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Preparation of linamarin from cassava leaves for use in a cassava cyanide kit

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Abstract

A simple method is described for the preparation, from very young cassava leaves, of a dilute hydrochloric acid solution of linamarin. Linamarin extraction from the leaves is virtually quantitative. The linamarin solution is used in the preparation of standard linamarin filter paper discs that are needed to monitor the performance of picrate kits for determination of the total cyanide content of cassava roots and cassava products. These standard discs are stable indefinitely if stored in the refrigerator, but very slowly lose linamarin activity if stored for more than one month at room temperature.

Keywords: Linamarin; Cassava leaves; Cyanide kit; Linamarase

1. Introduction

Cassava, the third most important food source in the tropics, produces two cyanogenic glucosides linamarin and a small amount of lotaustralin (methyl linamarin). These cyanogenic glucosides are hydrolysed in the presence of the enzyme linamarase (which is brought into contact with linamarin if the plant cells are broken) to a cyanohydrin, which breaks down further to hydrogen cyanide. This interesting mechanism is used by cassava and more than 2500 other plants to deter predators (Hosel, 1981; Moller and Seigler, 1998) and in the case of cassava, presents a significant safety problem for humans who eat it. Ingestion of cyanide from high cyanide (bitter) cassava may occasionally cause death (Akintonwa, Tunwashe, & Onifade, 1994), exacerbates goitre and cretinism (Delange, Ekpechi, & Rosling, 1994), causes tropical ataxic neuropathy (TAN) in older persons (Osuntokun, 1994) and also produces konzo, an irreversible paralysis of the legs, which occurs mainly in children (Ernesto et al., 2002; Howlett, Brubaker, Mlingi, & Rosling, 1990; Ministry of Health, Mozambique, 1984).

In order to allow non-chemists in the tropics to monitor the cyanide content of cassava food sources a simple picrate kit method was developed, that required only a small amount of water and could be used in the field, or with more accuracy in the laboratory (Bradbury, Egan, & Bradbury, 1999; Cardoso, Ernesto, Cliff, Egan, & Bradbury, 1998; Egan, Yeoh, & Bradbury, 1998). Determination of urinary thiocyanate measures the recent intake of cyanide (Carlsson, Mlingi, Juma, Ronquist, & Rosling, 1999); hence another picrate kit was developed to measure urinary thiocyanate (Haque, & Bradbury, 1999). These picrate kits are available free of charge to health workers and agriculturalists in developing countries.

In using the cassava cyanide kit, a routine check on the methodology is made with a small filter paper disc loaded with a known amount of linamarin (Bradbury et al., 1999). In this paper we have developed a simple method for preparation, from cassava leaves, of an acid-stabilised solution of linamarin, suitable for preparation of standard linamarin filter paper discs.

2. Materials and methods

Cassava leaves and roots were obtained from four different cultivars of cassava growing in pots in the Plant Culture Centre at the Australian National Uni-

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versity. Preliminary extractions were done using cassava roots and leaves in organic solvents, such as ethanol and acetone (King & Bradbury, 1995), but the final aqueous method used was as follows:

A 5 g sample of very young cassava leaves was cut up with scissors and immediately ground in a glass pestle and mortar with 5 ml 0.1 M HCl. A further 5 ml of 0.1 M HCl were added with further grinding and the pasty solution was poured through a cloth which was squeezed. The pink-coloured, cloudy solution was then centrifuged and the clear, supernatant liquid (about 7 ml) removed with a Pasteur pipette. This solution, which also contained linamarase (inactivated in the 0.1 M HCl) and linamarin, was stored frozen in a deep freeze cabinet at −20 °C. Experiments have shown that the frozen linamarin solution is stable for at least 5 months.

The linamarin solution was assayed in triplicate by adding 100 μl of the pink solution and 0.5 ml water to a small plastic bottle, followed by a 2.1 cm diameter filter paper disc previously loaded with buffer at pH 6 and linamarase. A picrate paper was placed in the bottle, which was closed with a screw cap and left at 30 °C overnight. The brownish picrate paper was removed from the bottle and immersed in 5.0 ml water for 30 min and the absorbance of the solution measured at 510 nm using a spectrometer. The cyanide content, in ppm, was obtained by multiplying the absorbance by 396 (Bradbury et al., 1999; Egan et al., 1998).

Standard linamarin discs, for use in the cassava cyanide kits, were prepared by adding a known amount (calculated beforehand from the result of the above assay) of the pink solution to small Whatman 3MM filter paper discs and allowing the HCl and water to evaporate off. To assay the standard linamarin discs, a buffered enzyme disc was placed in a plastic bottle; 0.5 ml water was added, followed by a standard linamarin disc. A picrate paper was placed in the bottle, which was closed and left overnight. The remainder of the method followed that given above.

To check the efficacy of the extraction of linamarin by grinding leaves in 0.1 M HCl, an experiment was done in which very young leaves from MCol 1468 were crushed in a pestle and mortar and weighed amounts (about 50 mg) placed in a small plastic bottle at times from 3 to 17 min. Phosphate buffer (0.5 ml of 0.1 M, pH 6) was added, followed immediately by a picrate paper and closure of the bottle with a screw lid. The remainder of the method is as described above.

3. Results and discussion

3.1. General

In preliminary studies, cassava peel from roots was macerated in ethanol in a mixer, but the plant material was difficult to break up. Cassava leaves were also ground in a pestle and mortar, in acetone. Very young leaves were chosen for two reasons: first because they were very soft and easy to grind up and second because we found that they contained more linamarin than young or mature leaves. Although an organic solvent has previously been used for extracting linamarin (King & Bradbury, 1995), it was found that concentration by rotary evaporation of an acetone solution containing linamarin caused considerable loss of linamarin, probably because of the presence of linamarase, extracted by the aqueous acetone solution. A simpler method was developed using water, which avoided organic solvents and rotary evaporation. Since both linamarin and linamarase are freely soluble in water, dilute hydrochloric acid was used to inactivate linamarase and hence prevent rapid hydrolysis of linamarin that would have occurred if a neutral aqueous solution had been used.

3.2. Amounts of linamarin obtained from cassava leaves of different cultivars

The amount of linamarin in the pink solution produced from crushed leaves was assayed using 100 μl aliquots and a calculation gave the amount of linamarin present in mg HCN equivalents/g leaf. These results are shown in Table 1 for leaves from four different cassava cultivars collected over one growing season.

The amount of linamarin of any particular cultivar did not vary greatly across the season, from September to April. The linamarin contents of very young leaves of cultivars MCol 1468 and MAus 7 were about twice as much as that of TMS 50395 and SM1-150. The linamarin solutions from all of these cultivars were concentrated enough to prepare standard linamarin 50 ppm discs used in the cassava cyanide kit (Bradbury et al., 1999).

The loss of HCN from very young leaves of MCol 1468, crushed in a mortar and pestle, followed a linear graph and, after 9 min, only 41% of the cyanide remained in the leaves, after which there was little fur-

Table 1
Yields of linamarin from very young cassava leaves of different varieties

Cassava variety	Yields of linamarin (mg HCN equivalents/g leaf) over a season				
	September	October	January	April	Mean value and S.D. (brackets)
Mcol 1468	0.63	0.53	0.63	0.54	0.58 (0.05)
M Aus 7	0.55	0.47	0.53	0.48	0.51 (0.04)
TMS 50395	0.18	0.22	0.31	0.36	0.27 (0.08)
SM1-150	0.20	0.21	0.27	0.31	0.25 (0.05)

The young leaves were harvested over a season at about the middle of the month from spring (September) through to autumn (April).

ther loss of cyanide from the leaves up to 17 min. By linear extrapolation, the linamarin content of the leaf at zero time was calculated and this agreed satisfactorily with the amount of linamarin obtained by grinding the leaf in 0.1.M HCl. The HCl extraction technique therefore recovers virtually 100% of the linamarin in very young leaves.

3.3. Stability of linamarin in filter paper discs

Standard linamarin discs were prepared by adding a known amount of the linamarin solution to a small Whatman 3MM filter paper and allowing the water and HCl to evaporate. There is a possibility that linamarase present in the solution may catalyse hydrolysis of linamarin. Standard linamarin discs were therefore stored in closed plastic bottles at 30 °C, at room temperature (about 20 °C), in a refrigerator at about 5 °C and in a deep freeze cabinet at about −20 °C, and assayed after periods from 1 to 7 months. There was no loss of linamarin content after one month, but after 2 months, the linamarin content of the discs kept at room temperature had dropped to 91% and, after 7 months, those kept at 30 °C had dropped to 87%. The standard linamarin discs stored in the refrigerator and the deep freeze cabinet showed no change in linamarin content after 7 months. To ensure the constancy of these standard linamarin discs over time it is therefore necessary to store them in a refrigerator.

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References

Akintonwa, A., Tunwashe, O., & Onifade, A. (1994). Fatal and non-fatal acute poisoning attributed to cassava-based meal. *Acta Hort*., *375*, 285–288.

Bradbury, M. G., Egan, S. V., & Bradbury, J. H. (1999). Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *Journal of the Science of Food and Agriculture*, *79*, 593–601.

Cardoso, A. P., Ernesto, M., Cliff, J., Egan, S. V., & Bradbury, J. H. (1998). Cyanogenic potential of cassava flour: field trial in Mozambique of a simple kit. *International Journal Food Science and Nutrition*, *49*, 93–99.

Carlsson, L., Mlingi, N., Juma, A., Ronquist, G., & Rosling, H. (1999). Metabolic fates in humans of linamarin in cassava flour ingested as stiff porridge. *Food Chemistry Toxicology*, *37*, 307–312.

Delange, F., Ekpechi, L., & Rosling, H. (1994). Cassava cyanogenesis and iodine deficiency disorders. *Acta Hort*., *375*, 289–293.

Egan, S. V., Yeoh, H. H., & Bradbury, J. H. (1998). Simple picrate paper kit for determination of the cyanogenic potential of cassava flour. *Journal of the Science of Food and Agriculture*, *76*, 39–48.

Ernesto, M., Cardoso, A. P., Nicala, D., Mirione, E., Massaza, F., Cliff, J., Haque, M. R., & Bradbury, J. H. (2002). Persistent konzo and cyanide toxicity from cassava in Northern Mozambique. *Acta Tropica*, *82*, 357–362.

Haque, M. R., & Bradbury, J. H. (1999). Simple method for determination of thiocyanate in urine. *Clin. Chem*., *45*, 1459–1464.

Hosel, W. (1981). The enzymatic hydrolysis of cyanogenic glucosides. In B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, & F. Wissing (Eds.), *Cyanide in biology* (pp. 217–232). London: Academic Press.

Howlett, W. P., Brubaker, G. R., Mlingi, N., & Rosling, H. (1990). Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain*, *113*, 223–235.

King, N. L. R., & Bradbury, J. H. (1995). Bitterness of cassava: identification of a new apiosyl glucoside and other compounds that affect its bitter taste. *Journal of the Science of Food and Agriculture*, *68*, 223–230.

Moller, B. L., & Seigler, D. S. (1998). Biosynthesis of cyanogenic glycosides, cyanolipids and related compounds. In B. K. Singh (Ed.), *Plant amino acids biochemistry and biotechnology* (pp. 563–609). Marcel Dekker.

Ministry of Health Mozambique. (1984). Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. *Bull. World Health Organ*, *62*, 477–484.

Osuntokun, B. O. (1994). Chronic cyanide intoxication of dietary origin and a degenerative neuropathy in Nigerians. *Acta Hort*., *375*, 311–321.