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An enzyme-based dip-stick for the estimation of cyanogenic potential of cassava flour

Hock-Hin Yeoh[a]* & Sylvia V. Egan[b]

[a]*School of Biological Sciences, National University of Singapore, Kent Ridge, Singapore 119260, Singapore*
[b]*Division of Botany and Zoology, The Australian National University, Canberra, ACT 0200, Australia*

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An enzyme-based dip-stick for cyanogenic potential determination was constructed by gluing three pieces of chromatography paper impregnated with cassava leaf linamarase, phosphate buffer pH 8 and alkaline picrate solution, respectively, onto a plastic strip measuring 10 mm×45 mm. Using different concentrations of linamarin as reference (equivalent to 0.5–40 μg HCN), the change in colour of the picrate paper was readily distinguishable. Beyond 40–80 μg HCN, it was difficult to differentiate the colour of the picrate paper with accuracy. For quantitative estimation, the colour from the picrate paper was eluted with water and its absorbance measured at 510 nm. Using the dip-stick in conjunction with different types of reaction vessels, it was possible to determine the cyanogenic potential (expressed as mg HCN kg^{-1}) of cassava flour. The method was simple to use. It could easily handle large numbers of samples and it seems suitable for field work. © 1997 Elsevier Science Ltd

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important food crop for more than 500 million people in the developing countries (Cock, 1985). The plant contains linamarin as the major cyanogenic glucoside. Hydrolysis results in the release of hydrogen cyanide (HCN). It is also common to find processed cassava products such as gari and flour that contain variable amounts of residual cyanide arising from the glucoside, free cyanide and acetone cyanohydrin (Aletor, 1993; Mlingi *et al.*, 1993; Kemdirin *et al.*, 1995). Various methods have been developed to estimate the linamarin/cyanide content (cyanogenic potential) in cassava and cassava products (Cooke, 1978; Bradbury *et al.*, 1991, 1994; Bradbury & Egan, 1992; Yeoh, 1993; Yeoh & Truong, 1993; Brimer, 1994; Yeoh & Tan, 1994; Tatsuma *et al.*, 1996). Recently, an enzyme-bound linamarin indicator paper strip was reported (Yeoh *et al.*, 1996). Using a similar concept, it might be possible to develop it into a dip-stick which could be used for semi-quantitative or even quantitative measurement of cyanogenic potential in cassava flour. In this paper, we describe its construction and provide a simple protocol for its use. Using different types of reaction vessels, the performance of the dip-stick was evaluated with different samples of cassava flour from Mozambique and Indonesia.

MATERIALS AND METHODS

Preparation of dip-stick

Cassava leaf linamarase was prepared as previously described (Yeoh, 1989). The enzyme was mixed with gelatin and polyvinylpyrrolidone-10 to give a final concentration of 1% and 5% (w/v), respectively (Yeoh *et al.*, 1996). An aliquot of the enzyme mixture (30 μl) containing approximately 0.1 U linamarase activity was applied on a 10 mm×15 mm Whatman chromatography paper grade 17Chr. To another piece of the paper, 100 μl of 1 M phosphate buffer pH 8 was applied. They were allowed to dry at room temperature (25–28°C). The Whatman 3MM chromatography paper was soaked in alkaline picrate solution (Williams & Edwards, 1980) and allowed to dry at room temperature before cutting it into 10 mm×12 mm pieces.

The enzyme-impregnated paper was then glued at one end of a plastic strip (10 mm×45 mm) cut from overhead transparency plastic sheet. The buffered paper was glued on the reverse side. The picrate paper was fixed on the same side as the enzyme-paper about 3–4 mm from the other end of the plastic strip. The glue used was a water-based commercial glue.

*To whom correspondence should be addressed.

Determination of cyanogenic potential

Samples of cassava flour were sent from Mozambique and Indonesia to Australia by Dr J. Cliff and Dr M. Dzajuli, respectively. Upon arrival they were kept at −20°C. Samples from Mozambique were sieved before use. Cassava flour (100 mg) was weighed directly into a suitable reaction vessel, followed by the addition of 0.5 ml of water. The contents were mixed by gentle agitation and a dip-stick was then placed inside the vessel. Depending on the type of reaction vessel, it was sealed or capped and incubated at 30°C for 20–22 h. For the purpose of estimating the cyanogenic potential in the cassava flour, a standard curve was prepared with different concentrations of linamarin solution (0.5 ml). In this study, three types of reaction vessel were used. One vessel (plastic bag) was made of clear plastic, heat-sealed on three sides and measured 19 mm×60 mm to fit the 10 mm×45 cm strip. The other two were bottles with tight-fitting caps: a glass bottle measuring 50 mm×23 mm (i.d.) and a plastic specimen bottle measuring 95 mm×14 mm (i.d.). With the plastic bag, the open end was sealed with tapes, then held together with clip binders to ensure that it was air-tight. When the 23 mm (i.d.) bottle was used, the enzyme section of the dip-stick was cut off and laid flat inside the bottle.

Depending on the amount of HCN present, the picrate paper turned from yellow to orange to brick red. For semi-quantitative estimation, the colour formed was compared against known amounts of linamarin (equivalent to 0.5–40 μg HCN). For quantitative measurement, the picrate paper was removed and soaked in 2.5 ml of water with occasional agitation for 15–30 min to allow the colour to leach out. Its absorbance at 510 nm was measured and compared against those prepared from known amounts of linamarin. The results were expressed as mean ± SD, based on at least four replicate analyses.

Acid hydrolysis method

The cyanogenic potential of the cassava flour was also determined using the acid hydrolysis method (Bradbury *et al.*, 1991) as modified by Bradbury *et al.* (1994). Duplicate analyses were carried out and the average values given.

RESULTS AND DISCUSSION

The principle of the dip-stick was based on the hydrolysis of linamarin, resulting in the release of HCN which was then detected by the picrate paper. As the pH of the reaction mixture was alkaline, acetone cyanohydrin present in the cassava flour would also undergo spontaneous hydrolysis to give HCN. A closed system was therefore required and the efficiency of detecting the HCN might depend on the dimensions of the reaction vessels used, among other factors (Williams & Edwards, 1980). Three types of vessels were explored. One was a simple rectangular plastic bag designed to fit the dip-stick. It was made from locally available clear plastic sheets and was intended for single-use. This would be convenient for field work and where large numbers of samples are involved. Two other vessels used were bottles of different dimensions; one measured 50 mm×23 mm (i.d.), the other, a disposable specimen bottle, measuring 95 mm×14 mm (i.d.). These bottles could be recycled. Although they could be bulky to carry around for field work, they are more convenient to use. With the plastic bag, one has to ensure that it is air-tight by carefully sealing the open end.

It was observed that the colour of the picrate paper changed from yellow to brick-red with increasing HCN, irrespective of the type of reaction vessel. However, the colour of the picrate paper was consistently less intense when the plastic bag was used as the reaction vessel, compared to using the bottles. This was confirmed by spectrophotometric measurement of the colour eluted from the picrate paper (Fig. 1). Furthermore, in the case of the plastic bag method, the relationship between the absorbance and the amount of HCN was not linear between 0 and 10 μg HCN. Within this range for both types of bottles, a linear relationship between absorbance and HCN content was observed. Nonetheless, a slight deviation in absorbance at the higher HCN levels was seen between them. Overall, by visual inspection, the dip-stick could easily detect from as little as 0.5 μg HCN to about 40 μg HCN. Beyond this amount up to 80 μg HCN, it was not easy to distinguish the different shades of brick-red. In fact, spectrophotometric measurements showed the values started to taper off from 20 to 80 μg HCN (Fig. 1, inset).

The cyanogenic potential of cassava flour could be estimated semi-quantitatively by comparing the colour

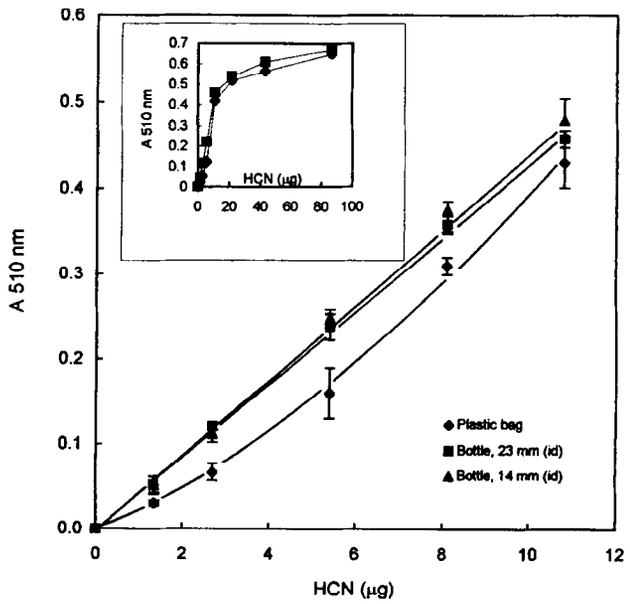

Fig. 1. Relationship between absorbance and HCN content.

Table 1. Cyanogenic potential (mg HCN kg^{-1} cassava flour) of cassava flour obtained from Mozambique and Indonesia

Sample number	Source of cassava flour	Dip-stick method using different reaction vessels			Acid hydrolysis method
		Plastic bag[a]	Bottle 1[b]	Bottle 2[c]	
1[d]	Mozambique (Nampula)	< 1	n.d.[e]	< 1	3
2	Mozambique (Nampula)	4 ± 1	4 ± 1	3 ± 0.3	6
3	Mozambique (Nampula)	40 ± 4	62 ± 4	48 ± 3	72
4	Indonesia (Bogor)	22 ± 2	22 ± 2	18 ± 1	30
5	Indonesia (Bogor)	38 ± 5	43 ± 1	40 ± 4	46
6	Indonesia (Bogor)	59 ± 6	66 ± 7	60 ± 7	70
7	Indonesia (Bogor)	66 ± 4	74 ± 7	72 ± 8	79

[a]Bag size: 19 mm × 55 mm.
[b]Bottle size: 50 mm × 23 mm (i.d.).
[c]Bottle size: 95 mm × 14 mm (i.d.).
[d]Not all samples tested positive; values were based on positive results.
[e]Not detected.

of the picrate paper against those from known amounts of linamarin (expressed as μg HCN). However, by eluting the colour from the picrate paper in water and measuring its absorbance at 510 nm, one could make a quantitative estimate of the cyanogenic potential. Table 1 shows the cyanogenic potential for seven samples of cassava flour obtained from Mozambique and Indonesia. For comparison, the HCN content of the same batch of cassava flour estimated by acid hydrolysis method was included (Bradbury *et al.*, 1991 as modified by Bradbury *et al.*, 1994). With the plastic bag, the cyanogenic potentials of three cassava flour samples (i.e. samples 3, 6 and 7) were slightly lower than values obtained by the acid hydrolysis procedure. Results obtained with 23 mm (i.d.) and 14 mm (i.d.) bottles were closely similar, except for one sample (i.e. sample 3). For this sample, analyses carried out with 23 mm (i.d.) bottles gave higher values (62 ± 4 mg HCN kg^{-1} cassava flour) than those using 14 mm (i.d.) bottles (48 ± 3 mg HCN kg^{-1} cassava flour). Overall, a better correlation with estimates from the acid hydrolysis method was obtained with bottle of 23 mm (i.d.). Nonetheless, for semi-quantitative estimation, these differences were perhaps not crucial if the estimates were only needed to provide an idea of the cyanogenic potential in these samples.

The data showed that a suitable reaction vessel must be used in conjunction with the dip-stick. Since both types of bottles gave similar results, it would be more convenient to use the 14 mm (i.d.) bottle, particularly if semi-quantitative estimation was required. With this bottle, the dip-stick was simply inserted into it whereas, if the bottle of 23 mm (i.d.) was used, the enzyme section of the dip-stick had to be cut off and placed flat at the bottom of the bottle. This additional step might be an important consideration if large numbers of samples are being handled.

Overall, the enzyme-based dip-stick method provides a simple and convenient way of determining the cyanogenic potential in cassava flour. It could also be used to determine the cyanide content in cassava roots; the root extract could be prepared using existing procedures (Cooke, 1978; Yeoh & Truong, 1993) and the dip-stick used to estimate the amount of HCN present. For semi-quantitative estimation, no equipment is needed. Furthermore, the dip-sticks are easy to prepare in bulk and could be stored for several months at room temperature. These features will be useful for field studies and for laboratories lacking facilities to carry out existing spectrophotometric methods. Linamarase prepared by other methods or from cassava peel could be used to prepare the dip-sticks. However, as the dip-sticks are stable and not bulky, they could also be made by established laboratories and posted to those who may require them.

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