

## PROTOCOL G: DETERMINATION OF TOTAL CYANIDE IN

### FLAX SEED (LINSEED)

1. Flax seed (linseed) contains the cyanide containing compounds linustatin, neolinustatin and linamarin as well as the enzyme linustatinase which catalyses the hydrolysis of these cyanogenic glucosides to liberate HCN. The flax seed or flax seed meal, must be ground up in a suitable grinder (a coffee grinder will do).
2. Weigh out 100 mg of the ground up flax seed (linseed) in a flat bottomed plastic bottle supplied in the kit. If an analytical balance is not available, it is possible to provide a small plastic portable balance with the kit.
3. Add 0.5 mL of 0.1 M phosphate buffer at pH 6<sup>a</sup>.
4. Add a yellow picrate paper attached to a plastic strip<sup>b</sup>. The picrate paper must not touch the liquid in the bottle. (STORE PICRATE PAPERS IN THE DEEP FREEZE OF THE REFRIGERATOR).
5. Immediately close the bottle with a screw capped lid.
6. Prepare another sample as above but with no ground flax seed, to serve as a blank.
7. As a control (or standard) to check on the method, place a Whatman filter paper disc loaded with buffer and linamarase (marked with a black spot) in a bottle, add a pink linamarin paper (see below), 0.5 mL water and a yellow picrate paper. Immediately close the bottle with a screw cap lid.
8. Allow the bottles to stand for 16-24 hour at room temperature (20-35° C).
9. Open the bottles and match the colour of the paper against the colour chart supplied.
10. From the colour chart supplied, read off the amount of total cyanogens in ppm in the ground flax seed. Also check that the blank corresponds to zero and the control gives the expected value.

### FOR USE IN A LABORATORY EQUIPPED WITH A SPECTROPHOTOMETER

11. Carefully remove the plastic backing sheet (it may be washed and used again) from the picrate paper.
12. Immerse the picrate paper in 5.0 mL of water (measured accurately with a pipette) for about 30 min with occasional gentle shaking.
13. Take the blank picrate paper (see 6 above), remove its plastic sheet and immerse the yellow picrate paper in 5.0 ml of water for about 30 min with occasional gentle shaking.
14. Measure the absorbance at 510 nm of the picrate solution from 12 against the blank from 13.
15. The total cyanogen content in ppm is calculated by the equation<sup>1</sup>  
$$\text{total cyanogen content (ppm)} = 396 \times \text{absorbance.}$$
16. The cyanogen content obtained for the same sample of ground flax seed from both measurements 10 and 15, should be in reasonable agreement. Also check that the control value from 7 agrees using both methods.

### FOOTNOTES -PREPARATION OF BUFFER AND PICRATE PAPERS

<sup>a</sup> A solution of approximately 0.1 M phosphate buffer may be prepared by taking 8 mL of concentrated phosphoric acid (88% H<sub>3</sub>PO<sub>4</sub>) and adding about 750 mL of water. A solution of 1 M sodium hydroxide is prepared by dissolving 10 g of sodium hydroxide pellets in water and making up to 250 mL. The sodium hydroxide solution is now added to the phosphoric acid solution with stirring until the pH measured using a pH meter increases up to 6.0. Alternatively the 0.1 M buffer may be prepared as follows: prepare two 0.1 molar solutions of sodium dihydrogen phosphate and disodium hydrogen phosphate by dissolving the calculated amounts of solid NaH<sub>2</sub>PO<sub>4</sub> and Na<sub>2</sub>HPO<sub>4</sub> to make 0.1 M solutions. Add the acidic sodium dihydrogen phosphate solution to the disodium hydrogen phosphate solution until the pH decreases to 6.0.

b Moist picric acid (1.4 g of BDH Lab Reagent) is weighed out and dissolved in 100 ml of 2.5 % (w/v) sodium carbonate. A square (about 10 cm x 10 cm) of Whatman 3MM filter paper is immersed in the yellow picrate solution for about 20 sec and hung up to dry in air. *Note. Wash off with water any yellow picric acid on hands. Wear gloves if available when handling picric acid solution and papers.* Any unevenly coloured sections of the paper 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, so that the upper end of the yellow paper is about 5 mm from one end of the strip. Picrate papers must not be exposed to bright sunlight and should not be left in laboratory light for long periods. STORE THEM IN THE DARK IN THE DEEP FREEZE OF THE REFRIGERATOR WHERE THEY ARE STABLE FOR MANY YEARS<sup>1</sup>. At room temperature they gradually darken and should not be used for more than ONE MONTH ONLY AT ROOM TEMPERATURE.

Every time that you run a set of experiments, it is important to run a linamarin control sample (step 7). The linamarin is a stable cyanide compound which is broken down at pH 6 by the enzyme linamarase to produce HCN. The enzyme linamarase is immobilised in small filter paper discs which also contain phosphate buffer at pH 6 and are marked with a black spot. In the kit there are pink filter paper discs containing linamarin which gives 50 ppm on the colour chart.

#### TROUBLE SHOOTING

If the cyanide content is much less than that expected from using the pink linamarin control sample, then there is clearly some problem with the methodology. Possible causes could be:

- (1) Picrate paper is old (has been stored at room temperature for more than 1 month) or has been exposed to bright light or long exposure to laboratory light.<sup>2</sup>
- (2) Loss of linamarase activity in the round Whatman 3MM discs included in the kit.
- (3) Breakdown of linamarin in the pink control paper<sup>2</sup>.
- (4) Use of a non-air tight container (e.g. screw cap is cracked), which would allow HCN gas to escape.

#### COMPONENTS OF KIT G

The kit has the following components:

1. Protocol G, which gives a detailed stepwise method for cyanide analysis of ground flax seed.
2. Bottles, clear plastic, flat-bottomed, with screw lids (25 mm diam., 50 mm high).
3. Small, graduated 1 ml, plastic pipette.
4. Squares of filter paper (Whatman 3MM) and of plastic overhead transparency sheets.
5. Picrate papers glued to strips of clear plastic with PVA 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.
6. Colour chart containing 10 entries from yellow to brown.
7. Pink filter papers containing linamarin for controls, equivalent to 50 ppm.
8. Filter paper discs, 21 mm, which contain buffer at pH 6 and linamarase. These papers are identified by a small black spot.

#### References

<sup>1</sup> 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, (1999).

<sup>2</sup>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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