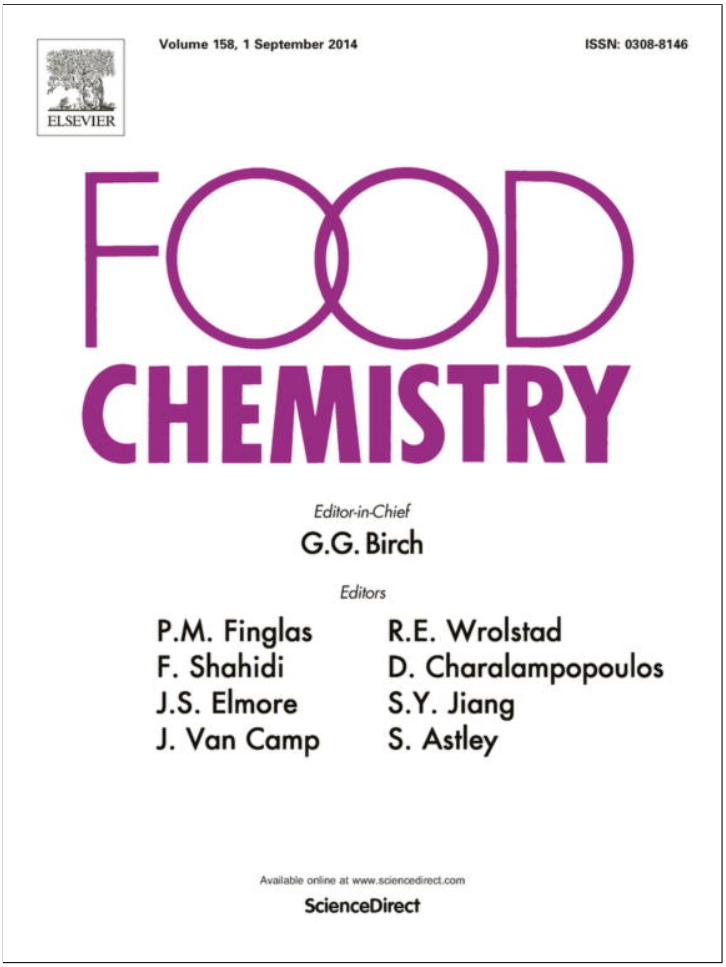

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Analytical Methods

Mild method for removal of cyanogens from cassava leaves with retention of vitamins and protein

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ABSTRACT

A mild method was developed to remove cyanogens from cassava leaves that involved three consecutive steps (1) pounding, (2) standing for 2 h in the sun or 5 h in the shade in the tropics and (3) washing three times in water. Four cassava cultivars were used and the mean residual total cyanide content after steps 1, 2 and 3 was 28%, 12% and 1%, respectively. The pounded cassava leaves retained their bright green colour and texture. The traditional method for removing cyanogens from pounded cassava leaves is by boiling in water which removed all cyanogens in 10 min. However this method caused the pounded leaves to become dull green in colour and would cause considerable losses of vitamins, protein and methionine, which are already in short supply in the diet of poor village people in tropical Africa.

1. Introduction

Cassava is the staple food of tropical Africa (Nhassico, Muquingue, Cliff, Cumbana, & Bradbury, 2008). The starchy cassava root is complemented nutritionally by the leaves which are often used as a sauce and are rich in protein, vitamins and minerals (Lancaster & Brooks, 1983). Cassava leaves are rich in protein, but the protein is limiting in the S-containing amino acids methionine and cysteine/cystine (Diasolua Ngudi, Kuo, & Lambein, 2003; Lancaster & Brooks, 1983; Yeoh & Chew, 1976). Cassava leaves may be harvested throughout the year and are used particularly by the Congolese population of Central Africa and in Liberia, Sierra Leone and Guinea, and there is moderate use in other tropical African countries (Achidi, Ajayi, Bokanga, & Maziya-Dixon, 2005). By contrast, cassava leaves are not used at all in the South Pacific, because of their high levels of toxic cyanogens and the ready availability of other greens (Bradbury & Holloway, 1988, chap. 4). Cassava leaves contain large amounts of the cyanogenic glucosides, linamarin and a small amount of lotaustralin (methyl linamarin), which are broken down by the enzyme linamarase present in the leaves to produce cyanohydrins that are further decomposed by another enzyme hydroxynitrile lyase also present, to give hydrogen cyanide (HCN) and a ketone (Bradbury & Denton, 2011; Cardoso et al., 2005; White, McMahon, & Sayre, 1994).

The intake of large amounts of cyanogens from consumption of high cyanide cassava roots, high cyanide cassava flour and poorly processed cassava leaves can lead to cyanide poisoning with symptoms of headache, nausea, dizziness, diarrhoea, vomiting and sometimes death (Nhassico et al., 2008). It can also cause konzo, an irreversible paralysis of the legs that occurs mainly in children and women of child bearing age. Konzo is associated with a high cyanogen intake from a monotonous diet of bitter cassava and an insufficient supply of essential S-containing amino acids, which are used up in the body to convert poisonous cyanide (CN) to thiocyanate (SCN), that is removed in the urine (Cliff, Martensson, Lundquist, Rosling, & Sorbo, 1985; Howlett, Brubaker, Mlingi, & Rosling, 1990). In all cases in which konzo has occurred there have been high intakes of cyanogens, whether as a result of (1) war (Cliff, Muquingue, Nhassico, Nzwalo, & Bradbury, 2011; Cliff et al., 1997), (2) drought, (Mlingi, Nkya, Tatala, Rashid, & Bradbury, 2011) when the cyanogen content of the plant is greatly increased due to water stress (Bokanga, Ekanayake, Dixon, & Porto, 1994) or (3) short processing of cassava roots (Banea et al., 1992). The association of high cyanogen intake with konzo has been confirmed by two recent studies (Banea et al., 2012; Banea et al., 2013) in which konzo has been controlled in four villages by the continued use by the village women of the wetting method that removes cyanogens

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from cassava flour (Bradbury, 2006; Bradbury & Denton, 2010; Cumbana, Mirione, Cliff, & Bradbury, 2007).

The need for an adequate supply of protein to control konzo was shown in three different konzo outbreaks with prevalence rates up to 7% in Mozambique, Tanzania and the Democratic Republic of Congo (DRC). However amongst people of the same ethnic group who lived only 5 km away the incidence of konzo was nearly zero, because in Mozambique they had access to fish from the sea (Ministry of Health, 1984), in Tanzania they had access to fish from lake Victoria, (Howlett, Brubaker, Mlingi, & Rosling, 1992) and in DRC they had animal protein from the forest (Banea Mayambu, 1993). The supply of protein is inadequate for poor village people living in the savanna and subsisting on a monotonous diet of bitter cassava roots, but is improved somewhat by consumption of cassava leaves as the main subsidiary food (Banea et al., 2012). The leaves are usually pounded for 10–15 min, which allows the enzymes to come in contact and catalyse hydrolysis of the cyanogens, followed by boiling in water for 10–120 min (Achidi et al., 2005; Lancaster & Brooks, 1983) to remove all cyanogens, but boiling pounded leaves for 30 min was found to reduce the protein content by 58% and the methionine content by 71% (Diasolua Ngudi et al., 2003). This large loss of protein and methionine due to boiling pounded cassava leaves in water means that less than one half of the original protein and methionine would be available to detoxify ingested cyanide. Furthermore there are large losses of vitamin C, thiamin, riboflavin and nicotinic acid due to boiling (Bradbury & Holloway, 1988; Lancaster & Brooks, 1983).

In a previous publication two mild methods were described for removal of cyanogens from pounded cassava leaves at room temperature and intact cassava leaves at 50 °C, which produced residual total cyanide contents of 3–8% (Bradbury & Denton, 2011). In this paper we report a much improved mild method that removes virtually all cyanogens from pounded cassava leaves.

2. Materials and methods

2.1. Materials

Cassava leaves were obtained from four cultivars of cassava plants grown in a glasshouse at the Plant Culture Facility, Australian National University. Three cultivars were from the Australian collection held at the University of Queensland and one (TMS 50395) was from IITA, Ibadan, Nigeria. The petioles and leaves were broken off the main stem of the plant, numbering the leaves from the first fully expanded leaf (leaf 1), counting sequentially down the stem. To obtain a reproducible sample of leaves from a particular cultivar, we collected leaf 4 and used the central section of the leaf blade (Bradbury & Denton, 2011).

2.2. Methods

2.2.1. Pounding treatment of leaves followed by standing at 30 or 50 °C and washing

Approximately 10 g leaf samples plus 10 mL of water were pounded in a pestle and mortar for 10–15 min until the leaves were finely ground. The pounded leaves were divided into two equal parts, which were placed in open dishes in two ovens at 30 °C for 5 h or at 50 °C for 2 h. These temperatures of 30 and 50 °C approximated to ambient temperatures in the shade in the tropics and standing in the sun, respectively (Bradbury & Denton, 2010). The pounded leaves were then washed with 15 mL water with swirling and standing for about 5 min. After each washing the mixture was poured through a fine metal sieve and the pounded leaves were dried between filter papers for total cyanide analysis. Three water washings were made, with pounded leaf samples dried between filter paper taken for total cyanide analysis after each washing. Duplicate samples were also taken for total cyanide analyses (see Section 2.2.3) of the initial cassava leaf samples before and after pounding, after standing for 2 and 5 h at 30 °C and after standing for 2 h at 50 °C.

2.2.2. Pounding of leaves followed by boiling in water

Approximately 10 g leaf samples of the central section of the leaf blades of leaf 4 of a particular cassava cultivar plus 10 mL water were pounded for 10–15 min in a pestle and mortar until finely ground. The sample was divided into five portions and placed in five small beakers with 60 mL boiling water for periods of 1, 2, 5, 10 and 20 min. After each treatment the boiling mixture was poured into 200 mL of cold water and then passed through a fine metal sieve to separate out the pounded leaf matter. The samples of boiled, pounded leaves were dried between filter papers before analysis for total cyanide.

2.2.3. Total cyanide analysis of leaves and pounded leaves

Duplicate 100 mg samples of the middle section of cassava leaf blades or of pounded leaves before or after various treatments were added to a small plastic bottle, a buffer/enzyme paper was added followed by 1 mL of 1 M phosphate buffer at pH 6.5, a picrate paper and a screw cap lid. The bottles stood overnight at 30 °C, then the plastic support was removed from the picrate papers, which were placed in test tubes and 5.0 mL water was added. After 30 min the absorbance of the coloured solutions was measured at 510 nm in a spectrophotometer and the total cyanide content in ppm calculated by multiplying the absorbance by 396 (Bradbury, Egan, & Bradbury, 1999; Egan, Yeoh, & Bradbury, 1998, http://biology.anu.edu.au/hosted_sites/CCDN/).

3. Results

In Tables 1 and 2 are shown the results for the removal of cyanogens by mild methods from cassava leaves of four different cultivars by pounding, standing at 30 or 50 °C for 2–5 h and washing three times in water. After these treatments the bright green colour of freshly pounded leaves was retained. In Table 3 are the results for removal of cyanogens from pounded cassava leaves followed by boiling the pounded leaves for 1–20 min. In this case the bright green colour of pounded leaves gradually faded with increased time of boiling and became dull green after 10–20 min boiling.

4. Discussion

The results in Tables 1 and 2 show that cyanogens can be removed from cassava leaves by a three step process of pounding followed by standing in an open dish for 2 h at 50 °C or 5 h at 30 °C and washing three times with water. The removal of

Table 1
% Total cyanide remaining after pounding, standing for 2 h at 50 °C[a] and washing at ambient temperature.

Treatment	% Total cyanide remaining after pounding, standing and washing for cvs				
	MAus7	TMS50395	MCol1468	SM1-150	Mean [b]
Pounding	27	36	33	15	28 (9)
Standing, 2 h	4	14	16	8	11 (6)
Wash 1	2	7	6	2	4 (3)
Wash 2	0	4	4	0	2 (2)
Wash 3	0	2	–	0	1 (1)

[a] This is achieved by standing for 2 h in the sun (Bradbury and Denton, 2010).
[b] Standard deviation in brackets.

Table 2
% Total cyanide remaining after pounding, standing for 2 and 5 h at 30 °C[a] and washing at ambient temperature.

Treatment	% Total cyanide remaining after pounding, standing and washing for cvs				
	MAus7	TMS50397	MCol1468	SM1-150	Mean [b]
Pounding	27	36	33	15	28 (9)
Standing, 2 h	12	18	19	14	16 (3)
Standing, 5 h	9	15	14	12	13 (3)
Wash 1	2	8	8	2	5 (3)
Wash 2	1	3	4	0	2 (2)
Wash 3	0	2	–	0	1 (1)

[a] This can be achieved in the tropics by standing in the shade (Cumbana et al., 2007).
[b] Standard deviation in brackets.

Table 3
% Total cyanide remaining after pounding and boiling in water.

Treatment	% Total cyanide remaining after pounding and boiling in water for cvs				
	MAus7	TMS50397	MCol1468	SM1-150	Mean [a]
Pounding	27	36	33	15	28 (9)
Boiling in water					
1 min	6	8	6	2	5 (3)
2 min	3	2	2	1	2 (1)
5 min	1	1	2	0	1 (1)
10 min	1	0	0	0	0
20 min	0	0	0	0	0

[a] Standard deviation in brackets.

cyanogens occurs gradually over each step, pounding, standing and washing (three times) and every step is important in securing the final removal of cyanogens. The method is an improvement on our earlier pounding method (Bradbury & Denton, 2011) in which only two of these three steps were used, which gave a mean residual total cyanide content after three washes of 6%. In this paper, the introduction of the period of standing for 2 h at 50 °C or 5 h at 30 °C, which is the basis of the successful "wetting method" that removes cyanogens from cassava flour (Banea et al., 2012; Bradbury, 2006; Bradbury & Denton, 2010; Cumbana et al., 2007), has allowed the reduction of the mean total cyanide content to less than one half of its value before standing (see Tables 1 and 2). The standing period is needed to allow the enzymes linamarase to hydrolyse linamarin to acetone cyanohydrin and hydroxynitrile lyase to hydrolyse acetone cyanohydrin to HCN, which escapes as a gas. The final water washings are needed to remove the last 11–13% of residual water-soluble cyanogens (linamarin, acetone cyanohydrin and HCN) which are still held in the pounded leaves. The final residual mean total cyanide content of the pounded leaves in Tables 1 and 2 is 1%. Thus, if the initial total cyanide content of leaves is high at say 1000 ppm (Lancaster and Brooks (1983) give a range of 80–1460 ppm cyanide for cassava leaves) then the amount remaining would be 10 ppm, which is within the safe level of 0–10 ppm for cyanide in cassava flour (FAO/WHO, 1991).

The results in Table 3 show that the removal of cyanogens by boiling pounded cassava leaves in water occurs rapidly and is complete in 10 min. Boiling pounded leaves in water for 10–120 min is the traditional method that is used in Africa to remove cyanogens from pounded cassava leaves (Achidi et al., 2005; Lancaster & Brooks, 1983) and the results in Table 3 show that boiling is very effective indeed. The use of the longer, more time consuming mild method to remove cyanogens conserves important vitamins, protein and methionine that are destroyed by boiling. Thus boiling cassava leaves for 10 min reduced the vitamin C content by 60% (Lancaster & Brooks, 1983). Losses from root crops of thiamin, riboflavin, nicotinic acid and vitamin C by boiling in water were very considerable (Bradbury & Holloway, 1988). Boiling of pounded cassava leaves for 30 min to dryness reduced the protein content by 58% and the methionine content by 71% (Diasolua Ngudi et al., 2003). The loss of methionine is particularly unfortunate, because it is necessary to detoxify cyanide to thiocyanate in the body. Losses of vitamins, protein and methionine would be avoided by using the mild method of pounding, standing and washing three times in water, although there could be some loss of water soluble vitamins during washing. An advantage of the mild method is that there would be less wood used and a reduction in the amount of time spent by the women working over a smoky wood fire inside the house.

5. Conclusion

A mild method was developed that removes virtually all cyanogens from cassava leaves, which involved pounding followed by standing for 2 h in the sun or 5 h in the shade, followed by three washes in water. All cyanogens were also removed by boiling pounded cassava leaves in water for 10 min, but the boiling method reduced greatly the content of water soluble B and C vitamins, protein and methionine. These nutrients are important in the diet of the poor village people of tropical Africa. We therefore recommend that the mild method for removing cyanogens from pounded cassava leaves be introduced as an alternative to boiling in water.

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