Journal of the National Science Council of Sri Lanka, **1** (1973), 207—210

SHORT COMMUNICATION

A Method for Assay of Total Potential Cyanide in Manioc Flour

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(Accepted for publication : October 12, 1973)

Manioc and its products usually contain small amounts of free cyanide and larger amounts of 'bound' cyanide. 'Bound' cyanide occurs mainly in the form of cyanogenic glucosides. Any method of determination of cyanogenic glucosides in plant material consists of three stages :

(1) Hydrolysis of the cyanogenic glucoside to give hydrogen cyanide (Liberation).
(2) Isolation of hydrogen cyanide (Recovery).
(3) Quantitation of the hydrogen cyanide.

Methods for determination of the cyanogenic glucosides (linamarin and lotaustralin) in manioc have been reported as early as 1944[2]. Nearly all the early methods relied either on acid hydrolysis or spontaneous autolysis by the endogenous enzyme linamarase, contained in the plant tissue, to liberate the bound cyanide[5]. These methods have been found wanting because linamarin is not readily hydrolysed by acid and because there is generally insufficient endogenous enzyme. Although Wood[7], using a model system of purified glucosides and linamarase, obtained a recovery of 87% after steam distillation, the critical factors that determine the reproducibility of the technique, *viz*, the total release and isolation of the cyanide and the prevention of losses due to secondary reactions have received insufficient attention.

For the development of a standard and uniform analytical procedure, factors including sample size, pH, addition of exogenous linamarase, time of incubation, recovery by aspiration with air or steam distillation and the effect of temperature and time on recovery, were studied. After liberation and isolation, cyanide was quantitatively determined by the colorimetric picric acid method[4,6].

Manioc was processed into flour by the traditional method of chipping, washing, drying and grinding. Samples (10 g) of this flour (moisture content 10.0%), were used for the assays.

The rate of distillation and volume of distillate for maximum recovery of liberated cyanide were determined for both aspiration with air and steam distillation. Aspiration was found to be less efficient than steam distillation (*Table* 1).

Table 1. Comparative recovery of HCN by steam distillation and aspiration with air

Method of hydrolysis	Maximum Recovery (HCN liberated, ppm)		Relative efficiency of aspiration
	Steam distillation	*Aspiration with air*	
Endogenous enzyme only (2 h)	49	36	0.73
With added exogenous enzyme (2 h)	112	81	0.71
2*N* H_2SO_4	57	39	0.68

Table 1 also shows that hydrolysis by the endogenous enzyme, or by acid is relatively inefficient. This point is further illustrated in the next two tables. *Table* 2 shows that hydrolysis by the endogenous linamarase requires the use of long incubation times. *Table* 3 shows that acid hydrolysis results in only one third to half the yield obtained with the exogenous enzyme. This data is compatible with studies on the acid hydrolysis of pure linamarin.[1] Use of an acid medium for distillation appears to give slightly lower values of cyanide ; this is similar to an observation by Zitnak[8].

Table 2. HCN liberated by endogenous enzyme

Weight of sample (g)	Time of incubation (h)	HCN liberated (ppm)
20	2	33
10	2	39
20	18	93
20	24	93

Table 3. HCN recovered (ppm) under different conditions

Hydrolysis with $2N$ H_2SO_4	Hydrolysis with added exogenous enzyme	
	Distillation at tissue pH	*Distillation in 2N acid medium*
59	157	156
65	140	145
57	146	132
	156	143
	163	140

The efficiency of the method was tested by recovery of added linamarin (instead of the added cyanide as done previously[3]). Recoveries of 88 — 90% were obtained. A further 5 — 6% can be obtained by a two step process i.e. distillation after the action of exogenous enzyme, followed by addition of $2N$ acid and re-distillation.

Unsieved manioc flour was used in the above assays. On sieving, much better reproducible results were obtained ; a sample of sieved manioc flour yielded values of 140, 142 and 141 ppm cyanide in successive assays. Free cyanide in the flour used was of the order of 5 ppm.

In view of the results obtained, the following method can be recommended for the determination of total potential cyanide in manioc flour.

Manioc flour (10 g) is introduced into a distillation flask, water (200 ml) and excess linamarase (approx. 450 μg/min.) is then added. The sample is incubated for 2h. at room temperature and distilled ; 250 to 300 ml of distillate is collected in a solution of 0.5g sodium hydroxide in 50 ml of water. Cyanide is then estimated by the colorimetric picric acid method[6].

The assay of total potential cyanide in other forms of processed and unprocessed manioc is in progress.

Acknowledgements

The authors thank the Director, Ceylon Institute of Scientific and Industrial Research (CISIR), for the facilities provided and Mr. S. F. Laurentius, Deputy Director (Research), CISIR, for his advice and encouragement.

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