

## The safety of dark, moulded Cassava Flour compared with white- a comparison of traditionally dried Cassava pieces in North-East Mozambique

A. J. Alexander Essers & M. J. Robert Nout

### Abstract

Fresh cassava roots (9) were split into segments to obtain pieces with similar levels of cyanogenic glucosides. 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 analyzed individually for moisture, cyanogenic potential and cyanohydrins plus HCN, pH, brightness, aflatoxins and the number and genus of fungal propagules.

Mean (+SD) initial cyanogenic potential was 399 (+273) mg CN equivalent per kg on dry weight basis. By the traditional processing and storage a considerable (range 92.3 - 99.5%) loss in cyanogenic potential was achieved. Levels of cyanohydrins plus HCN together ranged from 19 to 89% of the total cyanide. There was no correlation between the initial and residual cyanogenic potential. However, darker flours had significantly lower levels of cyanogenic potential, as well as cyanohydrins plus HCN. Similarly, darker flours showed a higher pH. No aflatoxins could be detected. It is concluded that safety of cassava flour cannot be judged by colour or extent of fungal growth.

### Introduction

Cassava (*Manihot esculenta* Crantz) is a shrub whose starchy storage roots 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 from cassava played an etiological 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 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 to 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 encourage mould growth prior to dehydration as follow: The fresh, peeled cassava pieces are kept covered with leaves for three days prior to exposing them directly to the sun. This diminishes the toxic effect of 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. Cyanogens 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 roots 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 cyanogens (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 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.

### Analyses

Analyses of cyanogen 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 16-20 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 Bruiji 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 segment 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 extracted by 8 minutes of gentle shaking of an Erlenmeyer flask containing 1.00 g flour in 20.0 ml 0.1 M orthophosphoric acid.

Total cyanogens, as well as cyanohydrins plus HCN, all expressed as mg of CN equivalent per 100 g on dry weight basis, were determined by the enzymic assay and

**Table 1.** Means (±SD) of the different parameters in fresh or sun-dried and stored cassava root pieces

| Parameters                                       | Fresh root (n=9) | Flour (n=14) |
|--|------------------|--------------|
| Moisture (%)                                     | 65.0 ± 2.4       | 14.2 ± 0.6   |
| Total cyanogens (mg CN eq./kg dry weight)        | 399 ± 91         | 12 ± 14      |
| Cyanohydrins plus HCN (mg CN eq./kg dry weight)  |                  | 7 ± 8        |
| Cyanohydrins, plus HCN as a % of total cyanogens |                  | 50.5 ± 22.5  |
| pH   |                  | 8.5 ± 0.8    |
| Fungal counts (cfu/g)                            |                  | 400 ± 530    |

**Table 2.** Indication of correlations between the different parameters of cassava flours

|                              | ngCN | ngCN% | pH  | Brightness | Fungal count |
|------------------------------|------|-------|-----|------------|--------------|
| Total cyanogens              | +++  | +     | --- | +++        | 0            |
| Cyanohydrins + HCN (ngCN)    |      | +++   | --- | +++        | 0            |
| NgCN as % of total cyanogens |      |       | 0   | 0          | ⊕            |
| pH                           |      |       |     | ---        | +            |
| Brightness                   |      |       |     |            | 0            |

Spearman rank correlation: +++, ++, +: Positive Correction; ---, --, -: Negative correction, statistically significant at, respectively,  $P < 0.01$ ,  $P < 0.05$  and  $P < 0.1$  (two-tailed); ⊕ and ⊖: positive or negative tendency ( $P < 0.1$ , one-tailed); 0: no correlation.

the pyridine/pyrazolone colourimetric technique as described by Cooke (1979). Determination were carried out in stoppered test-tubes. Brightness (Y) was determined by light reflection, using a Hunterlab model D25 optical head and served for the ranking of the flour suspended in 20.0 ml freshly boiled water. Moisture content was determined by oven-drying of 2.0 g sample at 110 ± 2°C until constant weight was reached. The origin of the dark colour was examined microscopically. Fungal counts were determined using oxytetracycline glucose yeast extract agar (Oxiod CM 545), incubated at 22°C for 5 days. Identification of fungi analysed semi-quantitatively as follows: A 2 g sample was extracted with 10 µl chloroform for 2 h. 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 h 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 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** summarises the analytical results (means + SD). **Table 2** summarises the relationships observed between the parameters of the flour samples, based upon Spearman's rank correlation test (Snedecor & Cochran, 1980). With the same test, no relationship could be detected between (initial) cyanogenic potential of reference samples, and residual cyanogen contents in flour samples of the same root. Since a ranking technique was used, the use of different analytical method has been applied within each array of reference or experimental samples. Likewise, no relationship between cyanogen 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 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

Table 1 shows that the darker the flour, the less total cyanogens it contained. Moreover, a lower level of total cyanogens was associated with an even lower level of cyanohydrins plus HCN. 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 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 glucosides, releasing cyanohydrins and HCN, 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 HCN is assumed to escape easily through evaporation, this is regarded as the main pathway for the loss of cyanogenic potential in cassava during the process of drying and storage. The "non-glucosidic cyanogens" are thought to contain cyanohydrins, HCN, and other cyanogenic compounds which are measurable without enzymatic or strong acid action. The range of non-

glucosidic cyanogens (29–89% of total cyanogens) is quite high. A consequence of this finding, for public health, might be that a large part of the cyanogens in dried cassava remains in a form that is readily available to the body than has been assumed up until now.

No aflatoxins or aflatoxin-producing fungi were detected. Care should be taken with aflatoxin determination, as some compound 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 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 cyanogenic potential than white pieces, whilst aflatoxin was absent from all samples. The different residual cyanogenic levels could not be accounted for by the initial ones. A casual relationship between the presence of the moulds and diminishing cyanogen 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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