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 cyanoglycosides 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 replicates analysis 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 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. © 2001 Published by Elsevier Science Ltd. All rights reserved.

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1. Introduction

There are at least 2500 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, which rapidly decomposes to hydrogen cyanide and an aldehyde or a ketone (Hofel, 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, horehound and white flax which have four types of plants that contain either both cyanoglycoside and enzyme (GE), the cyanoglycoside but no enzyme (G−), the enzyme but no cyanoglycoside (−E) and finally neither cyanoglycoside 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, Gort, & Hill, 1987), flax (Palmer, Olson, Halverson, Miller, & Smith, 1980; Oomah, Mazza, & Kenatchuk, 1992), giant taro (Bradbury, Egan, & Matthews, 1995; Nabrudst, 1973) and bamboo (Schwarzmaier, 1976, 1977).

The picrate method (Adersen, Andersen, & Briner 1983) and the Périg/Angers spot test (Van Wyck, 1987) have been used to survey for cyanogenesis a wide range of plants. The latter method depends on endogenous enzyme to catalyse hydrolysis of cyanoglycoside to cyanohydrin which then breaks down to hydrogen cyanide. If the enzyme is not present as in case (G−) above, or...
if the enzyme is inhibited by tannins (Goldstein & Spencer, 1983), then such methods would give a nega-
tive or low result.

In an attempt to develop a general method to deter-
mine 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 cy-
noglucosides. 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, Yeash, & Bradbury,
1999). Fortunately, as mentioned above most cyanog-
ogenic 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, amygda
date (from almonds) were obtained from Sigma Che-
mical Co, St Louis, MO, USA. Picric acid, barbituric
acid and isoamyl alcohol were from BDH Ltd, Poole,
UK. Linumin and neolinumin were gifts from Pro-
fessor I.S. Palmer of South Dakota State University,
Brookings, USA. Chloral hydrate and potassium cyanide
were from Prolabo, Paris, France and acetone
(95%) from Aldrich Chemical Co, Sydney,
Australia.

Fresh cassava roots, giant taro leaves and young sor-
gham leaves were obtained from plants growing in the
Plant Culture Facility at Australian National Uni-
versity. Samples of flax seed meal were obtained from
various health food stores in Australia. Ripe peaches,
apricots, plums and nectarines were purchased in Can-
berra 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, amygda
date (from almonds) were obtained from Sigma Che-
mical Co, St Louis, MO, USA. Picric acid, barbituric
acid and isoamyl alcohol were from BDH Ltd, Poole,
UK. Linumin and neolinumin were gifts from Pro-
fessor I.S. Palmer of South Dakota State University,
Brookings, USA. Chloral hydrate and potassium cyanide
were from Prolabo, Paris, France and acetone
(95%) from Aldrich Chemical Co, Sydney,
Australia.

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 break-
down of the bamboo cyano
glucoside (laxshylin) to HCN it was important to process the ground material immedi-
ately. 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, amygda
date, prunasin, and linumin, 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 acetonitrile 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 acetic buffer at pH 5.0. Chloramine-T
(0.4 ml) was added and after 5 min later 1.6 ml of iso-
propionic acid/barbituric acid. After one hour the
absorbance was measured at 600 nm (Bradbury et al.,
1994). A calibration curve was obtained using a stan-
dard 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 amygda
date 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, apri-
cots, plums and nectarines, were taken immediately
after grinding and made up to 100 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 extrap-
olation 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 pro-
duct, a single hydrolysis time was used, which corre-
sponded to the maximum recovery for that plant.

2.2. Picrate method

Either a small amount of the cyano
glucoside (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
112
Table 1

<table>
<thead>
<tr>
<th>Plant</th>
<th>Plant part and product</th>
<th>Cyanoglicoside</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cassava (Manihot esculenta)</td>
<td>Roots, leaves and cassava products (flour, gari, etc.)</td>
<td>Linear amines and small amount of toxic amines</td>
</tr>
<tr>
<td>Sorghum (Sorghum vulgare)</td>
<td>Young leaves</td>
<td>Dihydroxynitrile</td>
</tr>
<tr>
<td>Flex (Linum usitatissimum)</td>
<td>Seed (linseed or meal)</td>
<td>Linen and minor amounts of nitrile and linamarin</td>
</tr>
<tr>
<td>Giant tare (Alocasia macrorrhiza)</td>
<td>Stem, leaf</td>
<td>Triglycine</td>
</tr>
<tr>
<td>Bambus (Bambusa arundinacea Wild)</td>
<td>Young shoot</td>
<td>Tetraglycine</td>
</tr>
<tr>
<td>Apple (Malus sp.)</td>
<td>Seed</td>
<td>Amlygallin</td>
</tr>
<tr>
<td>Peach (Prunus persica)</td>
<td>Kernel</td>
<td>Amlygallin</td>
</tr>
<tr>
<td>Apricot (Prunus armenicae)</td>
<td>Kernel</td>
<td>Amlygallin</td>
</tr>
<tr>
<td>Plum (Prunus sp.)</td>
<td>Kernel</td>
<td>Amlygallin</td>
</tr>
<tr>
<td>Nectarine (Prunus persica var. nucipersica)</td>
<td>Kernel</td>
<td>Amlygallin</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Model compound/cyanoglicoside</th>
<th>Recovery of cyanide (%) using <em>Piricate</em> method</th>
<th>Acid hydrolysis method</th>
<th>Time of maximum recovery (min)<em>k</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium cyanide</td>
<td>102</td>
<td>Acid hydrolysis</td>
<td>0.39</td>
</tr>
<tr>
<td>Azetone cyanhydrin</td>
<td>100</td>
<td>Acid hydrolysis</td>
<td>0.16</td>
</tr>
<tr>
<td>Linamarin</td>
<td>102</td>
<td>Acid hydrolysis</td>
<td>0.17</td>
</tr>
<tr>
<td>Linustain</td>
<td>102</td>
<td>Acid hydrolysis</td>
<td>0.15</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>100</td>
<td>Acid hydrolysis</td>
<td>0.15</td>
</tr>
<tr>
<td>Prunasin</td>
<td>100</td>
<td>Acid hydrolysis</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Mean     | 97.5 (4.0)*

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

Table 3

<table>
<thead>
<tr>
<th>Plant material/food/feed</th>
<th>Time of maximum recovery (min) during acid hydrolysis</th>
<th>Total cyanogen content (ppm) using acid hydrolysis and <em>Piricate</em> method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flex seed meal</td>
<td>75</td>
<td>0.10</td>
</tr>
<tr>
<td>Cassava root</td>
<td>60</td>
<td>0.17</td>
</tr>
<tr>
<td>Sorghum leaf</td>
<td>55</td>
<td>0.17</td>
</tr>
<tr>
<td>Peach stone</td>
<td>55</td>
<td>0.12</td>
</tr>
<tr>
<td>Plum stone</td>
<td>50</td>
<td>0.12</td>
</tr>
<tr>
<td>Nectarine stone</td>
<td>50</td>
<td>0.13</td>
</tr>
<tr>
<td>Apricot stone</td>
<td>50</td>
<td>0.12</td>
</tr>
<tr>
<td>Apple seed (Fuji)</td>
<td>50</td>
<td>0.10</td>
</tr>
<tr>
<td>Giant tare leaf</td>
<td>15</td>
<td>0.29</td>
</tr>
<tr>
<td>Bamboo shoot</td>
<td>10</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* The gradient of the linear sections of the graphs of recovery of cyanide vs. time.
* No additional enzymes needed except for sorghum leaf where 5.0 EU of beta-glucosidase was added.
* See Bradbury et al. (1995).
4-10) was added, followed by exogenous enzyme in the
cases of cyanoglycoside solutions and with some plant
products. A plicrate 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 plicrate paper was removed and immersed
in 3.0 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 x absorbance x 100/z,
where z = weight (mg) of ground powder or leaf (Brad-
bury 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 cyanoglycoside it is neces-
sary to show that it works with a range of each. Ten
different plants and eight cyanoglycosides 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
(r² = 0.997) shows that the loss of HCN through the

glass stopper is a zero order process, fitting the simple
equation

% recovery cyanide = 98.6 - 0.393 time (min) (1)

The extrapolation to zero time gives a recovery of
98.6%, within experimental error of 100%. By sub-
stituting% recovery = 0 in Eq. (1), all HCN would be
lost in 254 min. Eq. (1) is rearranged substituting x
and n which are the percentage recoveries of cyanide at
time t and t = 0 respectively, and k₂ = zero order rate
constant, to give

x₂ - x = k₂t. (2)

The value of k₂ is dependent on the rate of loss of
HCN from the B14 stopped test tube, which is not
exactly the same from one experiment to the next.
The extrapolated % recovery of HCN at zero time of
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 lin-
er 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 k₂
tables 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 cy-

ano-glycosides linamarin, amygdalin and prunasin. Ten
replicate analyses of amygdalin were made with extrap-
olation to zero time with a mean percentage recovery

Fig. 1. Percentage recovery of cyanide vs time of hydrolysis in 2 M
sulfuric acid in a stopped B14 test tube at 100 °C of KCN (●), ace-
tone cyanohydrin (○) and linamarin (○)

Fig. 2. Percentage recovery of cyanide vs time of hydrolysis in 2 M
sulfuric acid in a stopped test tube at 100 °C of the cyanogenic gly-
co-side amygdalin (●), prunasin (○) and linamarin (○)
of 90.6 (S.D. 2.2). The recoveries are given in Table 2 of
9% cyanide obtained by extrapolation to zero time in
Figs. 1 and 2. The mean % recovery of 97.5% (S.D. 4.0)
over six moderately pure cyanogamins 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 cyanoglycosides from
plant and food/seed sources
The hydrolysis of the cyanoglycosides 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 hydrolysates of the
cyanoglycosides 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 ($k_{d}$) recorded in
Table 3. A similar procedure was carried out for nine
other plants and the $k_{d}$ values and maximum recovery
times are given in Table 3.
The relative stabilities to acid hydrolysis of the cya-
nglycosides 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 (cas-
sava) < linustatin, neoolinustatin and linamarin (flax).
Linustatin and neoolinustatin are the most stable to
hydrolysis presumably because they both have a dis-
accharide group. The least stable is taxiphyllin, which is
a stereoisomer of dhurrin.
3.3. Picate method applied to cyanoglycosides and plant
material
Ten replicate analyses by the picate method on
amygdalin gave a mean recovery of 101.9 % (S.D. 0.64).
The good % recoveries of cyanide from all model com-
pounds in Table 2 shows the accuracy of this method.
Fortunately, in determination of cyanoglycosides in
different plants by the picate method, the plant mate-
rial normally contains enough of the enzyme required to
hydrolyse the cyanoglycoside 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 picate 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 cyanoglo-
side which involves three steps: (1) enzyme catalysed
hydrolysis of the cyanoglycoside to cyanoxyhydrin, (2)
base catalysed breakdown of cyanoxyhydrin to cyanide
and (3) liberation of HCN from the solution and its reac-
tion with the picate 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 opti-
mum 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 (Yuen, 1969).
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

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Source</th>
<th>Type of sample</th>
<th>Total cyanide content (ppm) by</th>
<th>Acid hydrolysis method*</th>
<th>Piscara method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Australia</td>
<td>Seed</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Canada</td>
<td>Seed, ground</td>
<td>140</td>
<td>140</td>
<td>140</td>
</tr>
<tr>
<td>3</td>
<td>New South Wales</td>
<td>Seed, ground</td>
<td>360</td>
<td>360</td>
<td>360</td>
</tr>
<tr>
<td>4</td>
<td>New South Wales</td>
<td>Seed, ground</td>
<td>210</td>
<td>210</td>
<td>210</td>
</tr>
<tr>
<td>5</td>
<td>Australia</td>
<td>Meal, ground</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Australia</td>
<td>Seed, meal</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>New South Wales</td>
<td>Seed, meal</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>Queensland</td>
<td>Seed, ground</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>9</td>
<td>Australia</td>
<td>Seed, ground</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>10</td>
<td>Victoria</td>
<td>Seed, meal</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
<tr>
<td>11</td>
<td>South Australia</td>
<td>Seed, ground</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>12</td>
<td>South Australia</td>
<td>Soy &amp; limed bread</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>13</td>
<td>South Australia</td>
<td>Soy &amp; limed bread</td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td>14</td>
<td>South Australia</td>
<td>Soy &amp; limed bread</td>
<td>290</td>
<td>290</td>
<td>290</td>
</tr>
<tr>
<td>15</td>
<td>Wet. seed</td>
<td>12</td>
<td>9</td>
<td>9</td>
<td>9</td>
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<tr>
<td>16</td>
<td>Fourth, meal</td>
<td>210</td>
<td>220</td>
<td>220</td>
<td>220</td>
</tr>
<tr>
<td>17</td>
<td>Meal, ground</td>
<td>250</td>
<td>240</td>
<td>240</td>
<td>240</td>
</tr>
</tbody>
</table>

* mg HCN equivalent/kg flax seed (limed) and flax seed meal ppm.

* Acid hydrolysis results obtained by heating in 2 M sulfuric acid at 100 °C for 10 min; no extrapolation to zero time.

* Results obtained in phosphate buffer at pH 5 with no added enzyme.

### Table 6

<table>
<thead>
<tr>
<th>Number of sample</th>
<th>Part of bamboo shoot</th>
<th>Cysteine content (ppm) determined by</th>
<th>Acid hydrolysis method*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tip</td>
<td>920</td>
<td>1020</td>
</tr>
<tr>
<td>2</td>
<td>Middle</td>
<td>710</td>
<td>820</td>
</tr>
<tr>
<td>3</td>
<td>Base</td>
<td>114</td>
<td>122</td>
</tr>
<tr>
<td>4</td>
<td>Tip</td>
<td>1040</td>
<td>1150</td>
</tr>
<tr>
<td>5</td>
<td>Middle</td>
<td>620</td>
<td>700</td>
</tr>
<tr>
<td>6</td>
<td>Base</td>
<td>250</td>
<td>300</td>
</tr>
<tr>
<td>7</td>
<td>Tip</td>
<td>1460</td>
<td>1600</td>
</tr>
<tr>
<td>8</td>
<td>Middle</td>
<td>1140</td>
<td>1300</td>
</tr>
<tr>
<td>9</td>
<td>Base</td>
<td>460</td>
<td>460</td>
</tr>
</tbody>
</table>

* mg HCN equivalent/kg bamboo shoots ppm.

* Acid hydrolysis in 2 M sulfuric acid at 100 °C was carried out for 10 min due to rapid hydrolysis of tatipholin (Table 5) and no extrapolation was made to zero time.

* Bamboo shoot was crushed in a mortar and pestle and 23 mg samples were added to 0.5 ml phosphate buffer at pH 5, a piscara 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.

### 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 piscara and acid hydrolysis methods. The piscara 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 100-600 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 contents of sorghum leaves

There was no cyanoglycoside (dihydrin) present in ground sorghum seed but sorghum leaves 1, 2, 3 and 4 weeks after germination gave total cyanide levels of 745, ppm.
Ten replicate analyses of amylodalin 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 (Northbury et al., 1997; Egan et al., 1996).

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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