

PROTOCOL B2: DETERMINATION OF TOTAL CYANIDE

IN CASSAVA PRODUCTS (FLOUR, GARI, ETC)

1. Place the small portable balance supplied on its U-shaped plastic mount (see sketch 1) so that it swings freely. It has a 100 mg weight glued inside one spoon.
2. If the cassava product is large or has hard lumps in it such as in roasted gari, it must be ground into a powder using a pestle and mortar. The powdered cassava product which may be flour is added evenly to the empty spoon until balance is achieved as shown in sketch 1.
3. Follow sketch 2. Place a round paper disc containing buffer at pH 6 and enzyme^a (identified by a black spot) in a flat-bottomed plastic bottle and pour the 100 mg of powdered cassava product on top of it.
4. Add 0.5 ml of clean water using the plastic pipette (sketch 2).
5. Immediately add a yellow picrate paper attached to a plastic strip (sketch 2)^b. 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 2).
7. As a **blank**, prepare another sample as shown in sketch 2 but with no cassava product present.
8. Every time you run a set of experiments you should check that the method works OK, by using a **standard** pink linamarin paper from the kit as follows.
9. Follow sketch 3. Place a round paper disc with a black spot containing buffer and enzyme in the bottle. Add a **standard** pink 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 product. Also check that the **blank** is zero and that 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 equation¹
total cyanide content (ppm) = 396 x absorbance
19. The total cyanide content obtained for the same sample of cassava product, 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 BUFFER / ENZYME PAPERS AND PICRATE PAPERS

^a The enzyme linamarase may be prepared from cassava sap² using Kit C or it may be purchased from BDH Ltd, Poole, UK. The linamarase solution prepared by Protocol C is mixed with an equal volume of a solution containing 10% polyvinylpyrrolidone-10 (PVP-10) and 2% gelatin obtained from Sigma Chemical Co, St Louis, USA. Add one drop from the plastic pipette of 1 M phosphate buffer at pH 6 (see ^c below for preparation) to the paper discs supplied on request and allow to air dry. Apply 2 drops of the mixed linamarase/gelatin/PVP-10 solution using the plastic pipette to the paper disc and allow to air dry. This paper is identified in the kit by a small black spot.

^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 INDEFINITELY¹. 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.³

^c There are 2 different methods of preparation of the 1 M phosphate buffer as follows:
(1) Add 750 ml of water to 80 mL of concentrated (about 88%) phosphoric acid. 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.

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 likely that there is something wrong with the picrate paper. If the picrate paper has been left at room temperature then it gradually becomes darker and after more than one month its colour will match with about 20 ppm on the colour chart. When used with the **standard** pink paper the darkened picrate paper may match with about 70 ppm on the colour chart. If the picrate paper has been left in bright sunlight it becomes bleached on one side and is no good. 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 B2

The kit has the following components:

1. Protocol B2, which gives full instructions for total cyanide analysis of cassava products, particularly cassava flour.
2. A plastic balance with a 100 mg weight glued into one spoon, for weighing 100 mg of powdered cassava product (see sketch 1).
3. 30 flat-bottomed plastic bottles with screw lids.
4. Two graduated 1 ml, plastic pipettes.
5. Bottle containing 100 pH 6 buffer / enzyme papers, identified by a small black spot.
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. Filter paper and plastic overhead transparency sheets for making more picrate papers.

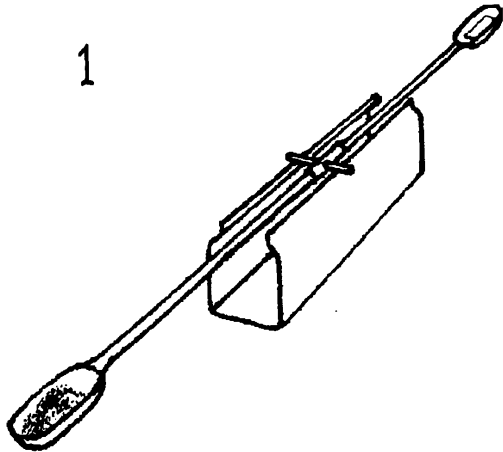
References

¹Bradbury, 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.

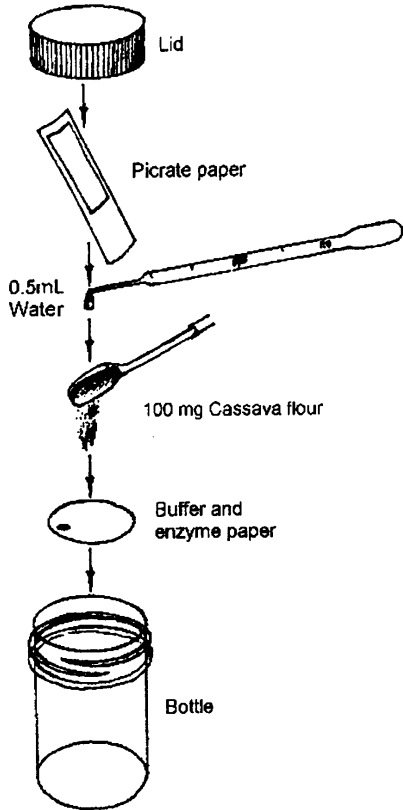
² Haque, M. R. and Bradbury, J. H. (1999) Preparation of linamarase solution from cassava latex for use in the cassava cyanide kit. *Food Chem.*, 67, 305-309.

³Egan, S.V., Yeoh, H.H. and Bradbury, J.H. (1998) Simple picrate paper kit for determination of cyanogenic potential of cassava flour. *J.Sci.Food Agric.* 75, 258-262.
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