flour. This can be used in a field situation by a relatively unskilled person. A complete kit has been developed including a simple protocol for its use. In a laboratory situation with access to a spectrophotometer, the determination may be made more quantitative (±20% compared with the acid hydrolysis method) by measuring the absorbance at 510 nm of the eluted picrate–HCN compound. We hope that this methodology may be useful for monitoring the cyanogenic potential of cassava flour in developing countries. The method may also be readily adapted to cassava tubers.

The chemical nature of the compound formed between picric acid and HCN was hypothesised nearly 100 years ago (reviewed by Williams and Edwards 1980) and it would be interesting to study the chemistry again using modern methods. The ageing of picrate paper on exposure to air has presented a problem in the development of this method, in that papers that are older than about one month should not be used. A detailed chemical study of the ageing process may well resolve this difficulty.

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