ELSEVIER

Food Chemistry 53 (1995) 29–33

0308-8146/95/$9.50

Critical stages in cyanogen removal during cassava processing in southern Tanzania

N. L. V. Mlingi,[a] Z. A. Bainbridge,*[b] N. H. Poulter[b] & H. Rosling[c]

[a]*Tanzania Food and Nutrition Centre, PO Box 977, Dar es Salaam, Tanzania*
[b]*Natural Resources Institute, Central Avenue, Chatham Maritime, Kent ME4 4TB, UK*
[c]*International Child Health Unit, University Hospital, S-751 85 Uppsala, Sweden*

(Received 29 September 1993; revised version received and accepted 14 July 1994)

Cassava processing was studied in villages in southern Tanzania. The established method of 17 days sun-drying of longitudinally split roots only reduced cyanogenic glucosides to 27–37%, leaving more than 100 mg HCN equiv per kg dry wt of flour, that is 10 times the safe level set by FAO/WHO. The cyanohydrins yielded remained high during sun-drying but fell to safe levels in dry products. Short-cut processing by alternating pounding and drying to make flour in a day removed glucosides more effectively but cyanohydrin levels ranged between 23 and 124 mg HCN equiv per kg dry wt, in such flour. Ingested cyanohydrins will yield cyanide in the alkalinic environment of the gut and may be much more toxic than equimolar amounts of glucosides, because high levels of glucoside are found to be excreted intact in humans ingesting flour with high glucoside content.

INTRODUCTION

Bitter cassava varieties have become a staple crop in many drought-prone areas of East Africa; probably because they yield better than other staples under adverse environmental conditions (Essers *et al.*, 1992). The designation of bitter and sweet varieties depends on taste that is associated with the levels of cyanogenic glucosides, mainly linamarin (Sunderesan *et al.*, 1987). The bitter or sweet distinction is relative since roots can become bitter with an increase in glucoside levels because of environmental factors such as drought, pests and diseases. To avoid dietary cyanide exposure, the glucosides and their breakdown products, jointly known as cyanogens, must be removed by processing before consumption.

Effective cassava processing methods disintegrate the root tissue completely, thereby releasing an endogenous enzyme, linamarase, that hydrolyses the glucosides to corresponding cyanohydrins. These will decompose above pH 6 to volatile hydrogen cyanide (HCN) that is rapidly lost from the system (Cooke & Maduagwu, 1978). Processing can reduce all cyanogens in cassava products to below the safe level of 10 mg HCN equiv per kg dry weight set by FAO in 1988 (FAO/WHO, 1988). Consumption of cassava products with high cyanogen levels may cause acute intoxications (Mlingi *et al.*, 1992), aggravate goitre (Bourdox *et al.*, 1982) and, in severe circumstances, induce paralytic diseases (Tylleskär *et al.*, 1992).

Masasi district in southern Tanzania experienced drought and an outbreak of acute intoxications in 1988. The intoxications were attributed to consumption of insufficiently processed cassava. Due to food shortage the established processing by sun-drying peeled cassava roots for 2 weeks to a product known as makopa was replaced by a short-cut method yielding a product known as chinyanya. This method provided flour the same day by alternate pounding and sun-drying of peeled roots. Remaining levels of cyanohydrins in the flour obtained were held responsible for the cyanide exposure found in the populations affected by the acute intoxications (Mlingi *et al.*, 1992).

To identify the critical stages in removing cyanogens during normal and short-cut methods of cassava processing in the affected area, a field study was carried out in three villages of Masasi district in September 1991.

MATERIALS AND METHODS

The study area

Masasi district is located in Mtwara region in southern Tanzania. In 1988 the population was 335 000 corresponding to 38 inhabitants per km^2. Mean annual precipitation was 940 mm and temperatures varied

*To whom correspondence should be addressed.

from a minimum of 18°C in July to a maximum of 35°C in December. Maize is the primary staple while cassava is secondary during favourable years but in drought years cassava becomes a primary staple.

District authorities were asked to select villages in different parts of the district that were known to have high cultivation of bitter cassava varieties and that had reported cases of acute intoxications from consumption of insufficiently processed cassava in the previous years. The three selected villages that met these criteria were situated 40–70 km east, south and west of the district headquarters. In each village representative households were selected for the study with the assistance of village leaders. General information on cassava processing was obtained through informal interviews and observations in all villages studied.

Makopa processing

To ascertain cyanogen levels at different stages of sun-drying makopa, two batches of cassava roots from mixed bitter varieties were harvested according to local practice. Longitudinal quarter sections of randomly selected fresh roots were cut in pieces of approximately 1 cm^3 dimensions, a sample for chemical analysis taken and the cyanogens extracted immediately in the field (O'Brien *et al.*, 1991). The remaining peeled roots were sun-dried in the villages. After 8 and 17 days, samples of roots were pounded and sieved into flour from which a representative sample was taken and stored frozen before analysis.

Makopa products were also sampled from 31 households belonging to one 10-cell unit (the smallest administrative unit of about 10 households) in each of the villages. Each household was visited and requested to provide a sample of makopa pieces which were ready for consumption. The woman in each household pounded and sieved the makopa into flour from which a sample was drawn. Each woman was asked how many days or weeks the makopa pieces had been sun-dried and stored.

Chinyanya processing

To assess cyanogen removal during chinyanya processing, three batches of roots were harvested from bitter cassava varieties. Samples of fresh roots were taken as described earlier. The remaining peeled roots from each batch were pounded into small pieces and sun-dried for 2–3 h before repounding. One sample was taken from the first pounding after sieving. The unsievable pieces were again sun-dried for 2-3 h and then repounded and sieved into flour, from which another sample was drawn.

The variation of cyanogen levels in chinyanya flour was studied by asking two women in each village on two consecutive days to process a total of 11 batches of chinyanya according to their individual practices. They were required to complete the processing the same day. Fresh roots were sampled as described above and samples of chinyanya flour were collected in the evening and stored frozen before analysis.

Chemical analysis

Determination of cyanogen levels in extracts of cassava samples collected from the households and in other experimental treatments was made according to the method described by Cooke (1978), as modified by O'Brien *et al.* (1991). As earlier reported, cyanide exposure was estimated in nine men, 13 women and 10 children from 12 households in Masasi district by determination of urinary thiocyanate and cyanogen levels in makopa flour eaten the same day (Mlingi *et al.*, 1992). The frozen 32 urines were then assayed for linamarin with a new enzymatic diffusion method with solid state detection on picrate impregnated sheets as described by Brimer and Rosling (1993).

RESULTS

Makopa processing

Interviews consistently revealed that the population had a clear belief that makopa should be well dried before pounding into flour for consumption and that it should be possible to hear a brittle sound on breaking makopa when sufficiently sun-dried. Depending on size and type of roots as well as weather, makopa would be ready for pounding after about 2 weeks of drying.

The multiple stage sampling during sun-drying of two batches of makopa showed that glucoside levels had dropped to 53 and 24% of original values after 8 days and 37 and 27%, respectively, after 17 days. The cyanohydrin levels remaining increased after 8 days but, after 17 days when a low moisture level was reached, cyanohydrin levels dropped to below 10 mg CN equiv per kg dry wt in both batches (Table 1).

Makopa flour samples collected from 31 households had a wide variation in cyanogen levels (Table 1). The mean glucoside and HCN levels in these samples were in the same range as those obtained for day 17 in the two-batch multiple stage study. The duration of drying period for makopa ranged between 1 and 9 weeks. Out of the 12 makopa samples dried for 1–2 weeks five had moisture levels above 13% and, of the 18 samples dried for longer,

Table 1. Cyanogen levels (mg HCN equiv per kg dry wt) at different stages of sun-drying makopa and in makopa flours

	Glucoside	Cyanohydrin	HCN	Moisture (%)
Batch 1				
Fresh root	1039	35	16	60·3
After 8 days	548	25	18	14·4
After 17 days	383	8	10	11·1
Batch 2				
Fresh roots	462	26	5	71·4
After 8 days	109	31	5	11·2
After 17 days	123	5	4	11·0
Households[a] (mean ± SD, n = 31)	145 ± 26 (67–757)	17 ± 3 (0–61)	6 ± 0·4 (3–11)	12·1 ± 0·3 (9·4–16·1)

[a]Ranges are given in parentheses.

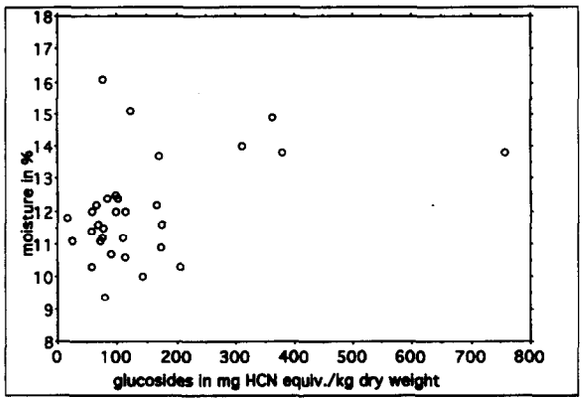

Fig. 1. Cyanogenic glucoside levels versus moisture in 31 makopa samples from households.

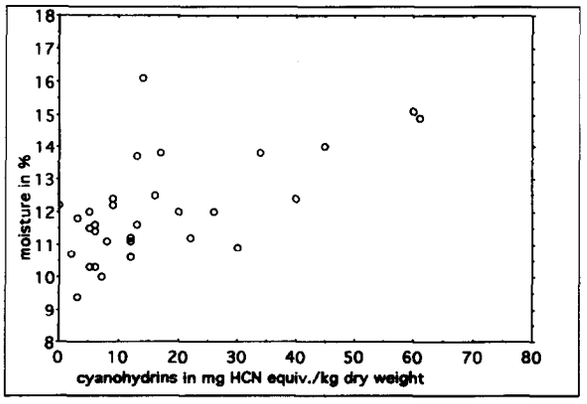

Fig. 2. Cyanohydrin levels versus moisture in 31 makopa samples from households.

only one had a moisture above 13%. Figure 1 shows that only makopa samples with moisture above 13% had glucoside levels above 200 mg CN equiv per kg and that glucoside levels did not continue to fall below this limit. Figure 2 shows that cyanohydrin levels also tend to be higher when moisture was still high but, in contrast to glucoside levels, the levels of this cyanogen continued to fall to negligible levels with falling moisture content.

Chinyanya processing

The short-cut processing method of making chinyanya was known to all women but it was only used in food shortage situations without any clearly established procedures. The multiple stage sampling of three batches of chinyanya processing indicated a very rapid decrease of glucoside levels. The remaining glucosides in the flour obtained were 7–14% of initial levels in the fresh roots. The cyanohydrin levels found were mostly higher than glucoside levels and they correlated with the moisture level (Table 2).

The mean ± SD in the fresh roots used for chinyanya processed by individual women was 503 ± 231 mg HCN equiv per kg and the large variation in cyanogen level found in the flour obtained from the 11 batches of chinyanya is shown in Table 2. Glucoside levels did not correlate with moisture but cyanohydrins were above 25 mg HCN equiv per kg in four of five flours with moisture above 13% and below that level in all the six flours with lower moisture.

Table 2. Cyanogen levels (mg HCN equiv per kg dry wt) at different stages of chinyanya processing and in chinyanya samples

	Glucoside	Cyanohydrin	HCN	Moisture (%)
Batch 1				
Fresh roots	1518	28	7	60·3
First flour	90	124	12	23·7
Second flour	216	56	4	19·4
Batch 2				
Fresh roots	463	26	5	71·4
First flour	34	35	4	19·5
Second flour	41	23	3	9·7
Batch 3				
Fresh roots	484	22	7	62·0
First flour	6	39	3	21·4
Second flour	35	37	3	12·2
Households[a] (mean ± SD, n = 31)	95 ± 60 (22–232)	23 ± 14 (5–49)	4 ± 1 (1–5)	13·4 ± 2·8 (10·4–18·4)

[a]Ranges are given in parentheses.

Urinary excretion of ingested glucosides

Mean ± SEM urinary linamarin was 263 ± 52 μmol/litre in the frozen urines collected from 32 subjects in a village in Masasi district in 1989 when they consumed makopa flour as the dominant staple food. Mean ± SEM of the main cyanide metabolite thiocyanate was 68 ± 9 μmol/litre in these urines which is slightly higher than the 42 ± 5 μmol/litre observed in non-smoking healthy Swedish adults (Lundquist *et al.*, 1979).

DISCUSSION

Makopa processing

Prolonged sun-drying of bitter cassava roots into makopa considerably reduces the amount of cyanogenic glucosides. However, independently of the rate and completeness of drying, it seems that about 10% of initial glucosides remain in the flour as indicated by both the experiment and the makopas collected from households. Our findings of about 100 mg HCN equiv per kg dry wt of glucosides correspond to an earlier observed level of 128 mg HCN equiv per kg dry wt in makopa flour collected during a previous study in the same district in 1989 (Mlingi *et al.*, 1992). A similar level of 90 mg HCN equiv per kg dry wt was found in flour from sun-dried bitter cassava roots in Northern Mozambique (Ministry of Health, Mozambique, 1984).

The relationship between moisture and glucoside levels in the flours obtained from multiple stage sampling and household survey of makopa indicates that enzymatic hydrolysis of glucosides halts when moisture

drops to below 12 or 13%. A possible explanation is that the remaining glucosides were never exposed to the enzyme until moisture became too low for enzymatic hydrolysis. If roots are cut into small finger size pieces, they dry faster than whole or half-split roots but higher glucoside levels will remain (Mlingi *et al.*, 1992). The implication is that sun-drying of fresh roots with a cyanogenic potential of greater than 100 mg HCN equiv per kg dry wt will not reduce glucoside levels to below 10 mg HCN equiv per kg dry wt.

Cyanohydrin levels remained high during sun-drying of makopa but were reduced to negligible levels when moisture content dropped below 12 or 13%. Moderately high levels of cyanohydrin were observed in makopa samples from some households but this could be explained by a weakness in the study design. Households which did not have makopa 'ready for consumption' gave the best available makopas, which were sometimes still insufficiently dried.

The low cyanohydrin and HCN levels found in sufficiently dried flours are in accordance with findings in other cassava products (Banea *et al.*, 1992). The practical importance of these findings is that completeness of disintegration before drying determines glucoside removal whereas cyanohydrin removal only depends on completeness of subsequent drying. Thus in flour from sufficiently sun-dried makopa and chinyanya, low cyanohydrins will be found even if glucosides remain much higher. HCN is always rapidly lost and will not constitute a dietary cyanogen.

A previous study in Masasi district indicated a very low cyanide exposure as estimated by urinary thiocyanate levels in the normal range in people who consumed makopa flour with high glucoside levels (Mlingi *et al.*, 1992). A new analytical method for determination of urinary linamarin could now be used which showed four times higher linamarin than thiocyanate in the urines of these people. This shows that ingested cyanogenic glucosides, in great part, may be absorbed and excreted intact in the urine, thus explaining why flour with 10 times 'safe cyanogen levels' can be consumed without clinical or chemical effects. That these glucosides can pass the body unchanged has been shown in animal experiments (Barret *et al.*, 1977) and the same tendency was also recently reported from Mozambican cassava-eating subjects (Brimer & Rosling, 1993). An improved understanding of the fate in humans of ingested cyanogenic glucosides is needed for definition of safe levels in food products (Speijers, 1993).

Chinyanya processing

Chinyanya processing rapidly reduced glucoside content to low levels. A reduction of about 80% was attained in batches processed by individual women. This reduction was mainly a result of the initial pounding stage. The high degree of cellular disintegration achieved in this procedure released the linamarase enzyme that immediately hydrolysed the glucosides to release the corresponding cyanohydrins. Variation in cyanohydrin levels observed in these flours is probably mainly explained by variations in the technique of sun-drying which was done on rocks, mats, the ground or on raised platforms. Sufficient time and utensils for sun-drying and storage could reduce cyanohydrins to a low level and thereby render this type of flour safe for consumption. Chinyanya processing can in fact be regarded as a rudimentary form of gari processing, a common cassava processing method in West Africa that is unknown in East Africa. Gari processing involves grating of peeled cassava roots, dewatering, fermenting for a required number of days, pressing and finally roasting.

Chinyanya processing in its present form should be discouraged or improved by the following modifications: grating peeled roots to obtain uniform tissue disintegration for release of the hydrolytic enzyme linamarase, followed by dewatering, fermentation, pressing and lastly heat-drying or, as an option, extensive sun-drying to reduce moisture level for cyanohydrin removal and improved storability.

CONCLUSION

The prolonged sun-drying of cassava roots used in southern Tanzania will not reduce cyanogenic glucosides in bitter cassava roots to the safe limit set by FAO (FAO/WHO, 1988).

The short-cut method of alternate pounding and drying of cassava roots resulted in a sharp decline in glucoside levels but high cyanohydrin levels may remain if the products are not sufficiently dried.

Ingestion of cyanohydrins is probably more important for cyanide exposure than ingestion of intact glucosides since the latter substance may be largely excreted unchanged in the urine.

The short-cut processing of cassava in southern Tanzania could be improved by sufficient sun-drying to obtain flour with low moisture, and hence low cyanohydrin content.

ACKNOWLEDGEMENT

The study was supported by the International Programme in Chemical Sciences, Uppsala University Sweden, the Natural Resources Institute, Chatham, Kent UK and UNICEF Dar es Salaam, Tanzania. The authors thank all villagers and leaders in Masasi who participated in the study; staff of the Natural Resources Institute, Tanzania Bureau of Standards and Tanzania Food and Nutrition Centre for participating in the study; and Lena Carlsson for urine analysis.

REFERENCES

Banea, M., Poulter, N. & Rosling, H. (1992). Short cuts in cassava processing and risk of dietary cyanide exposure in Zaire. *Food Nutr. Bull.*, **14**, 137–43.

Barret, M. D., Hill, D. C., Alexander, J. C. & Zitnak, A. (1977). Fate of orally dosed linamarin in the rat. *Can. J. Physiol. Pharmacol.*, **55**, 134–6.

Bourdox, P., Seghers, P., Mafuta, M., Vanderpas, J., Vanderpas-Rivera, M., Delange, F. & Ermans, A. M. (1982). Cassava products: HCN content and detoxification processes. In *Nutritional Factors Involved in the Goitrogenic Action of Cassava,* IDRC Monograph 184e) eds F. Delange, F. B. Iteke & A. M. Ermans. IDRC, Ottawa, Canada, pp. 51–8.

Brimer, L. & Rosling, H. (1993). A micro diffusion method with solid state detection of cyanogenic glucosides from cassava in human urine. *Food Chem. Toxicol.*, **31**(8), 599–603.

Cooke, R. D. (1978). An enzymatic assay for the total cyanide content of cassava (*Manihot esculenta*, Grantz). *J. Sci. Food Agric.*, **29**, 345–52.

Cooke, R. D. & Maduagwu, E. N. (1978). The effect of simple processing on the cyanide content of cassava chips. *J. Food Technol.*, **13**, 299–306.

Essers, A. J. A., Alsen, P. & Rosling, H. (1992). Insufficient processing of cassava induced acute intoxications and the paralytic disease konzo in rural area in Mozambique. *Ecol. Food Nutr.*, **27**, 17–27.

FAO/WHO Codex Alimentarius Commission (1988). Report of the Eighth session of the Codex Coordinating Committee for Africa. Cairo, Egypt.

Lundquist, P., Mårtensson, J., Sörbo, B. & Öhman, S. (1979). Method for determining thiocyanate in serum and urine. *Clin. Chem.*, **25**, 678–81.

Ministry of Health of Mozambique (1984) Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic content of cassava products. *Bull. WHO*, **62**(3), 485–92.

Mlingi, N. L. V., Poulter, N. & Rosling, H. (1992). An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. *Nutr. Res.*, **12**, 677–87.

O'Brien, G. M., Taylor, A. J. & Poulter, N. (1991). An improved enzymatic assay for cyanogens in fresh and processed cassava. *J. Sci. Food Agric.*, **56**, 277–89.

Speijers, G. (1993) Cyanogenic glucosides. *WHO Food Additives Series*, **30**, 299–337.

Sunderesan, S., Nambisan, B. & Easwari Amna, C. S. (1987). Bitterness in cassava in relation to cyano-glucoside content. *Indian J. Agric. Sci.*, **57**, 34–40.

Tylleskär, T., Banea, M., Bikangi, N., Cooke, R. D., Poulter, N. & Rosling, H. (1992). Cassava cyanogens and konzo, an upper motor neuron disease found in Africa. *Lancet*, **339**, 208–11.