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Short Communication

Simple wetting method to reduce cyanogen content of cassava flour

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Abstract

Ten samples of cassava flour from Mozambique, Indonesia and Australia and one sample of gari from Mozambique were thoroughly mixed with water in the ratio 1:1.25. All the water was absorbed by the flour and the mixture was left in an open beaker at 30 °C. It was found that, providing that there was a reasonable amount of linamarase in the flour, the total cyanide content reduced about three-fold over 5 h. Addition of exogenous linamarase increased greatly the rate of breakdown of linamarin in the flour. There was no breakdown of linamarin from a gari sample at pH 4.2, but breakdown occurred when the pH was increased to 6 with buffer.

The rationale for using this wetting method is that it is simple: the cassava flour is thoroughly mixed with water and allowed to stand in an open vessel for about 5 h, and then it is used for cooking. If the results in this brief paper are confirmed in the field and the method is acceptable to women, then it should decrease substantially the cyanide intake of those people in eastern, central and southern Africa who consume cassava flour, and thus reduce the incidence of cyanide poisoning and konzo.

Keywords: Casssava flour; Wetting method; Reduces cyanide intake; Konzo; Linamarase; Linamarin

1. Introduction

Cassava is the staple food for at least 500 million people in the tropics (Cock, 1985). The starchy roots are the main food source, and the processed leaves are also used as a protein-rich food in Africa and elsewhere. Two cyanogenic glucosides, linamarin and a small amount of methyl linamarin—located inside the plant cells together with a specific hydrolytic enzyme, linamarase, located in the cell wall (Mkpong et al., 1990), form an effective biological system to deter predators.

Common methods of processing cassava roots to produce flour in eastern, central and southern Africa involve either sun drying of the peeled root followed by pounding and sieving, or heap fermentation (Cardoso et al., 1998). Because these methods cause only limited disruption of plant cells with limited contact between linamarin and linamarase, the amount of residual total cyanide in the flour amounts to 25–33% of that originally present for sun drying, and 12.5–16.5% for heap fermentation (Cardoso et al., 2005). This leads to average values of 40–46 mg HCN equivalents/kg flour (ppm) in normal years (Ernesto et al., 2002) compared with the WHO safe level of 10 ppm (FAO/WHO, 1991). In years of low rainfall the average value increases greatly to >100 ppm (Ernesto et al., 2002). Ingestion of high cyanide cassava flour is thought to be the major contributing cause of konzo, an irreversible paralysis of the legs in women of child-bearing age and children, which occurs in Mozambique, United Republic of Tanzania, Democratic Republic of the Congo, Central African Republic and Cameroon, but has not yet been reported from western Africa.

In western Africa and central and southern America cassava parenchyma is ground, grated or crushed up into small pieces, which causes disruption of many plant cells and allows good contact between linamarin and linamarase. The moist mash is left for several days, the water-soluble cyanogens squeezed out and the residual

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HCN gas removed by roasting. The retention of cyanogens in gari or farinha is only 1.8–2.4% (Oke, 1994; Dufour, 1994), which is an order of magnitude lower than that in the flour produced by sun drying and heap fermentation in eastern, southern and central Africa. In order to eliminate konzo from this region, much better methods are needed to reduce the total cyanide content of flour (Cardoso et al., 2005). This short communication describes a simple wetting method used just prior to cooking the cassava flour, which considerably reduces the total cyanide content of cassava flour.

2. Materials and methods

2.1. Materials

Samples of cassava flour and gari had been collected from various sources in Mozambique, Indonesia and Australia and stored at −20 °C for some years.

Under these conditions the flour was quite stable and no change in its total cyanide content was observed over a period of years. Flour was also produced from cassava plants grown in the Plant Culture Facility at the Australian National University. The peeled roots were sliced longitudinally and dried in an oven at 50 °C for 4–5 days, followed by pounding in a pestle and mortar. The flour was sieved using three Endicott metal sieves into fine- (<425 μM), medium- (425–850 μM) and coarse- (>825 μM) sized flour samples. Ten samples of flour and one of gari were used. Linamarase solution was prepared from cassava latex and its activity assayed by the method of Haque and Bradbury (1999).

2.2. Preliminary experiments

Five cassava flour samples from cvs. SM1-150, MAus 7, MCol 1468, TMS 50395(1), and TMS 50395(2), which had been left in open dishes in the laboratory for 6 months under ambient conditions (15–27 °C, 20–80% R.H) with moisture content about 7.2% (Cardoso et al., 2004), showed no decrease in their total cyanide contents. Using the same flour samples in closed glass desiccators over excess water at 100% RH gave steady decreases in total cyanide content over time. After 14 days the cyanide remaining was 42 (23)%. Mold growth commenced in one sample after 17 days and gradually spread to the other samples.

2.3. Rate of loss of cyanide from flour

The standard procedure involved mixing thoroughly 5 g of flour with 6.25 mL water (pH ca 5.7) in a 100 mL beaker. The water was absorbed by the flour. Duplicate 100 mg samples of wet flour were removed at 0, 1, 2, 3, 5 and 24 h for total cyanide analysis from the open beaker, which was kept in a constant-temperature oven at 30 or 35 °C. The flour gradually dried out, and after 5 h it was necessary to add about 2 mL water and mix thoroughly before it was left overnight. The 100 mg samples were placed in a small plastic bottle, 0.5 mL water and normally a buffer/linamarase small filter paper were added, then a picrate paper, and the bottle was closed with a lid. (In most cases the flour contained sufficient linamarase so that the addition of the buffer/enzyme paper was found not to be necessary). The bottle was left at 30 °C for about 24 h, the picrate paper removed from the plastic backing sheet, placed in a test tube and 5.0 mL water was added. The absorbance of the solution was measured at 510 nm and the total cyanide content in ppm calculated by multiplying by 396 (Egan et al., 1998; Bradbury et al., 1999). The total cyanide content was converted to % of total cyanide remaining using the zero time value as 100% and plotted versus time of treatment. From the curved graph, the initial % loss of total cyanide over the first hour was determined. A plot of log(% cyanide remaining) versus time did not produce a straight line, hence the loss of cyanide is not described by a first-order rate process.

Additional experiments were carried out as follows:

- In one case, 100 g of flour (Mozambique) was mixed thoroughly with 125 mL water and then the standard procedure was followed.
- The rate of loss of cyanide from M Aus 7 flour was measured with different ratios of flour to water, viz. 1:1, 1:2 and 1:3.
- A linamarase solution (0.25 mL) of activity = 1.6 U/mL was added to 6 mL of water, mixed thoroughly with 5 g Bogor A flour and the standard procedure followed.
- The gari sample from Mozambique when wetted had a pH of 4.2 and the cyanide level did not decrease over time using the standard procedure even after 0.25 mL linamarase solution was added. The pH was increased to 6 by mixing gari thoroughly with a mixture of 5 mL 1 M phosphate buffer at pH 6 and 2 mL water. In another experiment, 0.25 mL linamarase solution was also added.

2.4. Linamarase activity of flour

To 1 g of flour was added 4 mL of water with stirring, and the mixture was left for about 30 min followed by centrifugation. The following amounts of the supernatant solution from the centrifugation, viz. 0, 100, 200, 300, and 500 μL, were added to small plastic bottles and water added to make up to a total volume of 500 μL. Experiments were done in duplicate. Fifty microliters of 1 M phosphate buffer at pH 6 was added followed by 100 μL linamarin solution (Haque and Bradbury, 2004). The solution was mixed, a picrate paper was added, the

lid closed immediately and the bottle kept at 30 °C. After exactly 15 min, 200 mg of guanidine hydrochloride was added with stirring to inactivate the enzyme, the lid closed and the bottle kept at 30 °C for 3 h (Haque and Bradbury, 1999). The picrate paper was removed and the total cyanide content determined. A graph was plotted of the amount of HCN liberated in μg versus volume of linamarase solution used in μL. The gradient of the line (gr) was obtained from the graph and the linamarase activity of the flour was calculated by the equation (Haque and Bradbury, 1999)

$$\text{Activity of flour(U/g flour)} = \text{gr} \times 4000/27tw. \quad (1)$$

One enzyme unit, U, is defined as the amount of linamarase required to hydrolyse one micromole of linamarin to HCN in 1 min, t = time in minutes over which the linamarase is active (15 min) and w is the weight of flour = 1 g.

3. Results and discussion

The 10 samples of cassava flour, one from Mozambique, Bogor A and Bogor 3 from Indonesia and the remainder from Australia and one sample of gari from Mozambique had total cyanide levels ranging from 40–250 ppm. The initial % loss of cyanide from TMS 50395(1), which contained 200 ppm total cyanide, was about the same as that from the Mozambique flour, which contained 42 ppm total cyanide (see Table 1). The initial loss of cyanide in the first hour recorded in Table 1 is obtained from a graph such as that shown in Fig. 1. The cyanide loss over a 24 h time period is shown in Fig. 2, and it was found that the major amount of cyanide loss occurs during the first 5 h after mixing. Hence Table 1 records the % cyanide remaining 5 h after thorough mixing with water. If one excludes the two results in which exogenous enzyme was added and averages the 16 results in Table 1, the mean value (standard deviation in brackets) is 45(27)%.

What is the reason for the differences in the results in Table 1, particularly between Bogor A, Bogor 3, gari (Moz) and the other samples? There are no consistent differences between the use of temperatures of 30 or 35 °C for TMS 50395(1) and MAus 7 samples in Table 1. The particle size of the flour was compared for a sample of TMS 50395(3) sieved to three different sizes, which resulted in essentially the same rate of breakdown for fine, medium and coarse flour (see Table 1). However, the importance of the enzyme concentration was shown by the fact that the initial rates of breakdown of TMS 50395(3) > Mozambique MAus 7 > Bogor A, which is the same order as the enzyme activities of the flour samples (Table 1). To confirm the importance of enzyme concentration, linamarase solution was added to Bogor A and pH-adjusted gari (Moz), which greatly increased the rates of breakdown of linamarin in both samples (Table 1).

Table 1
Effect of various factors on initial rate of breakdown of linamarin in flour and gari

Sample	Wt. of sample (g)	Particle size[a]	Temperature (°C)	Enzyme activity of sample (U/g)[b]	Initial rate of breakdown (% per hour)	% cyanide left after 5 h
Mozambique	5	m	30	0.049	19	35
Mozambique	100	m	30	0.049	28	27
TMS	5	f	30	nm	14	46
50395(1)	5	f	35	nm	25	36
M Aus 7	5	f	30	0.049	19	28
M Aus 7	5	f	35	0.049	16	35
TMS	5	f	30	0.12	30	20
50395	5	m	30	0.12	34	18
(3)	5	c	30	0.12	35	14
Indonesia	5	f	30	0.0055	2	91
Bogor A	5	f	30	0.0055	60[c]	10
M Col 1468	5	f	30	nm	10	51
M Aus 7(2)	5	f	30	nm	20	40
Bogor 3	5	f	35	nm	3	85
Bogor A	5	f	35	0.0055	3	87
TMS50395(2)	5	f	30	nm	31	20
Gari (Moz)	5	c[d]	30	nm	11[e]	85
Gari (Moz)	5	c[d]	30	nm	20[c,e]	58

[a]Particle size fine (f) <425 μm, medium (m) 425–850 μm, coarse(c) >825 μm.
[b]nm = not measured.
[c]0.25 ml linamarase solution added of activity 1.6 U/ml.
[d]Roasted granulated product from Southern Mozambique.
[e]pH of gari increased from 4.2 to 6 by addition of pH 6 phosphate buffer.

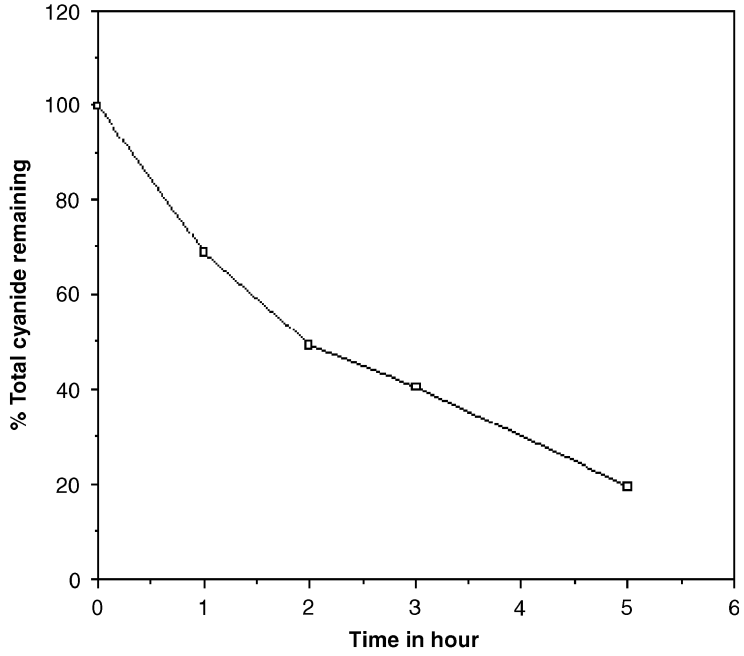

Fig. 1. Graph of % total cyanide remaining versus time in hour, for 5 g cassava flour sample TMS 50395(3) fine, mixed thoroughly with water (1:1.25) and left in an open beaker at 30 °C.

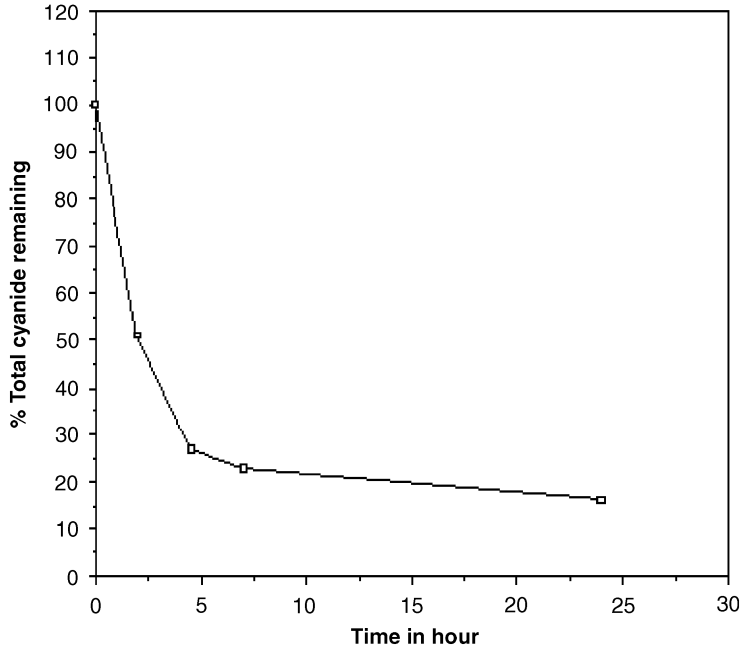

Fig. 2. Graph of % total cyanide remaining versus time in hour, for 100 g cassava flour from Mozambique mixed thoroughly with 125 mL water and left in an open beaker at 30 °C.

3.1. Effect of moisture content on breakdown of linamarin catalysed by linamarase

The breakdown of linamarin in flour was negligible under ambient laboratory conditions, but at 100% RH at room temperature the % cyanide remaining over five samples of flour was 42% after 14 days (see above). A similar mean value of 45% is obtained after 5 h using flour to water ratios of 1:1.25 (Table 1). No difference was observed in the rate of breakdown of linamarin when flour to water ratios of 1:1, 1:2 and 1:3 were used. Clearly, the water present in the flour rapidly swells the flour particles and allows linamarase to come into contact with linamarin and hydrolysis proceeds. Once the flour is fully swollen, hydrolysis occurs at the maximum rate for that particular enzyme concentration

and the presence of excess water, such as occurred with a flour:water ratio of 1:3, did not increase the rate of breakdown of linamarin. Nevertheless it is important that the flour does not dry out on standing at 30–35 °C, because this will prevent further reaction of linamarase with linamarin.

3.2. Effect of particle size of substrate on breakdown of linamarin catalysed by linamarase

As already mentioned (see above) the sun drying and heap fermentation of large pieces of peeled roots does not allow good contact between linamarase and linamarin; hence the residual total cyanide is 12.5–33%, compared with 1.8–2.4% for the methods in which the roots are grated into small pieces (Cardoso et al., 2005). It is also possible that in the former methods, the roots may dry out too quickly, reducing the moisture content to less than 13%, below which no breakdown occurs (Mlingi and Bainbridge, 1994). However, for the range of particle sizes of flour and gari used in these experiments, it was found that the particle size was not important.

3.3. Effect of temperature on breakdown of linamarin catalysed by linamarase

Experiments carried out at 30 and 35 °C gave the same rate of breakdown, so 30 °C was used as the standard temperature in all experiments, which is considered to be an appropriate temperature for tropical countries. Further work needs to be done at lower temperatures, because of decreased rates of reactions and slower removal of HCN, which has a boiling point of 26 °C.

3.4. Effect of linamarase content of flour on rate of breakdown of linamarin

The results in Table 1 show that for sample Bogor A, for which the enzyme concentration is more than 20 times lower than TMS 50395(3), the breakdown of linamarin is very slow, but is increased greatly by adding linamarase solution to the water used for wetting the flour. This follows basic enzymology since the rate of reaction can be increased by augmenting the enzyme concentration. A similar result was obtained with gari at pH 6. The linamarase concentration in cassava parenchyma has been found to vary over a 20-fold range in the roots of different cultivars (Bradbury and Egan, 1994), a range similar to the one found in this study. Hence it is possible that there may be some samples of flour found in Africa, which, like the Indonesian samples used in this study, do not contain enough enzyme for the wetting method to work effectively. This will require the testing of many samples in eastern, central and southern Africa.

3.5. Effect of pH of substrate on breakdown of linamarin by linamarase

The pH optimum for hydrolysis of linamarin by linamarase to give acetone cyanohydrin is about 6 (Yeoh, 1989). This is suitable for flour samples, because the pH of a number of wet samples (including those from Indonesia) was found to be about 5.7. Below pH 5, catalysed breakdown to acetone cyanohydrin is much slower. The uncatalysed breakdown of acetone cyanohydrin to HCN is much slower as pH falls below 5 and ceases at pH 4 (White et al., 1994). Thus, the wet gari sample at pH 4.2 did not break down to HCN, but at pH 6 linamarin did break down (see Table 1). During the preparation of gari, fermentation occurs with production of lactic acid and a reduction of pH to about 4.2, which is a problem for use of the wetting method. However, total cyanide levels in gari are 0–40 ppm (Oke, 1994; Aletor, 1993; Adindu et al., 2003), which is much lower than with flour; hence the wetting method is not needed as much with gari, particularly as the pH would have to be increased to 5–6 by adding some material such as, for example, baking soda (sodium bicarbonate) to the water used for wetting, which may make the gari less acceptable for cooking.

3.6. Application of simple wetting method

The simple wetting method involves thorough mixing of cassava flour with water, standing the mixture in an open vessel for about 5 h and then using the wetted flour for cooking. Provided that this flour has similar linamarase content to the Mozambique flour sample in Table 1, then the linamarin content will be reduced to less than one-third of its previous value after 5 h of mixing with water. The simple wetting method would have the effect of reducing the mean value of total cyanide in flour in Mozambique in a normal year from its current value of 40–46 ppm to 12–14 ppm when it is consumed, and in a year of low rainfall from 100–148 ppm (Ernesto et al., 2002) to 30–44 ppm when it is consumed. Providing that the linamarase content of the flour is adequate, this approximately three-fold reduction of total cyanide content on consumption of the flour could alleviate cyanide poisoning, konzo and other cyanide-related conditions.

4. Conclusion

Providing that cassava flour contains an adequate amount of residual linamarase, it may be mixed

thoroughly with water in the ratio of about 1:1.25 in the morning, allowed to stand in an open vessel for about 5 h at about 30 °C, and the total cyanide content will be reduced to about three-fold. The wetted flour should then be used in the evening for cooking. If this method is found to be acceptable to women and becomes commonplace in eastern, central and southern Africa, it has the potential to reduce greatly the incidence of cyanide poisoning and konzo.

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