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Studies on Some Local Legumes

II. Cyanogenic Glucosides

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Abstract : The seeds of nearly 50 selected legumes (mainly those commonly found in Sri Lanka) were screened for the presence of cyanide and cyanogenic glucosides. Only four of these contained significant amounts of cyanogenic glucosides ; all were varieties of *Phaseolus lunatus* (sieva bean or lima bean). Methods of processing to eliminate the cyanogenic glucoside was investigated with success. Local strains of *Vigna sinensis*, *Phaseolus vulgaris* and *Pisum sativum* were found to contain less cyanogenic glucoside than previously reported for strains grown elsewhere.

1. Introduction

The presence of cyanogenic glucosides have been reported in some plants of Family Leguminoseae. Its occurence in lima bean (*Phaseolus lunatus*) and sieva bean (*Phaseolus lunatus* L.) has been well documented.[1,5,7,8,9,13] The glucoside was identified as linamarin as early as 1907,[5] and since then there have been many studies on its distribution in the plant[10] and some on the effect of processing.[3,12] The only studies in Sri Lanka have been those of Charvanapavan[3] who in 1944 gave some data on the analysis of a locally available variety of lima bean but the account bears no description of either the variety or the method of analysis used.

Very little work has been conducted on cyanogenic glucosides of other legume seeds although Jaffe[6] and Montgomery[10] have reported that varieties of *Phaseolus vulgaris* (kidney or navy bean) *Pisum sativum* (garden pea) and *Vigna sinensis* (black-eye pea) contain cyanide in the order of 20 p.p.m. Cyanogenic glucosides have also been reported in *Cicer arietinum* and *Lathyrus sativus*.[9]

As in the case of the cyanogenic glucoside content of manioc, the methods of assay previously reported relied upon were either autolysis[2] or acid hydrolysis. The former assay is probably valid for most fresh samples but cannot be applied to processed material. The latter assay, as in the case of manioc, gives low results and is therefore not valid. In addition to the methods mentioned, another assay based on the use of sweet almond extract[2] (a source of glucosidase to decompose the amygdalin type of cyanogenic glucoside) has been recommended, but this would be ineffective on the linamarin type of cyanogenic glucoside which is generally present in the Phaseolae ; cyanogenic glucosides can be classified into four groups according to the chemical structure of the aglycone.[4]

Our studies have been mainly based on the application of the enzymic assay worked out for the cyanogenic glucosides of manioc, viz. the use of exogenous linamarase to hydrolyse bound cyanide. The assay has been found to work successfully on lima bean. In studies on screening, our work has been based on the reasoning that all cyanogenic glucosides except the linamarin type are decomposed by acid to give high yields of cyanide. Due to this, an initial screening was done by acid

hydrolysis and further tests were done only on those giving at least a slight positive reaction or those previously suspected for containing linamarin. The studies have shown that : (1) nearly all the types tested contained little (less than 5 p.p.m) or no cyanide, (2) varieties of lima bean could contain up to 800 p.p.m. total cyanide, (3) it is possible to process the lima bean so that only a small fraction of the total cyanide is present in the cooked material.

2. Experimental

2.1. Plant Material

2.1.1. *General*

Seed material was obtained from a variety of sources. Varieties of *Dolichos*, *Psophocarpus* (introduced) and *Phaseolus lunatus* (white) were obtained from the Agriculture Department Dry Zone Research Station (Mahailluppallama). Seeds of cover crops were obtained from Mr. Roy Bandaranaike, while common varieties of grain legumes were purchased in the open market or from Agriculture Department sales outlets. The remaining seeds were collected during field trips or from home gardens. The last category included *Canavalia gladiatus* (awara) *Cajanus cajan* (tur dhal) *Phaseolus lunatus* (lima bean-black varieties) and *Psophocarpus tetragonolobus* (local variety).

2.1.2. *Lima bean* (black variety).

The seeds tested were obtained from plants grown in home gardens. The plant is a climber with leaves and flowers typical of the Phaseolae. Pods are 6 to 7cm long and green when tender and generally bear 3 black seeds (due to a dark purple pigment in the testa). The seeds are flat with an average weight of 0.33g (at 13.5% moisture) bearing the typical markings of *Phaseolus lunatus* varieties.

2.2. Preliminary screening of samples for cyanogenic glucoside

The seeds (20g, 10% to 15% moisture) were coarsely ground and hydrolysed with 4N H_2SO_4 during steam distillation (45 min). The distillate (175 ml) was collected in 50 ml of 6.25% Na_2CO_3 and tested for cyanide by the picric acid test.[14]

2.3. Tests with manioc linamarase

The samples were finely ground in an edge runner mill (350μ particle size) and known weights (20, 10 or 5g) suspended in 100ml of 0.02M citrate buffer (pH 5.4) was incubated with manioc linamarase (100 units) for 4 to 8h and total cyanide determined as described previously.[11] Autolysis experiments were carried out in the absence of manioc linamarase. Free cyanide was determined as described previously.[11] Manioc linamarase was prepared by the method of Wood.[15]

3. Results and Discussion

3.1. Screening tests for cyanide

Screening tests for cyanide in the legumes using acid hydrolysis gave negative results (sensitivity 10 p.p.m. for the linamarin type and 5 p.p.m. for other types of bound glycosidic cyanide) for most of the seeds tested (Table 1). Possible trace quantities were detected in a *Mimosa* and *Crotolaria brownei*, while definite cyanide positive reactions were observed with 4 varieties of *Phaseolus lunatus* (Table 1).

TABLE 1—Screening of legumes for bound cyanide

Code	Plant Species	Cyanide (Acid hydrolysable)
PB1a	Atylosia	—
PB2a	Cajanus cajan	—
PD1a	Crotalaria anagyroides	—
PD1b	Crotalaria brownei	+
PD1c	Crotalaria usaramoensis	—
PD2a	Trigonella foenum graecum	—
PE1a	Centrosema pubescens	—
PE2a	Clitoria ternatea	—
PE3a	Glycine max	—
PF1a	Arachis hypogea	—
PF2a	Desmodium gyroides	—
PF2b	Desmodium incenatum	—
PF2c	Desmodium ovalifolium	—
PG1a	Calpagoniam mucunoides	—
PG2a	Canavalia gladiatus	—
PG3a	Dolichos bi florus	—
PG3b	Dolichos lablab (yellow)	—
PG3c	Dolichos lablab (brown)	—
PG4a	Erythrina	—
PG5b	Phaseolus lathyroides (Pueraria javanica)	—
PG5c	Phaseolus lunatus (black)	++++
PG5d	Phaseolus lunatus (white, small)	++
PG5dl	Phaseolus lunatus (white, small)	+++
PG5e	Phaseolus lunatus (white, large)	++
PG5f	Phaseolus mungo	—
PG5g	Phaseolus mungo (var. radiatus)	—
PG5h	Phaseolus vulgaris (butter bean)	—
PG5i	Phaseolus vulgaris (navy bean)	—
PG5j	Phaseolus vulgaris (bush bean)	—
PG5k	Phaseolus vulgaris (wal bonchi)	—
PG6c	Psophocarpus tetragonalobus (light brown)	—
PG7a	Pueraria kudzu	—
PG7b	Pueraria phaseoloides	—
PG8a	Stizolobium nivea (mottled)	—
PG8b	Stizolobium nivea (grey)	—
PG8c	Stizolobium nivea (black)	—
PG8d	Stizolobium nivea (black, large)	—
PG9a	Vigna sinensis (black)	—
PG9b	Vigna sinensis (brown)	—
PG9c	Vigna sinensis (yellow)	—
PG9d	Vigna sinensis (panduru me)	—
PH1a	Tephrosia candida	—
PH1b	Tephrosia candida belga	—
PH1c	Tephrosia candida belga S	—
PH1d	Tephrosia vogelii	—
PI1a	Pisum sativum	—
MB2a	Mimosa I	+
CA1a	Cassia species	—

+, Trace 5—10 p.p.m. cyanide
++, 20—50 p.p.m. cyanide
+++, 50—100 p.p.m. cyanide
++++, > 100 p.p.m. cyanide

Studies using linamarase as the cyanide releasing agent on those seeds giving cyanide positive reactions and also those legumes previously reported[6,10] to contain glucosidic cyanide showed that the mimosa variety contained 14 p.p.m. total cyanide and *C.brownei* less than 5 p.p.m. Contrary to reports in the literature (which have quoted values of the order of 20 p.p.m.) the total cyanide levels in the edible legumes, *Pisum sativum*, *Vigna sinensis* (4 varieties: black, brown and yellow medium sized seed varieties and 'panduru me') and *Phaseolus vulgaris* (4 varieties : Navy bean, butter bean, bush bean and 'wal bonchi', all sold by Agriculture Department sales outlets) were very low, being present only in trace quantities (less than 5 p.p.m.)

Cyanide levels of *Phaseolus lunatus* varieties are given in Table 2. All results quoted refer to one batch of seeds. These studies showed agreement with the literature on the point that smaller, darker seed types contain more bound cyanide.

TABLE 2—Cyanogenic glucoside content of some varieties of lima bean

	Variety	Source	Total cyanide (p.p.m.)
1.	Black small PG5c	Kegalle	460
2.	Black small PG5c	Grown in Colombo	500
3.	Black small PG5cl	Matara	792
4.	White small PG5d	Mahailluppallama	93
5.	White small (Henderson Bush) PG5dl	Mahailluppallama (Nigerian)	110
6.	White large (Burpees Bush) PG5e	Mahailluppallama (Nigerian)	46

Assays done using autolysis and exogenous linamarase. For experimental details see 2.3. Each experimental point refers to one batch of seeds.

3.2. Conditions of assay for total cyanide of *P. Lunatus* (PG5c).

Before initiating detailed studies on the effect of processing and cooking on the cyanogenic glucosides of the seeds, it was decided to compare the efficiency of the various methods of analysis available. It was found that the best results were obtained either by homogenising the fresh seed followed by autolysis or by use of exogenous linamarase on an extract of the powdered seed (Table 3). For the latter purpose the seed must be dried to less than 8% moisture at a temperature below 70°C before powdering in an edge runner mill. It was found that drying at 110°C caused some lowering of the total cyanide value possibly due to quick autolysis of the tissue and the resulting loss of free cyanide formed. Drying also reduces linamarase activity rendering autolysis ineffective (Table 4). However, the results of most of the methods used in Table 2 are comparable considering that sampling of seeds for assay results in an almost 10% standard deviation (90% probability) when random samples of 20 seeds are used for the assay (done six times in parallel). This was no doubt, due to the variation of total cyanide content from seed to seed and this deviation could be reduced by vastly increasing sample size. However, it was decided to slightly increase sample size to 30 seeds for the experiments described below, due to : (1) the limited quantity of seed material available (2) the fact that the effects studied showed relatively large changes (much larger than the standard deviations observed).

TABLE 3—Assay of total cyanide in lima bean by various methods

Method of Assay	Temp. of drying of seed (°C)	Total cyanide (p.p.m.)
1. Acid hydrolysis	—	158
2. Autolysis (4h)	—	460
3. Autolysis (15 min) + Exogenous linamarase (5h)	70	473
4. Autolysis (1½h) + Exogenous linamarase (3h)	70	464
5. Autolysis + Exogenous linamarase (4h)	70	492
6. Autolysis (4h)	70	423
7. Autolysis + Exogenous linamarase (overnight)	110	396
8. Autolysis + Exogenous linamarase (4h)	110	418

In 3 and 4 autolysis and exogenous linamarase hydrolysis were carried out in a two step process with a distillation step inbetween. See Table 3 for autolysis values. In other cases (5.7 and 8.) the reaction was stimultaneous.

For further experimental details see 2.2, 2.3. One batch of such was used of the above set of results.

TABLE 4—Effect of temperature of drying on the autolysis of cyanogenic glucosides

Time (h)	Temperature (°C)	Cyanide released (p.p.m.)
0.25	70	90
0.75	70	150
1.5	70	279
3	110	48
4	70	423
4	110	66
5	110	83

For experimental details see 2.3. Results refer to one batch of seeds.

The results of Table 2 show that use of manioc linamarase is a valid method of assaying linamarin in lima bean. This was important as this method has to be used for assaying processed lima bean where autolysis cannot be used and acid hydrolysis results in abnormally low values for bound cyanide content.

3.3. Location of cyanogenic glucosides

In many cyanogenic tissues the outer layers of the tissue contain larger quantities of glucoside. Separation of the testa (13.5% by weight) from the rest of the seed showed that the reverse was true in this case ; the testa contained only 90 p.p.m. total cyanide compared to 582 p.p.m. for the rest of the seed. In other words, only 2.5% of the total cyanide was present in the testa. This variation of cyanogenic glucoside content of the different regions of the seed might account (in part) for the deviations observed in the assay.

3.4. Effect of soaking and boiling

There are several methods of cooking lima bean (in Sri Lanka). Most of these methods involve a soaking stage prior to boiling. A number of variations were tried out and it was noticed that swelling up of the seed markedly affected the final total cyanide content of the boiled seed (Table 5). Whereas soaking in water or dilute Na_2CO_3 resulted in only about half the seeds swelling and considerable residual total cyanide, induced swelling caused by slightly damaging the seeds or by removal of the testa (of undried fresh seed) resulted in considerably greater loss of total cyanide. However, the most promising results were obtained by boiling germinated seeds ; here only 3% of the original total cyanide remained.

All the above observations were true for seeds that were freshly harvested (within one month). However old seeds (15 months after harvest) are much easier to detoxify (Table 6). These seeds which are 80% to 90% non-viable swell up abnormally.

TABLE 5—Effect of soaking and boiling on total cyanide content of lima bean seeds

Treatment	Seeds swollen (%)	Residual total cyanide (p.p.m.)	Total cyanide remaining (%)
1. S — B — R — B	47	348	70
2. D — S — B — R — B	100	87	17
3. S* — B — R — B	47	306	61
4. R — S — B — B	100	196	25
5. G — R — B — B	100	15	3

S, Soak, (24h) ;
B, Boil 15 min ;
R, Remove testa ;
D, Damage ;
G, Germinate (5 days moistening)
S*, Soak in Na_2CO_3 (24h)

For details see 3.4: Results refer to one batch of seeds.

TABLE 6—Effect of soaking and boiling on total cyanide content of old lima bean seeds

Treatment	Residual total cyanide (p.p.m.)	% Remaining
1. S — B — B (over swollen)	<5	<1
2. S — R — B — B (over swollen)	<5	<1
3. S — B — B (selected unswollen)	160	35
4. B — R — B	40	09
5. B — B	127	28

See Table 4 for abbreviations.
For details see 3.4. Results refer to one batch of seeds.

3.5. Fate of cyanide in seed on soaking

Studies on the effect of soaking showed that there was very little free cyanide in the soak water. Next, it was decided to find out at which stage the cyanide was lost. In order to determine this, two sets of experiments were designed.

Experiment 1.

The seeds were soaked in a closed system (a cyanide determination steam distillation apparatus). After the soaking period (3 days) the seeds were boiled (during the steam distillation process). The boiled seeds were then separated from the aqueous medium and both medium and seed tested for bound cyanide. The results (Table 7) showed that although more than 50% of the original total cyanide was present in the soak-boil water in the form of bound cyanide, very little free cyanide was present. Further, a cyanide balance calculation showed that about 20% of the original total cyanide was unaccounted for. This suggested the possibility that some cyanide (free ?) was converted to other products, probably by a natural detoxification mechanism in the seed. The next set of experiments resulted in a similar conclusion.

TABLE 7—Fate of bound cyanide in lima bean on soaking and boiling

		Cyanide	
		p.p.m.	%
A			
1.	Free cyanide released on soaking and boiling	13	2.6
2.	Total cyanide in seed	120	24
3.	Bound cyanide released on soaking and boiling	265	53
B			
1.	Free cyanide in soak water	0	0
2.	Bound cyanide in soak water	0	0
3.	Total cyanide in seed	154	31
4.	Bound cyanide in boil water	169	33

For details see 3.5. Results refer to the batch of seeds.

Experiment 2.

In this experiment, free and bound cyanide in the soak water and bound cyanide in the boil water was determined. As no free cyainde is formed during boiling stage (Table 7A), the results (Table 7B) again showed some of the original cyanide was unaccounted for. The results also showed that release of bound cyanide takes place not during the soaking stage but in the boiling stage.

3.6. Detoxified flours

An attempt was made to prepare detoxified lima bean flour using the data accumulated above. The seeds were dried at 60°C and then ground into a fine flour. Water was added until a thick paste was formed and the mixture incubated for 4h after

which the paste was dried first at 60°C (to 15% moisture) and then at 100°C for 2h. The total cyanide content of the resulting flour was found to be 85 p.p.m. (17% of original total cyanide). Although it was considered possible to remove a larger fraction of the cyanide by (1) increasing amount of water added to flour and (2) by increasing incubation time (for autolysis), further work was not carried out as the flour obtained had a highly undesirable purple colouration arising from the pigment of the testa and because the removal of the testa was an operation which is unlikely to be accomplished easily on a large scale.

4. Conclusions

Most edible legumes contain little or no free and bound cyanide. The same is true also for legumes that are not usually eaten. The only exception appears to be the lima bean which can contain dangerous levels of bound cyanide. However these studies show that proper cooking procedures can be used to reduce the total cyanide content to relatively safe levels. The availability of safe methods of processing may provide sufficient impetus for the cultivation of this bean, which is a high yielder and whose cyanogenic character protects it from insect attack.

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References

1. ANON. (1912) *Bull. imp. Inst. Lond.* **10** : 653.
2. British Standards (1976) 4317 Part II, ISO 2164-1975.
3. CHARAVANAPAVAN, C. (1944). *Trop. Agric.* (Cey) **100** : 164.
4. EYJOLFSSON, R. (1970). *In "Progress of the Chemistry of Natural Products"* **28** : 74.
5. GUIGNARD L. (1907). *Bull. Sci. Pharmacol.* **14** : 556.
6. JAFFE, W. G. (1950). *Acta Client Veuezolana* **1** : 62.
7. KOHN—ABREST, E. (1906). *Comp. Rend. Acad. Sci.* **142** : 586.
8. LANGE, W. (1907). *Arb. Baiserl. Gesundh.* **25** : 478.
9. LIENER, I. E. (1962). *Am. J. clin. Nutr.* **11** : 281.
10. MONTGOMERY, R. D. (1964) *W. Indian med. J.* **13** : 1.
11. PIERIS, N., JANSZ, E. R. & KANDAGE, R. (1974). *J. Natn. Sci. Coun. Sri Lanka* 2 : 67.
12. RAZAFIMAHERY, R. (1954) *Bulletin de l' Academic Malagasy Republic.*
13. VIEHOEVER, A. (1940). *Thai. Sci. Bull.* **2** : 1.
14. WOOD, T. (1965) *J. Sci. Fd. Agric.* **16** : 300.
15. WOOD, T. (1966) *J. Sci. Fd. Agric.* **17** : 85.