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The safety of dark, moulded cassava flour compared with white—a comparison of traditionally dried cassava pieces in North East Mozambique

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Abstract *Fresh cassava tubers were split into segments to obtain pieces with similar cyanide levels. From each root, one segment was deep-frozen immediately and analysed to serve as a reference. Remaining segments were dried and stored for 8 months under traditional household conditions in rural North East Mozambique. In these pieces, a varying extent of fungal growth occurred. They were ground and analysed individually for moisture, total and free cyanide, pH, brightness, aflatoxins and the number and genus of fungal propagules. By the traditional process a considerable (92·3–99·5%) loss in total cyanide was achieved. Free cyanide levels ranged from 19 to 89% of the total cyanide. There was no correlation between the initial (fresh root) and final (after drying and storage) total cyanide levels. However, darker flours had lower levels of total and free cyanide. Similarly, darker flours showed a higher pH. No aflatoxins could be detected.*

It is concluded that judgement of the consumer safety of cassava flour cannot adequately be carried out by analysis of colour or extent of fungal growth.

Keywords: cassava, *Manihot esculenta*, cassava flour, processing, storage, toxicity, cyanide, fungi, aflatoxin, Mozambique

Introduction

Cassava (*Manihot esculenta* Crantz) is a shrub whose tubers form the main staple food for an estimated 500 million people in the third world (Lancaster *et al.* 1982). In North-East Mozambique, cassava is grown mainly on family plots and produces the main staple food for the rural population (Casimiro 1972). In 1981 there was an epidemic of spastic paraparesis in which cyanide intoxication from cassava played an aetiological role (Ministry of Health of Mozambique, 1984). This aroused alertness on the toxicological aspects of cassava.

The predominant method of processing the roots is as follows: during the main harvest in August/September in the dry season, nearly all mature cassava plants are uprooted. The roots are peeled, cut into pieces of about 20 cm length and split

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longitudinally if these are regarded as being too thick. These pieces are exposed to the sun by spreading them out neatly on the ground (coastal zone) or on a platform (interior). After about 2–3 weeks of sun-drying, the cassava is transferred to a store, which may be a separate building or, in the coastal zone, a loft in the house or kitchen. Eventually, the cassava being consumed from immediately after the drying up to the next year's harvest, the pieces are drawn from the store and pounded into flour for consumption as a stiff porridge on the same or the next day.

Although the harvest takes place during the dry season, occasional showers may cause the drying pieces to become wet, enabling a more or less profuse mould growth to take place. This may also happen to stored dried cassava during the rainy seasons, due to inadequate roof protection.

Infestation with various storage pests is common, but was reported by local peasants to occur later in bitter than in sweet cultivars. In one region, the Murrupula district, part of the rural population encourages mould growth prior to dehydration as follows: the fresh, peeled cassava pieces are kept covered with leaves for 3 days prior to exposing them directly to the sun. This diminishes the toxic effect of fresh bitter cassava, according to the local peasants. The presence of moulds in the cassava pieces and the products made from them—flour and porridge—usually results in a grey and sometimes greenish grey colour. Sometimes, dark cassava flour is preferred over white by the rural inhabitants of the coastal zone.

In a period when food was rather scarce in the urban areas, the Ministry of Health (to which one of the authors was attached) declared a large quantity of heavily infested and moulded dry cassava as unfit for human consumption. The workers at the mill and other citizens complained about the decision, some of them stating that this was the proper appearance of cassava. Health officials clearly had a different opinion about suitability for consumption from the potential consumers.

Can dried cassava pieces or flour be judged solely by their appearance? In other words, is dark cassava flour less safe than white? There are many aspects to verify the nutritional value of cassava flour. In this study we limited ourselves to items of toxicological relevance, viz. cyanide and aflatoxins, and some other parameters in order to understand some of the relationships and processes that lead to higher or lower toxicity.

Materials and methods

Sample preparation

The preparation of the cassava samples was carried out using traditional methods: the handling and treatment of the cassava roots were carried out by local peasants with a minimum of instruction, but under observation by one of the authors.

In a coastal village, nine tubers of the bitter cassava cultivar, 'Gurue', were harvested in the morning. They were peeled immediately and cut longitudinally into four segments in order to obtain pieces containing similar levels of cyanide (De Bruijn 1971). Each segment was labeled.

One segment from each root was put immediately into a polyethylene bag and stored in a deep-freeze to serve as a reference. Two segments of each root were spread out on the humid, sandy soil, together with other cassava pieces of the family, to be sun-dried. The pieces were looked after and sometimes turned over by a peasant woman, until they were found to be satisfactorily dried (this was after two weeks) and subsequently stored in her loft, together with the other dried pieces. The cassava pieces were not affected by rain during the drying period, but during storage some of them became wet during the rainy season because of roof leakage. After 8 months, 14 experimental samples were recovered from the loft, put into polyethylene bags and transferred to a deep-freeze, where they remained until analysis. Most of the samples showed mould growth; none was infested by insects.

Analysis

Analyses of cyanide and moisture contents of the fresh reference segments were carried out 2 weeks after sampling, in the National Laboratory of Water and Food Hygiene in Mozambique, as follows: The samples were homogenized in cold water with a blender, after which a sub-sample of the suspension was incubated for at least 16 hours at 37°C for autolysis. The cyanide was then expelled by steam distillation, until at least 200 ml condensate had been collected in 25 ml of 0·1 M KOH (De Bruijn 1971). Colouration of the cyanide was achieved by forming a complex with pyridine, barbituric acid and hypochlorite (Lundquist *et al.* 1979) and was measured at 580 nm with a Varian Spectrophotometer.

Moisture was measured by incubation of a 5–10 g sub-sample at 110 ± 2°C, until constant weight was reached.

Analysis of the experimental segments was carried out after 1 year's storage at −18°C in the laboratories of the Agricultural University, Wageningen, The Netherlands. After having reached room temperature, each segment was unpacked and milled in a mini-hammer mill (Culatti, DFH 48) with a 1 mm mesh sieve, overheating being avoided. The homogenized flour samples were analysed as follows:

1. *Cyanide* (expressed as mg of CN per 100 g dry weight):
   a. *Total cyanide* was determined by enzymatic assay and the pyridine/pyrazolone colourimetric technique as described by Cooke (1978).
   b. *Free cyanide*, in comparison with total cyanide, was determined as directly measurable CN, after quenching with alkali and without previous enzymatic action (Cooke 1979).

   Extraction was carried out by 8 minutes of gentle shaking of an Erlenmeyer flask containing 1·00 g flour in 20·0 ml 0·1 M orthophosphoric acid. Determinations were carried out in stoppered test-tubes.

   % Free cyanide was calculated as: (free cyanide/total cyanide) × 100%
2. *Brightness (Y)* was determined by light reflection, using a Hunterlab model D25 optical head and served for the ranking of the flours.

   The origin of the dark colour was examined microscopically.

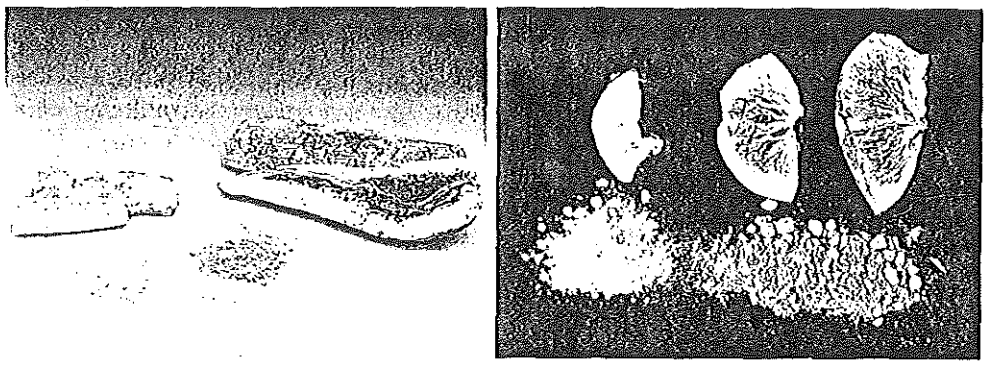

Figure 1a and 1b. Clean white and dark moulded cassava pieces, their cross-sections and respective flours

3. *pH* was established according to Williams (1984) with 2·00 g of flour suspended in 20·0 ml freshly boiled water.
4. *Moisture* content was determined by oven-drying 2·0 g samples at 110±2°C until constant weight was reached.
5. *Fungal counts* were determined using oxytetracycline glucose yeast extract agar (Oxoid CM 545), incubated at 22°C for 5 days.
6. *Identification of fungi* was based upon their cultural and morphological characteristics.
7. *Aflatoxin:* A semi-quantitative analysis was carried out as follows: A 2 g sample was extracted with 10 ml chloroform for 2 hours. The chloroform extract (1–10 μl) was spotted on HPTLC plates (Art. 5631 Merck, Darmstadt) together with quantitative references of aflatoxins. After developing with diethyl ether, the plates were developed with chloroform: acetone (90:10, v/v) in the same direction. Prior to use, the plates were activated by drying for 2 hours in an oven at 103–105°C, and allowed to cool for at least 1 hour in a dessicator. Confirmation of the identity of aflatoxin was carried out according to EEC norm nr. 76-372-EEC. L102 (1976).

All chemical analyses were executed in duplicate. All samples were handled in one analytical run, to allow for comparison between the results.

Results

Most of the dried cassava pieces, as shown in Figures 1a and 1b, had dots or larger areas with dark grey and slightly greenish moulds on the surface, as well as dark grey streaks within the pieces. The colour of watery suspensions of the ground pieces, ranged from white to dark grey.

Subjective (visual) ranking of the flours corresponded very well to the determinations by brightness measurements. Inspection by microscope revealed mycelium fragments in the flour samples. These were significantly more abundant in the darker flours.

Table 1 summarizes the analytical results (means and standard deviations). Table 2 summarizes the relationships observed between the parameters of the flour samples, based upon Spearman's rank correlation test (Snedecor & Cochran, 1980).

Table 1. Mean values and their standard deviations (SD) of the different parameters in the cassava samples

Sample	Parameter	Mean	SD
Fresh Root (n=9)	Moisture (%)	65·0	2·4
	Total CN (mg/100 g dry wt.)	39·9	9·1
Flour (n=14)	Moisture (%)	14·2	0·6
	Total CN (mg/100 g dry wt.)	1·2	1·4
	Free CN (mg/100 g dry wt.)	0·7	0·8
	Free CN as % of total CN	50·5	22·5
	pH	8·5	0·8
	Brightness (Y)	57·2	12·1
	Fungi counts (cfu/g)	400	530

Table 2. Indication of correlations between the different parameters

	Free CN	Free CN%	pH	Brightness	Fungal count
Total CN	+++	+	−−−	+++	0
Free CN		+++	−−−	+++	0
Free CN%			0	0	⊕
pH				−−−	+
Brightness					⊖

Spearman rank correlation:
+++, ++, + = Positive correlation
−−−, −−, − = Negative correlation (statistically significant at $p<0{\cdot}01$, $p<0{\cdot}05$ and $p<0{\cdot}1$ (two sided) respectively)
⊕ and ⊖: positive or negative tendency ($p<0{\cdot}1$, one sided)
0: no correlation

With the same test, no relationship could be detected between (initial) cyanide contents of reference samples, and (final) cyanide contents in flour samples of the same root. Since a ranking technique was used, the use of different analytical methods is not likely to influence the probability for correlation, as the same analytical method has been applied within each array of reference or experimental samples. Likewise, no relationship between cyanide contents of flour samples from the different segments of the same root were observed.

The moisture content of the flours showed marginal differences at the time of analysis and no correlation with other parameters was observed.

The fluorescent spots that developed on the HPTLC plates were initially mistaken for aflatoxin. The size of the spots varied among the flour samples. A positive correlation with the flour brightness was observed: the brighter the flour, the higher the level of the

fluorescent compound. However, confirmation of the identity of aflatoxin with the EEC test was negative.

Various genera of fungi could be identified, including *Rhizopus, Mucor, Penicillium* and *Fusarium* spp. No relationship was observed between the occurrence of any of these fungi and other parameters.

Discussion and conclusions

Table 1 shows that the darker the flour, the less cyanide it contained. Moreover, a lower level of total cyanide was associated with an even lower level of free cyanide.

In the bioconversion of the experimental samples, the mould growth is the principal factor and independent variable. One of the results of mould growth is the darkening of the flour. This may be caused by fungal spores or by disruption of the integrity of the cells, causing the formation of blue-black streaks (CIAT 1973), or both. Although not all fungi produce dark coloured spores, the fact that the darker flours contained more mycelial fragments than the whiter flours supports this relationship. So does the weak negative correlation between brightness and the count of fungal propagules.

The poor relationship, in general, between fungal counts and the extent of mould growth (Nout 1984), may interfere with a statistical analysis of relationships between fungal counts and other quality parameters. In the tested cassava, a solid-state fermentation of a predominantly fungal nature has taken place. Various fungal enzymes, e.g. proteases, deaminases and decarboxylases, may have contributed to a pH increase. In addition, fungal activity may also have contributed to a more effective decomposition of cyanogenic glycosides, releasing 'free cyanide', either by the excretion of glucosidases, or by disintegration of the cell wall, thereby facilitating the contact between linamarase from the cell wall and the linamarin, or both. As free cyanide is assumed to escape easily through evaporation in the form of HCN, in contrast to the glycosidic form, this is regarded as the main pathway for the loss of total cyanide in cassava during the process of drying and storage.

The 'free cyanide' is thought to exist in three forms: cyanohydrins, HCN, and other cyanide compounds which are measurable without enzymatic or strong acid action. At pH > 5–6 the equilibrium between cyanohydrin and HCN will shift to HCN (Cooke 1978) which, with a boiling point of 26°C, is likely to evaporate rapidly. Therefore, low levels of free cyanide were expected in all experimental samples, since pH levels were from 7·0 upwards. In this respect the range of free cyanide (29–89% of total cyanide) is considered to be high. Apparently, the so-called 'free cyanide' in this dry cassava matrix is not as free as the name suggests. A consequence of this finding, for public health, might be that a larger part of the cyanide in dried cassava than has been assumed up until now remains in a form that is readily available to the body.

No aflatoxins nor aflatoxin-producing fungi were detected. Care should be taken with aflatoxin determination, as some compounds in cassava can apparently be mistaken for aflatoxin in a certain analytical setting. Coker and Tomlins (1986) mention scopoletin as an interfering agent in this respect and make suggestions for a confirmation test. As high

levels of aflatoxin contamination in cassava flour from Mozambique have been reported (Mota & Lourenço 1974), the possible presence of aflatoxin, as well as its determination merit alertness. Although aflatoxins were not detected in the present study, some of the moulds identified are capable of elaborating mycotoxins under some conditions. Consequently, traditional products should be screened for mycotoxins in future investigations.

Although several hypotheses on the processes in the contaminated drying of cassava that led to our findings remain to be tested, this experiment indicates that dark, moulded cassava pieces had much lower cyanide levels than white pieces, whilst aflatoxin was absent from all samples. The different cyanide levels could not be accounted for by the initial concentrations. A causal relationship between the presence of the moulds and diminishing cyanide levels is likely and merits further research.

In conclusion, we postulate that although dark cassava flour may not always be safer than white, the latter is not necessarily safer than dark flour. Consequently, a judgement of toxicity, based on only colour or mould growth, is certainly not appropriate.

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