

**SHORT COMMUNICATION**

**PROBLEMS ASSOCIATED WITH THE ESTIMATION OF CYANIDE IN RUBBER SEED KERNEL**

**G. V. MALLIKA, E. R. JANSZ, NIRMALA M. PIERIS**

*Ceylon Institute of Scientific and Industrial Research, P.O. Box 787, Colombo.*

AND

**A. M. ABEYSEKERA**

*Department of Chemistry, University of Sri Jayewardenepura, Nugegoda.*

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**INTRODUCTION**

The cyanogenic glucosides of cassava (mainly linamarin) have been reliably and reproducibly estimated using an exogenous enzyme (linamarase) assay for almost two decades.<sup>1,2</sup> Rubber seed has the same cyanogenic glucoside<sup>3,4</sup> but previous work with this material had either used trichloroacetic acid as a cyanide liberator<sup>3</sup> or yielded very variable results with autolysis.<sup>4</sup> This study is an attempt to understand the lack of reproducibility in total cyanide determinations and to validate the techniques used previously to estimate total cyanide content in rubber seed.

**METHODS AND MATERIALS**

Details are given in the legends of tables.

**RESULTS AND DISCUSSION**

**Sampling :**

Collection of random samples of rubber seed and analysis by the autolysis technique<sup>5</sup> yielded total cyanide content varying from 100 - 4000 mgkg<sup>-1</sup>. These variations can be attributed to (i) inherent differences in the sample and (ii) experimental error. Differences in the sample may be due to among others (a) genetic variation (b) effect of maturity and (c) the history of seeds after seed fall.

To limit such variations samples seeds were collected from the same field one day after seed fall. Results from six such samples gave 1512, 1177, 2214, 2072, 2176 and 1763 mgkg<sup>-1</sup> respectively i.e.  $1819 \pm 414$  (mean  $\pm$  standard deviation). Analysis of seeds from the same fruit collected from a tree gave surprisingly wide disparities (Table 1), indicating that in addition to the factors listed above, other factors possibly affecting the cyanophoric system such as autolytic cyanide release, could be operating even while the fruit is still on the tree. To an analyst this has serious implications especially since rubber has a relatively large seed and therefore might require a relatively large sample weight if variations were to be averaged out to give an analytically significant result. However analysis of large samples present other problems, viz., loss of cyanide during handling (experimental errors) due to the seed having large quantities of cyanide liberating enzymes.<sup>5</sup>

**Table 1 : Variation of total cyanide in the seeds of the same fruit**

Sample No.	Total Cyanide (mg/kg on dry basis)
Fruit 1	1897
	1754
	15
Fruit 2	484
	609
	670
Fruit 3	2055
	1592
	1713
Fruit 4	2306
	2240
	1885
Fruit 5	1905
	1458
	231
Fruit 6	1199
	1621
	1918

Fruits (trilocular) No's 1 to 6 were collected from the tree and each of the three seeds analysed separately.<sup>5</sup> Total cyanide was determined by the picrate method<sup>2,7</sup> in individual seeds of the same fruit by homogenization and autolysis<sup>5</sup> (24h).

In practice it has been found that least deviations were obtained by using 6 - 8 g samples at 0 - 5<sup>o</sup> C while minimising handling time. However a coefficient of variation of 15 - 25 % can yet be observed.

### Hydrolysis of cyanogenic glucosides

When trichloroacetic acid was used, release was less than 3% of the available total cyanide. Results obtained previously using this method are therefore grossly misleading.<sup>3</sup> Use of 2M H<sub>2</sub>SO<sub>4</sub> at room temperature also released very little cyanide. This acid required 30 h refluxing to completely release total cyanide from rubber seed. These experiments also show that low yields obtained with short reflux times did not reflect a hydrolysis of the C ≡ N triple bond as previously theorized<sup>6</sup> as the full complement of total cyanide could be released from this reaction mixture by the action of added linamarase (exogenous) after pH adjustment (Table 2).

**Table 2: Total cyanide in rubber seed kernel and meal determined by acid hydrolysis followed by exogenous enzyme hydrolysis**

Type of sample	Total cyanide (mg/kg on dry basis)	
	Acid hydrolysis (i)	Exogenous enzyme hydrolysis after (i)
Immature Kernel	169	12,363
Mature kernel	22	1546
Mature kernel	32	2032
Mature kernel	69	2404
Meal	20	213

Acid hydrolysis<sup>1</sup> was carried out at room temperature (30<sup>0</sup> C) and the reaction mixture distilled.<sup>6</sup> The residue from distillation was adjusted to pH 6 (10 ml of 0.1 M phosphate buffer) and incubated with linamarase<sup>2</sup> (EC 3.2.1.21) for 4 h and cyanide estimated.<sup>7</sup>

Two hours of autolysis was sufficient to completely release total cyanide. Longer autolysis times could be used for release, provided cyanide utilizing systems (e.g. rhodanese) are not active. Results of this study show that 24h incubation at ambient temperature resulted in a loss of approximately 10% cyanide (Table 3).

Addition of exogenous linamarase to the distilled autolysate residue did not yield significant additional cyanide in processed material like rubber seed meal, indicating that the activity of endogenous enzyme was adequate for the release of cyanide even after processing.

Table 3 : Cyanide utilization during incubation period

Incubation time (h)	Total cyanide recovered (mg)	Cyanide utilized (%)
0	320	0
2	310	3
4	295	8
24	284	11
48	281	12

A sample of extract was prepared by homogenizing rubber seed kernel (60g) in water (600ml). CO<sub>2</sub> was passed through the filtrate until all cyanide was removed (4h). The solution was made up to 1 l and KCN (0.4g) was introduced. Samples were incubated separately and cyanide content determined over 48 h.

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#### References

1. Pieris N.M. (1976). Studies on the cyanogenic glucosides of *Manihot esculenta* Crantz. Ph.D Thesis. University of Colombo.
2. Pieris N. M. M., Jansz E. R. & Kandage R. (1974). Cyanogenic glucoside content of manioc. *Journal of the National Science Council of Sri Lanka* 2: 67-76.
3. UNIDO (1985). Project Document US/GLO/81/103. Phase 1. Development of rubber seed processing technology for the production of vegetable oil and animal feed. Edited by KH Todt and HFT Vogt Institute für Naturwissenschaftlich - Technische Dinstet. Federal Republic of Germany. p. 1-85.
4. UNIDO (1985). Project Document US/GLO/81/103, Phase 11 Rubber seed processing for the production of vegetable oil and animal feed, CISIR, Sri Lanka pp. 121.
5. Mallika G. V. (1991). Applied chemical studies on rubber seed to support its industrial utilization. M.Phil thesis of University of Sri Jayewardenepura.
6. Jansz E. R. & Nethsingha C. L. M. (1973). Manioc; selected topics. *Journal of the National Science Council of Sri Lanka* 1: 83-96.
7. Wood T. (1966). The Isolation, properties and enzymic break down of linamarin from cassava. *Journal of Science, Food and Agriculture* 17: 85-91.