PROTOCOL A: DETERMINATION OF TOTAL CYANIDE IN CASSAVA ROOTS

1. Place the small plastic balance on its U-shaped mount (see sketch 1) so that it swings freely. It has a 100 mg weight glued inside one spoon.
2. A 1-2 mm thick cross section of the clean cassava root is cut across the middle of the root as shown in sketch 2. The peel is removed and a sector is cut (see sketch 2 and 3) and its weight adjusted to 100 mg by cutting off small pieces along the straight edge of the sector and weighing on the small balance.
Note. The total cyanide content varies between different roots from the one plant and between different plants of the same variety. To get a more reliable average result you can determine the total cyanide content of two or more different roots from different plants of the same variety.
3. Please follow sketch 3. Place a round paper disc containing buffer at pH 6[a] in a flat-bottomed plastic bottle and place a 100 mg sector of cassava root on top of it. *Note. Do not leave these sectors standing in the air for more than one hour before setting up the analysis .*
4. Add 0.5 ml of clean water using the plastic pipette (sketch 3).
5. Immediately add a yellow picrate paper attached to a plastic strip[b] (sketch 3). The picrate paper must not touch the liquid in the bottle.(WHEN NOT IN USE STORE PICRATE PAPERS IN THE DEEP FREEZE OF THE REFRIGERATOR).
6. IMMEDIATELY close the bottle with a screw capped lid (sketch 3).
7. As a **blank**, prepare another sample as shown in sketch 3 but with no cassava present.
8. Every time you run a set of experiments you should check that the method works OK, using a **standard** pink linamarin paper from the kit.
9. Follow sketch 4. Place a round paper disc with a black spot containing buffer and enzyme in the bottle. Add a pink **standard** paper and then 0.5 ml water from a pipette and the picrate paper. IMMEDIATELY close the bottle with the screw capped lid.
10. Allow the bottles to stand for 16-24 hour at room temperature.
11. Open the bottles and match the colour of the picrate papers against the shades of colour of the colour chart supplied.
12. Read off from the colour chart the total cyanide content in ppm in the cassava root. Also check out that the **blank** is zero and the **standard** gives a colour of about 50 ppm.

THIS SECTION TO BE FOLLOWED IF YOU HAVE A SPECTROPHOTOMETER

13. Carefully remove the plastic backing sheet (it may be washed and used again) from the picrate paper.
14. Place the picrate paper in a test tube and add 5.0 ml of water measured accurately with a pipette.
15. Leave the test tube at room temperature for about 30 min with occasional gentle stirring.
16. Take the **blank** picrate paper (see 7 above), remove its plastic sheet and place the yellow picrate paper in 5.0 ml of water for about 30 min with occasional gentle stirring.
17. Measure the absorbance at 510 nm of the picrate solution from 15 against the blank from 16.
18. The total cyanide content in ppm is calculated by the equation[1]
total cyanide content (ppm) = 396 x absorbance
19. The cyanide content obtained for the same sample of root, from both measurements 12 and 18, should be about the same. Also check that the **standard** value agrees using both methods.

FOOTNOTES: IF YOU WISH TO PREPARE YOUR OWN PHOSPHATE BUFFER AND PICRATE PAPERS

[a] For field work it is best to have the phosphate buffer inside the paper discs. However, in the laboratory it is simpler not to use a paper disc. Simply add 0.5 ml of 0.1 M phosphate buffer at pH 6. There are 2 different methods of preparation of the phosphate buffer as follows:
(1) Add 750 ml of water to 80 mL of concentrated phosphoric acid (88% H_3PO_4). A solution of 10 M sodium hydroxide is prepared by dissolving 100 g of sodium hydroxide pellets in

water and making up to 250 mL. The sodium hydroxide solution is added to the phosphoric acid solution slowly with stirring until the pH measured with a pH meter increases up to 6.0.
(2) Prepare two one molar solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate by dissolving the calculated amounts (check the labels on the bottles) of each solid to make 1 M solutions. Carefully add the acidic sodium dihydrogen phosphate solution to the disodium hydrogen phosphate solution until the pH decreases to 6.0.
To make 0.1 M phosphate buffer mix 100 ml of 1.0 M phosphate buffer with 900 ml water.

b To prepare your own picrate papers you need your own bottle of moist picric acid purchased from BDH or another supplier. Weigh out 1.4 g of moist picric acid and add 100 ml of sodium carbonate solution, made by dissolving 2.5 g of sodium carbonate in 100 ml of water. Using a filter paper sheet supplied in the kit, cut about a 10 cm x 10 cm square of paper and place it in the yellow picrate solution in a dish for about 20 sec and hang it up to dry in air. *Note. Wear gloves if available when handling picric acid papers. Wash off with water any yellow picric acid on hands.* Unevenly coloured sections of the paper particularly at the edges are cut off. The paper is cut into 30 mm x 10 mm rectangular pieces. Each piece is glued using one small drop of PVA hobby glue to a plastic strip (10 mm x 50 mm), cut from overhead transparency plastic sheet supplied in the kit. It is glued so that the upper end of the yellow paper is 5-10 mm from one end of the plastic strip (see sketch 3). Picrate papers must not be left in bright sunlight and should not be left in laboratory light for long periods. STORE PICRATE PAPERS IN THE DARK IN THE DEEP FREEZE OF THE REFRIGERATOR WHERE THEY ARE STABLE INDEFINITELY[1]. At room temperature they gradually darken and after one month cannot be used with the colour chart but may still be used with the spectrometer method, because the darker colour cancels out. [2]

TROUBLE SHOOTING

The total cyanide content from using the pink **standard** paper should be about 50 ppm. If it is not between 40 and 60 ppm then it is most likely that there is something wrong with the picrate paper. If the picrate paper has been left at room temperature for more than one month then it will have gradually become darker, and will look like about 20 ppm on the colour chart. When used with the **standard** it will get darker still and could look like about 70 ppm on the colour chart. If the picrate paper has been left in bright sunlight it will become bleached on one side and will be spoiled. If you use a bottle which is not gas tight (e.g. the screw cap is cracked) then HCN gas would escape and this would give a low result.

LIST OF COMPONENTS OF KIT A

The kit has the following components:
1. Protocol A, which gives full instructions for total cyanide analysis of cassava roots.
2. A plastic balance with a 100 mg weight glued into one spoon, for weighing 100 mg of cassava root.
3. 30 flat-bottomed plastic bottles with screw lids.
4. Two graduated 1 ml, plastic pipettes.
5. Bottle containing 100 buffer papers at pH 6.
6. 100 yellow picrate papers glued to strips of clear plastic with hobby glue. STORE IN THE DEEP FREEZE OF THE REFRIGERATOR. STABLE FOR ONE MONTH ONLY AT ROOM TEMPERATURE. Picric acid is not supplied because it cannot be sent by air.
7. Colour chart with 10 shades of colour which correspond to 0 – 800 ppm total cyanide.
8. Ten pink **standard** papers containing linamarin equal to 50 ppm cyanide.
9. Ten paper discs which contain buffer at pH 6 and the enzyme linamarase. These papers have a small black spot, so that they will not be confused with buffer papers.
10. Filter paper and plastic overhead transparency sheets for making more picrate papers.

References

[1] Bradbury, M G., Egan, S.V. and Bradbury, J H (1999) Determination of all forms of cyanogens in cassava roots and cassava products using picrate paper kits. J.Sci. Food Agric., 79, 593-601.

[2]Egan, S.V., Yeoh, H.H. and Bradbury, J.H. (1998) Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. J. Sci. Food Agric.76, 39-48

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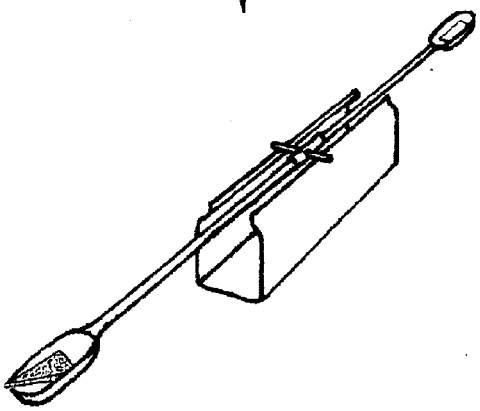
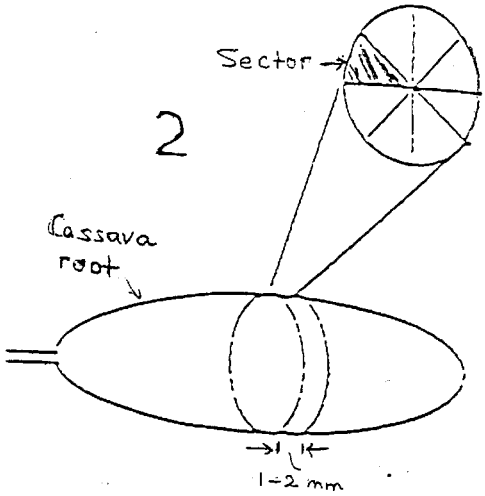
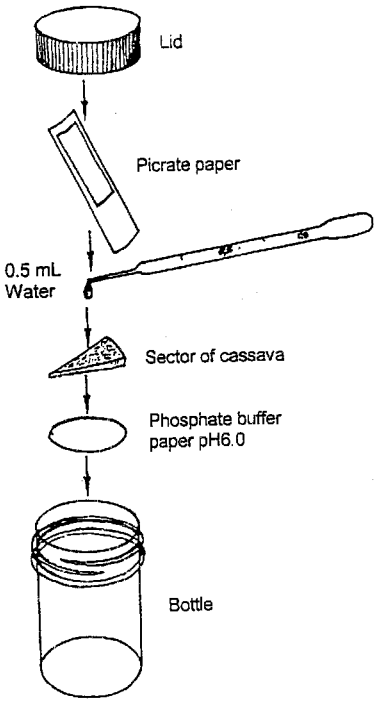
1



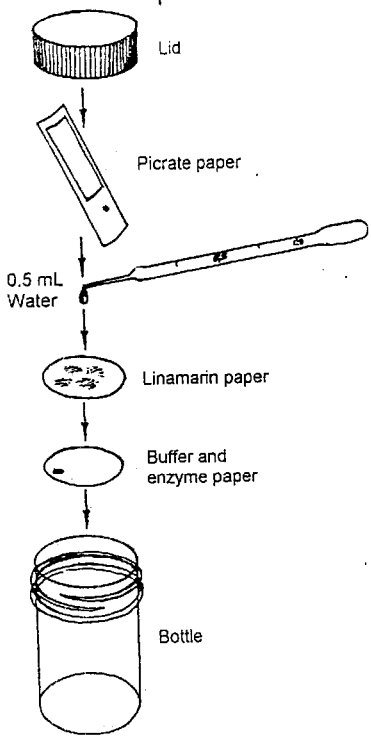
2


Sector
Cassava root
1-2 mm

3


Lid
Picrate paper
0.5 mL Water
Sector of cassava
Phosphate buffer paper pH6.0
Bottle

4


Lid
Picrate paper
0.5 mL Water
Linamarin paper
Buffer and enzyme paper
Bottle