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Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods

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Abstract

A general method has been developed for determination of the total cyanide content of all cyanogenic plants and foods. Ten cyanogenic substrates (cassava, flax seed, sorghum and giant taro leaves, stones of peach, plum, nectarine and apricot, apple seeds and bamboo shoot) were chosen, as well as various model compounds, and the total cyanide contents determined by the acid hydrolysis and picrate kit methods. The hydrolysis of cyanoglucosides in 2 M sulfuric acid at 100°C in a glass stoppered test tube causes some loss of HCN which is corrected for by extrapolation to zero time. However, using model compounds including replicate analyses on amygdalin, the picrate method is found to be more accurate and reproducible than the acid hydrolysis method. The picrate kit method is available free of charge to workers in developing countries for determination of cyanide in cassava roots and cassava products, flax seed, bamboo shoots and cyanide containing leaves. For eleven different samples of flax seed and flax seed meal the total cyanide content was 140–370 ppm. Bamboo shoots contained up to 1600 ppm total cyanide in the tip reducing to 110 ppm in the base. The total cyanide content of sorghum leaves was 740 ppm 1 week after germination but reduced to 60 ppm 3 weeks later. The acid hydrolysis method is generally applicable to all plants, but is much more difficult to use and is less accurate and reproducible than the picrate method, which is the method of choice for plants of importance for human food.

Keywords: Total cyanide; Picrate method; Acid hydrolysis; Cyongenic plants; Cassava; Sorghum; Flax; Bamboo shoot

1. Introduction

There are at least 2650 species of plants that produce cyanoglycosides and usually also a corresponding hydrolytic enzyme (beta-glycosidase), which are brought together when the cell structure of the plant is disrupted by a predator, with subsequent breakdown to a sugar and a cyanohydrin, that rapidly decomposes to hydrogen cyanide and an aldehyde or a ketone (Hosel, 1981; Moller & Seigler, 1999). This combination of cyanoglycoside and hydrolytic enzyme is the means by which cyanogenic plants are protected against predators (Jones, 1998; Moller & Seigler, 1999). Any particular species produces one or more related cyanoglycosides. Most plant species are either cyanogenic (like cassava) or acyanogenic. There are a few species such as white clover, bird's foot trefoil, yarrow, harebell and white flax which have four types of plants that contain either both cyanoglucoside and enzyme (GE), the cyanoglucoside but no enzyme (G−), the enzyme but no cyanoglucoside (−E) and finally neither cyanoglucoside nor enzyme (−−) (Hughes, 1991; Jones, 1988; Till, 1986).

Many methods have been developed for determination of the total cyanogen (total cyanide) content of cassava (Bradbury, Bradbury, & Egan, 1994; Bradbury, Egan, & Lynch, 1991; Cooke, 1978), sorghum (Haskins, Gorz, & Hill, 1988), flax (Palmer, Olson, Halverson, Miller, & Smith, 1980; Oomah, Mazza, & Kenaschuk, 1992), giant taro (Bradbury, Egan, & Matthews, 1995; Nahrstedt, 1975) and bamboo (Schwarzmaier, 1976, 1977).

The picrate method (Adsersen, Andersen, & Brimer 1988) and the Feigl-Anger spot test (Van Wyck, 1989) have been used to survey for cyanogenesis a wide range of plants. The latter method depends on endogenous enzyme to catalyse hydrolysis of cyanoglucoside to cyanohydrin which then breaks down to hydrogen cyanide. If the enzyme is not present as in case (G−) above, or

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if the enzyme is inhibited by tannins (Goldstein & Spencer, 1985), then such methods would give a negative or low result.

In an attempt to develop a general method to determine the total cyanide content of any plant material one possibility is to use acid hydrolysis (Bradbury et al., 1991, 1994), since this does not require the presence of a specific enzyme and would be effective with all cyanoglucosides. However, there is some loss of HCN gas during acid hydrolysis at 100 °C (Bradbury et al., 1994). Another possibility is the picrate method, recently developed as a kit and available free of charge to health workers and agriculturalists in developing countries for analysis of cassava roots and cassava products (Bradbury, Egan, & Bradbury, 1999; Egan, Yeoh, & Bradbury, 1998). Fortunately, as mentioned above most cyanogenic plants, especially those used as food sources (Jones, 1998) contain the appropriate enzyme to ensure hydrolysis of the cyanoglucoside. In this paper we have generalised the picrate and acid hydrolysis methods to allow determination of the total cyanide content of any plant and food.

2. Materials and methods

Linamarin. amygdalin, prunasin, and beta-glucosidase (from almonds) were obtained from Sigma Chemical Co, St Louis, MO, USA. Picric acid, barbituric acid and isonicotinic acid were from BDH Ltd, Poole, UK. Linustatin and neolinustatin were gifts from Professor I.S. Palmer of South Dakota State University, Brookings, USA. Chloramine-T and potassium cyanide (AR) were from Prolabo, Paris, France and acetone cyanohydrin (99%) from Aldrich Chemical Co, Sydney, Australia.

Fresh cassava roots, giant taro leaves and young sorghum leaves were obtained from plants growing in the Plant Culture Facility at Australian National University. Samples of flax seed meal were obtained from various health food stores in Australia. Ripe peaches, apricots, plums and nectarines were purchased in Canberra supermarkets, the stones were cracked open and the soft kernels were ground in a pestle and mortar and used. Apple seeds were obtained from ripe apples.

Linamarin, amygdalin, prunasin and linustatin were accurately weighed to make 10 ml of aqueous solution containing 1.00 g HCN equivalents /l. An aliquot was used in the picrate and acid hydrolysis methods. Cassava roots were sampled as described previously (Bradbury et al., 1999) and a sector (about 12 g) was cut up and ground in a pestle and mortar. Flax seed (linseed) meal was ground in a coffee grinder. Leaves of giant taro and sorghum grown in a glasshouse were cut up with scissors and ground in a pestle and mortar. Bamboo shoots grown at Whitsunday, Queensland, were obtained from Mr. S. Keilar within 24 h of harvesting. The shoot was cut longitudinally and the leaves were removed. The half section was cut transversely at the tip, middle and base and a small portion was sliced and ground in a pestle and mortar. Because of rapid breakdown of the bamboo cyanoglucoside (taxiphyllin) to HCN it was important to process the ground material immediately. Bamboo shoots were stable in the refrigerator at 4 °C for 1 week, but could not be stored in the deep freeze.

2.1. Acid hydrolysis method

For linamarin, amygdalin, prunasin, and linustatin, 100 μl of 1 g HCN equivalents/l solutions were added to 0.1 M phosphoric acid and made up to 25 ml in a standard flask. Standard solutions of 1.00 g HCN equivalents/l of KCN and acetone cyanohydrin were prepared and added to 0.1 M phosphoric acid. To duplicate aliquots (2.00 ml) of these solutions was added 2.0 ml of 4 M sulphuric acid and the mixture heated for different times in a B14 stoppered test tube in boiling water, which just covered the liquid level in the test tube. Each sample was cooled in ice cold water, with the stopper loosely in place, 5.0 ml of 3.6 M sodium hydroxide was added and after 5 min, 1 ml was added to 7 ml of 0.2 M acetate buffer at pH 5.0. Chloramine-T (0.4 ml) was added and about 5 min later 1.6 ml of isonicotinic acid/barbituric acid. After one hour the absorbance was measured at 600 nm (Bradbury et al., 1994). A calibration curve was obtained using a standard solution of KCN (Bradbury et al., 1994). The amount of total cyanide present was obtained by linear extrapolation to zero time of the data. Ten replicate analyses were made in duplicate with amygdalin with linear extrapolation to zero time.

Finely ground material (usually 100 mg) from giant taro and sorghum leaves, bamboo shoot, cassava roots, flax seed meal, apple seeds and kernels of peach, apricots, plums and nectarines, were taken immediately after grinding and made up to 10.0 ml with 0.1 M phosphoric acid. The mixture was centrifuged and duplicate 2.00 ml taken for analysis as described above. The total cyanide content was obtained by linear extrapolation to zero time. The time of heating which gave maximum recovery was recorded in Table 3. In further experiments on different samples of the same plant product, a single hydrolysis time was used, which corresponded to the maximum recovery for that plant.

2.2. Picrate method

Either a small amount of the cyanoglucoside (see above) was pipetted out or 25–100 mg of the ground powder or leaf was weighed out (immediately after grinding) into a small flat bottomed plastic vial (Egan et al., 1998). Phosphate buffer (0.5 ml of 0.1 M at pH

Table 1
Plants studied in this paper and their cyanoglucosides

Plant	Plant part and product	Cyanoglucoside
Cassava (*Manihot esculenta*)	Roots, leaves and cassava products (flour, gari, etc.)	Linamarin and small amountof lotaustralin
Sorghum (*Sorghum vulgare*)	Young leaves	Dhurrin
Flax (*Linum usitatissimum*)	Seed (linseed) linseed meal	Linustatin, neolinustatin and linamarin
Giant taro (*Alocasia macrorrhizos*)	Stem, leaf	Triglochinin
Bamboo (*Bambusa arundinacea* Willd)	Young shoot	Taxiphyllin
Apple (*Malus* spp)	Seed	Amygdalin
Peach (*Prunus persica*)	Kernel	Amygdalin
Apricot (*Prunus armeniace*)	Kernel	Amygdalin
Plum (*Prunus* sp.)	Kernel	Amygdalin
Nectarine (*Prunus persica var nucipersica*)	Kernel	Amygdalin

Table 2
Recovery of cyanide (%) from model compounds including cyanoglucosides using acid hydrolysis[a] and picrate methods

Model compound/cyanoglucoside	Recovery of cyanide (%) using		Acid hydrolysis method	
	Picrate method	Acid hydrolysis	Time of maximum recovery (min)[b]	k_0[c]
Potassium cyanide	102	99	0	0.39
Acetone cyanohydrin	100	99	20	0.18
Linamarin	102	103	60	0.17
Linustatin	102	97	60	0.15
Amygdalin	100	91	60	0.12
Prunasin	100	96	60	0.13
Mean	101(1.1)[d]	97.5 (4.0)[d]		

[a] Hydrolysis in 2 M sulfuric acid at 100 °C.
[b] Obtained from Figs. 1 and 2.
[c] The gradient of the linear sections of the graphs, after the occurrence of the maxima, in Figs. 1 and 2 [see Eq. (2)].
[d] Standard deviation.

Table 3
Comparison of the total cyanide contents (mg HCN equivalents/kg plant material = ppm) of plants/foods/feeds using acid hydrolysis and picrate methods of analysis

Plant material/food/feed	Time of maximum recovery (min) during acid hydrolysis	k_0[a]	Total cyanogen contents (ppm) using	
			Acid hydrolysis	Picrate[b]
Flax seed meal	75	0.10	390	360
Cassava root	60	0.17	27	25
Sorghum leaf	60	0.17	750	790
Peach stone	50	0.13	710	720
Plum stone	50	0.12	696	764
Nectarine stone	50	0.13	196	209
Apricot stone	50	0.13	785	813
Apple seed (Fuji)	50	0.10	690	790
Giant taro leaf[c]	15	0.29	29	32
Bamboo shoot	10	0.24	1010	1060

[a] The gradient of the linear sections of the graphs of% recovery of cyanide vs time.
[b] No additional enzyme needed except for sorghum leaf where 5.0 EU of beta-glucosidase was added.
[c] See Bradbury et al. (1995).

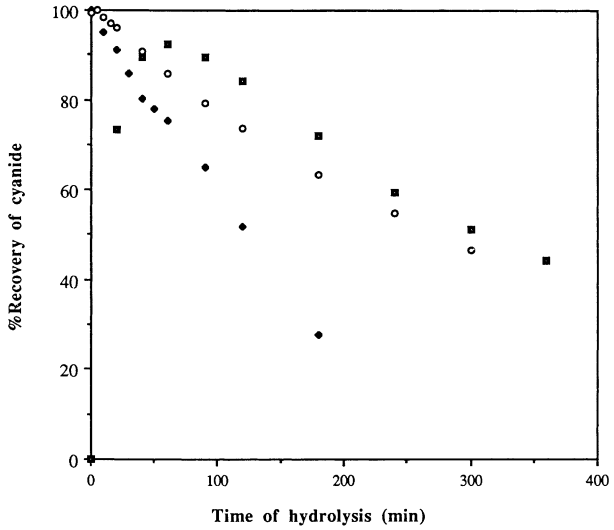

Fig. 1. Percentage recovery of cyanide vs time of hydrolysis in 2 M sulfuric acid in a stoppered B14 test tube at 100 °C of KCN (◆), acetone cyanohydrin (○) and linamarin (⊡).

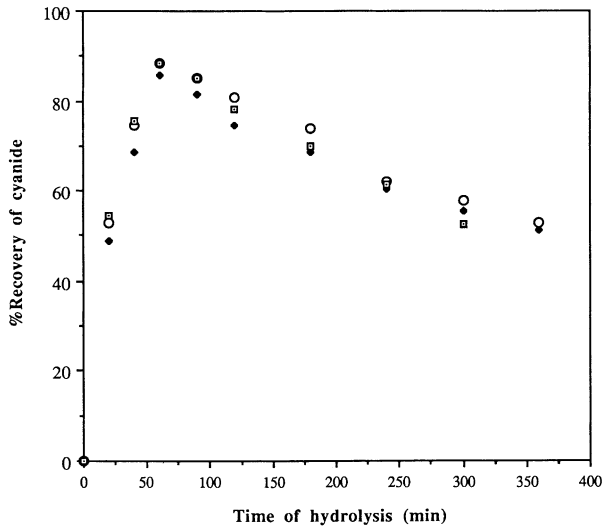

Fig. 2. Percentage recovery of cyanide vs time of hydrolysis in 2 M sulfuric acid in a stoppered test tube at 100 °C of the cyanogenic glucosides amygdalin (◆), prunasin (○) and linustatin (⊡).

4–10) was added, followed by exogenous enzyme in the cases of cyanoglucoside solutions and with some plant products. A picrate paper attached to a plastic backing strip (Bradbury et al., 1999) was added and the vial immediately closed with a screw stopper. After about 16 h at 30 °C, the picrate paper was removed and immersed in 5.0 ml water for not less than 30 min The absorbance was measured at 510 nm and the total cyanide content (ppm) determined by the equation

total cyanide content (ppm)

$$= 396 \times \text{absorbance} \times 100/z,$$

where z = weight (mg) of ground powder or leaf (Bradbury et al., 1999). Ten replicate analyses were made in duplicate with amygdalin to check the reproducibility of the method.

3. Results and discussion

To establish that these methods are applicable to any cyanogenic plant and to any cyanoglucoside it is necessary to show that it works with a range of each. Ten different plants and eight cyanoglocosides were studied (see Table 1).

3.1. Acid hydrolysis of model compounds

The loss of HCN from heating KCN in 2 M sulfuric acid at 100°C under the conditions of the experiment is shown in Fig 1.

The linear relation between % recovery and time ($r^2 = 0.997$) shows that the loss of HCN through the glass stopper is a zero order process, fitting the simple equation

$$\%\ \text{recovery cyanide} = 98.6 - 0.393\ \text{time (min)} \qquad (1)$$

The extrapolation to zero time gives a recovery of 98.6%, within experimental error of 100%. By substituting% recovery = 0 in Eq. (1), all HCN would be lost in 254 min. Eq. (1) is rearranged by substituting x and x_0 which are the percentage recoveries of cyanide at time t and $t = 0$ respectively, and k_0 = zero order rate constant, to give

$$x_0 - x = k_0 t. \qquad (2)$$

The value of k_0 is dependent on the rate of loss of HCN from the B14 stoppered test tube, which is not exactly the same from one experiment to the next.

The extrapolated % recovery of HCN at zero time of heating on hydrolysis of acetone cyanohydrin shown in Fig. 1 and Table 2 is 99%. With linamarin there are two hydrolysis steps since linamarin is hydrolysed first to glucose and acetone cyanohydrin and then the latter is hydrolysed to cyanide. This causes a maximum in the curve at about 60 min. Extrapolation to $t = 0$ of the linear section of the graph after the maximum gives the % recovery of cyanide from linamarin. The gradient of the linear sections of the lines in Fig. 2 are recorded as k_0 values in Table 2 and show that the loss of HCN through the stopper from KCN is greater than from acetone cyanohydrin and linamarin.

Fig. 2 shows the results of the hydrolysis of the cyanoglucosides linustatin, amygdalin and prunasin. Ten replicate analyses of amygdalin were made with extrapolation to zero time with a mean percentage recovery

of 90.6 (S.D. 2.2). The recoveries are given in Table 2 of % cyanide obtained by extrapolations to zero time in Figs. 1 and 2. The mean % recovery of 97.5% (S.D. 4.0) over six moderately pure cyanogens shows that the acid hydrolysis method gives reasonably accurate results providing that an extrapolation is made to zero time.

3.2. Acid hydrolysis of various cyanoglucosides from plant and food/feed sources

The hydrolysis of the cyanoglucosides of flax seed meal is shown in Fig. 3. The total cyanide content obtained by extrapolation to zero time is 390 ppm. To allow comparisons between the acid hydrolyses of the cyanoglucosides from ten different plants, the data set for the linear section of the flax hydrolysis in Fig. 3 is normalised by setting the value of 390 ppm equal to 100%. This normalised data is then replotted ($r^2 = 0.977$) and the gradient of the line (k_0) recorded in Table 3. A similar procedure was carried out for nine other plants and the k_0 values and maximum recovery times are given in Table 3.

The relative stabilities to acid hydrolysis of the cyanoglucosides from the various cyanogenic plants is shown by the maximum recovery times (Table 3) which increase in the series taxiphyllin (bamboo) < triglochinin (giant taro) < amygdalin (apple, peach, apricot, plum and nectarine) < dhurrin (sorghum) = linamarin (cassava) < linustatin, neolinustatin and linamarin (flax). Linustatin and neolinustatin are the most stable to hydrolysis presumably because they both have a disaccharide group. The least stable is taxiphyllin, which is a stereoisomer of dhurrin.

3.3. Picrate method applied to cyanoglucosides and plant material

Ten replicate analyses by the picrate method on amygdalin gave a mean recovery of 101.9 % (S.D. 0.64). The good % recoveries of cyanide from all model compounds in Table 2 shows the accuracy of this method. Fortunately, in determination of cyanoglucosides in different plants by the picrate method, the plant material normally contains enough of the enzyme required to hydrolyse the cyanoglucoside present in that plant. This is shown by the results in Table 3 in which no additional enzyme was needed except for sorghum leaves where the recovery increased from 70% (with no added enzyme) to 100% if 5.0 EU of beta-glucosidase was added.

3.4. Effect of pH on cyanide recovery using picrate method

The data shown in Table 4 result from experiments made from pH 4–10 on different plant materials with no added enzyme. The data is expressed as a percentage of the highest value obtained taken as 100%. There is a pH optimum for the liberation of HCN from the cyanoglucoside which involves three steps: (1) enzyme catalysed hydrolysis of the cyanoglucoside to cyanohydrin, (2) base catalysed breakdown of cyanohydrin to cyanide and (3) liberation of HCN from the solution and its reaction with the picrate paper. Given that the precision of these duplicate experiments is about 5% it is clear that the optimum pH is 5–6, except for apple seed and sorghum leaf where it is about 8. In earlier work we used a different approach which showed that the optimum pH for cassava was 8 (Egan et al., 1998), but these results show that pH 5–6 is optimal, which agrees with a pH optimum of 6 for linamarase (Yeoh, 1989).

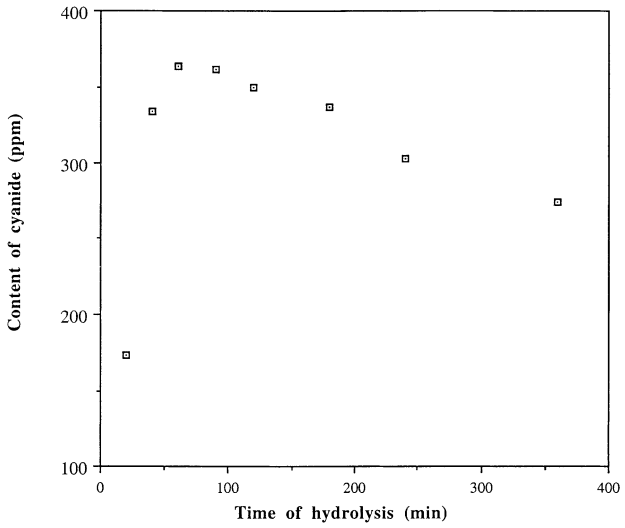

Fig. 3. The cyanide content (ppm) of flax seed meal vs time of hydrolysis in 2 M sulfuric acid in a B14 stoppered test tube at 100 °C.

Table 4
The effect of pH on cyanide yield from different plants using the picrate method[a]

Plant material	Cyanide yield (%) at pH						
	4	5	6	7	8	9	10
Cassava	93	97	100	89	86	82	78
Flax seed meal	97	100	97	93	85	–	–
Peach stone	67	100	96	78	77	74	–
Bamboo shoot	–	100	94	88	88	–	–
Apple seed (Fuji)	75	85	90	97	100	98	–
Sorghum leaf	–	25	61	80	100	89	86

[a] The cyanide yield (%) is calculated for comparison purposes by defining the maximum value in the pH series as 100%.

3.5. Total cyanide content of flax seed products from health food shops

Analyses of the intact seed gave very low results (see Table 5) but the values increased greatly with ground seed. Increases in cyanide content also occurred after grinding flax seed meal. For finely ground flax seed and

Table 5
Total cyanide content in ppm[a] of flax seed (linseed) and flax seed meal

Sample number	Source	Type of sample	Total cyanide content (ppm) by	
			Acid hydrolysis method[b]	Picrate method[c]
1	Australia	Seed	–	2
		Seed, ground	140	140
2	Canada	Seed		2
		Seed, ground	360	360
		Seed meal	–	210
		Meal, ground	–	360
3	New South Wales	Seed	–	3
		Seed, ground	360	350
		Seed meal	–	180
		Meal, ground	–	340
4	New South Wales	Seed, ground	360	370
5	Queensland	Seed	–	120
		Seed, ground	300	310
		Seed meal	–	330
6	Aust. Cap. Terr.	Seed, ground	170	160
7	Aust. Cap. Terr.	Seed, ground	–	160
8	Victoria	Seed cake	–	240
		Seed cake, ground	250	250
9	South Australia	Seed, ground	290	300
10	South Australia	Soy & linseed bread		
		Premix. flour	12	9
		Flour, ground	31	18
11	New Zealand	meal	210	220
		Meal, ground	250	240

[a] mg HCN equivalents/kg flax product = ppm.
[b] Acid hydrolysis results obtained by heating in 2 M sulfuric acid at 100 °C for 50 min; no extrapolation to zero time.
[c] Results obtained in phosphate buffer at pH 5 with no added enzyme.

Table 6
Total cyanide content in ppm[a] of bamboo shoots

Number of sample	Part of shoot	Cyanide content (ppm) determined by	
		Acid hydrolysis method[b]	Picrate method[c]
1	Tip	920	1010
1	Middle	730	820
1	Base	114	123
2	Tip	1040	1150
2	Middle	620	700
2	Base	280	300
3	Tip	1460	1600
3	Middle	1140	1330
3	Base	380	440

[a] mg HCN equivalents/kg bamboo shoots = ppm.
[b] Acid hydrolysis in 2 M sulfuric acid at 100 °C was carried out for 10 min due to rapid hydrolysis of taxiphyllin (Table 3) and no extrapolation was made to zero time.
[c] Bamboo shoot was crushed in a mortar and pestle and 25 mg samples were added to 0.5 ml phosphate buffer at pH 5, a picrate paper was added and the plastic bottle immediately closed and left for 16 h at 30 °C. Liberation of HCN occurred rapidly after crushing the bamboo shoot.

meal the range of values is 140–370 ppm. The WHO safe level for total cyanide content of cassava flour is 10 ppm (FAO/WHO, 1991) and values in excess of 100 ppm are considered a danger to health (Cardoso, Ernesto, Cliff, Egan, & Bradbury, 1998; Ernesto et al., 2001). Since cassava is a staple food in many developing countries there is a considerable daily intake, whereas flax seed products are a health food and hence the daily intake would be much smaller.

3.6. Total cyanide contents of bamboo shoots

The results in Table 6 give the total cyanide content of tip, middle and base of samples of bamboo shoot determined by the picrate and acid hydrolysis methods. The picrate results are on average 10% higher than the acid hydrolysis results which were obtained from a single time of hydrolysis. The total cyanide levels are highest in the tip and lowest at the base of the bamboo shoot. The values are in the range 100–1600 ppm and represent a potential health hazard, but since the loss of HCN after cutting is quite rapid it may perhaps not present a problem for consumers.

3.7. Total cyanide content of sorghum leaves

There was no cyanoglucoside (dhurrin) present in ground sorghum seed but sorghum leaves 1, 2, 3 and 4 weeks after germination gave total cyanide levels of 740, 280, 40 and 60 ppm, respectively, by the acid hydrolysis and picrate methods. The picrate result for brownish 4 week old sorghum leaves was only 4 ppm but increased to 60 ppm on addition of the enzyme beta-glucosidase. The high levels of total cyanide and its decrease over time accounts for the practice of Australian farmers in not grazing cattle for some weeks in new regrowth of sorghum.

3.8. Comparison of acid hydrolysis and picrate methods

Ten replicate analyses of amygdalin by the acid hydrolysis and picrate methods gave mean percent recoveries (standard deviations in parentheses) of 90.6 (2.2) and 101.9 (0.64) respectively. In Table 2,the mean percent recovery from model compounds is 97.5 (4.0) and 101.1 (1.1) for the acid hydrolysis and picrate methods, respectively. These results show that the picrate method is more reproducible and accurate than the acid hydrolysis method. In Table 3, the acid hydrolysis results are lower than the picrate results in nine cases out of eleven. Having regard to the large differences between the chemical procedures used in the two methods the agreement between them is reasonable. The advantage of the *acid hydrolysis method* is that acid hydrolysis works in principle for all cyanogenic glucosides and has been successful in all cases examined thus far. However, to get accurate results, it requires measurements at different heating times and extrapolation to zero time. The advantages of the *picrate method* are that it is more accurate and very simple to use compared with the acid hydrolysis method. It is currently available in kit form for cassava roots and products, sorghum leaves, bamboo shoots and flax seed meal, which are available free of charge to agriculturalists and health workers in developing countries (Bradbury et al., 1999; Egan et al., 1998).

3.9. General method for determination of total cyanide in plants

The acid hydrolysis method is generally applicable to determine the total cyanide content of all plants. However, it is much more difficult to use, less accurate and normally gives slightly lower results than the picrate method, which is the method of choice to determine the total cyanide content of plants of importance for human food.

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