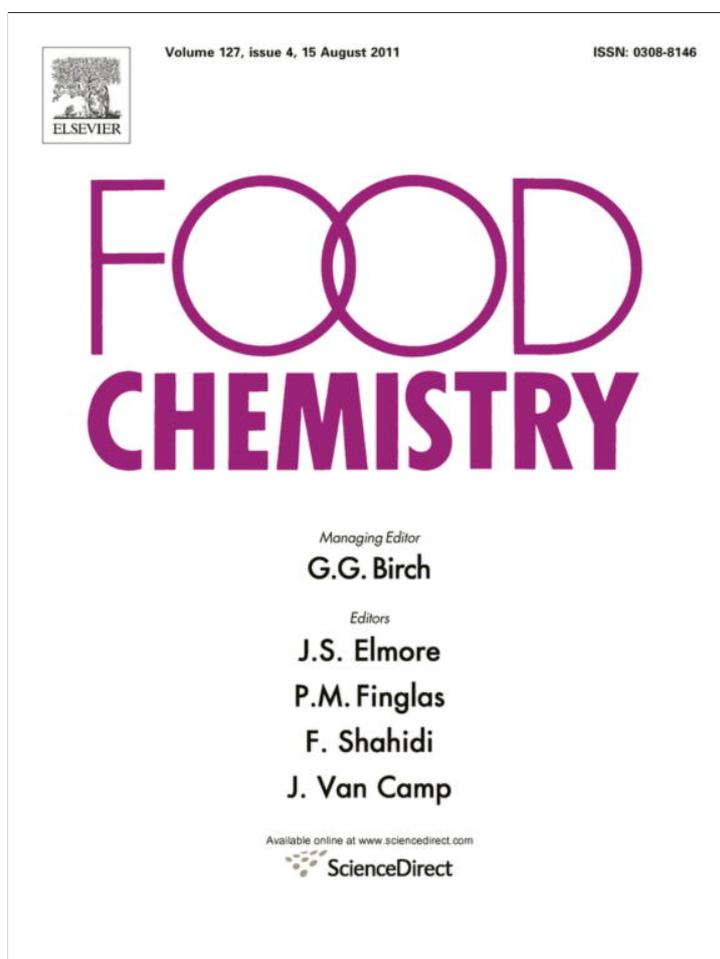


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Mild methods of processing cassava leaves to remove cyanogens and conserve key nutrients

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ARTICLE INFO

Article history:

Received 2 December 2010
Received in revised form 9 February 2011
Accepted 13 February 2011
Available online 17 February 2011

Keywords:

Cassava leaves
Processing
Cyanogens
Poisoning
Konzo
S-containing amino acids
Vitamins

ABSTRACT

Current methods for processing cassava leaves to remove cyanogens involve pounding followed by boiling in water or boiling intact leaves for 30 min or longer. Boiling in water rapidly removes cyanogens but also breaks down vitamins, proteins and S-containing amino acids, which are necessary to detoxify ingested cyanide. Two methods have been developed to remove cyanogens whilst conserving these key nutrients present in cassava leaves. The first method involves pounding leaves in a pestle and mortar for a minimum of 10 min until the leaves are well macerated, followed by washing the pounded leaves twice in twice their weight of water at ambient temperature, which reduces the total cyanide remaining to 8%. Two further washes reduce the total cyanide to 3%. The second method is to immerse cassava leaves in ten times their weight of water at 50 ± 3 °C for 2 h followed by one change of water and further immersion for 2 h at 50 °C which reduces the total cyanide remaining to 7%.

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1. Introduction

Cassava is the major staple food of tropical Africa. The starchy roots are complemented nutritionally by the leaves, which are a good source of protein, vitamins and minerals (Lancaster & Brooks, 1983). Achidi, Ajayi, Bokanga, and Maziya-Dixon (2005) noted that cassava leaves are available throughout the year and believe that they should be accorded as much importance as the roots. The Congolese consider that cassava is “all sufficient” because they get “bread from the roots and meat from the leaves”. Cassava leaves are used widely throughout tropical Africa, but particularly by the Congolese population of Central Africa and also in Liberia, Sierra Leone and Guinea. There is also moderate use in Senegal, Cameroon, Chad, Uganda, Tanzania, Zambia, Mozambique and Madagascar. Cassava leaves contain large amounts of the cyanogenic glucosides linamarin and a small percentage of lotaustralin (methyl linamarin) and two enzymes: (1) linamarase that catalyses hydrolysis of these cyanogens to glucose and cyanohydrins and (2) hydroxynitrile lyase that catalyses hydrolysis of the cyanohydrins to hydrogen cyanide (HCN) and a ketone. On the other hand, the starchy roots contain very little hydroxynitrile lyase and much less linamarin and linamarase than the leaves (White, Arias-Garzon, Mc Mahon, & Sayre, 1998).

Consumption of cassava roots and leaves containing large amounts of cyanogens can cause cyanide poisoning, with symptoms of headache, nausea, dizziness, diarrhoea and vomiting, sometimes leading to death (Nhassico, Muquingue, Cliff, Cumbana, & Bradbury, 2008). It can also cause epidemics of konzo during war or as a result of drought, when the linamarin content of the root increases greatly (Bokanga, Ekanayake, Dixon, & Porto, 1994). Konzo is an irreversible paralysis of the legs that occurs mainly in children and young women, and is associated with a very high intake of cyanogens over several weeks, combined with low protein intake (Cliff, Martensson, Lundquist, Rosling, & Sorbo, 1985; Howlett, Brubaker, Mlingi, & Rosling, 1990). Studies in Mozambique (Ministry of Health Mozambique, 1984), Tanzania (Howlett, Brubaker, Mlingi, & Rosling, 1992) and Democratic Republic of Congo (DRC) (Banea Mayambu, 1993) have shown that, during war or drought, konzo outbreaks have occurred with a prevalence rate of up to 7% amongst very poor people, who have consumed insufficiently processed bitter cassava roots as their staple food. But in each case there have been people of the same ethnic group with frequent daily contacts but located only 5 km away, who have a konzo prevalence close to zero. Those with near zero prevalence in Mozambique lived close to the sea, in Tanzania they lived close to Lake Victoria and in the DRC they lived in the forest, adjacent to the savannah areas where there was konzo. In every case the near-zero prevalence resulted from a better diet that included fish or animal protein as well as bitter cassava (Banea Mayambu, 1993; Howlett et al., 1992; Ministry of Health Mozambique, 1984).

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Clearly these people had a better diet containing more protein and this had protected them from getting konzo, compared with those of their own people who had no access to animal protein and many of which had contracted konzo. Since ingested cyanide (CN) is converted in the body to thiocyanate (SCN) by a reaction that consumes essential S-containing amino acids methionine and cysteine, a poor supply of the latter because of a low protein diet would slow the detoxification of cyanide to thiocyanate. This could cause increase of the concentration of cyanide in the blood to a level at which konzo would occur. Compared with leaves of other plants, cassava leaves are high in protein, but the protein is limiting in the S-containing amino acids which are required for the detoxification of cyanide (Diasolua Ngudi, Kuo, & Lambein, 2003a; Lancaster & Brooks, 1983; Yeoh & Chew, 1976).

The most common method of processing cassava leaves is to pound them in a wooden pestle and mortar for about 15 min followed by boiling in water for 10–120 min (Achidi et al., 2005; Diasolua Ngudi, Kuo, & Lambein, 2003b; Diasolua Ngudi et al., 2003a; Lancaster & Brooks, 1983). Exposure to boiling water for 30 min was found to reduce the protein content by 58%, the methionine content by 71% (Diasolua Ngudi et al., 2003a); 10 min boiling reduced the vitamin C content by 60% (Lancaster & Brooks, 1983). Boiling kidney beans for 3 h reduced the methionine content by 65% whereas treatment at 65 °C for 3 h caused no significant decrease in methionine (Candela, Astiasaran, & Bello, 1997). Losses from root crops of vitamin A (β -carotene) and the water-soluble B group vitamins thiamine, riboflavin and nicotinic acid, as a result of boiling in water, are considerable (Bradbury & Holloway, 1988, chap. 4). It is important to prevent these very large losses of key nutrients, especially methionine and cysteine; cysteine is present at about one half the amount of methionine (Eggum, 1970; Yeoh & Chew, 1976). Boiling cassava leaves in water also denatures both linamarase and hydroxynitrile lyase, which eliminates the possibility of catalytic breakdown of linamarin and acetone cyanohydrin, respectively (Bradbury & Denton, 2010a).

In this paper, we report the development of two mild methods to remove cyanogens from (1) pounded leaves at ambient temperature and (2) intact leaves at 50 °C, which conserve key nutrients such as vitamins and particularly S-containing amino acids.

2. Materials and methods

2.1. Materials

Cassava leaves were obtained from the cultivars of cassava plants grown in a glasshouse at the Plant Culture Facility at Australian National University. The petioles and leaves were broken off from the main stem of the plant from very young leaves (leaf 0), the first fully expanded leaf (leaf 1) and counting sequentially down to leaf 8.

2.2. Methods

2.2.1. Variability of total cyanide content within a leaf and between leaves of different ages

Each leaf normally had about 6–8 leaf blades each of about 300–700 mg weight. Leaf blades were broken off from the leaves and torn into three sections: the tip of the blade, a central section and the stalk end section, where the blade is attached to the full leaf and the petiole. Samples of these sections (100 mg) were taken for total cyanide analysis (see Section 2.2.4). Leaves and petioles numbered 1–8 were also taken from cassava plants for analysis. For consistency of analysis, the central sections of these leaf blades were taken for total cyanide analysis.

2.2.2. Pounding treatment of leaves followed by washing at 30 °C

The central section (100 mg) of the leaf blade was taken for total cyanide analysis. Samples (10 g) of leaf blades from a particular cultivar were pounded in a pestle and mortar for 10 min and 100-mg samples taken for total cyanide analysis. The pounded leaves were then washed in 20 mL of water at about 30 °C, with slight stirring for about 5 min, and filtered through a fine metal sieve. The green pounded leaves were dried between filter paper to remove excess water and duplicate 100-mg samples were taken for analysis. The remaining pounded leaves were washed for a second time with 20 mL water at about 30 °C for about 5 min, filtered and a sample dried between filter papers and taken for analysis. The washing procedure was repeated for a total of four washes with total cyanide analyses after each wash.

2.2.3. Treatment of intact leaf blades with water at different temperatures

A 10-g sample of leaf blades from a particular cultivar (M Aus7) was immersed in 100 mL water at various temperatures from 30 to 100 °C with or without stirring. Duplicate analyses of 100 mg of the central section of the leaf blade were made at the beginning of the experiment and after various times of treatment of the leaf blades. Excess water was dried from the leaf blades by pressing between filter papers before weighing duplicate 100-mg samples for analysis.

In another series of experiments done on all cultivars, two 10-g samples of leaf blades of a particular cultivar were each immersed in 100 mL water at 50 ± 3 °C with mixing at the beginning. Duplicate 100-mg samples of the central section of the leaf blades were taken for analysis at the beginning of the experiment and after 2 h. The first sample of leaves was heated at 50 °C, with leaf blades withdrawn for analysis after 3, 4 and 5 h. With the second sample of leaves after 2 h treatment the water was removed, the leaves squeezed, and fresh water added at 50 ± 3 °C and leaf blades withdrawn for analysis after 3, 4 and 5 h. The water from each of these immersions of cassava leaf blades contained the cyanogens from the leaves and 1-mL samples of the water were taken for total cyanide analysis.

2.2.4. Total cyanide analysis of leaves

Duplicate 100-mg samples of intact leaf blades (broken into several small pieces to fit into a small plastic bottle) or pounded leaves or 1-mL samples of aqueous cyanogen extracts were added to a small plastic bottle, a small 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. Because of the rapid loss of hydrogen cyanide gas from broken or pounded leaves this operation was done as rapidly as possible after weighing out the sample. The bottles were allowed to stand overnight at 30 °C, the picrate papers were removed from the plastic support and 5.0 mL of water added to elute the colour. The absorbance was measured in a spectrophotometer at 510 nm and the total cyanide content in ppm calculated by multiplying the absorbance by 396 (Bradbury, Egan, & Bradbury, 1999; Egan, Yeoh, & Bradbury, 1998). For the aqueous cyanide solutions 1 mL was taken instead of 100 mg, hence the multiplying factor in this case was 39.6 instead of 396.

3. Results and discussion

3.1. Differences in total cyanide content within a single leaf blade and between leaves of different ages

The results given in Table 1 show a gradient of increasing total cyanide content from the tip to the stalk end of the leaf blade for each of the four cultivars. In order to get a representative sample

Table 1
Total cyanide content (ppm) of different sectors across cassava leaf blades of leaf 4.

Leaf sector	Total cyanide content (ppm) in leaf blades from different cultivars			
	SM1–150	MCol 1468	TMS 50395	MAus 7
Tip end	23	81	110	100
Middle	61	185	202	180
Stalk end	92	192	303	293

of the leaf in studies on cassava leaf blades, we used the middle section of the leaf.

The total cyanide content of mid-sections of leaf blades of very young leaves (leaf 0), the first fully extended leaf (leaf 1), leaf 4 and leaf 8 of the four cultivars is shown in Table 2. The range of total cyanide results in Table 2 is 64–490 ppm, which is at the low end of the range of results summarised by Lancaster and Brooks (1983) of 80–1860 ppm. Very young leaves and the first fully expanded leaves have the highest total cyanide content and the values decrease as the leaves age. Generally cassava leaves 1–10 are picked and used in Africa (Achidi et al., 2005), and for consistency we have used the central section of the leaf blades of leaf 4 in subsequent analyses on intact leaves.

3.2. Pounding of cassava leaf blades followed by washing in water

A very common method of processing cassava leaves involves pounding for 15 min followed by boiling in water for 15–60 min (Bokanga, 1994; Bradbury & Denton 2010a; Lancaster & Brooks, 1983). Boiling pounded cassava leaves in water for 30 min reduced the protein content by 58% and the methionine content by 71% (Diasolua Ngudi et al., 2003a). Boiling kidney beans for 3 h reduced the methionine content by 65%, whereas treatment for 3 h at 65 °C caused no significant decrease in methionine content (Candela et al., 1997). Boiling also reduces the vitamin content (Bradbury & Holloway, 1988).

The results in Table 3 show that it is possible to reduce the total cyanide content of pounded leaves to 8% by pounding for 10 min, followed by washing twice for 5 min in twice their weight of water at ambient temperature. After four washings in twice their weight of water the total cyanide content is reduced to 3%. In considering how many washes should be needed, we note that cassava leaves contain 60–1860 ppm total cyanide (Section 3.1; Lancaster & Brooks, 1983). For example, if cassava leaves originally contained 1000 ppm total cyanide (twice the maximum amount found in this study, see Table 2) then 3% retention of cyanide in pounded leaves would be 30 ppm cyanide, which is three times the World Health Organisation safe level for cassava flour of 10 ppm (FAO/WHO, 1991). It is interesting that the total cyanide content of pounded cassava leaves is reduced to 3% after four washings with water, because it was not possible to reach such low percentage values on treatment of cassava flour, where the residual cyanogen is acetone cyanohydrin (Bradbury, 2006; Bradbury & Denton 2010b; Cumbana, Mirione, Cliff, & Bradbury, 2007). The nearly complete loss of HCN from pounded leaves is due to two reasons. Firstly,

Table 2
Total cyanide content (ppm) of mid-sections of leaf blades 0, 1, 4 and 8^a.

Cultivar	Total cyanide content (ppm)			
	0	1	4	8
MAus 7	445	306	152	88
TMS 50395	490	470	347	332
MCol 1468	353	245	213	64
SM 1–150	282	298	139	133

^a Leaves are numbered from 0 for very young leaves, 1 for the first fully expanded leaf with increasing numbers down to leaf 8.

the ground leaves have a more open structure than cassava flour. Secondly, hydroxynitrile lyase enzyme, which catalyses hydrolysis of acetone cyanohydrin to HCN, is present in the leaves but absent from the flour.

The finely ground cassava leaf concentrate is fresh green in colour and because the whole treatment is done at ambient temperature it retains nearly all the protein, essential S-containing amino acids and vitamins of the original cassava leaves.

3.3. Treatment of intact cassava leaves in water

Although pounding of cassava leaves followed by boiling in water is the most common current method of processing, cyanogens are also removed from intact leaves by boiling them in water. Leaves can be blanched by boiling in water for 5 min or by heating on a hot plate for 3–10 min (Achidi et al., 2005) or sun-drying (Lancaster & Brooks, 1983). Cassava leaves may be cooked in water, which softens the leaves, and then pounded in a pestle and mortar, but we have found no loss of total cyanide due to pounding of already cooked leaves (Bradbury & Denton, 2010a). Faced with a multiplicity of different methods for treating leaves, we have focused on the loss of cyanogens from intact leaves in water at different temperatures and the effect of stirring.

As shown in Fig. 1, in boiling water there was a rapid loss of total cyanide content of leaf blades, leaving only 4% cyanide remaining after 40 min, whereas in water at 55 °C with continuous slow stirring, using a mechanical stirrer which entangled the leaf blades and caused some to break, 100 min was required to reach 3% cyanide remaining. Leaf blades immersed in water (unstirred) at 55 °C needed 4 h to leave only 5% cyanide remaining. The loss of cyanide was increased with stirring, due to disruption of leaf structure with liberation of more linamarin and enzymes than in the absence of stirring. Immersion in water at 30 °C caused very slow loss of cyanide with 14% total cyanide remaining after 40 h. The quickest method to remove cyanogens from intact leaves was to boil them in water, but this caused a large loss of proteins, S-containing amino acids and vitamins. The loss of these nutrients was greatly reduced by immersing leaves in water at about 50 °C.

Fig. 2 shows the results averaged over four cultivars of immersing cassava leaf blades in 10 times their weight of water at 50 ± 3 °C for (1) 5 h and (2) using the same conditions as in (1), but changing the water after 2 h treatment, to get rid of the cyanogens present in the water. It is clear that changing the water after 2 h treatment at 50 °C is very effective in reducing the total cyanide remaining in the leaves to 7% after 4 h, compared with 17% remaining after 4 h, when there is no change of water. In this method of treatment the cyanogens (linamarin, acetone cyanohydrin and hydrogen cyanide) are extracted from the leaves in the warm water and remain dissolved in the water, except for some loss of hydrogen cyanide gas. This was confirmed by total cyanide analyses of the aqueous solutions from the 0–2 h, 2–5 h and 0–5 h immersions. There was a considerable amount of total cyanide present in the aqueous solutions from the 0–2 h and 0–5 h immersions, but always at a concentration lower than that remaining in the leaves and only 10% of that amount was present in the 2–5 h aqueous solution.

Table 3

% Total cyanide remaining after pounding followed by washing at about 30 °C^a.

	% Total cyanide remaining after pounding and washing for cultivars				
	MAus 7	TMS 50395	MCol 1468	SM 1–150	Mean ^b
After pounding (10 min)	34	30	32	19	29 (7)
Wash 1	10	13	18	12	13 (3)
Wash 2	7	8	10	6	8 (2)
Wash 3	5	7	8	3	6 (2)
Wash 4	5	6	0	1	3 (3)

^a For detailed method see Section 2.2.2.

^b Standard deviation in brackets.

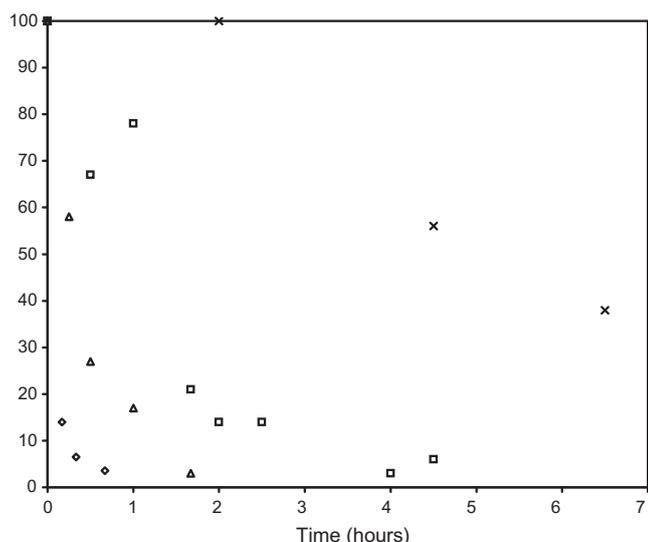


Fig. 1. Graphs of % total cyanide remaining vs time in h for MAus 7 cassava leaf blades (leaf 4) boiled in water (◇), stirred at 55 °C (△), immersed at 55 °C (□) and immersed at 30 °C (X). At 30 °C after 24 h and 30 h immersion there was 17% and 14% cyanide remaining, respectively.

A suitable treatment is to immerse cassava leaf blades in 10 times their weight of water at 50 ± 3 °C for 2 h, followed by a change of water and continuation of the treatment at about 50 °C for another 2 h. The aqueous solutions which contain most of the cyanogens from the leaves are poisonous and must be discarded. In the absence of a thermometer, a simple test of water temperature is to put a finger into the water and check the time until it gets too hot and must be removed. At 50 °C it is greater than 2 min and at 54 °C it is only 15 s.

4. Conclusions

Two methods have been developed to remove cyanogens from cassava leaves while at the same time conserving key nutrients; proteins, vitamins and S-containing amino acids, which are vital to detoxify ingested cyanide. The first method is to pound leaves for at least 10 min until they are well macerated and then wash them twice with twice their weight of water at ambient temperature, removing the wash water between each washing. This reduces the total cyanide remaining to 8% and further washes reduce the total cyanide content even more (Table 3). This is the preferred method because it produces fresh-ground green leaves that retain nearly all the protein, S-containing amino acids and vitamins of the original leaves. The second method is used for intact leaves. Leaves are immersed in ten times their weight of water, with initial mixing to wet the leaves, at about 50 °C for

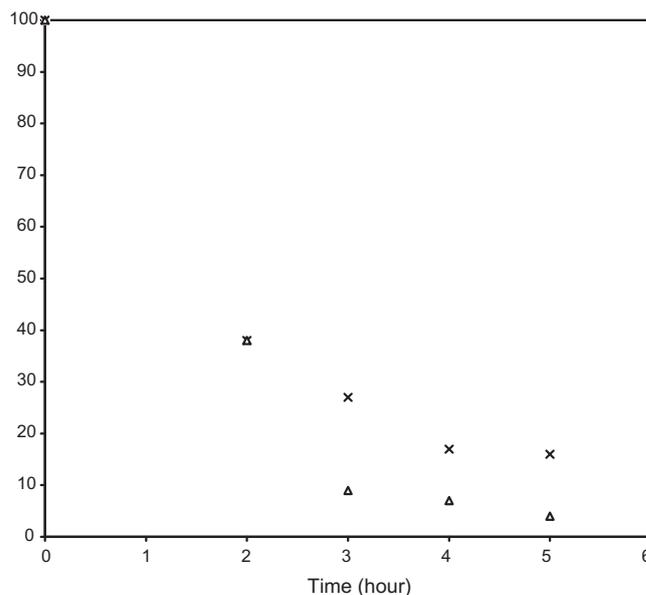


Fig. 2. Graph of % total cyanide remaining vs time in h for the mean of four cultivars (1) immersed in 10 times their weight of water at 50 ± 3 °C (X) and (2) immersed in 10 times their weight of water for 2 h at 50 ± 3 °C, followed by a change of water and immersion in 10 times their weight of water at 50 °C for 2–5 h (△).

2 h, with a change of water and further heating at about 50 °C for another 2 h, which reduces the total cyanide remaining to 7%. The aqueous solutions from these treatments and the washing treatments used in the first method contain poisonous cyanogens and must be discarded. The application of these mild methods will conserve important nutrients in cassava leaves and improve the nutrition of the people. Used in conjunction with the wetting method that removes cyanogens from cassava flour (Bradbury, 2006; Bradbury, Cliff, & Denton, 2011), it is another tool to help prevent konzo (Banea et al., 2010).

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