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# Variations in cyanogen content of cassava during village processing in Cameroon

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A study of the traditional processing of two cassava foods, 'farine de manioc' and 'baton de manioc', was undertaken in Cameroon. The influences of various stages of the processes on the amounts of the different cyanogenic components (cyanogenic glucosides, cyanohydrins and hydrogen cyanide) were studied. The traditional water fermentation, followed by pressing and sun-drying or boiling, reduced the amount of total cyanogens in fresh roots (91–1515 mg $kg^{-1}$) to more acceptable levels (0·0–11·3 mg $kg^{-1}$) in foods ready for consumption. Fermentation temperature and the extent of root comminution increased the rates of glucoside hydrolysis giving a temporary increase in the levels of the intermediate product, cyanohydrin. Despite the low pH in the fermentations, hydrolysis of cyanohydrins to hydrogen cyanide was still in evidence in the later stages of fermentation, the latter compound being removed during pressing, sun-drying and cooking.

## INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a root crop on which over 500 million people rely as a major source of calories (Cock, 1985) and its use as a human food as well as an industrial raw material is increasing. World production of cassava in 1988 was estimated to be about 138 million tonnes, of which 57 million tonnes was produced in Africa (FAO, 1988).

Many of the cassava varieties grown in Africa are of the so-called 'bitter' or high cyanide types. Due to the high levels of toxic cyanogenic components in the harvested crop together with its perishable and bulky nature, processing (usually by fermentation) is required prior to consumption (Lancaster *et al.*, 1982).

In the processing of cassava roots, the cyanogenic glucosides are hydrolysed by the endogenous enzyme linamarase to form cyanohydrins, which in turn are hydrolysed by the enzyme hydroxynitrile lyase, to form hydrogen cyanide (HCN). This hydrolysis occurs spontaneously at pH 4 and upwards (Fomunyam *et al.*, 1985). The water-solubility and volatility of HCN facilitate easy removal from the cassava tissues during soaking, fermentation and drying.

In Cameroon, cassava is an important food crop and is processed into a wide range of products (FAO, 1990; Ambe & Foaguegue, 1990). The objective of this study was to investigate the efficiency of village processing with regard to cyanogen removal.

## MATERIALS AND METHODS

### Farine de manioc

'Farine' is a dried cassava flour and was studied at Nkoumetou, 30 km north of Yaounde. Fresh cassava was peeled, cut and split into 60–90 mm chunks and washed. The cassava pieces were soaked for 2 days under water in large basins during which time they fermented and softened. Excess water was removed from the fermented pieces by squeezing in the hand. The pieces

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were roughly chopped using a machete and placed in old fertilizer sacks weighted with stones to remove more water overnight. In the morning, there was further sun-drying on ricks or on roofing sheets. The final product was prepared by pounding into a fine flour then boiling with water to form a thick porridge.

### Baton de manioc

'Baton' is a method for preparing cassava for more immediate consumption and was studied at Nega, 100 km north of Yaounde. Cassava roots were peeled, split and the central fibres removed. Cubes 20–40 mm in size were washed, placed in basins, covered with water and left for 2 days, sometimes near the fire. After fermentation, excess water was squeezed out of pieces by hand and/or they were placed in a sack weighted with stones for 1 h. The pulp was pounded in a mortar and ground between a stone and a wooden slab to yield a fine paste which was wrapped in young leaves, bound with raffia and boiled for 20–30 min.

### Sampling of cassava processing

Three households from each village were selected at random. In each case, the cassava was weighed and the local name for the variety determined. A random sample of 10 roots was taken for subsequent laboratory evaluation for cyanogen content. Discs 1 cm thick were taken from each root at the centre, proximal and distal ends, peeled and cut into 1 cm cubes, which were then thoroughly mixed together, prior to extraction and analyses.

During cassava processing, temperatures and pH of soak-waters were monitored at 24-h intervals, and samples were taken and removed to the laboratory; after draining to remove surface water, each sample was cut into 1 cm cubes which were thoroughly mixed prior to extraction and analyses.

### Analyses of samples

All analyses were carried out in sextuplicate. All reasonable steps were taken to assure sample homogeneity. Moisture-content and pH analyses of cassava used in the village processes were carried out at the laboratory in Yaounde. Extracts were prepared for subsequent analysis of cyanogenic components in the UK, using the methodology of Cooke (1978, 1979), as modified by O'Brien *et al.* (1991): cassava samples (50 g) were homogenized in 160 ml 0·1 M orthophosphoric acid containing 25% ethanol. Centrifugation (5000 *g*/20 min) followed; the supernatant fraction was subsampled and stored in a 15 ml screw-top sample bottle, labelled and stored at +4°C in a domestic refrigerator, prior to refrigerated transit. All results given in this paper for cyanogens are presented on a dry weight basis to enable a better comparison to be made between samples with widely differing moisture contents.

Because of difficulties associated with the measurement of low levels of cyanogens, and because glucosides and cyanohydrins are measured by subtraction, negative values are sometimes obtained for these two cyanogens. This problem is discussed elsewhere (O'Brien *et al.*, 1991). The tables of results discussed below show these negative values where they occurred, but they can in all cases be considered to approximate to zero.

## RESULTS AND DISCUSSION

### Stability of extracts

The results shown relate to extracts assayed up to 45 days after extraction; results for total and non-glycosidic cyanogens do not vary by more than 10% (upwards or downwards) after extraction, although free cyanide (HCN) results may decrease by 15–53% over a 2-month period (O'Brien *et al.*, 1991). The free cyanide contents of the cassava batches studied represent only a very small proportion of total cyanogen content in the early-to-middle stages of processing, and in most of the final products the free cyanide content was very low (less than 5 mg $kg^{-1}$); any adjustment to allow for losses of free cyanide would not significantly affect the findings of this experimental work.

### Cyanogens in fresh cassava roots

The total cyanogen content of freshly harvested and peeled cassava roots, expressed on a dry weight basis, ranged from approximately 91 mg $kg^{-1}$ to 1515 mg $kg^{-1}$ (Tables 1 and 2). This wide range was to be expected from the number of different varieties used by the processors, and the values quoted fall within the range of what has been reported to date (Coursey, 1973; Nambisan & Sundaresan, 1985; Asiedu, 1986). In the current study it is clear that virtually all of the total cyanogens present in the fresh roots were in the form of cyanogenic glucosides, although a limited amount of enzymic hydrolysis may have occurred during sample preparation, resulting in a small amount of the two other components being produced.

### Effects of processing on total cyanide

The processes studied in each of the villages were highly effective in substantially reducing the levels of total cyanogens found in fresh roots to more acceptable levels.

The processes used in the preparation of 'baton de manioc' were marginally more effective than those used

**Table 1. Cyanogen contents of cassava processed to 'farine de manioc' in Nkoumetou village**

| Sample time | pH | % Total cyanogens | Cyanogen content (HCN mg $kg^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | Total | Glucoside | Cyanohydrin | HCN |
| (a) *Household No. 1 — 'Bitole' variety (Batch A)* | | | | | | |
| Day 0 | 6·83 | 100·0 | 476·6 | 447·1 | 22·3 | 7·2 |
| | (0·08) | | (17·4) | (18·9) | (3·1) | (1·2) |
| Day 1 | 6·73 | 80·3 | 382·9 | 337·2 | 36·6 | 9·1 |
| | (0·15) | | (20·1) | (20·3) | (2·0) | (1·0) |
| Day 2 | 4·69 | 30·3 | 144·6 | 4·4 | 134·6 | 5·7 |
| | (0·03) | | (6·2) | (7·0) | (2·3) | (0·5) |
| Day 3 | 4·20 | 18·6 | 88·5 | −9·8 | 91·4 | 6·9 |
| | (0·03) | | (3·0) | (1·5) | (2·2) | (1·2) |
| Day 4 | 4·48 | 3·9 | 18·6 | −1·6 | 15·9 | 4·3 |
| | (0·04) | | (1·1) | (1·0) | (1·1) | (0·9) |
| (b) *Household No. 1 — 'Six Mois' variety (Batch B)* | | | | | | |
| Day 0 | 6·69 | 100·0 | 754·4 | 694·6 | 46·1 | 13·6 |
| | (0·07) | | (76·9) | (72·4) | (4·9) | (2·8) |
| Day 1 | 6·35 | 93·1 | 702·2 | 652·0 | 43·4 | 6·9 |
| | (0·09) | | (25·7) | (24·0) | (2·4) | (1·1) |
| Day 2 | 4·96 | 23·2 | 175·1 | 20·3 | 149·0 | 5·9 |
| | (0·04) | | (13·9) | (14·3) | (2·1) | (0·8) |
| Day 3 | 4·38 | 12·4 | 93·2 | −7·2 | 91·0 | 9·4 |
| | (0·01) | | (4·1) | (2·0) | (3·9) | (2·2) |
| Day 4 | 4·61 | 3·7 | 27·9 | 4·3 | 16·8 | 6·8 |
| | (0·02) | | (6·8) | (4·8) | (5·7) | (3·6) |
| (c) *Household No. 2 — 'Bitole' variety* | | | | | | |
| Day 0 | 6·83 | 100·0 | 407·9 | 377·9 | 23·3 | 6·7 |
| | (0·08) | | (37·8) | (38·7) | (3·0) | (1·5) |
| Day 1 | 6·98 | 95·9 | 391·3 | 357·7 | 27·3 | 6·4 |
| | (0·17) | | (19·6) | (17·8) | (1·6) | (0·4) |
| Day 2 | 4·82 | 47·4 | 193·4 | 26·9 | 160·4 | 6·2 |
| | (0·02) | | (5·5) | (8·2) | (3·6) | (1·0) |
| Day 3 | 4·32 | 30·7 | 125·2 | −4·9 | 122·1 | 8·4 |
| | (0·02) | | (2·7) | (3·9) | (1·2) | (1·2) |
| Day 4 | 4·58 | 8·8 | 35·7 | −0·1 | 32·4 | 3·3 |
| | (0·03) | | (3·1) | (2·3) | (1·1) | (0·5) |
| (d) *Household No. 3 — 'Six Mois' variety* | | | | | | |
| Day 0 | 6·43 | 100·0 | 1515·0 | 1428·0 | 75·5 | 11·5 |
| | (0·11) | | (136·1) | (126·2) | (15·3) | (3·1) |
| Day 1 | 6·20 | 76·8 | 1163·0 | 1095·0 | 58·5 | 9·5 |
| | (0·14) | | (95·4) | (98·0) | (5·3) | (1·6) |
| Day 2 | 4·71 | 38·0 | 575·2 | 246·9 | 309·2 | 19·1 |
| | (0·05) | | (23·6) | (24·1) | (3·3) | (2·8) |
| Day 3 | 4·40 | 22·7 | 344·5 | −4·7 | 328·8 | 20·4 |
| | (0·02) | | (7·0) | (8·6) | (5·9) | (1·5) |
| Day 4 | 4·54 | 6·3 | 94·9 | 27·6 | 63·4 | 3·9 |
| | (0·06) | | (15·6) | (13·5) | (3·3) | (0·4) |

Values presented are mean values of six replicates with estimated sample standard deviation ($\sigma_{n-1}$) in parenthesis.
All values are given on a dry weight basis.
All process stages as follows: day 0, fresh roots; days 1–2, soaking; day 3, overnight pressing; day 4, dried.

in the preparation of 'farine de manioc', with a mean overall reduction in total cyanogens of 99·6% (range 99·3–100%) and 94·4% (range of 91·3–96·6%), respectively (Tables 1 and 2). However, the final stage in the preparation of the 'baton de manioc' involved boiling in water, which was not the case with the flour product.

The results for total cyanogen content of the 'baton de manioc' samples just prior to cooking indicate a decline to levels not very different from those in the uncooked flour (97·4%, range 95·6–97·8%). Analysis of the raw data, using the arcsin transformation (commonly used in statistical analysis of percentage change) followed by a *t*-test, did not show a significant difference between the two sets of figures at 5% significance level. However, a trend towards difference was indicated. The results confirm that unmodified village-processing of even high cyanogen varieties of cassava is capable of extensive detoxification.

**Table 2. Cyanogen contents of cassava processed to 'baton de manioc' in Nega village**

| Sample time | pH | % Total cyanogens | Cyanogen content (HCN mg kg$^{-1}$) | | | |
|---|---|---|---|---|---|---|
| | | | Total | Glucoside | Cyanohydrin | HCN |
| (a) *Household No. 1 — 'Menyo' variety* | | | | | | |
| Day 0 | 6·68 | 100·0 | 217·9 | 200·6 | 14·2 | 3·1 |
| | (0·07) | | (63·8) | (60·5) | (3·2) | (0·8) |
| Day 1 | 5·54 | 38·8 | 84·5 | 48·3 | 32·9 | 3·4 |
| | (0·14) | | (16·1) | (15·0) | (2·5) | (0·4) |
| Day 2 | 4·97 | 10·0 | 21·9 | 1·2 | 16·4 | 4·3 |
| | (0·06) | | (1·8) | (1·5) | (1·2) | (0·6) |
| Day 3a | 4·51 | 4·4 | 9·6 | −0·4 | 8·7 | 1·3 |
| | (0·06) | | (0·2) | (0·2) | (0·4) | (0·3) |
| Day 3b | 4·51 | 3·4 | 7·5 | −0·7 | 6·5 | 1·7 |
| | (0·04) | | (0·2) | (0·4) | (0·2) | (0·3) |
| Day 4 | 4·48 | 0·2 | 0·5 | −0·9 | 0·3 | 1·1 |
| | (0·05) | | (0·3) | (0·2) | (0·5) | (0·6) |
| (b) *Household No. 2 — 'Awono' and 'Menyo' varieties* | | | | | | |
| Day 0 | 6·77 | 100·0 | 90·9 | 80·8 | 8·5 | 1·6 |
| | (0·06) | | (9·0) | (8·1) | (1·2) | (0·4) |
| Day 1 | 4·86 | 15·0 | 13·7 | 1·5 | 9·9 | 2·3 |
| | (0·04) | | (1·8) | (1·2) | (0·8) | (0·5) |
| Day 2 | 4·42 | 5·7 | 5·2 | −0·1 | 3·4 | 1·9 |
| | (0·01) | | (0·4) | (0·5) | (0·4) | (0·2) |
| Day 3 | 4·19 | 4·5 | 4·1 | −0·7 | 2·8 | 1·9 |
| | (0·01) | | (0·2) | (0·6) | (0·9) | (0·7) |
| Day 4a | 4·32 | 2·2 | 2·0 | −0·6 | 1·4 | 1·2 |
| | (0·06) | | (0·2) | (0·3) | (0·4) | (0·6) |
| Day 4b | 4·74 | 0·0 | 0·0 | −0·3 | 0·2 | 0·1 |
| | (0·02) | | | (0·2) | (0·1) | (0·1) |
| (c) *Household No. 3 — 'Senegalais' variety* | | | | | | |
| Day 0 | 6·62 | 100·0 | 120·4 | 111·5 | 6·3 | 2·6 |
| | (0·09) | | (16·3) | (16·4) | (0·4) | (0·4) |
| Day 1 | 5·02 | 46·1 | 55·5 | 30·2 | 23·3 | 2·0 |
| | (0·05) | | (4·9) | (4·8) | (1·4) | (0·4) |
| Day 2 | 4·25 | 7·9 | 9·5 | −0·6 | 9·4 | 0·7 |
| | (0·04) | | (0·4) | (0·6) | (0·4) | (0·1) |
| Day 3a | 4·09 | 2·2 | 2·7 | −0·7 | 2·9 | 0·4 |
| | (0·03) | | (0·4) | (0·1) | (0·5) | (0·3) |
| Day 3b | 4·29 | 0·7 | 0·9 | 0·4 | −0·4 | 0·9 |
| | (0·05) | | (0·4) | (0·4) | (0·4) | (0·5) |

Values presented are mean values of six replicates with estimated sample standard deviation ($\sigma_{n-1}$) in parenthesis.
All values are given on a dry weight basis.
Processing: inclusion of suffix 'a' or 'b' denotes sampling before and after a given stage (either the pressing or the cooking stage).

**Inter-relationships of cyanogens**

In all of the processes studied, the period of greatest decline in the content of the cyanogenic glucosides was during the first 2 days. This decline in glucoside coincided with a rise in the levels of the intermediate product of hydrolysis, cyanohydrin. During this period the decrease in glucoside was considerably greater (around 2·5 times) than the corresponding increases in both the intermediate product and the final product of hydrolysis, HCN. Hence an overall loss of cyanogens from the system is indicated (Figs 1 and 2).

These changes in the relative proportions of the different cyanogens may be attributed to the fact that under mildly acid conditions such as those found in fermenting cassava, cyanohydrins will spontaneously breakdown to yield free cyanide, (it is, however, noted that, with sampling every 24 h, one cannot speculate overmuch about rates of hydrolysis, etc.).

The production of acid which causes a lowering of pH, has been attributed to lactic acid bacteria in the fermentation mixture (Okafor *et al.*, 1984; Nwachukwu & Edwards, 1987). The hydrolysis of cyanogenic glucosides is attributed to linamarase (linamarin β-D-glucoside glycohydrolase; EC 3.2.1.21), which is endogenous in cassava tissues (Coursey, 1973). In all of the batches studied, with one notable exception (Table 1, Section (d)), the cyanogenic glucoside contents were reduced to 5 mg kg$^{-1}$ or less; this indicates the efficacy of the wet fermentation process in facilitating contact between

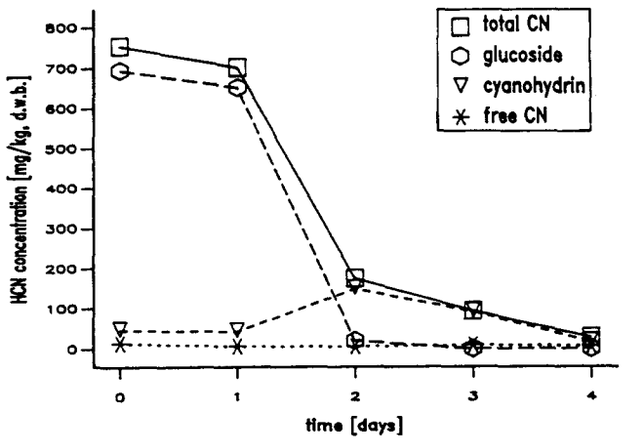


**Fig. 1.** Variations in cyanogen content of cassava processed to 'farine de manioc' in Nkoumetou village (from Table 1 Section (b)).

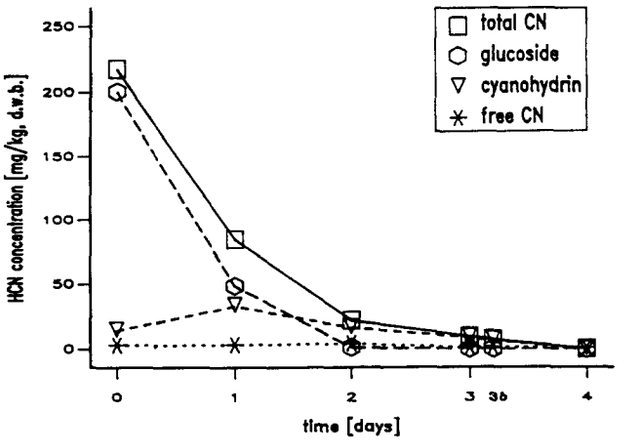


**Fig. 2.** Variations in cyanogen content of cassava processed to 'baton de manioc' in Nega village (from Table 2 Section (a)).

linamarase and the glucoside substrate. Also, in all of the completed 'farine' batches and in all of the 'baton' batches prior to cooking, the majority of the cyanogen content was in the form of cyanohydrins. Given the pH range involved in the latter stages of fermentation (4·09–4·51), and given the relative stability of cyanohydrins at acidic pH (Fomunyam *et al.*, 1985), this is not surprising.

### The role of cellular disruption

In cassava root tissues, enzymic disruption of cellular structure seems concurrent with root-softening (Okafor *et al.*, 1984; Okolie & Ugochukwu, 1988). In the 'farine' and 'baton' processes, some differences were observed. During the initial 24 h of fermentation of cassava destined for 'baton de manioc', steep declines in both pH and cyanogenic glucoside content of the samples were noted (Table 2). In the processes for 'farine de manioc', there was little change in either of these parameters during this period, although similar effects were observed to have occurred during the second day (Table 1). This difference would seem to indicate that the chopped cassava roots used in the 'baton de manioc' processes were more readily susceptible to the actions both of bacteria (as indicated by the fall in pH) and of endogenous linamarase enzyme. The initial preparation of roots for processing was different for each of the two processes: roots used for 'farine de manioc' were peeled, then chopped transversely into chunks 6–9 cm thick, whilst those used for 'baton de manioc' were peeled, split to remove central fibres and then chopped into cubes of 2–4 cm thickness.

It has been asserted that the degree of comminution of cassava roots is directly related to rates of softening, pH reduction and fermentation (Okafor *et al.*, 1984). It would appear that the delayed fermentation of root pieces in the 'farine de manioc' process, relative to that observed in the 'baton de manioc' process, is attributable to the larger-sized pieces and the lesser physical damage done to the roots.

In addition, it is reasonable to assume for the 'baton de manioc' process that the observed effects were further

**Table 3. Temperature and pH of soaking water during fermentation of cassava in Nkoumetou and Nega villages**

| Household number | Temperature (°C) and pH | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Day 0 | | Day 1 | | Day 2 | | Day 3 | |
| *Nkoumetou* | | | | | | | | |
| 1 Batch A | 23 | 6·05 | 27 | 4·85 | 27 | 4·74 | — | — |
| 1 Batch B | 23 | 6·06 | 26 | 4·40 | 26 | 4·21 | — | — |
| 2 | 25 | 5·59 | 28 | 4·30 | 28 | 4·08 | — | — |
| 3 | 25 | 5·95 | 28 | 4·31 | 28 | 4·45 | — | — |
| *Nega* | | | | | | | | |
| 1 | 23 | 6·69 | 27 | 4·46 | 26 | 4·61 | 29 | 4·69 |
| 2 | 37[a] | 6·50 | 29 | 4·63 | 31 | 4·60 | 29 | 4·56 |
| 3 | 25 | 6·11 | 30 | 4·48 | 29 | 4·28 | — | — |

[a] Warm water heated over the fire was added to the partially filled basins containing cold water and root pieces. Temperature was measured once each day in the morning, at the surface and bottom of the basins. The average temperature was calculated.

compounded by the practice of soaking the root pieces in warm water, and storage of fermentation basins close to the fire (Table 3). The raised temperatures of the ferments would assist in encouraging more rapid microbial growth and enzymic activity.

### The influence of product storage

Two samples of 'farine de manioc' prepared by women in Nkoumetou village were returned to the UK and stored at ambient temperatures for 3 months prior to re-analysis. It was found that total cyanogen levels in the flours had fallen quite dramatically during storage (from 18·6 mg $kg^{-1}$ to 7·41 mg $kg^{-1}$ for batch 1, and from 27·9 mg $kg^{-1}$ to 3·66 mg $kg^{-1}$ for batch 2). These findings confirm those reported by Mahungu *et al.* (1987), which indicated similar declines in Nigerian gari stored at ambient temperatures for 4 months. Rosling, H. and Milingi, N. (ICHU, Uppsala University & The Tanzania Food & Nutrition Centre; pers. comm.) have studied the controlled-temperature storage of cassava foods prepared in Zaire and Tanzania, and have also reported reduction in total cyanogen content during storage.

## CONCLUSIONS

The fermentation of peeled and chopped roots in water was found to be an effective means of cyanogen-removal from cassava in the villages where this study took place in Cameroon. This was true even of high-cyanogen varieties of cassava. The rates of linamarase activity and breakdown of the intermediate hydrolysis product, cyanohydrin, are influenced by the degree of comminution of the fermenting root pieces. The greater the extent of comminution, the more rapid is the enzymic hydrolysis of cyanogenic glucosides. Whilst the pH of the roots and soak-water remains close to neutral, the pH-mediated breakdown of cyanohydrins to HCN will ensue. However, when the pH of the system becomes more acidic, a greater proportion of cyanohydrin is likely to be retained in the cassava food. The final cooking stage is therefore important in removing most of the retained cyanohydrin.

Storage of sun-dried 'farine de manioc' flours for 3 months at UK ambient temperatures was effective in further reducing the levels of any residual cyanogens. This was especially true of cyanohydrins, and there may be important implications regarding the handling of fermented, low-pH cassava foods. Boiling of 'baton de manioc' has also been shown to be beneficial.

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