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Cassava Processing and Dietary Cyanide Exposure in Tanzania

BY

NICHOLAS L.V. MLINGI

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ABSTRACT

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The relationship between dietary cyanide (CN) exposure from the cyanogenic glucoside linamarin in cassava and the methods used to process this important root crop were studied in Tanzania.

An outbreak of acute intoxications in southern Tanzania coincided with a drought in 1988. The affected population attributed intoxications to short-cuts made in the sun-drying of cassava roots. Processing experiments showed that these short-cuts yielded flour with high residual levels of cyanohydrin, a linamarin metabolite that is transformed to CN in the gut. A causal role of CN in the intoxications was supported by hundred-fold higher mean urinary levels of the main metabolite thiocyanate (SCN) in affected (1120 µmol/l) compared to non-affected (7 µmol/l) population groups.

An epidemic of the paralytic disease konzo, that has been attributed to CN exposure from cassava, occurred during a drought in northern Tanzania in 1985. Qualitative and quantitative interviews revealed that the established way of heap-fermenting crushed cassava roots was shortened during the drought due to food shortage and intensive trade. Experiments showed that this chain of events resulted in high levels of cyanohydrin in flour. Low protein intake may have enhanced CN toxicity due to low supply of sulphur for CN to SCN conversion.

Studies of 217 women from an iodine deficient area in western Tanzania showed that the total goitre rate of 73 % could be explained by iodine deficiency as verified by a median urinary iodine of 3.6 µg/dl. Although 98% ate cassava roots daily their mean urinary SCN was only 128 µmol/l. Use of mechanical milling was associated with low SCN, probably because milling ensures complete drying of roots, which removes cyanohydrin.

A mean urinary SCN of 36 µmol/l indicated low CN exposure in 193 schoolchildren studied in Dar es Salaam. They consumed cassava roots without prior processing that effectively removes cyanogens. The low CN exposure can be explained by the use of non-bitter varieties with low linamarin levels and low consumption frequency and that ingested linamarin is partly excreted in the urine without releasing cyanide in the body.

Key words: Cassava, food processing, cyanogens, cyanide, thiocyanate, linamarin, intoxication, konzo, goitre, iodine deficiency disorders

Nicholas L.V. Mlingi, Department of Pediatrics, Unit for International Child Health, Uppsala University Children's Hospital, S-751 85 Uppsala, Sweden and Tanzania Food and Nutrition Centre (TFNC), P.O. Box 977, Dar-es-Salaam, Tanzania

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Literarum radices amaras, fructus dulces

The roots of knowledge are bitter, but its fruits are sweet

Marcus Tullius Cicero
(106-43 B.C.)

To my better-half Anna,
our children Bob, Lilly and Hans

PAPERS INCLUDED IN THE THESIS

This thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Mlingi N, Poulter N, Rosling H. An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. *Nutrition Research* 1992;12:677-687.

II. Mlingi N, Kimatta S, Rosling H. Konzo, a paralytic disease observed in southern Tanzania. *Tropical Doctor* 1991;21:24-25.

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IV. Mlingi N, Assey V D, Swai A B M, McLarty D G, Karlén H, Rosling H. Determinants of cyanide exposure from cassava in a konzo-affected population in northern Tanzania. *International Journal of Food Sciences and Nutrition* 1993;44:137-144.

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VI. Mlingi N, Abrahamsson M, Yuen J, Gebre-Medhin M, and Rosling H. Low cyanide exposure from cassava consumption in Dar-es-Salaam, Tanzania. Submitted for publication.

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Cover:
Cassava roots as illustrated in "Tropical Agriculturalist: Cassava" by Pierre Silvestre. Courtesy of Macmillan Publishers Ltd, London and Basingstoke, U.K.

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ABBREVIATIONS

CIAT	Centro Internacional de Agricultura Tropical
CN	Cyanide
CSPD	Child Survival, Protection and Development
DMO	District Medical Officer
FAO	Food and Agriculture Organization
HCN	Hydrogen Cyanide
ICCIDD	International Council for the Control of IDD
ICH	Unit for International Child Health
IDD	Iodine Deficiency Disorders
IITA	International Institute of Tropical Agriculture
IGT	Impaired Glucose Tolerance
IPICS	International Program In the Chemical Sciences
ISP	International Science Programs
KCMC	Kilimanjaro Christian Medical Centre
KCN	Potassium Cyanide
MCH	Maternal and Child Health
MMC	Muhimbili Medical Centre
NaCN	Sodium Cyanide
NRI	Natural Resources Institute
OSCN	Hypothiocyanate
PEM	Protein Energy Malnutrition
RAP	Rapid Assessment Procedures
RRA	Rapid Rural Appraisal
RMO	Regional Medical Officer
SCN	Thiocyanate
SEM	Standard Error of the Mean
TAN	Tropical Ataxic Neuropathy
TBS	Tanzania Bureau of Standards
TFNC	Tanzania Food and Nutrition Centre
TGR	Total Goitre Rate
VGR	Visible Goitre Rate
UNICEF	United Nations Children's Fund
WHO	World Health Organization

PREFACE

I grew up on the slopes of Mount Kilimanjaro where the culture of consuming cassava was confined to a few places in the lower parts of the mountain. On the slopes, banana was grown as the main staple. Children were cautioned by parents to avoid eating cassava, especially the raw roots. This warning was due to poisonings and deaths which had been experienced in this area years earlier. Parents emphasized that the best food was banana and if somebody wanted to eat stiff porridge, in Swahili known as *ugali*, it should be made from maize flour.

My brother used to cultivate a field in the lower parts of the mountain slopes where he learned how to grow cassava from other farmers. When he harvested cassava, it was brought home, peeled and spread on the roof top of the house to sun-dry. When sufficiently sun-dried, my brother mixed the dried cassava pieces with maize before milling into flour. One day I was invited to eat *ugali* made from this mixture at my brother's house. I immediately liked cassava *ugali*, because I found it more palatable than maize *ugali*. That is how my interest in cassava started.

When I got employed as a food chemist at the Tanzania Food and Nutrition Centre (TFNC) in 1978, I used to analyse cassava flour samples for moisture content in the laboratory. The nice smell of cassava flour during drying in the oven impressed me and contributed to my interest in cassava. In 1982, I got a chance of carrying out a nutritional evaluation of fermented cassava flour, as part of my Master's degree at the Department of Nutrition, Uppsala University, Sweden.

After returning to Tanzania, I learnt in 1985 about an epidemic of paralysis in Tarime district, Mara region which was attributed to consumption of insufficiently processed cassava during a food shortage period (Howlett et al. 1990). My involvement in the investigations of this epidemic prompted me to continue to investigate the relationship between processing of cassava roots and dietary cyanide exposure. With these studies my ambition is to introduce and promote effective processing methods in Tanzania to secure safe cassava consumption in the country.

MUHTASARI

Utafiti ulifanyika wa kuchunguza kama sumu itokanayo na mihogo ambayo haikusindikwa vizuri ilileta madhara ya kiafya nchini Tanzania. Utafiti huo ulifanyika katika sehemu nne nchini zilimwazo mihogo kwa wingi.

Kulipuka kwa ugonjwa wa konzo wilayani Tarime, Mkoa wa Mara mwaka 1984/85 kulitokana na sumu ya mihogo. Upungufu wa chakula wakati wa ukame uliwafanya watu wafupishe njia ya kusindika mihogo. Pia biashara ya mihogo katika kijiji kilichoathirika sana ilichangia kufupisha njia hiyo. Sumu kwenye unga wa kuchachusha kwa siku moja badala ya nne ilikuwa kwa wastani wa milligramu 57 kwa kila kilogramu moja. Sumu hiyo ilipatikana kwenye mikojo ya walaji kwa wastani wa µmol 490 kwa kila lita. Pia kulipuka kwa konzo kulisababishwa na ulaji duni wa utomwili wenye madini ya sulfa (sulphur) ambayo hutumika kuondoa sumu hiyo mwilini.

Mlipuko wa kuenea kulewa na kutapika uliowapata watu wilayani Masasi, Mkoa wa Mtwara mwaka 1987/88 ulitokana na sumu ya mihogo wakati wa upungufu wa chakula ulioletwa na ukame. Kulewa huko kulitokana na kula unga wa mihogo ambayo haikusindikwa vizuri. Mihogo ilisindikwa kwa siku moja kupata unga uitwao chinyanya kwa kutwanga mihogo mibichi na kuanika juani huku wakirudiarudia kutwanga na wakichekecha unga wa ugali. Sumu hiyo ya mihogo ilipatikana kwa wingi sana kwenye damu kwa wastani wa µmol 335 na kwenye mikojo kwa µmol 1120 kwa lita kwa waliokula ugali huo. Sumu iliyokuwemo kwenye unga ulioliwa ni kiasi cha milligramu 48 kwa kila kilogramu ya unga.

Uchunguzi uliofanyika Wilaya ya Kigoma kijijini, Mkoa wa Kigoma ulionyesha kuwa sumu itokanayo na mihogo haihusiani na kuenea kwa uvimbe wa tezi la shingo katika wilaya hiyo. Kuwepo kwa ugonjwa huo huko Kigoma ni kwa ajili ya upungufu wa madini joto ardhini. Lakini usindikaji mzuri wa mihogo kama kusagisha mashine hupunguza tatizo la kuenea kwa sumu hiyo, kwani kina mama 74% kati ya 217 waliokuwa wakisaga mihogo walikuwa na sumu kidogo (chini ya µmol 100 kwa lita) kwenye mikojo yao.

Utafiti jijini Dar-es-Salaam ulionyesha kuwa kuenea kwa sumu ya mhogo kwa walaji wa vitongoji vya jiji ni kidogo sana kwani ulaji wa mihogo ni mdogo kwa kiasi cha 49% na hutumika zaidi mihogo baridi (mitamu). Sumu hiyo ilikuwemo kwenye mikojo ya watoto wa shule kwa wastani wa µmol 36 kwa lita.

INTRODUCTION

Cassava as a food crop

Cassava (*Manihot esculenta* Crantz) is the most important tropical root crop. Its starchy roots are a major source of dietary energy for more than 500 million people (Cock 1985). It is known to be the highest producer of carbohydrates among staple crops. According to FAO (1989) cassava ranks fourth on the list of major food crops in developing countries after rice, wheat and maize. In Sub-Saharan Africa cassava is mainly a subsistence crop grown by small-scale farmers, and it feeds about 200 million people daily, which is nearly half of the continent's population (Madeley 1993).

The cassava plant is a perennial, woody shrub that grows 1 to 3 metres high. It was domesticated many thousand years ago in tropical America. It is referred to as "manioc" in French and American English, "mandioca" in Portuguese and "yuca" in Spanish. Cassava was introduced to West and Central Africa by the Portuguese in the 16th century. It was cultivated around the mouth of the River Zaire as early as 1558 (Jones 1959). The introduction of cassava in East Africa is poorly documented but it is assumed that it was introduced from the Portuguese trading stations in Mombasa, Zanzibar, Mozambique and Sofala. It was grown in Madagascar by the end of 18th century (Carter et al. 1992). Cassava was introduced in Asia and the South Pacific territories during the 17th and 18th century. At present cassava is grown between 30º N and 30º S of the Equator up to an altitude of 1,500 metres.

In Africa cassava alleviates recurring food crises because of its agricultural advantages. The main advantages are higher yields per unit area of land as well as per unit of labour compared to cereals under similar conditions. It is tolerant to drought and produces on poor soils where other staples would fail. It is flexible in planting and harvesting time. Further advantages are the use of its non-edible stem cuttings as planting material and the limited need for weeding. Being a perennial plant its roots can be left in the soil for several seasons to compensate for one poor agricultural year (Hahn et al. 1985, 1987).

Cassava production

World production of fresh cassava roots grew from 70 million tons in 1960 to 154 million tons in 1991 (CIAT 1993). Cassava is produced in 92 tropical and sub-tropical countries. The five major cassava-producing countries in the world are Brazil (25 million tons), Nigeria (20), Thailand (20), Zaire (18) and Indonesia (16). Tanzania is estimated to produce 6.3 million tons. The total area harvested in the world is about 16 million hectares, with 57% in Africa, 25% in Asia and 18% in Latin America. About 15% of the world's production of cassava is exported to Europe and Japan as either chips or pellets, and starch. The starch is used in the food industry, for textiles, in the paper industry and for beer brewing. The remaining 85% of the world's production is used within the producing countries for food (58%), animal feed (28%), and industrial uses (3%), and the wastage is 11% (CIAT 1993).

The area of land planted with cassava is greatest in Africa, but yields are lower than in other continents (Silvestre and Arraudeau 1983). Africa is the only part of the world where per capita food production has been declining in the last two decades although cassava production has nearly doubled during this period (de Bruijn and Fresco 1989). Most of the cassava in Africa is produced by female farmers for human food and is consumed near to where it is grown. There is a growing commercial market for cassava in Africa, and men are gradually being involved in the production of cassava in Nigeria, Ghana and Zaire (FAO 1990).

Human cassava consumption is greatest in Africa, averaging 96 kg fresh weight per capita. The highest consumption is found in Zaire with 391 kg annually per capita or 1,123 calories per day (CIAT 1993). The starchy roots are the most commonly consumed part, but the leaves are also consumed as a preferred green vegetable in many cassava-growing communities, especially in Central Africa (Hahn 1989).

Cassava have long been recognized by many African farmers as an insurance against famine, but its value as a food security crop has been internationally undervalued until recently. In 1986 the late Executive Director of UNICEF, James Grant, described cassava as a poor man's cinderella. He took the initiative of promoting cassava as a household food security crop in Africa (Power 1986). In Central African countries where cassava constitutes over 50% of the staple food (Nweke 1988) and in West Africa the attitude towards cassava is positive. It forms part of the culture as expressed in poetry (Nwapa 1986).

Maize is the dominant staple in East Africa, but cassava is crucially important in Mozambique, Tanzania, Uganda, Rwanda and Burundi (Nweke 1988). Cassava is a secondary staple in almost half of the twenty regions in Tanzania, where it contributes between 9 and 60% of the total energy intake per day (Jonsson 1986). However, during drought years cassava has a long history of providing food security to a large part of the country. The four main cassava-producing zones in Tanzania are the Lake Victoria zone (Mwanza, Shinyanga and Mara regions) and Lake Tanganyika zone (Kigoma and Tabora regions). Others are the southern zone (Mtwara, Lindi and Ruvuma regions) and the eastern coastal zone, which includes Tanga, Dar-es-Salaam, Coast and Morogoro regions on the mainland and the Zanzibar Islands.

Cassava in human nutrition

Cassava roots are rich in carbohydrates, but other nutrients are in low levels as shown in table 1. Fresh cassava roots contain 30 to 40% dry matter and have a starch content of approximately 85% of dry matter. The roots are either directly boiled or processed into flour used for making stiff porridge. The protein levels of cassava roots are lower than those of cereals, legumes and even other root and tuber crops such as yams and potatoes. Its protein is deficient in some of the essential amino acids, especially cystine, methionine and tryptophan (Lancaster et al. 1982, Gomez et al. 1985).

Table 1. Composition of 100 g of fresh cassava roots and leaves.

	Roots[a]	Leaves[b]
Water (g)	62.5	71.7
Carbohydrates (g)	34.7	18.3
Protein (g)	1.2	7.0
Fat (g)	0.3	1.0
Fibre (g)	0.0129[c]	4.0
Calcium (mg)	33.0	303.0
Iron (mg)	0.7	-
Thiamine (mg)	0.06	0.09 - 0.33[d]
Riboflavine (mg)	0.03	0.22 - 0.43[d]
Niacin (mg)	0.6	0.80 - 3.5[d]
Vitamin C (mg)	36.0	231.0 - 742.0
Vitamin A (IU)	trace	336.0 - 17445[d]

Source: [a]Cock 1985, [b]Lancaster and Brooks 1983, [c] Oke 1968, [d]Terra 1964.

Cassava leaves are, however, richer in crude proteins than the roots. The leaves also have a high content of iron and vitamins A and C. It has been shown that protein levels vary from 18 to 40%

of the leaf dry matter (Eggum 1970). Levels of the sulphur-containing amino acids cysteine and methionine are comparable to those of the FAO reference protein (Gomez and Valdivieso 1985). For this reason cassava leaf protein is claimed to be superior to soya bean protein. Combinations of cassava roots and leaves with groundnuts, legumes and fish provide a balanced diet (Okigbo 1980).

Vitamins are low in raw fresh cassava roots, and only vitamin C is found in appreciable quantities (Oyenuga 1968, FAO 1968). Furthermore, all the vitamins are considerably decreased by processing. In some types of cassava flour no vitamin C remains and most of the B vitamins and minerals found in fresh roots are lost during processing. Only calcium and iron have been found in higher quantities in some cassava products, such as starch and lafun (a cassava product in Nigeria), probably because of contamination (Favier et al. 1971, Joseph 1973). Riboflavin may be found in higher quantities in processed products than in fresh cassava roots (Ankrah 1972, Watson 1979). It has been suggested that riboflavin is synthesised during fermentation of cassava roots (Favier et al. 1971).

The problem of low protein content in cassava roots can be solved during industrial processing through fortification or by consumption of protein rich supplementary foods. Solid state heap fermentation carried out at household levels or on a large scale in factories has been found to increase protein content by three to eight times (Amey 1987, Sauti et al. 1987, Mlingi 1984). The nitrogen is assumed to be trapped from the air by various microorganisms during processing.

Young children in communities which use cassava as their main staple sometimes suffer from protein deficiency if cassava is eaten without supplementary foods rich in proteins, such as beans, meat, fish and pulses. It is unfortunate that in several areas where cassava is produced and utilized for human food, the supply of animal protein is inadequate. Hence a high percentage of preschool children, pregnant and lactating mothers suffer from protein energy malnutrition (Maletnlema 1978).

Another nutritional drawback of cassava is its dietary bulk properties, i.e. factors that make it difficult for an individual to consume sufficient amounts to meet his/her energy and nutrient requirements. In young children the volume of a traditional diet of low energy density may be too large to allow the child to ingest all the food necessary to cover energy needs, particularly if the number of feeds offered is low (Ljungqvist et al. 1981, Svanberg 1987, Lorri 1993).

The two essential dietary bulk properties, nutrient density and consistency in starch-rich foods, are interrelated. A porridge can be made more liquid and easier to feed an infant by adding water but this means that the nutrient density decreases and the amount that must be consumed becomes larger. If the volume is diminished by less dilution of the food with water, "the thickness" (viscosity) of the food will increase, thus making it less easy to ingest. It is difficult for preschool children to meet the daily energy requirements by consuming large volumes of cassava porridge. Young children have high nutrient requirements in relation to their body size, and relatively small stomach volumes (Cameron and Hofvander 1983). Protein energy malnutrition (PEM) is prevalent among children on such diets (Mosha and Svanberg 1983, Rutishauser et al. 1974).

The dietary bulk problem can be solved by adding energy-rich supplements such as sugar and oils, by use of germinated cereal flour as an addition in preparation of gruels and by the use of fermentation that improves starch characteristics (Svanberg 1987, Lorri 1993). Modification of starch structure such as during extrusion or roasting processes may also alleviate the dietary bulk problem.

Cassava cyanogenesis

Cassava is one of the 2,500 plant species known to contain cyanogenic glycosides (Conn 1994). A total of 75 different cyanogenic glucosides are known. The well-studied ones are amygdalin found in bitter almonds, dhurrin in sorghum and linamarin as well as lotaustralin that are found in cassava, bamboo and linseed (Nahrstedt 1988). Bamboo shoots and cassava are the plants known to contain the highest levels of cyanogenic glucosides. Observational studies indicate that bitterness or toxicity may play an important role in the prevention of damage to the crop by animals that feed on the roots (Essers et al. 1992).

The toxicity of cassava was first reported in 1605 (de Bruijn 1971). All varieties of the species *Manihot esculenta* Crantz contain varying amounts of linamarin [2-(ß-D-glucopyranosyloxy)-isobutyronitrile] and lotaustralin [2-(ß-D-glucopyranosyloxy)-methylbutyronitrile]. Linamarin is more abundant (about 90%) than lotaustralin (Nartey 1973). The primary precursors of these glucosides are the amino acids valine and isoleucine respectively (Conn 1994). Genetic characteristics of a variety and several environmental factors such as soil, rainfall, degree of shading and type of fertiliser applied controls the level of cyanogenic glucosides in cassava (de Bruijn 1971, Bokanga et al. 1994). The identification and characterization of the enzymes and the genes involved in the biosynthesis opens future possibilities for biotechnological optimization of glucoside levels in cassava (Koch et al. 1994).

Cyanogenic glucosides are not uniformly distributed in the various tissues of the cassava plant. The highest concentration is usually found in the peel and the lowest in the central pith (Kojima et al. 1983). The leaves often contain the next highest concentration (de Bruijn 1971), but the correlation between the glucoside content of the roots and the leaves is not very clear (Cooke et al. 1978a). It is suggested that linamarin is synthesised in the leaves and transported to other tissues of cassava, including the roots, as the diglucoside linustatin (Selmar 1994). Younger tissues contain higher levels of cyanogenic glucosides than the older ones. In the root, the section closest to the stem (proximal) contains more glucosides than the middle and distal sections.

Higher concentrations of glucosides correlate to the bitter taste of the fresh roots (Sundaresan et al. 1987, Bokanga 1994). However, some varieties with extreme expression of either trait do not follow the general trend. A variety producing non-bitter roots in one area may yield bitter roots in another area because of water stress, soil type or the nitrogen content of the soil (Pereira et al. 1981). Taste is used to distinguish cassava varieties into "bitter" and "non-bitter" (often referred to as "sweet"). It is thought that the more elaborate processing methods were first applied to bitter cassava roots in order to remove the bitter taste. The bitter taste often remains even after boiling the un-processed roots. The compounds responsible for bitterness are not yet known (Bokanga 1993, 1994). Some consumers enjoy chewing fresh peeled cassava root from non-bitter cultivars, since it has a sweet taste.

Cassava plant cells contain an endogenous enzyme linamarase (linamarin ß-D-glucoside glucohydrolase, EC 3.2.1.21) that is capable of hydrolysing the cyanogenic glucosides. The glucosides and the enzyme are present in all tissues of cassava, except probably in the seeds (Nartey 1973). The enzyme is stored in the walls of the plant cells, while the glucosides are stored in the vacuoles. When the cell structure is destroyed the enzyme is brought into contact with the glucosides resulting in rapid hydrolysis with formation of the corresponding cyanohydrins. The cyanohydrins spontaneously decompose to acetone and hydrogen cyanide (HCN) (Conn 1969) as shown in figure 1. It has been postulated by analogy with a similar reaction in sorghum that a second enzyme, α-hydroxynitrile lyase, may catalyse the dissociation of the cyanohydrins.

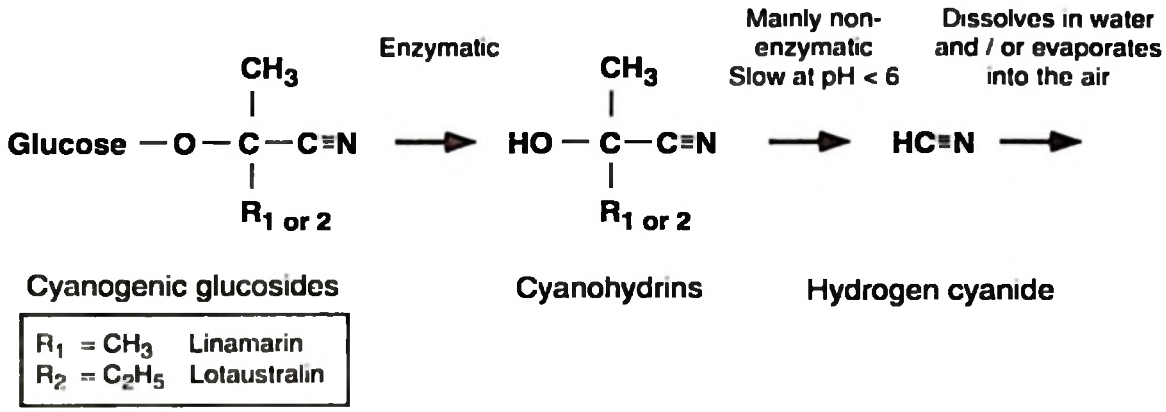

Figure 1. The breakdown of glucoside in cassava into cyanohydrin and hydrogen cyanide.

The cyanohydrins are relatively stable in acidic conditions, but above 30°C and pH 6, the cyanohydrins have a half-life of less than 30 minutes since the alkaline pH favours their dissociation. Processed cassava root products contain three forms of cyanogens that can yield cyanide ions, viz (i) the glucosides, linamarin and lotaustralin (earlier referred to as bound cyanide), (ii) cyanohydrins and (iii) hydrogen cyanide, a gas above 26°C. Total cyanogens implies the sum of all three components.

Cassava processing

The three main reasons for processing cassava roots are to increase shelf life, facilitate transport and remove cyanogens. The Amerindians who first cultivated cassava several thousand years ago devised processing methods which could achieve all the three goals (Dufour 1994). The fresh roots rot within 3-4 days after harvest if not processed. Transportation of roots to urban markets is simplified by processing the roots due to reduced water content. Processing also makes the roots and the products more palatable by providing a variety of products which are convenient to cook, prepare and consume.

A great diversity of processing methods are found in cassava-growing communities (Lancaster et al. 1982, Hahn 1989). Processing methods include a combination of several procedures that are performed during specific time periods and in a specific sequence (Nweke 1992). These procedures include peeling, crushing, milling, slicing, grating, chipping and expression of water. Others involve sun or smoke drying, frying in oil, fermentation by soaking in water or by heaping. Others are stacking, sedimentation, sieving, cooking, boiling or steaming. During processing a wide range of storable intermediate products are yielded in which some do not need more processing while others need further cooking during meal preparation.

Cassava processing methods currently in use in Africa might have been introduced with the plant, or invented locally at a later date. Soaking appears to have been developed independently in Africa, based on original methods used to prepare the toxic species of yam (*Dioscorea sp.*) (Hahn 1989). The processing methods vary both between and within countries, depending on food culture and cassava varieties used. Environmental factors such as availability of water, sunshine and firewood as well as processing equipment and technologies available are the most important determinants (Nweke 1992). The main cassava processing methods practised in Africa can be categorised into the following four groups:

Soaking - involves fermentation by submerging peeled or unpeeled roots in water for several days. After subsequent sun-drying, products are obtained with names such as *lafun* in Nigeria, *kivunde, kondowole* and *makopa* in Tanzania, *water fufu* in Cameron, *placali* in Ivory Coast and *cossettes* in Zaire. The soaking method can be modified by pounding and steaming of the soaked roots to get a paste known as *chikwangue* in Zaire and *agbelima* in Ghana.

Heaping - involves air or solid state fermentation of peeled roots for some days followed by sun-drying and pounding into flour. The intermediate products obtained include *makopa*, *udaga*, *nyange* and *bada* in Tanzania and *fufu* in Nigeria.

Direct sun-drying of peeled roots or root pieces is done in several areas in Africa. The dried product is called *kokonte* in Ghana and *makopa* in Tanzania and can be pounded into flour.

Grating and moist fermentation, followed by roasting or toasting, is known as *gari* processing in some West African countries and as *atoukpou* in Ivory Coast. This involves grating peeled cassava roots, collection of the mash in a sack and pressing out the juice while the mash is left to ferment for a number of days before roasting.

The intermediate products can be grouped into pastes, dried pieces or chips and toasted granules (Nweke 1992). Cassava roots are also widely used in fresh form in almost all countries. The roots from non-bitter cassava varieties can be directly boiled or fried and also chewed raw as a snack (FAO 1990). Other cassava products in Africa not included in the above categories include starch and alcohol.

Cassava leaves are preferred as a green vegetable throughout Central Africa and in some parts of Tanzania. Cassava leaves are made into a relish called *sakasaka* or *pondu* in Zaire, the

Congo, Central African Republic and Sudan, *kizaka* in Angola, *mathapa* in Mozambique, *chigwada* in Malawi, *chombo* or *gwada* in Zambia, *gwen* in Cameroon, *kisamvu* in Tanzania, *cassada* leaves in Sierra Leone, *banankou boulou nan* in Mali, *mafe haako bantare* in Guinea and *isombe* in Rwanda.

Cassava-processing methods are more or less effective in reducing the cyanogen content. They lead to the hydrolysis of the glucosides to give cyanohydins and subsequently hydrocyanic acid which is either volatilized or leached out through dissolution in water. When cassava is peeled and grated before fermentation and subsequently heated when being roasted into gari, it is possible to obtain a product with negligible cyanogen levels, irrespective of the cassava variety used (Oke 1994). Grating is found to be effective in bringing the enzyme and the substrate into contact. In the production of gari, which involves grating, fermentation and roasting, there is reduction of the total amount of cyanogens by 80-95%, relative to fresh peeled roots (Mahungu et al. 1987). When gari is cooked into *eba*, the cyanogens are further reduced to even safer levels. Fermentation of cassava roots by soaking in water is also an effective technique for cyanogen removal (Hahn 1989).

Processing the roots to *chikwangue* results in 90 to 100% reduction of cyanogen content (Bokanga 1989). The methods which do not involve a detoxification step are normally applied to non-bitter roots from low cyanogen cultivars while methods which involved cyanogen reduction are applied to bitter and toxic roots (Coursey 1973).

Cyanide metabolism in humans

If cyanogens are not effectively removed during processing, the consumption of cassava may result in dietary cyanide exposure. The source of dietary cyanide from cassava may be ingested glucosides or cyanohydrins which in the gut may be broken down to hydrogen cyanide.

There are several sources of cyanide exposure to humans beside consumption of insufficiently processed cassava. This include consumption of other cyanogenic plants such as linseeds or bitter almonds. Fire gases from combustion of nitrogen-containing materials and tobacco smoke may also result in cyanide exposure. Other sources are the occupational exposures from alkyl-cyanides used as solvents or cyanide salts used industrially for metal cleaning and polishing. The anti-hypertensive drug sodium nitroprusside consists of 44% cyanide, which is liberated following its administration (Schultz 1984).

The human body has two defence mechanisms against cyanide. When cyanide enters the blood stream from either the lungs or the gastro-intestinal tract, it is trapped by the methemoglobin fraction in the red blood cells. This fraction normally constitutes about 1% of all haemoglobin, and it can reversibly bind about 10 mg of HCN as cyanomethaemoglobin (Schultz 1984, Lundquist 1989). Cyanide can passively be released according to the law of mass action. The second defence mechanism is the conversion of cyanide to thiocyanate (SCN) catalysed by the enzyme rhodanese, also called thiosulphate sulphur transferase EC 2.8.1.1, that was crystallized by Sörbo (1953). Rhodanese converts cyanide (CN) to thiocyanate (SCN) (figure 2). The conversion requires a sulphane sulphur substrate, i.e. a divalent sulphur (-S-) covalently bonded to another sulphur atom. The substrate through different metabolic pathways is provided from dietary sulphur amino acids. Substrate availability is the rate-limiting factor (Lundquist 1992). The detoxification process mainly takes place in the liver and the kidneys.

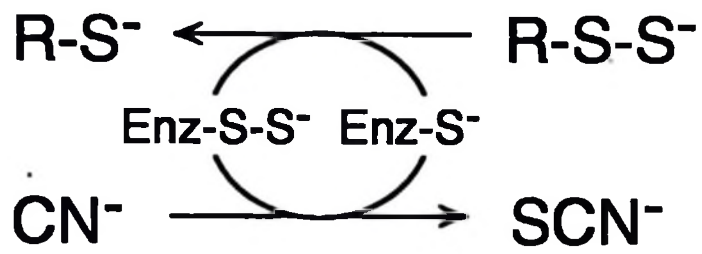

Figure 2. The conversion of cyanide to thiocyanate in the human body.

Thiocyanate is mainly found extracelluarly in blood, urine, saliva and in gastric juice (Ruddell et al. 1977). The thiocyanate ion is a pseudo-halide that is handled as chloride and iodide. It is rapidly filtered by the glomerulus and efficiently reabsorbed by the tubule (Schultz 1984). Thiocyanate was used for controlling hypertension in the 1950's. Toxic manifestations occur at serum levels above 1,300-2,000 µmol/l, and includes bone marrow depression, hypothyroidism, fatigue, anorexia and nausea (Barnett et al. 1951).

When cyanide exposure rates are greater than the conversion rate and cyanide saturates the methemoglobin pool, acute cyanide intoxication occurs. In this situation cyanide rapidly accumulates in plasma and attacks target organs such as the brain (Lundquist et al. 1985). Fatal toxicity of cyanide is believed to be due to energy failure of cells as cyanide inhibits cytochrome c oxidase, the terminal enzyme of the mitochondrial electron transport chain and blocks the oxidative phosphorylation (Pettersen & Cohen 1993).

The central nervous system is considered particularly sensitive to cyanide because of its limited anaerobic metabolism, low energy reserves, high energy demands and high respiratory rate. Cyanide seems to act directly on the neuronal energy metabolism (Patel et al. 1992). The non-specific clinical symptoms and signs of acute sub-lethal doses of cyanide are light-headedness, headache, drowsiness, tremor and coma. Acute inhalation of 2 mmol (50 mg) HCN can be lethal, while ingestion of 200 mg NaCN or 300 mg KCN can be lethal, which on a molar basis is twice as much as the lethal dose when inhaled (Labianca 1979, Ansell and Lewis 1970). Lethal blood levels of cyanide in man are estimated to be 180 µmol/l in whole blood and 20-30 µmol/l in plasma. The rate of endogenous detoxification of cyanide in well-nourished adults is around 1 µg (0.04 µmol)/kg per minute. This corresponds to a lethal dose rate in an adult of about 3-4 mmol/-24 hours (Schulz 1984).

Health effects attributed to cassava toxicity

Cyanide exposure from cassava has been associated with several health disorders in humans. However, due to the methodological limitations of the few studies done, the causal relationship is not fully established between cyanide exposure and the conditions mentioned below (Rosling 1994).

Acute poisoning following cassava meals is rare in relation to the extensive use of cassava as human food. The published reports are very scarce, especially for fatal poisonings (Akintonwa 1992, Espinoza 1992). According to Clark (1936), acute cyanide poisoning due to cassava used to be common in West Africa. The clinical features are not specific, and main symptoms are vomiting, headache, dizziness and collapse, described by Nicholls et al. (1960).

Several anecdotal documentations of cassava poisoning exist in East Africa (Bontinck 1974, Parke 1891, Casati 1990). Two lethal cases of poisoning following eating of sweet cassava (*Manihot aipi*) were reported from the Pemba Island, Tanzania by von Queisser (1966). Sporadic poisonings were reported in Kenya between 1980 and 1986 (Imungi 1986). These case reports from eastern, central, Nyanza and western provinces included a total of 13 deaths and 14 hospitalized patients. A rural outbreak of acute poisoning following cassava meals was documented during drought period in a cassava-growing community in northern Mozambique when insufficiently processed products were consumed (Essers et al. 1992).

Aggravation of Iodine Deficiency Disorders (IDD). Cyanide exposure from cassava has been associated to increased goitre frequency, the main manifestation of IDD. This effect has been attributed to increased thiocyanate levels, the end product of cyanide detoxification in the human body. Thiocyanate has been shown to competitively interfere with iodine uptake in the thyroid gland in experimental studies. The anti-thyroidal effect of thiocyanate has been explained by the pseudo-halide character of the ion which interferes with iodine transport and inhibition of the oxidation of iodine and by inhibiting iodination of tyrosine in the follicle (Virion et al. 1980). Field surveys in Zaire suggest that high SCN loads in cassava-eating populations may aggravate the effect of iodine deficiency through a similar mechanism (Bourdoux et al. 1978). However, it has been shown that populations with very high dietary SCN load from insufficiently processed cassava do not develop goitre if iodine intake is adequate (Delange et al. 1989, Cliff et al. 1986).

Tropical ataxic neuropathy (TAN) is a neurological syndrome in adults that is characterized by insidious onset of progressive symmetrical peripheral neuropathy combined with signs of myelopathy and sometimes optic neuropathy. The disease was extensively studied in some rural populations in Nigeria by Clark (1935), Monekosso and Wilson (1966) and Osuntokun (1981). They found an association between occurrence of TAN and several years of moderate dietary cyanide exposure from cassava and a low protein intake resulting in deficiency of sulphur needed for cyanide detoxification. Cyanide has been proposed as a causal factor but other dietary deficiencies were also believed to contribute to the disease (Osuntokun 1981). The same syndrome was also reported from Tanzania (Makene and Wilson 1972, van Heijst et al. 1994). Visual impairment was for the first time attributed to cassava in the last century by the anthropologist Mary Kingsley (1982), who reported that a population in West Africa suffered from blindness attributed to a diet that was too exclusively 'maniocan'.

Konzo is another neurological disease that has also been attributed to cyanide exposure from cassava. It is a form of spastic paralysis affecting both legs with an abrupt onset and a non-progressive course. The paralysis varies in severity but always mostly affects the distal parts of the legs. Konzo was recently identified as a distinct disease entity (Howlett et al. 1990) and it was named after the local designation among the population in Zaire where it was first reported (Trolli 1938). In the last decade epidemics of konzo have occurred in Mozambique, Zaire and Central African Republic, where several studies revealed a consistent association with high cyanide and low sulphur intake from a diet dominated by insufficiently processed bitter cassava (Ministry of Health 1984, Cliff et al. 1985, Essers et al. 1992, Tylleskär et al. 1992, 1994, Tylleskär 1994). The neuro-damage was attributed to the combined metabolic effect of high cyanide exposure and low sulphur intake.

Other diseases. Malnutrition-related diabetes is another disease of which cyanide exposure was thought to be an aetiological factor. A causal role of dietary cyanide exposure was proposed as a possible cause by animal experiments but epidemiological studies have failed to support a relationship in humans between cyanide exposure from cassava and diabetes (Swai et al. 1992).

Protein malnutrition is believed to be aggravated by cyanide intake in children consuming insufficiently processed cassava. The reason is that the limited intake of essential sulphur amino acids in the diet is used for conversion of CN to SCN instead of protein synthesis (Vis 1983).

Cassava and cyanide exposure in Tanzania

Tanzania is the third largest producer of cassava in Africa (CIAT 1993), but cassava is only an important staple in half of the country's twenty regions. The most common processing methods are direct sun-drying of peeled roots for weeks into a storable product known as *makopa*. Another method is fermentation of peeled roots by soaking in water for several days followed by sun-drying. A third method is fermentation of peeled root pieces in covered heaps to enhance mould growth. The different dried products obtained can be stored and subsequently milled or pounded into flour used for making the stiff porridge, *ugali*. The processing methods used in Tanzania have only been briefly documented in some of the cassava-growing communities (Sennappa and Mlingi 1988). They have not been studied in detail in any of the cassava-growing regions.

High cyanide exposure from cassava was implicated as the main cause of an outbreak of the paralytic disease konzo in northern Tanzania in 1984/85 (Howlett et al. 1990). This prompted further investigation of cassava processing in this area and other parts of Tanzania with special reference to cyanide exposure. In March 1988 during the planning phase of these studies reports of widespread acute poisonings in Masasi district in southern Tanzania were received. The poisonings were believed to be linked to cassava consumption and the need to investigate the cassava processing and consumption associated with these two outbreaks of disease was the starting point of this thesis.

AIMS OF THE STUDIES

The overall objective of the studies was to investigate the relationship between cassava processing methods and dietary cyanide exposure associated with public health problems in Tanzania.

The specific objectives were:

- To investigate how the outbreak of acute poisoning in southern Tanzania was associated with cyanide exposure and insufficient processing of cassava roots (Paper I).
- To study if cases of konzo occurred in the population exposed to dietary cyanide in southern Tanzania (Paper II)
- To elucidate the mechanism of cyanogen removal during sun-drying of cassava in southern Tanzania (Paper III).
- To investigate if and how the short-cuts in cassava processing resulted in cyanide exposure that was associated with the paralytic disease konzo in northern Tanzania (Paper IV).
- To elucidate if the thiocyanate load from cassava consumption was related to cassava processing and if it aggravates goitre in an IDD endemic district in western Tanzania (Paper V).
- To investigate if cassava consumption in Dar-es-Salaam may cause dietary cyanide exposure (Paper VI).

METHODOLOGICAL REVIEW

Different combinations of both qualitative and quantitative survey methods, as well as biomedical and chemical methods were used during the investigations reported in this thesis. A brief review of the methods used follows.

Explorative survey methods

The combined and interactive use of explorative and qualitative survey methods, also referred to as Rapid Assessment Procedures (RAP) or Rapid Rural Appraisal (RRA), is useful for elucidation of sensitive information, as well as practices and perceptions that are unknown to the investigators. Techniques used in RRA include secondary data review, direct observation in field situations, taking part in rural activities, semi-structured interviewing, workshops and brain-storming. Others are group walks, mapping, ranking, scoring, developing chronologies of local events and making portraits or case studies of people or situations. These techniques strive to be rapid and provide useful information through the use of multiple sources (Scrimshaw 1992). When conducted properly they can elicit a range of insights which is inaccessible through conventional quantitative survey methods (Chambers 1992).

Following the development of RRA techniques for agro-ecosystems analysis the techniques spread fast and by the end of 1980's they were already applied in a wide range of sectors in more than 25 countries (Heaver 1992). RAP procedures for health and nutrition evolved separately in the 1990's with surprisingly little cross-fertilization with practitioners of RRA. Instead experience was drawn from medical anthropology (Scrimshaw and Hurtado 1987).

RRA and RAP share a number of common characteristics, but they can also be regarded as two entirely independent processes. RRA constitute an interdisciplinary survey from fields such as sociology, anthropology, geography and journalism whereas RAP is more of an anthropological rapid assessment of human behaviour (Kachondham 1992). The RAP acronyms refer to a set of qualitative techniques among those used in RRA that have been applied especially for health and nutritional programme evaluation (Scrimshaw and Hurtado 1987). RAP should not replace established anthropology but can be seen as a selective application of some of its tools.

Quantitative surveys permit collection of data from large numbers of people in standardized ways, enabling comparison of representative data between individuals, communities, countries and time periods. On the other hand qualitative information generated on the same topics permits a more detailed and accurate understanding of the underlying social and cultural patterns of behaviour. The relevance and appropriateness of such information is verified by triangulation, which involves cross-checking data through the use of repeated questions, discussions and actual observations (Pelto 1978, Patton 1990).

RAP and RRA are inappropriate when statistically representative data is needed. The investigation may be biased due to distortions in the observed situations, unrepresentative observation periods and biased sampling of people sampled. As the techniques do not have standard methodology the qualified researchers must perform the collection of information. Quantitative and qualitative approaches should be considered as complementary to each other (Cook and Reichardt 1979).

Key informant interviews are one of the most widely used RAP/RRA techniques. Key informants are selected because they are expected to possess information, ideas or insights on a particular subject in a certain community (Kumar 1989). Key informant interviews are essentially qualitative interviews using interview guides that list issues to be covered. The interviewer frames the actual questions in the course of interviews and more than ten informants used to be interviewed.

Key informant interviews are useful when an understanding of the underlying motivations and attitudes of a target population is required, especially in determining what people do and why they do it. They may also be useful when quantitative data collected through other methods need to be interpreted or during preparation of a quantitative survey questionnaire. Key-informant interviews often provide data that cannot be obtained with other methods, especially confidential information that would not be revealed in other settings. They provide flexibility to explore new relevant ideas and issues that had not been anticipated in the planning of the study (Kumar 1987).

Key informant interviews are not appropriate when quantitative data is needed. The findings can be biased if the informants are not carefully selected from a wider pool of knowledgeable informants familiar with the local situation. The findings are also susceptible to interviewer biases.

Focus group interviews are qualitative research techniques used to explore feelings and opinions of small groups of participants on a given practice, experience, service or other phenomenon. A focus group session is a discussion in which 6 to 12 respondents under the guidance of a moderator, talk about topics of importance to an investigation (Folch-Lyon et al. 1981).

The session creates an opportunity for participants to interact and share ideas in an informal manner about a certain topic common to them. The participants are chosen from a specified target group whose opinions and ideas are relevant to the investigation. Several group sessions should always be conducted. As long as new information is obtained more groups should be interviewed to ensure adequate coverage of the topic.

The moderator plays a key role in creating a supportive climate that encourages all group members to share their views (figure 3). He should facilitate interaction among members to interject probing comments, transitional questions and summaries without interfering with dialogue among the participants (Basch 1987). He should cover topics listed in the prepared outline, while omitting those that seem not to be relevant. Physical setting and psychological climate are important. Audio-taped records or moderator's and assistant's notes constitute raw data used for analysis.

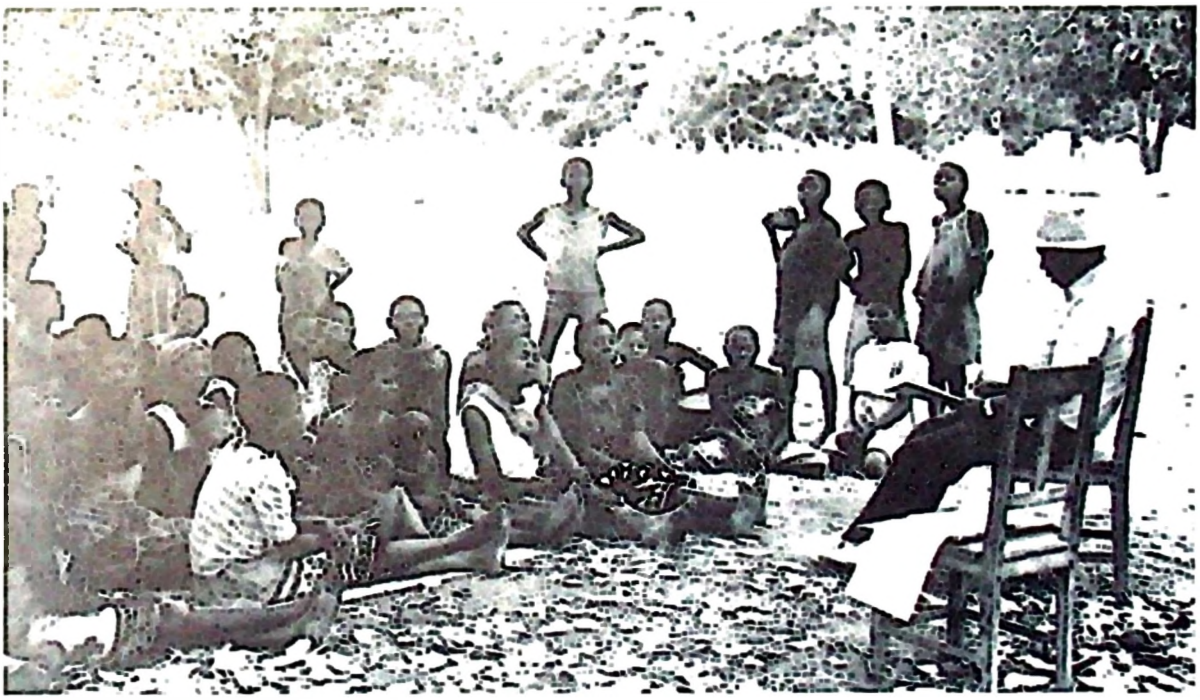

Figure 3. Focus group discussion in Ulungu village Masasi district, southern Tanzania.

Focus group interviews help to generate and pretest questions for quantitative surveys or other field data collection methods (Glik et al. 1988).

Advantages of focus group methods are that they are relatively easy for learning about respondent "subgroups'" ideas and opinions. The groups can be assembled according to stratification criteria (e.g. sex, age, educational level, etc.). This may provide a more stimulating and secure setting to express ideas. Other assets include reduced pressure on subjects to respond to every question, item or issues, relatively fast turn-around time, and ability to use 'leading arrangements' that are not feasible in individual interviews (Bellenger et al. 1976). Focus group interviews can give order, a rationale, and a logic to practices encountered in traditional cultures. To get what is in people's minds about their behaviour is a matter of posing the right questions.

Focus group interviews cannot be used for testing hypothesis in experimental study design nor to obtain representative quantitative data. Another limitation is that only individuals who are able and willing to verbalize their views can be studied. The focus group method may provide evidence to support preconceptions more easily than other methods, since findings rely heavily on the guidence by the moderator and the interpretations by the investigators. Conclusions from focus groups should be judged or checked through triangulation with other qualitative and quantitative survey methods.

Dietary assessment methods

Data on foods consumed can be obtained by 24-hour recall, dietary history and food frequency interviews (Cameron and Staveren 1988). When compared to prospective dietary records, these interview methods place minimal burdens on the subjects. The main disadvantage of these interview methods is that the food consumption data collected is based entirely on the subject's memory and judgement.

The 24-hour recall is a technique where each subject is asked to recall and describe all food and drinks consumed in 24 hours before the interview (Nelson 1991). The respondent is asked to recall diet from waking up to the time of going to bed. The interviewer must be thoroughly familiar with the food habits of the study population. Photographs or food portion models are needed for portion size estimation and respondents should be given a chance of describing the portions on their own terms. To enhance the completeness and accuracy of responses, the interviews should be conducted in the subject's native language. The interviewers should receive adequate training in the technique so that they do not bias the subject's responses through leading questions or by failing to probe adequately for items not mentioned. The 24-hour recall technique allows large number of subjects to be interviewed with a minimum of resources because of the small amount of information required from each respondent. However, the 24-hour recall does not provide a reliable estimate of an individual's intake during the preceding weeks or months because of day to day variation.

The dietary history method assesses an individual's total food intake and usual meal pattern over varying periods of time, usually the last month, last six months or last year. The dietary history is a detailed interview to establish "usual" food consumption patterns. Starting with a 24-hour recall, the interviewer probes carefully for food consumption meal by meal, seeking a day to day and seasonal variations, to build up the 'usual' pattern. As in 24-hour recall conceptualization in the form of food lists and photographs may be used. Interviews may last more than one hour, and are best recorded on prepared forms (Bingham and Nelson 1991).

The dietary history method has advantages over prospective dietary records and 24-hour recall methods as it provides detailed information about meal patterns, food consumption and data on the usual intakes of a wide variety of nutrients. Subject involvement is kept to a minimum with no literacy or numerical skills needed. Foods infrequently consumed that may be important sources of nutrient or highly seasonal are better assessed by dietary history than by other methods.

The disadvantages are common to all recall methods and the complexity of the procedure makes it particularly prone to interviewer bias. Diet histories may exaggerate the regularity of dietary habits and do not provide information about day to day variations in the diet. Repeatability and relative validity of diet histories suggest that the method is robust enough to be of value, either to compare group means or to rank subjects according to levels of current or recent food consumption or nutrient intake (Bingham and Nelson 1991).

Food frequency methods estimate how often certain foods are consumed during a specified period of time. The method is useful in classifying subjects into high, medium or low consumers of certain foods and/or nutrients. Most questionnaires used are pre-coded, which makes data collected simple to compile. The information sought is only on those foods or nutrients relevant to the aim(s) of the study (Cameron and Staveren 1988). The method has been used in studies of associations between diet and health, to evaluate nutrition education programmes and to examine dietary compliance (Räsänen 1982 and Willet et al. 1985).

The basic food frequency questionnaire consists of two components which include a food list and a frequency response section for subjects to report how often each food was eaten. The disadvantage of the method is the tedious work in the development and validation of the questionnaire. Food frequency questionnaires have become the primary method for measuring dietary intake in epidemiological studies (Willet 1990).

The underlying principle of food frequency approach is that the average long-term diet such as intake over weeks, months or years is the conceptually important exposure rather than intake on a few specific days. Food frequency categories should always be continuous, e.g. "Never", "Less than once per month", "Once or twice per month", "Three to four times per month", followed by categories indicating the number of days per week on which the item was consumed. Portion sizes used during interviews should reflect known consumption patterns in the population and the questionnaire should allow for a sufficient range of expression of portion size.

Biochemical analyses

Total cyanogens in cassava (glucosides, cyanohydrins and hydrogen cyanide) were earlier expressed as "cyanide content" per kg. The amount of glucosides and cyanohydrins are still expressed in mg hydrogen cyanide (HCN) equivalent per kg dry or wet weight because methods for cyanogen determination involves conversion to hydrogen cyanide (Singh and Wasi 1986). Earlier methods for determination of cyanogen content of cassava products depended on: (i) hydrolysing the cyanogenic glucosides, (ii) isolation of cyanide from the mixture (steam distillation or aspiration) and (iii) determination of the cyanide by colorimetric methods. The first stage of hydrolysing the glucoside was accomplished through strong acids or autolysis by the endogenous linamarase. Autolysis is sensitive to variations in endogenous enzyme activity (de Bruijn 1971) and the long incubations involved may yield errors due to secondary reactions.

The present most commonly used enzymatic assay method for cyanogens in cassava products enables separate determination of each type of cyanogen (Cooke 1978a). The method depends on inactivation of the endogenous linamarase during extraction of cassava samples in an acid/ethanol medium. Under these conditions the cyanohydrin present is stabilized. Sample extracts are analysed directly for HCN content, after being made alkaline to facilitate decomposition of the cyanohydrins and after incubation with exogenous purified linamarase to determine total cyanogen content (all three cyanogens). The amount of cyanohydrins and glucosides can be calculated. The method was modified by O'Brien et al. (1991) permitting an additional flexibility in sample preparation, storage and separate assaying of the three cyanogens. A further improvement was made by introduction of the chromogen isonicotinate/1,3-dimethyl barbiturate instead of pyridine/ pyrazolone (Essers 1993). The new chromogen is less toxic, easier to handle, has increased sensitivity and longer storability.

Thiocyanate is the main cyanide metabolite (70-80%) in humans (Lundquist 1992). It is excreted in urine within some days of its formation. Determination of thiocyanate in serum or urine give information about the exposure to cyanide in the previous week and it is the most widely used indicator for cyanide exposure in humans. Thiocyanate is stable in urine and samples can be transported to the laboratory and stored frozen for long periods before analysis is performed. The presently used specific method for determination of thiocyanate in serum and urine was described by Lundquist et al. (1979, 1983). Thiocyanate is separated from interfering compounds by adsorption to an anion exchange resin with affinity for thiocyanate, and elution with sodium perchlorate. The eluted thiocyanate is quantified by a modified König reaction, with sodium hypochlorite being used as a chlorinating reagent. Analytical recovery of thiocyanate added to serum and urine is quantitative and the coefficient of variation is 2.3% both within-day and between-day. The high affinity for thiocyanate of the anion-exchange resin was found not to be based on anion exchange mechanism but was probably related to the chaotropic effect of thiocyanate. Recently the method was modified to use a new chromogen, isonicotinate/1,3-dimethyl barbiturate instead of barbituric acid-pyridine reagent (Lundquist 1995).

Urinary linamarin can be estimated by a micro-diffusion method (Brimer and Rosling 1993) but a more sensitive and specific method has later been developed. (Carlsson et al. 1995). It is based on solid-phase extraction by adsorbing linamarin on a silica sorbent column and colorimetric determination of cyanide in the eluate after liberating the cyanide with the linamarase enzyme.

Urinary inorganic sulphate can be used to estimate dietary intake of sulphur amino acids. Methionine, cysteine and cystine not used for protein synthesis are degraded to inorganic sulphate and rapidly excreted in the urine (Young et al. 1958). There is a linear relationship between the urinary inorganic sulphate excretion and the dietary intake of sulphur amino acids (Sabry et al. 1965). Determination of inorganic sulphate in urine is based on turbidimetry of sulphate as barium sulphate in the presence of a small amount of preformed barium sulphate with polyethylene glycol as a stabilizing agent (Lundquist et al. 1980).

Urinary iodine is used for assessing iodine intake because 90% of all iodine is eventually excreted in the urine. The minimum daily iodine requirement is about 50 µg and a urinary iodine level of less than 5 µg/dl means iodine deficiency (Dunn and van der Haar 1990). Some degree of iodine deficiency may exist even when the urinary iodine is as high as 10 µg/dl per day. When the mean daily excretion in a population is less than 2.5 µg/dl, cretinism will frequently be found in the population.

Iodine in urine is stable and samples can be transported cooled to the laboratory and stored frozen until analysed. Urinary iodine levels vary greatly between individuals in the same population and between different days in the same person. A 24-hour urine collection is time consuming and hence not practical under field conditions.

Casual urine samples are easily collected and the concentration of iodine in the urine is expressed as µg per 100 ml and given as mean, median or frequency distribution. Iodine in urine can be determined using the principle of Sandell-Kolthoff (1937). This involves an initial step in which urine is either digested in strong acid or ashed at high temperature. Thereafter iodide is determined by its catalytic action on the reduction of the ceric ion (Ce^{4+}) to the cerous ion (Ce^{3+}) coupled to the oxidation of arsenite, As^{3+}, to As^{5+}. The ceric ion has yellow colour, while the cerous ion is colourless and so the reaction can be followed by disappearance of the yellow colour as the ceric ion is reduced (Bourdoux 1988).

Goitre grading

Goitre is the main manifestation of IDD and the goitre frequency is used to assess the severity of iodine deficiency in a given population (Perez et al. 1960). Goitre grading is the main basis for decision making on the type of prophylactic measures to be implemented and for evaluation of the intervention. For the purpose of goitre grading, primary school children are recommended as a convenient group to study. They reflect the recent status of iodine nutrition in the community and are the age group in which decrease in thyroid size can be first seen after correction of iodine deficiency (DeMaeyer et al. 1979, Hetzel 1989, Dunn and van der Haar 1990).

Goitre grading is done according to well defined criteria Concern has been raised on the subjectivity of goitre grading and classification, even in instances involving experienced examiners using the same grading system (MacLennan and Gaitan 1974). The inter-observer variation has been found to be as high as 20% in trained and experienced examiners (DeMaeyer et al. 1979, Crooks et al. 1964).

Goitre grading starts with thyroid inspection, in which the subject's neck is observed for thyroid gland enlargement (Perez et al. 1960, MacLennan et al. 1974). Inspection is followed by palpation from the front where the goitre is examined by touching the front of the neck with both thumbs. According to Perez et al. (1960), a thyroid gland whose lateral lobes have a volume greater than the terminal phalanges of the thumbs of the person being examined should be considered as goitrous. This definition has also been recommended and used by other workers (Delange et al. 1986, Dunn and van der Haar 1990) as shown in table 2.

Table 2. Classification of goitre.

Grade 0	No goitre.
Grade 1a	Thyroid lobes larger than ends of thumbs.
Grade 1b	Thyroid enlarged, visible with head tilted back.
Grade 2	Thyroid enlarged, visible with head in normal position.
Grade 3	Thyroid greatly enlarged, visible from about 10 meters.

Source: Dunn and van der Haar (1990).

From the classification total goitre and visible goitre rates are calculated as per Delange et al. (1986). Total Goitre Rate (TGR) include 1a + 1b + 2 + 3 + nodular and Visible Goitre Rate (VGR) include grades 2 +3.

MATERIALS AND METHODS

Study areas

The studies were performed in four districts situated in the corners of Tanzania and in the capital city, respectively (figure 4).

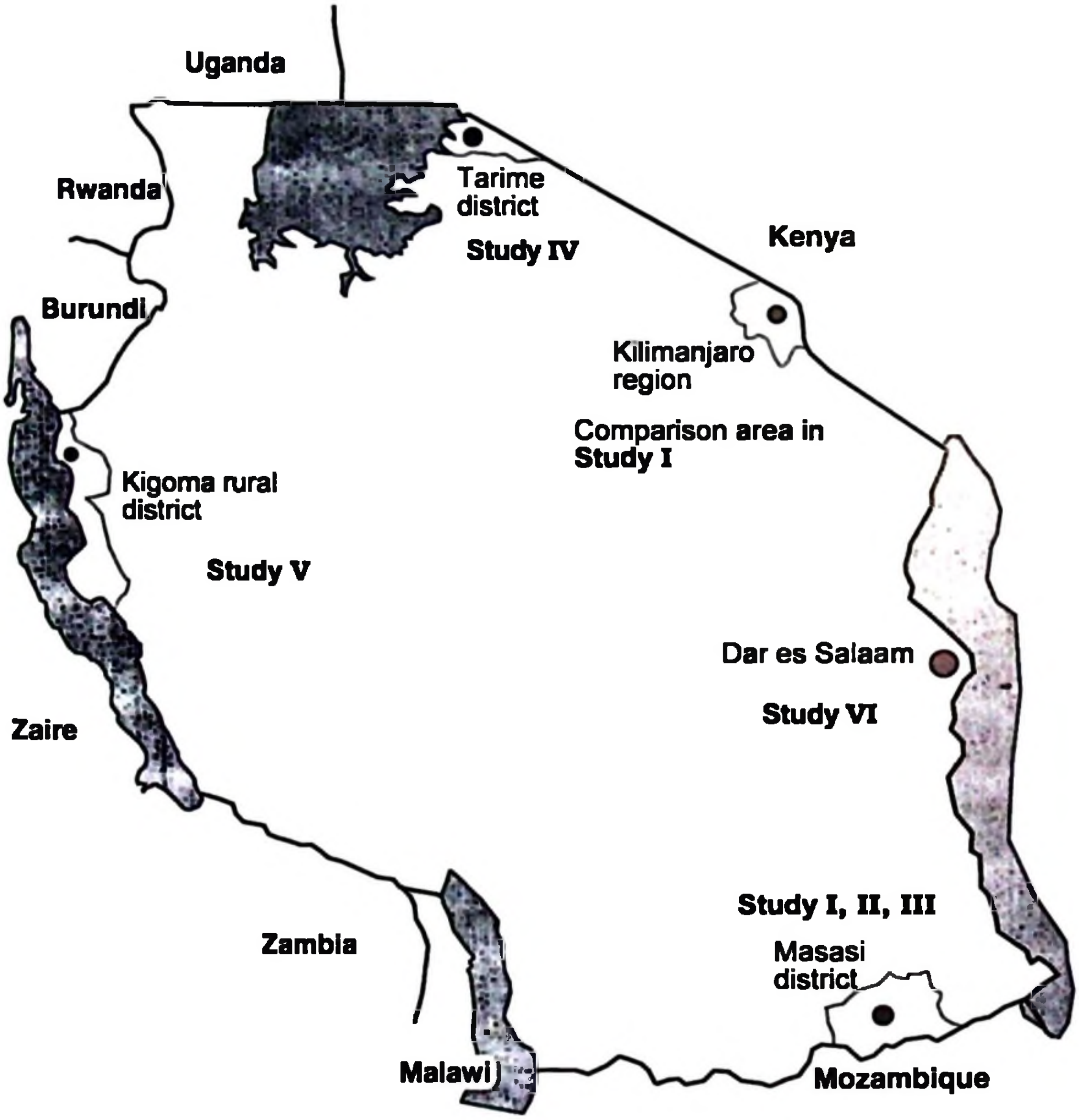

Figure 4. Map of Tanzania showing areas where the studies were performed.

Studies I-III were performed in Masasi district, southern Tanzania from which reports of acute intoxications were obtained in March 1988. The district had a population of 335,000 and 38 inhabitants per km^2 in 1988 (Bureau of Statistics 1990). The district is mainly inhabited by the Yao tribe who cultivate maize and cassava as their staple foods. Direct sun-drying of peeled roots is the main cassava-processing method in this area. When sufficiently sun-dried the root pieces, called *makopa,* can be pounded into flour for making *ugali*. Investigations on the outbreak of acute intoxications were carried out in Mtandi, Chanikanguo and Mumbaka villages in September 1988, and a follow-up was done one year later. Experiments to establish critical stages in cyanogen removal during cassava processing in southern Tanzania were carried out in Kanyimbi, Lipupu and Ulungu villages in September 1991. The villages were situated 40 to 70 km east, south and west of the district headquarter.

Study IV was carried out in Tarime district, northern Tanzania which experienced a konzo epidemic in 1984/85 (Howlett et al. 1990). The district borders Kenya in the north, Lake Victoria in the west and in the south east it borders on Serengeti National Park. The district is inhabited by Kuria, Luo and Gita tribes and the population was 260,000 in April 1989. The population density was 67 people per km^2 which was higher than the average of 26 people per km^2 in mainland Tanzania (Bureau of Statistics 1990). In the studied villages the main staple crop is cassava due to higher yields, but maize and sorghum are also grown. Study IV was done in April 1989 and February 1991 in the most affected village Nyambori and in the neighbouring unaffected village Masonga on the lake shore.

Study V was done in Kigoma rural district, western Tanzania in November 1991. The district borders on Lake Tanganyika in the west and Burundi in the north. The district lies in the western arm of the Great Rift valley, and its soils are iodine deficient. IDD is endemic in the district with a goitre prevalence (TGR) of 37% in school children (Kavishe 1990). Cassava is highly consumed in Kigoma district especially in the lowland zones. The village Bitale was selected for the study as it was reported to have both high goitre frequency and high cassava consumption.

Study VI was carried out in Dar-es-Salaam city in 1992 and 1993. This city, with approximately 2.0 million inhabitants (Bureau of Statistics 1990), lies in the eastern cassava-growing zone. Its suburbs can be categorized into high- and low-income areas. Six low-income suburbs, namely Buguruni, Kigamboni, Tandale, Tandika, Temeke-Yombo and Vingunguti were selected for the study.

Study design and methodology

The studies were carried out using different combinations of methods as shown in table 3.

Table 3. Methods used in the six studies.

	Qualitative survey methods	Quantitative survey methods	Biochemical analysis of plasma & urine SCN	Linamarin	SO_4	Iodine	Cyanogen assay of cassava flour from experiments and/or surveys
Study I	X	X	X				X
Study II		X	X				
Study III	X			X			X
Study IV	X	X	X		X		X
Study V	X	X	X			X	X
Study VI	X	X	X	X			X

Paper I:
Acute intoxications in southern Tanzania

Information about the intoxications in Masasi district was obtained through key-informant interviews (Kumar 1989), with regional leaders, UNICEF staff working with the Child Survival, Protection and Development programme, and district government staff. The three villages of Mtandi, Chanikanguo and Mumbaka were selected by Masasi district authorities because they reported more cases of acute intoxications than other villages. Focus group discussions regarding the intoxications were held with the village leaders. One affected ten-cell unit, the smallest administrative unit in Tanzania, was selected in each village for interviews with all 35 households. Blood and urine were obtained from 95 members of these households.

In 1989, one year after the intoxications two villages were re-visited and dietary interviews repeated in 12 households, from which also recently pounded cassava flour was collected for analysis. Urine and serum was collected from 32 subjects in these households, but the serum samples were lost due to theft of the freezer. Urinary thiocyanate and linamarin were determined. In the third village, six households which reported several acute intoxications in 1988, were extensively re-interviewed regarding symptoms experienced by a physician speaking the local language. Informed consent was obtained from district and village leaders and participating households regarding interviews and collection of blood and urine. For comparison, plasma and urinary thiocyanate were determined in specimens from 201 adults of both sexes randomly selected in another survey in a banana-eating population in Kilimanjaro region where cassava consumption is rare.

Information obtained through interviews was used in designing a cassava processing experiment in Mtandi village where an elderly lady was asked to simulate the short-cut processing methods practised during the food shortage period in 1988. One method was to process a product named *chinyanya* obtained by alternate pounding and sun-drying fresh roots while sieving out flour until a sufficient amount for *ugali* was obtained. The other short-cut method was to process a product nicknamed *small size makopa* obtained by slicing the roots into thin pieces to dry quickly. Nineteen cassava roots from a bitter variety locally known as *mreteta* were peeled and longitudinally split to form 19 pairs which were given to the lady to process in one day into each of the two short-cut products mentioned. Samples of the products obtained were collected after pounding into flour and stored frozen prior to analysis.

Paper II:
Konzo in southern Tanzania

Mtandi village with 2,100 inhabitants in Masasi district was screened for konzo in 1989. Two physicians were led by the village leaders to all households known to have a member with walking difficulty. An informal interview with a key informant led to the identification of an isolated household having three members disabled by spastic paralysis that were unknown to the village leaders. The two young patients identified were examined and diagnosed as konzo cases by the physicians. A subject suspected to be a konzo patient during the study in a neighbouring village in September 1988 had moved to district village where he was traced for examination by the physicians. Serum was collected in 1988 and 1989 from this subject for SCN determination.

Paper III:
Cyanogen removal during processing

With informed consent informal interviews and observations of cassava processing were done in the three villages. Thereafter experiments to ascertain critical stages of cyanogen removal in the established cassava processing and in the short-cut method were done. In each of the villages cassava roots from mixed bitter varieties were harvested from communal fields.

Two batches of roots were harvested and directly sampled for cyanogens in fresh roots. The batches were given to two women to peel and sun-dry into *makopa* products. Samples were collected on day 8 and 17 of sun-drying, and pounded into flour from which samples were taken, sealed in polyethylene bags and stored frozen until analysis. One sample of *makopa* was also collected from each of 31 households from one ten-cell unit in each village.

The short-cut method was studied by random sampling of fresh roots from 3 batches and at two steps during the repetitive pounding and sun-drying. In addition roots were assigned to 11 women for processing of *chinyanya* in their homes under supervision and flour was collected in the evening as described above.

Paper IV:
Cyanide exposure from cassava in northern Tanzania
The information on established and short-cut cassava processing methods obtained from key informants, focus group interviews and participatory observations, was used to design a processing experiment. This simulated the short-cut method and the established method for heap fermentation of cassava roots. Roots from a bitter variety known as *Ndiarye* were used by 15 women to process *udaga* in their households using the established procedure of fermenting for four nights. Another group of 15 women used the short-cut method with one day of fermentation. After fermentation the crushed and fermented root pieces were sun-dried for five or more days before pounding into flour. The flour samples in polyethylene bags, were stored frozen prior to analysis.

After informed consent, all subjects > 15 years in the village were invited to a study of glucose intolerance (Swai et al. 1992). Out of 1,067 subjects studied urine from 45 subjects with IGT and diabetes and 128 controls (selected by picking every fifth subject) was analysed for thiocyanate determination. From the same urines, 93 specimens from 36 males and 57 females had sufficient volume for determination of inorganic sulphate and were used for this study. Female subjects from the households which provided urine were interviewed on food frequency with a structured questionnaire, without registration of portion size.

A neighbouring village on the lake shore which was not affected by the konzo epidemic but also had a high cassava consumption was studied for comparison. Food frequency interviews were carried out with subjects in 20 randomly selected households and urine was collected from 33 subjects (16 males and 17 females aged 9-55 years).

In 1991 the influence of household economy on cassava processing was studied in the same two villages. Focus group interviews were held with 6-10 women and a few men in certain groups. Additional information on living conditions, agricultural practices, cassava processing and trade was gathered through participatory observations while living two weeks in the village. Structured interviews were subsequently done with 101 randomly selected women from each village.

Paper V:
Milling reduces the goitrogenic potential of cassava

Key informants from district health and agricultural departments provided information about cassava cultivation, processing, short-cuts in processing, diet and goitre frequency. The informants suggested the study to be done in a village located in the intermediate zone where both goitre frequency and cassava consumption are high. Processing methods were elucidated through 10 focus group interviews in Bitale and surrounding villages. In Bitale 217 women aged 15-45 years were randomly selected for food frequency interviews and structured interviews on cassava-processing practices. Goitre grading was also done and urine samples were collected for iodine and thiocyanate determination. In two subsequent days 3,791 subjects from the same village, were given two iodinated oil capsules for IDD prophylaxis and their goitres were graded.

A cassava-processing experiment was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria to compare the effect of milling and pounding on cyanogen removal. Roots from a high cyanogen cassava variety 81/01623 growing in the same field were peeled, split into quarters, grouped into four batches and soaked in water from different sources for three days. After three days sun-drying the dried pieces from each of the 21 batches were randomly divided into two parts where one part that was milled and the other pounded into flour. Samples were collected in polyethylene bags and cyanogens extracted the same or second day and extracts refrigerated before analysis.

Paper VI:
Low cyanide exposure from cassava in Dar-es-Salaam

To investigate if cassava consumption results in cyanide exposure in the population of the city suburbs cyanogen levels were determined in flour made from *makopa*, a cassava product sold in the food markets of the suburbs. From markets in six selected low-income suburbs 45 samples of *makopa* were bought and transported to the laboratory. Each batch was split into two, one was milled and the other pounded into flours which were stored frozen until analysed.

Two primary schools were selected from the six suburbs. Urine was collected from 193 primary five children aged 10 to 18 years for thiocyanate and linamarin determination. Each child was also interviewed with a structured food frequency questionnaire regarding cassava consumption in the previous week.

Statistical analysis

Statistical programmes used were Statview SE + graphics (Abacus Concepts, Inc., Berkeley, CA 1992), SPSS/PC+ Norusis 1986 and SAS (SAS INSTITUTE INC. 1982).

SUMMARY OF RESULTS

Paper I:
Acute intoxications in southern Tanzania

Key informants and focus group interviews revealed that the prolonged drought between June 1987 and September 1988 caused the most severe food shortage period ever experienced in the district. The interviews consistently revealed that many families grew both bitter and non-bitter cassava varieties, but during the drought all roots from both types tasted bitter. Among the 35 interviewed households, 71% cultivated only bitter varieties, while the remaining cultivated both bitter and non-bitter.

The groups stated that in normal years cassava was processed by prolonged direct sun-drying of whole peeled or split roots into *makopa* which when fully dried could be stored until pounded into flour. During the food shortage period the established processing method was replaced by two short-cut methods. The first was to process *chinyanya,* which involved pounding fresh peeled roots into small pieces and sun-drying them for one to two hours before repounding and sun-drying. This procedure was repeated several times until sufficient flour was sieved out for making *ugali* the same day.

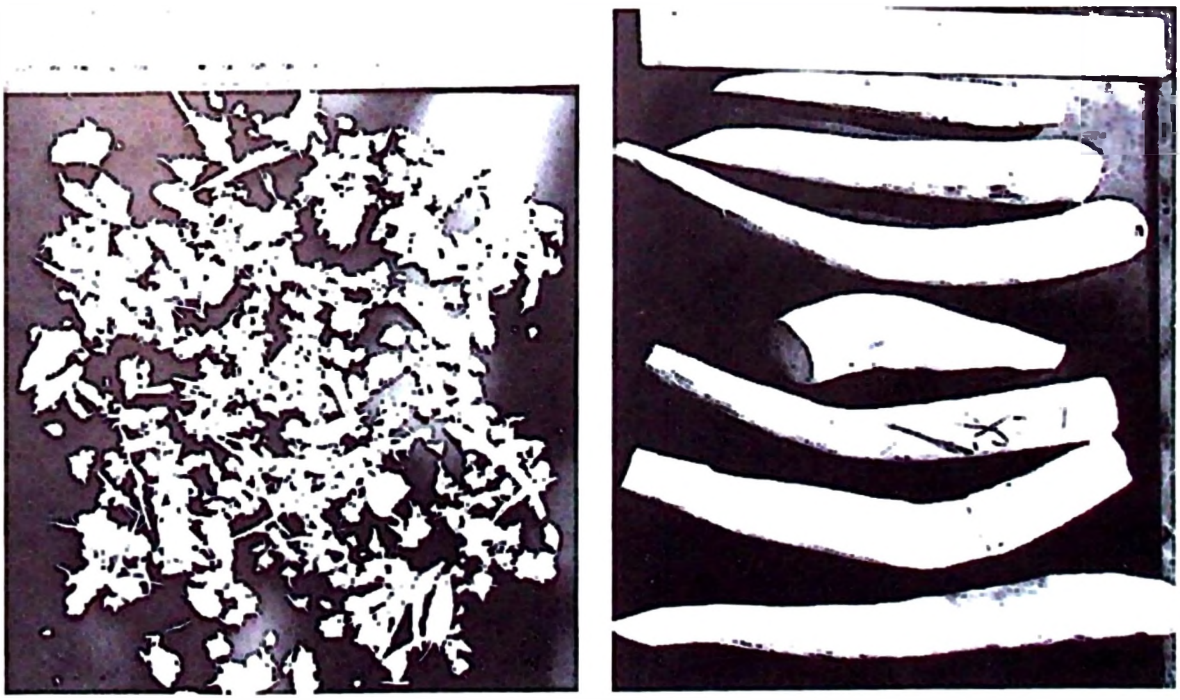

Figure 5. Chinyanya and makopa products processed in southern Tanzania.

The second short-cut method involved slicing fresh peeled roots into small finger-size pieces which were sun-dried on the rocks to dry fast. The small-size *makopa* could be pounded into flour within one or two days. During the food shortage a relish of cassava leaves (*kisamvu*) was frequently used to supplement *ugali*. Of the 35 interviewed households 100% consumed cassava in the last 24 hours and 29% maize. Out of 35 households 65% had processed and consumed *chinyanya*. Key informants stated that acute intoxications after cassava meals were frequent in several villages in Masasi district between March and September 1988. All subjects interviewed had seen or heard that neighbours were intoxicated after consumption of cassava *ugali* during the food shortage. Among the 35 households interviewed, 80% confirmed that family members had experienced acute intoxications on several occasions. The intoxications were manifested by vomiting, dizziness, nausea, palpitation, weakness, diarrhoea, headache and difficulty in vision. The pattern of symptoms obtained by extensive interviews in six households in Mumbaka village were the same

The processing experiment showed high total cyanogen levels in flour obtained from both *chinyanya* and small-size *makopa* (table 4) but cyanohydrin levels were higher in *chinyanya* and glucosides higher in small size *makopa* compared to levels in the flour from normal size *makopa* collected in the households one year after the drought.

Table 4. Cyanogen levels in different cassava products from Masasi.

	Processing experiment		Household flour normal year
	Chinyanya (n=19)	Small size *makopa* (n=19)	Normal size *makopa* (n=12)
Glucosides*	90 ± 17 (12 – 296)	768 ± 107 (121 – 1837)	120 ± 70 (3 – 879)
Cyanohydrins*	48 ± 5 (16 – 120)	15 ± 4 (0 – 61)	7 ± 2 (0 – 17)
Hydrogen cyanide*	6 ± 1.0 (2 – 12)	7 ± 0.5 (5 – 10)	6 ± 0.5 (4 – 9)
Total cyanogens*	144 ± 18 (56 – 336)	791 ± 107 (131 – 1855)	133 ± 71 (8 – 901)
Moisture (%)	13.5 ± 1 (6.0 – 23.6)	10.8 ± 0.2 (9.3 – 2.7)	10.6 ± 0.4 (9.5 – 14.4)

Values are given as mean ± SEM and range in brackets
* Cyanogen levels are in mg HCN equivalent/kg dry weight

Mean ± SEM of serum thiocyanate was 335 ± 12 µmol/l during the drought year compared to 28 ± 4 in a banana-eating population. Urinary thiocyanate levels were 1,120 ± 75 µmol/l during the drought and 68 ± 9 in the following year compared to 7 ± 1 in a banana-eating population.

In the year following the drought all 12 households interviewed still consumed cassava daily but claimed that the *ugali* was made from well-processed *makopa*. As reported in Paper III, mean ± SEM urinary linamarin was 263 ± 52 µmol/l in the 32 subjects studied in 1989.

Paper II:
Konzo in southern Tanzania

Of the estimated 2,100 inhabitants in the screened village 28 subjects with walking difficulties were identified. Two boys aged 7 and 17 and one woman aged 25 years fulfilled the criteria for konzo. They had an abrupt onset in July to August 1988. In a 5-year-old boy with suspected konzo in a neighbouring village cyanide exposure was estimated in 1988 by determination of thiocyanate levels in the month of onset. The urinary level was 1,730 µmol/l and the serum level 338 µmol/l. One year later the serum level had fallen to 178 µmol/l. The clinical history, symptoms and signs of all the four cases were typical of konzo with an abrupt onset during the drought season. The two older boys were reexamined in 1994 and the spastic paraparesis had remained unchanged.

Paper III:
Cyanogen removal during processing

Key-informants stated that the population was aware that *makopa* should be sufficiently sun-dried before pounding into flour for *ugali*. A brittle sound should be heard on breaking a piece of *makopa* to indicate that it was ready. Depending on size, type of roots and weather, *makopa* were ready for pounding into flour after one or more weeks of sun-drying. However, this principle was not adhered to by poor households during food shortage because they had no other alternative foods to eat. *Chinyanya* processing was known by all village women but they stated that it was only practised during food shortage periods. Processing into small size *makopa* was also practised during favourable years because cassava roots were split into smaller sizes so that they could dry faster.

The experiment carried out to identify critical stages in cyanogen removal from 2 batches showed that the established method of sun-drying longitudinally split cassava roots for *makopa* reduced glucosides within 17 days to 26 and 37% of the initial levels, leaving a glucoside level of more than 100 mg HCN equiv/kg dry weight in flour. The short-cut method of processing *chinyanya* flour within a day, reduced glucoside levels to 7-14% of the levels in fresh roots but *chinyanya* flour had higher *cyanohydrin* levels. In flour from *makopa* batches collected in 31 households the mean ± SD of cyanogenic glucosides was 145 ± 26, of cyanohydrins 17 ± 3 and of hydrogen cyanide 6 ± 0.4 mg HCN equiv./kg dry weight, respectively.

Table 5. Cyanogen levels (mean ± SEM) in fresh roots and flour obtained from 2 batches *makopa* and 3 batches *chinyanya* processing.

	Glucosides	Cyanohydrins	HCN	Moisture %
	(mg HCN equivalent/kg dry weight)			
Makopa 1				
Fresh roots	1039	35	16	60.3
Flour	383	8	10	11.1
Makopa 2				
Fresh roots	462	26	5	71.4
Flour	123	5	4	11.0
Chinyanya 1				
Fresh roots	1518	28	7	60.3
Flour	216	56	4	19.4
Chinyanya 2				
Fresh roots	463	26	5	71.4
Flour	41	23	3	9.7
Chinyanya 3				
Fresh roots	484	22	7	62.0
Flour	35	37	3	12.2

Note ( ) = Range

Paper IV:
Cyanide exposure from cassava in northern Tanzania

Key informants, focus groups and household interviews in the konzo-affected villages consistently revealed that food shortage forced people to shorten cassava-processing methods in 1984/85.

In the konzo-affected inland village, it was stated that short cuts were also due to intensive sale of cassava. People in most households, including families who were affected with konzo exclusively consumed *ugali* prepared from insufficiently processed cassava roots. In favourable years cassava was usually mixed with cereals such as sorghum and millets before milling into flour. During the food shortage period, no cereal was available and cassava flour was processed from young immature roots.

The established method of processing cassava into *udaga* includes making a starter by fermenting a few split peeled roots in a covered bowl inside the house for two nights during which they became covered with mould. The mouldy roots were mixed with a bigger batch of freshly harvested peeled roots and crushed together on a rock with a wooden mallet into pieces of 0.5 to 2.0 cm in size. The pieces were subsequently heaped, pressed with large stones and left to ferment for two nights. The pieces were re-mixed and left for a second fermentation period of one to five nights. The outer greyish mould layer which grew on the heap was removed before further crushing of the pieces on a flat stone. The lot was dried on a rock or mat and the final *udaga* product could be stored, sold or pounded into flour when required. During the food shortage period short-cuts were made in both fermentation and sun-drying before pounding.

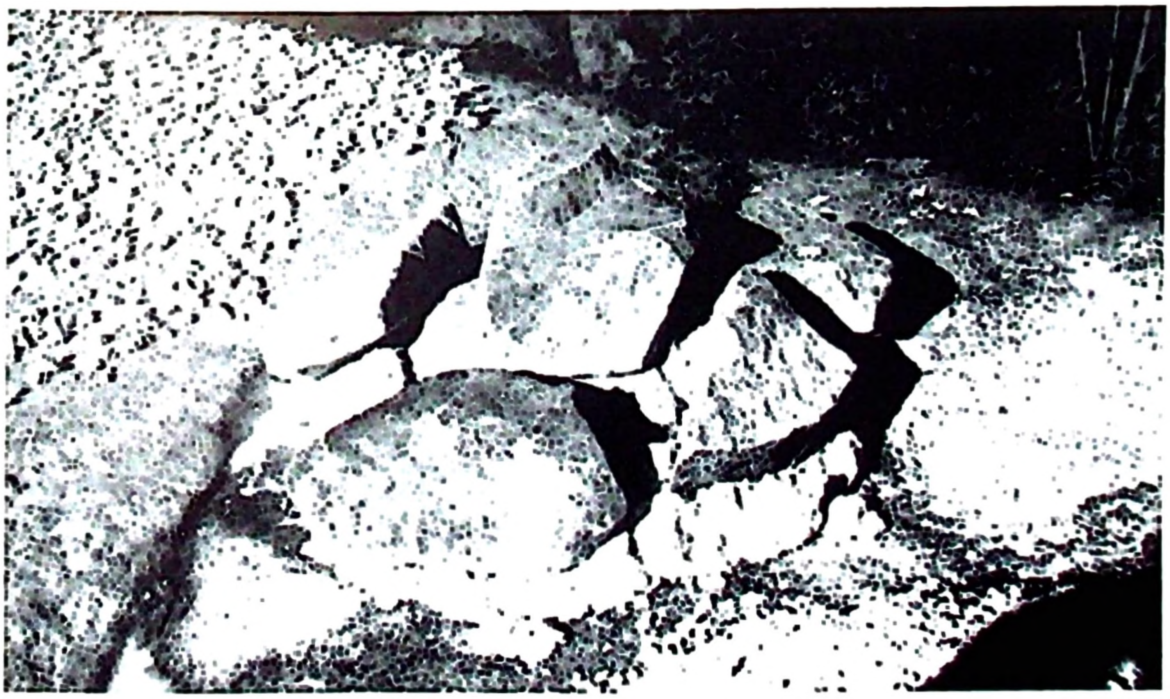

Figure 6. Udaga fermentation in Nyambori village, Tarime district.

The food consumption frequency at the end of the dry period in 1989 showed that cassava was the main staple consumed daily as *ugali* in almost all households in both villages (96-100%). Fish was the most frequently eaten protein-rich food in both villages. In the affected village 33% (95% confidence interval 25-41%) rarely or never consumed fish. This is statistically significantly different from the shore village where no one consumed fish at such low frequency (95% confidence interval 0-14%). The interviews did not estimate amounts consumed, but information from other sources indicated that the lower frequency of fish consumption in the inland village was also associated with smaller amounts consumed.

The household interviews in 1991 revealed that the un-affected shore village had a diversified economy and fish sales as a major source of income. Household incomes in the konzo-affected village were derived mainly from cassava sales (92%), whereas few households (14%) in the shore village sold cassava.

The cassava-processing experiment in the konzo-affected village showed that the established method of fermenting crushed cassava roots, effectively reduced levels of cyanogenic glucosides but significant amount of cyanohydrins remained in the flour even after four nights fermentation. Cyanohydrin levels (mean ± SEM) were higher in flour from short-cut processed roots fermented for one night, 57 ± 6 mg HCN equiv/kg dry weight compared to 36 ± 7 from those fermented for four nights ($p=0.04$). Glucoside levels were 13 ± 6 in one night fermentation and 3 ± 1 in the four nights. Hydrogen cyanide levels were 5 ± 1 and 3 ± 1 mg HCN equiv/kg dry weight in one and four nights respectively.

The mean ± SEM urinary thiocyanate level in 93 subjects from the inland village studied in April 1989 was 490 ± 48 µmol/l, the urinary inorganic sulphate level was 3,802 ± 369 µmol/l and mean ± SEM urinary ratio of thiocyanate/sulphate was 0.24 ± 0.03. In the non-affected shore village 33 subjects had a urinary thiocyanate level of 344 ± 39 µmol/l (not significantly different) while inorganic sulphate levels were 7,038 ± 855 µmol/l ($p < 0.001$), thiocyanate/sulphate ratio 0.07 ± 0.01($p < 0.001$).

Paper V:
Milling reduces the goitrogenic potential of cassava

Key-informants and focus group discussions revealed that the importance of cassava had grown over the last decades in Kigoma district, especially after the introduction of bitter varieties. Consistent information indicated the existence of three established processing methods and one short-cut method. In *nyange* processing the peeled roots were heaped together and covered with straw, cassava peels or leaves to ferment for 2-4 days depending on temperature conditions. After being covered by brown or orange mould the roots were sun-dried (figure 7). The second method was *kivunde* processing which included 3-5 days' soaking and 5-7 days' sun-drying.

The *lowe* method was said to be losing popularity because it was cumbersome. It involved two boiling steps and a lengthy period of pounding the wet mash in elongated wooden trays after boiling. Fist-sized portions of the mash obtained were wrapped in banana leaves and should be eaten within 1-2 days.

Figure 7. Nyange product in Kigoma district, western Tanzania.

A short-cut processing method yileded a product known as *mhondano,* which was considered not appropriate to consume. All groups declared that *ugali* from *mhondano* flour could induce acute intoxications within hours of consumption. No one admitted having used it and the method was embarrassing to discuss since it was practised by poor households during food shortage.

Food frequency interviews with 217 women revealed that cassava flour *ugali* was consumed twice per day by 81% of the households (table 6). Low urinary thiocyanate was statistically associated to frequent milling of cassava flour but not to any other of the practices presented in table 6. The association of low thiocyanate levels with milling did not disappear in multiple logistic regression analysis.

The experiment carried out on the effect of milling versus pounding showed a minor but statistically significant difference in cyanohydrin levels between the two procedures. The cyanohydrin levels (mean ± SEM) were 8.2 ± 2.0 and 11.2 ± 2.5 mg HCN equiv/kg dry weight in milled and pounded flours, respectively. Glucoside levels were 35.8 ± 7.5 in milled and 37.0 ± 8.1 in pounded. Hydrogen cyanide levels were 7.9 ± 0.4 and 7.3 ± 0.5 mg HCN equiv/kg dry weight in milled and pounded flours, respectively.

Table 6. Proportion of 217 women with low and high urinary thiocyanate in relation to cassava consumption, processing method, use of short-cuts and milling, respectively.

	Urinary thiocyanate Low <100 µmol/l	Urinary thiocyanate High ≥100 µmol/l	Total	p-values
Cassava consumption				
Once daily or less	27 (64%)	15 (36%)	42	n.s.
Twice daily	107 (61%)	68 (39%)	175	
Main processing method				
Nyange	96 (59%)	67 (41%)	163	n.s.
Others	38 (70%)	16 (30%)	54	
Use of short-cuts				
Sometimes	24 (60%)	16 (40%)	40	n.s.
Never	110 (62%)	67 (38%)	177	
Flour obtained by milling				
Often	61 (74%)	21 (26%)	82	p<0.01
Sometimes or never	73 (54%)	62 (46%)	135	
Total	134	83	217	

p - values calculated by Chi-square test

The total goitre rate in 217 women aged 15-45 years was 73% while visible goitre was 13%. As shown in table 7, the majority of women, 185 (85%) had urinary iodine levels below 10 µg/dl. The mean urinary iodine level was 5.6 µg/dl and the range was 0.8-30.5 µg/dl. The mean urinary thiocyanate level was 128 µmol/l and the range was 0-2,080 µmol/l. A total of 42 women had a urinary I/SCN ratio below 3 µg/mg. The mean I/SCN ratio was 26.3 µg/mg while the median was 9.4. Subjects with urinary iodine/thiocyanate ratios below 3 µg/mg had significantly more goitre.

Table 7. Relationship between goitre and high and low levels of urinary iodine, thiocyanate and iodine/thiocyanate ratio in 217 women from Kigoma district.

	No goitre	Goitre	Total	Chi-test
Urinary iodine				
High ≥ 10 µg/dl	14 (23%)	18 (12%)	32	< 0.05
Low < 10 µg/dl	46 (77%)	139 (88%)	185	
Total	60 (100%)	157 (100%)	217	
Urinary thiocyanate				
High ≥ 100 µmol/l	20 (33%)	63 (40%)	83	n.s.
Low < 100 µmol/l	40 (67%)	94 (60%)	134	
Total	60 (100%)	157 (100%)	217	
I/SCN ratio				
High ≥ 3 µg/mg	54 (90%)	122 (78%)	176	n.s.
Low < 3 µg/mg	6 (10%)	35 (22%)	41	
Total	60 (100%	157 (100)	217	

Paper VI: Low cyanide exposure from cassava in Dar-es-Salaam

Cyanogen levels in flour obtained from *makopa* products sampled from food markets in six suburbs of Dar-es-Salaam were low. Mean ± SEM total cyanogens was 9.4 ± 1.5 mg HCN equiv./kg dry weight. Glucosides and hydrogen cyanide had a mean of 8.2 and 3.2 mg HCN equiv./kg dry weight respectively, while cyanohydrins were lower, with a mean of 2.2 mg HCN equiv./kg dry weight.

Interviews with 193 school children aged 10-18 years revealed that the frequency of consuming cassava *ugali* in the suburbs was low i.e. 13% consumed cassava *ugali* at least once a week. Fried cassava pieces, *chips dume* in the streets or at school was most frequently consumed by the children; 17% consumed it daily, 43% several days per week and 22% once a week. Boiled cassava pieces were consumed for breakfast or as a snack between meals at least once a week by 49% of the children. Other cassava products such as cassava leaves and raw root pieces were not commonly consumed.

Urinary thiocyanate levels in the 193 children ranged from 0 to 177 µmol/l with a mean ± SEM of 36 ± 3 and a median of 26. There was an increase in thiocyanate levels with increased consumption of boiled cassava pieces. Multiple regression analysis revealed a statistically significant association between consumption of boiled cassava pieces and urinary thiocyanate levels ($p < 0.001$). It could be shown that each meal of boiled cassava pieces increased the levels of thiocyanate in urine by 6.7 µmol/l. There was no statistically significant correlation between urinary thiocyanate levels with consumption of other cassava foodstuffs.

Linamarin levels in urine ranged from 5 to 45 µmol/l and the mean ± SEM was 18 ± 1 while the median was 15. There was a positive correlation between linamarin levels and consumption of fried cassava pieces at 95% confidence intervals. Each meal of fried cassava pieces increased the levels of linamarin in urine by 1.5 µmol/l. Other cassava foodstuffs had no statistically significant effect on linamarin levels in urine.

DISCUSSIONS

If the food security provided by cassava is to have an optimal health impact, the nutritional drawbacks of cassava must be avoided or minimized. The results of this thesis indicate that cyanogenic glucosides in roots of bitter and toxic cassava varieties can be considerably reduced through processing, but effectiveness varies between different methods. Where less effective processing methods are used, toxicity may be avoided by exclusive cultivation of non-bitter varieties with low glucoside levels in the fresh roots. Hence the effectiveness of the processing technique needs to be related to the type of cassava cultivars grown in each area of a country. The studies in four widely separated cassava growing areas of Tanzania showed that there is a wide variation of the types of processing methods used within a country. The findings further indicate that the use of short-cuts in cassava processing due to food shortage seems to be the main determinant for high dietary cyanide exposure from cassava, rather than the type of processing method in regular use. The findings show a similar chain of events from food insecurity in poor households, short-cuts in cassava-processing methods, high residual levels of cyanogens in the flour consumed to dietary cyanide exposure, as reported from other parts of Africa (Banea 1993, Essers et al. 1992, Tylleskär et al. 1994). Diseases attributed to cassava toxicity seem to appear in very poor rural communities during periods with severe agricultural and economic problems. The studies in Tanzania were not based on representative samples from large cassava producing regions and therefore the results cannot be used to quantify the problem in the whole country. However, it can be concluded that many cassava growing regions of Tanzania are at risk of toxic effects of cassava and that prevention is feasible through promotion of effective processing and introduction of mechanical milling.

Methodological limitations

The qualitative survey methods used in the studies were useful because the cassava-processing practices could not be directly investigated through structured interviews. The reason was that many of these practices were unknown to the investigator at the start of the studies, especially the changes in cassava processing that were induced by drought and food shortage. An additional advantage was due to the fact that deviations from traditional processing methods was a taboo. The easily recognised embarrassment that was induced by asking individual women if they made short-cuts in processing of cassava indicated that answers to structured questions on this topic had a low validity. However, when asking groups of women about general practices in the community followed by probing about details, a much more coherent information was obtained on the use of short-cuts. As judged from women's reactions the information obtained from unstructured interviews and other qualitative methods was perceived as more valid . To avoid leaving out certain classes of people in the community, the focus group members were selected jointly with the village leaders and the survey team. However, it was sometimes found during focus group interviews that village leaders could hide sensitive information by influencing the discussions.

The limitations of qualitative interview methods regarding lack of representativeness were partly reduced by triangulation with several methods and partly by structured interviews with a sample of representative households. The number of people in the focus groups often spontaneously increased beyond 10 members and made discussions difficult to handle. Precautions were always taken to include poor households suffering from food shortage. Women were often interviewed in separate groups so that they could speak freely. Participatory observations combined with spontaneous discussions gave a chance of rapid learning about processing in the villages. When spending the night in the village many issues not discussed in the daytime were disclosed in the night and illegal trading of cassava could be informally observed. Key-informant and focus group interviews were mainly used in these studies to elucidate processing practices for design of questionnaires and community-based processing experiments. A more extensive use of anthropological methods was not possible in the different surveys but would undoubtedly have yielded a deeper, and perhaps different, understanding of the determinants of cassava processing.

The study populations statements about toxic effects of short-cuts in processing were confirmed by chemical determination of cyanogens in cassava flour from controlled food chemistry experiments and by use of biochemical markers of cyanide exposure. It is the coherent findings from this combined use of methods that indicate a causal relationship between short-cuts in cassava processing and dietary cyanide exposure.

The chemical analysis used required careful handling of both flour and urine specimens. It was found that cassava flour had to be cooled immediately on collection and frozen within hours to avoid further degradation of cyanogens during transport.

Similarly samples for thiocyanate determination were cooled and transported and stored frozen prior to analysis. Thiocyanate is relatively stable in urine, and it is a good indicator for dietary cyanide exposure because thiocyanate is the main cyanide metabolite. However, thiocyanate intake from foods such as cabbage and milk can elevate SCN levels, and cigarette smoking will also result in increased thiocyanate levels (Lundquist et al. 1979). Hence there is no standard reference for urinary thiocyanate levels in humans since the thiocyanate levels depend on the diet consumed (table 8). However, the populations who consumed insufficiently processed cassava had higher thiocyanate levels than smokers, and there is good reason to believe that cassava was the main source of thiocyanate in the populations on cassava-dominated diets. Urinary thiocyanate levels were determined in casual urine due to the logistic difficulties in collecting 24-hour urine for determination of thiocyanate excretion which is a more reliable indicator of the thiocyanate load. The urinary thiocyanate levels are still useful indicators since the range of exposure is so wide.

Table 8. Mean ± SEM urinary thiocyanate levels in population groups with different diets and smoking habits.

Country	Regular smokers	Cassava consumers	Konzo affected	U-SCN µmol/l	Reference
Tanzania	No	Yes	Yes	1120 ± 75	Paper I
Tanzania	No	Yes	Yes	490 ± 48	Paper IV
Tanzania	No	Yes	No	128 ± 13	Paper V
Tanzania	No	Yes	No	36 ± 3	Paper VI
Tanzania	No	No	No	7 ± 1	Paper I
Mozambique	No	Yes	Yes	1175 ± 130	Casadei et al.1990
Mozambique	No	No	No	8.9 ± 2.7	Lundquist et al. 1983
Zaire	No	Yes	Yes	757 ± 85	Tylleskär et al. 1992
Zaire	No	Yes	No	50 ± 7	"
Cuba	No	No	No	12 ± 2	Hemandez et al.1995
Cuba	No	Yes	No	22 ± 2	"
Cuba	Yes	No	No	119 ± 7	"
France	No	No	No	27 ± 2	Pré and Vassy 1991
France	Yes	No	No	146 ± 10	"
Sweden	No	No	No	31 ± 4	Tylleskär et al.1992

Cyanogen removal during processing

Studies by several workers have shown that cyanogens are reduced to negligible levels by processing methods such as grating and soaking, followed by sufficient heating or sun-drying. All procedures leading to tissue disintegration without heating lead to release of the endogenous linamarase enzyme and result in glucoside hydrolysis. Glucoside removal can also be enhanced by direct leaching into the soaking water (Nambisan and Sundaresan 1985, Vasconcelos et al. 1990, Hahn 1989).

It has been observed that fermentation in air is not as effective as that in water (Mahungu et al. 1987) but heap fermentation of whole or split roots studied in Uganda showed that the process was significantly more effective than sun-drying alone (Essers et al. 1994). The present studies support earlier findings that all processing methods to which cassava is subjected rapidly remove the hydrogen cyanide yielded, and the remaining cyanogens are mainly cyanohydrins and glucosides (Coursey 1973, Cooke and Maduagwu 1978, El Tinay et al. 1987, Ayernor 1985, Ezeala and Okoro 1986).

The direct sun-drying used in southern Tanzania is not effective in reducing cyanogens. The reasons is most probably that it does not achieve sufficient cell disintegration to bring the linamarase enzyme into contact with the glucosides to achieve hydrolysis. The breakdown of the cyanohydrins formed can be achieved by sufficient drying, and consequently flour from well-dried *makopa* contained low levels of both hydrogen cyanide and cyanohydrins, whereas considerable amounts of glucosides remained in the flour. When processing methods were shortened during food shortage the products were not sufficiently sun-dried which resulted in high remaining amounts of cyanohydrins. Such a situation happened in southern Tanzania during *chinyanya* processing. Pounding of fresh roots in a mortar followed by alternate sun-drying and repounding gave a product with high cyanohydrin levels. Slicing of roots causes minimum tissue damage. When followed by rapid drying it results in high retention of glucosides, probably because the linamarase enzyme is rapidly inactivated during the drying process as was demonstrated by the experiments. This was what happened when the small size *makopa* was processed in southern Tanzania.

It was found that milling of cassava pieces resulted in similar cyanogen levels as pounding. The lower cyanide exposure found in women who frequently milled their cassava can be explained by another factor. The use of hammer mills requires sufficient drying whereas flour can be produced from wet cassava pieces by pounding at home. The use of mechanized milling requires well-dried cassava products and these will have low cyanohydrin levels. Hence during cassava processing completeness of root disintegration before sun-drying determines glucoside removal, whereas cyanohydrin removal seems to mainly depend on completeness of subsequent sun-drying to lower moisture levels of less than 13%.

Cassava processing in Tanzania

Many processing methods practised in Tanzania are not effective in reducing cyanogens in roots from bitter cassava varieties with high initial glucoside levels. In few areas soaking was praised, but many cassava growing areas of the country have long dry seasons when water is not sufficient for soaking of cassava roots.

The studies in southern Tanzania showed that sun-drying peeled cassava roots to *makopa* products, reduced initial cyanogenic glucosides by about 70%. The final products contained ten times the safe levels of 10 mg HCN equiv./kg dry weight recommended by the Codex Alimentarius Committee of the FAO/WHO (1991).

The heap fermentation practised in western Tanzania is an improvement compared to direct sun-drying with regard to cyanogen removal (Essers et al. 1995). The reason is probably that moisture is maintained and that mould growth helps to disintegrate the cells. The crushing of peeled roots with a wooden mallet followed by fermentation in heaps as observed in the study in northern Tanzania is a further improvement because the hammering will further disintegrate the cells. It was also found that glucosides were reduced to low levels by this method. One may regard these methods as a gradual improvement of the cell disintegration phase of processing, leading to the careful grating of fresh roots before fermentation that was developed in tropical America thousands of years ago. This is the same principle as in *gari* processing in West Africa where mechanical graters are now used in many villages. Hence there is a need to advocate cassava grating in all those areas where various forms of heap fermentation is practised. The type of dewatering in Tarime district, by pressing crushed pieces on rocks using huge stones during fermentation would have been more effective if flat wooden logs were used to press the crushed cassava pieces collected together in a sack. This is an improved water expression procedure which could result in a product with minimum cyanogens.

The study in Dar-es-Salaam showed that roots from non-bitter varieties of cassava was eaten in the city and thereby most probably had relatively low glucoside levels. The traders and farmers selected non-bitter cassava varieties which had high demand in the urban market. Processing of these roots by the relatively ineffective sun-drying method yielded flour with low cyanogen levels. The low frequency of consuming cassava further contributed to low cyanide exposure in the city suburbs.

Short-cuts or deviations from established cassava-processing methods were found to be the main cause of cyanide exposure in two of the studied areas. The underlying cause of the short-cuts were food shortages caused by drought. In most areas incomplete sun-drying determined the residual cyanogens remaining since the products did not attain the required moisture level. The manner in which the final product was prepared and stored also influenced the exposure.

Mechanisms of dietary cyanide exposure

High dietary cyanide exposure from cassava was found in populations making short-cuts in the established cassava-processing methods. The short cuts were triggered by food shortage due to drought, crop failure and commercialization of cassava. The insufficiently processed products either contained intact glucosides and also cyanohydrins, whereas the levels of hydrogen cyanide were low.

The studies could not verify which of these cyanogens was the main source of cyanide but some findings indicate that the residual cyanohydrins may have been the most important source of dietary cyanide exposure. The year after the outbreak of intoxications in southern Tanzania the population were consuming cassava flour with ten times more glucosides per kg dry weight(100 mg HCN equivalents of glucosides per kg dry weight) than is considered safe level by Codex Alimentarius. Cyanohydrin levels were low and low urinary thiocyanate indicated a low dietary cyanide exposure. Secondly, the short-cut product *chinyanya*, to which the population attributed the intoxications, did not contain higher levels of glucoside but several times more cyanohydrins than the flour regularly consumed. The type of cassava flour that was associated with high cyanide exposure in northern Tanzania also contained high amount of cyanohydrins and relatively low amounts of glucosides.

Ingestion of linamarin will cause cyanide exposure if suitable microbial ß-glucosidases occur in the gut or if active linamarase is ingested together with linamarin. Animal studies have shown that a substantial proportion of ingested linamarin is absorbed from the gut and excreted unchanged in the urine (Barret et al. 1977). The finding of relatively high urinary linamarin levels in subjects from southern Tanzania and from the area studied in the northern part of the country (Carlsson et al. 1995) supports the hypothesis that this can also happen in humans. An enzyme responsible for hydrolysis of linamarin in the human gut has not yet been identified. Hence, although linamarin is a very stable compound that resists boiling, dietary cyanide exposure can be considerably lower than that expected from the amount of cyanogenic glucosides in the food products (Hernandez et al. 1995).

Cyanohydrins are relatively unstable, but most of the cyanohydrins in cassava flour have been found to remain in the stiff porridge that is made by rapidly mixing flour with boiling water taken off the fire (Tylleskär et al. 1993). Ingested cyanohydrins were expected to cause cyanide exposure because they are acid-stable and decompose above pH 5, releasing HCN (Formunyam et al. 1985). Little is known about the fate of different forms of cyanogens during digestion in humans, but with the alkaline pH in the small intestine, the cyanohydrins will rapidly decompose to yield cyanide that is absorbed, causing the exposure.

The study in Dar-es-Salaam showed that higher urinary thiocyanate was associated with consumption of boiled cassava pieces while higher linamarin levels were associated with consumption of fried pieces. This suggests that short-boiling of pieces may result in residual linamarase activity in the products that can release cyanide in the gut from the ingested glucosides. In contrast the frying may destroy the enzyme but the glucoside will remain and to a great extent pass the body unchanged.

Effects of dietary cyanide exposure

The acute intoxications which occurred in epidemic proportions in Masasi district in 1988 were probably caused by dietary cyanide exposure, but the evidence is only indirect since no blood cyanide levels were measured at the time of intoxications. In fact only two cases of acute cassava poisoning with measured blood cyanide have been possible to find in the literature (Akintowa and Tunwashe 1992). All cases interviewed linked to intoxications to consumption of short-cut processed cassava and the symptoms were compatible with cyanide intoxication. However, the symptoms of acute cyanide toxicity are not specific for this toxin. The levels of urinary thiocyanate found during the period of intoxications, more than 1100 µmol/l and serum thiocyanate above 300 µmol/l corresponded to a daily intake of 1-2 mmol of cyanide. This corresponds to the range where symptoms of acute intoxication is expected to occur. An interesting observation was that very few deaths could be documented in spite of many thousands of cases of acute intoxications. This may suggest that most of the intoxications are due to an acute but non-lethal intoxication of linamarin rather than hydrogen cyanide. There is a need for careful laboratory investigations of a number of subjects with acute cassava poisoning to verify that cyanide is the main toxic agent. Appropriate analytical methods are available for collection of blood for cyanide analysis even in remote health facilities (Lundquist et al. 1985, 1989)

The konzo outbreak in northern Tanzania has in earlier studies been associated with cyanide exposure (Howlett et al. 1990, 1992). The same association has been found in Mozambique, Zaire and the Central African Republic. Although the main aim of this thesis was to study the causes of dietary cyanide exposure, there are two observations that support a causal role of cyanide exposure in konzo. Firstly it was possible to identify 4 cases of konzo in the area in southern Tanzania with known high dietary cyanide exposure and very high cyanide exposure in the month of onset could be verified in one of these cases. Secondly there was a statistically significant lower urinary thiocyanate/sulphate ratio in the konzo-affected village in northern Tanzania compared to the neighbouring non-affected village on the shore of Lake Victoria. Paper IV also reports the same chain of events behind the konzo outbreak as has been found in all other foci of the disease, drought caused a food shortage which triggered short-cuts in the established cassava-processing methods. The short-cuts resulted in high levels of cyanohydrins in cassava products that probably caused the cyanide exposure associated with the konzo outbreak. It should be noted that the study was done in the same season of the year as the main epidemic and that sporadic cases of konzo still occurred in the affected village (Howlett et al. 1992). Low intake of protein-rich foods supplying sulphur needed for cyanide detoxification may thus have contributed to a high biologically effective dose of cyanide.

Milling and thiocyanate load in Kigoma district. Thiocyanate load resulting from dietary cyanide exposure from cassava has been implicated in aggravating Iodine Deficiency Disorders (IDD). Although a slight association was found between the urinary iodine/SCN ratios, the goitre frequency can be fully explained by the iodine deficiency as measured by urinary iodine levels. The most interesting finding was that frequent milling was associated to low urinary thiocyanate. The reason for this is believed to be that milling forced the women to sun-dry cassava sufficiently in order to be accepted by the mill owners to avoid clogging the mill and this secured removal of cyanohydrins from cassava products. Milling may therefore have contributed to decrease or eliminate a possible goitrogenic effect of thiocyanate. However, iodine supplementation is the main priority and sufficient iodine can counteract the suggested effect of thiocyanate load from cassava on the thyroid gland.

Prevention of dietary cyanide exposure

Diseases attributed to cyanide exposure from cassava have mainly been reported from populations in Sub-Saharan Africa. The exposure has been precipitated by the epidemics of acute poisonings and konzo, where the diets of affected populations before outbreak consisted of insufficiently processed cassava. The short-cuts practised by the rural communities when processing cassava were induced by food shortage. It has been argued that the underlying cause for the outbreaks of konzo and acute intoxications reported in Africa was food insecurity and rural poverty. These were triggered by an agro-ecological deterioration and rapid population growth coupled with natural disasters such as floods and drought which created a market demand for cassava. Thus improving the rural economy, production, diversification and accessibility to food would serve as a first step to avoid toxic effects from bitter cassava.

The potential toxicity of cassava is very well known to cassava-growing populations and though cassava roots may contain high levels of cyanogenic glucosides, most of them can be eliminated by effective processing. Sometimes certain advice is given to farmers but are not feasible to implement, such as growing of low cyanogen cultivars, carrying out of effective processing such as soaking and consumption of a balanced diet to supply essential sulphur amino acids needed for detoxification. The farmers often have strong preferences for bitter varieties. The advantages linked to toxicity could be protection against predators, theft and against certain cassava diseases. Advising farmers in a rural community to balance their diets might seem impractical because during food shortage there is no variety of foods for anyone to balance the diet.

A feasible way to avoid cyanide exposure appears to be promotion of effective processing methods in areas where they are unknown. The effectiveness of cyanogen removal varies among the cassava processing methods and their suitability at a certain location. Before introducing a new processing technology in an area, socio-economic and environmental factors should be considered; otherwise the new method might not be accepted or can be rejected. When promoting effective processing methods precautions have to be taken to distinguish between modification of the established processing methods or introducing new and effective ones. The new methods should be a complement to the other and not replacement of the established ones. For example the processing method practiced in northern Tanzania needs minor modifications in some stages such as grating the roots instead of crushing and dewatering by improved presses instead of using huge stones. Fermentation should be done in sacks rather than heaping the pieces on rocks. Hence, in some areas in Tanzania, advocacy of effective cassava processing should be to advise people to strictly adhere to their established processing methods (Banea 1993) and introduction of minor modifications.

The success of preventing cyanide exposure in communities will depend on the approach used to get the message through to the people. Proper and adequate health and nutrition education and technology on cassava processing and its effects on human health should be introduced and run parallel with the mentioned interventions. Awareness should be created to the public so that they can perceive the problem and accept changes or decide on their own what solution or technology should be adopted. With community participation the problem which is afflicting more rural communities than the urban ones could be solved.

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REFERENCES

Akintonwa A, Tunwashe OL. Fatal cyanide poisoning from cassava-based meal. *Hum Exp Toxicol* 1992;11:479.

Amey MA. Some traditional methods of cassava conservation and processing in Uganda. Paper presented at the Third East and Southern African Root Crops Workshop; Dec 1987; Mzuzu, Malawi.

Ankrah EK. Riboflavin content of some fermented foods of Ghana. *Ghana J Agric Sci* 1972;9:95-8.

Ansell M, Lewis FAS. A review of cyanide concentrations found in human organs. *J Forensic Med* 1970;**17**: 148-55.

Ayernor GS. Effects of retting cassava on product yield and cyanide detoxification. *J Food Technol* 1985;**20**: 89-96.

Banea M. Cassava processing, dietary cyanide exposure and konzo in Zaire [Master thesis]. Uppsala, Sweden: Uppsala University, 1993.

Barnett HIM. Jackson MV, Spaulding WB.Thiocyanate psychosis. *JAMA* 1951;**147**:1554-8.

Barret MD, Hill DC, Alexander JC, Zitnak A. Fate of orally dosed linamarin in the rat. *Can J Physiol Pharmacol* 1977;**55**:134-6.

Basch CE. Focus group interview: An underutilized research technique for improving theory and practice in health education. *Health Educ Q* 1987;**14**:411-48.

Bellenger DN, Bernhardt KL, Goldstucker JL. Qualitative Marketing Research. Chicago: American Marketing Association, 1976.

Bingham SA, Nelson M. Food consumption and nutrient intake. In: Margetts BM, Nelson M, eds. Design Concepts in Nutritional Epidemiology. New York: Oxford University Press, 1991: 153-71.

Bokanga M. Microbiology and Biochemistry of cassava fermentation [Doctoral thesis]. Ithaca, USA: Cornell University, 1989.

Bokanga M. The truth about cyanide in cassava: Is it bitter? *IITA Research* 1993;**6**:24-5.

Bokanga M, Ekanayake IJ, Dixon AGO. Genotype-environment interactions for cyanogenic potential in cassava. *Acta Horticulturae* 1994;**375**:131-9.

Bontinck F. L'autobiographie de Hamed ben Mohammed el-Murgebi Tippo Tip (1840-1905). Acadėmie Royale des Sciences d'Outre - Mer. Classe des Sciences Morales et Politiques. Brussels: Academic, 1974;Vol XLII-4:304.

Bourdoux P, Delange F, Gerard M, Mafuta M, Hanson A, Ermans AM. Evidence that cassava ingestion increases thiocyanate formation: A possible etiological factor in endemic goitre. *J Clin Endocrinol Metab* 1978;**46**:613-21.

Bourdoux P. Measurement of iodine in the assessment of iodine deficiency. *IDD Newsletter* 1988;**4**(1):8-12.

Brimer L, Rosling H. Microdiffusion method with solid state detection of cyanogenic glucosides from cassava in human urine. *Food Chem Toxicol* 1993;**31**:599603.

Bureau of Statistics. Tanzania National Census 1988. Dar-es-Salaam, Tanzania: Planning Commission, Preidents Office, 1990.

Cameron M, Hofvander Y. Manual on feeding infants and young children. Oxford: Oxford University Press, 1983.

Cameron M, Staveren WA. Manual on methodology for food consumption studies. New York: Oxford University Press, 1988.

Carlsson L, Mlingi N, Ronquist G, Rosling H. A specific and sensitive method for the determination of linamarin in urine. *Natural Toxins* 1995. In Press.

Carter S, Fresco L, Jones P, Fairbairn J. An atlas of cassava in Africa. Historical, agroecological and demographic aspects of crop distribution. Cali, Colombia: CIAT, 1992.

Casadei E, Cliff J, Neves J. Surveillance of urinary thiocyanate concentration after epidemic spastic paraparesis in Mozambique. *J Trop Med Hyg* 1990; 93:257-61.

Casati G. Ten Years in Equatorial Africa and return with Emin Pasha. Vol II. New York: Negro University Press, 1990.

Chambers R. Rapid but relaxed and participatory rural appraisal: Towards applications in health and nutrition. In: Scrimshaw NS, Gleason GR, eds. Rapid Assessment Procedures - qualitative methodologies for planning and evaluation of health related programmes. Boston (MA): International Nutrition Foundation for Developing Countries (INFDC), 1992.

CIAT. Cassava: The latest facts about an ancient crop. Cali, Colombia: CIAT, 1993.

Clark AJ. Report on effects of certain poisons in food plants of West Africa upon health of native races. *Trop Med Hyg* 1936;39:269-85.

Clark A. Aetiology of pellagra and allied nutritional diseases. *W Afr Med J* 1935;8:7-9.

Cliff J, Lundquist P, Rosling H, Sörbo B, Wide L. Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. *Acta Endocrinol* 1986;32:186-9.

Cliff J, Lundquist P, Mårtensson J, Rosling H, Sörbo B. Association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis. *Lancet* 1985;ii:1211-3.

Cock JH. Cassava: New potentials for a neglected crop. Colorado, USA: Praeger FA, Westview press, 1985.

Conn E. Cyanogenic glucosides. *J Agric Food Chem* 1969;17:519-526.

Conn E. Cyanogenesis - A personal perspective. *Acta Horticulturae* 1994;375:29-41.

Cooke RD, Howland AK, Hahn SK. Screening cassava for low cyanide using an enzymatic assay. *Exptl Agric* 1978a;14:367-72.

Cooke RD, Maduagwu EN. The effects of simple processing on the cyanide content of cassava chips. *J Food Technol* 1978;13:299-306.

Cook T, Reichardt CS. Qualitative and quantitative methods in evaluation research. Beverly Hills (CA), USA: Sage Publications, 1979.

Coursey DG. Cassava as food: Toxicity and technology. In: Nestel B, MacIntyre R, eds. Chronic cassava toxicity. Ottawa: IDRC, 1973: 27-36.

Crooks J, Aboul-Khair SA, Turnbull AC, Hytten FE. The incidence of goitre during pregnancy. *Lancet* 1964;ii:334.

de Bruijn GH. Etude du caractere cyanogenetique du manioc (Manihot esculenta, Crantz). Wageningen, The Netherlands: Veenman & Zonen, 1971.

de Bruijn GH, Fresco LO. The importance of cassava in the world food production. *Netherlands J Agricult Sci* 1989;**37**:21-34.

Delange F, Bastani S, Benmiloud M. Definitions of endemic goitre and cretinism. Classification of goitre size and severity of endemias and survey techniques. In: Dunn JT et al., eds. Towards the eradication of endemic goitre, cretinism and iodine deficiency. Washington: PAHO/ WHO 1986: 373-5.

Delange F. Cassava and the thyroid. In: Gaitan E, ed. Environmental goitrogenesis. Boca Raton, USA: CRC Press 1989: 173-94.

DeMaeyer EM, Lowenstein FW, Thilly CH. The control of endemic goitre. Geneva: WHO, 1979.

Dufour DL. Cassava in Amazonia: Lessons in utilization and safety from native peoples. *Acta Horticulturae* 1994;**375**:175-82.

Dunn JT, Medeiros-Neto GA. Endemic goitre and cretinism: Continuing threats to world health. PAHO Scientific Publication No 292, 1974.

Dunn JT, Van der Haar F. A practical guide to the correction of iodine deficiency. Technical Manual no 3. International Council for control of Iodine Deficiency Disorders. UNICEF & WHO, 1990.

Eggum BO. The protein quality of cassava leaves. *Br J Nutr* 1970;**24**:761.

El Tinay AH, Bureng PL, Yas EAE. Hydrocyanic acid levels in fermented cassava. *J Food Technol* 1987; **19**:197-202.

Espinoza OB, Perez M, Ramirez MS. Bitter cassava poisoning in eight children: A case report. *Vet Hum Toxicol* 1992;**34**(1):65.

Essers AJA, Alsen P, Rosling H. Insufficient processing of cassava induced acute intoxications and the paralytic disease konzo in a rural area of Mozambique. *Ecol Food Nutr* 1992; **27**:17-27.

Essers SAJA, Bosveld M, van der Grift RM, Voragen AGJ. Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *J Sci Food Agric* 1993;**63**:287-96.

Ezeala DO, Okoro N. Processing techniques and HCN content of cassava-based foodstuffs in Nigeria. *J Food Biochem* 1986; **10**:125-32.

FAO. Food composition tables for use in West Africa. Rome: FAO, 1968.

FAO. Utilization of tropical foods: roots and tubers. Rome: FAO, 1989.

FAO. Roots, tubers, plantains and bananas in human nutrition. Rome: FAO, 1990. FAO Food and Nutrition Series, No 24.

FAO/WHO. Joint FAO/WHO food Standards Programme, Codex Alimentarius Commission, XII, Supplement 4. Rome: FAO, 1991.

Favier JC, Chevassus-Agnes S, Gallon G. La technologie traditionnelle du manioc au Cameroun: Influence sur la valuer nutritive. *Ann Nutr Aliment* 1971;**25**:1-59.

Folch-Lyon E, Macora L, Shearer SB. Focus groups and survey research on family planning. *Stud Fam Plann* 1981;**12**:409-32.

Formunyam RT, Adegbola AA, Oke OL. The stability of cyanohydrins. *Food Chem* 1985;**17**:221-5.

Glik D, Gordon A, Ward W, Kouame K, Guessan M. Focus group methods for formative research in child health: An Ivorian example. *Int 'l Quarterly Community Health Education* 1988; 8(4):1987-8.

Gomez E, Valdivieso M. Cassava foliage: Chemical composition, cyanide content and effect of drying on cyanide elimination. *J Sci Food Agric* 1985;36:433-41.

Hahn SK, Keyser J. Cassava: a basic food for Africa. *Outlook Agric* 1985;14:95-9.

Hahn SK, Mahungu NM, Otoo JA, Msabaha MAM, Lutaladio NB, Dahniya MT. Tropical Root Crops - Root crops and the African food crisis. In: Terry ER, Akoroda MO, Arene OB, eds. Proceedings of Third Triennial Symposium of the ISTRC - Africa Branch; Owerri, Nigeria. Ottawa: IDRC 1987;258e:24-9.

Hahn SK. An overview of African traditional cassava processing and utilization. *Outlook Agric* 1989;18:110-8.

Hahn SK. Cassava research to overcome the constraints to production and use in Africa. In: Cassava toxicity and thyroid. Delange F, Ahluwalia R, eds. Ottawa: IDRC, 1983.

Heaver R. Participatory rural appraisal: Potential applications in family planning, health and nutrition programmes. In: Rapid Rural Appraisal (RRA) Notes, Series Number 16, Sustainable Agriculture Programme. London: International Institute for Environmental Development, 1992.

Hernandez T, Lundquist P, Oliveira L, Cristia RP, Rodriguez E, Rosling H. Fate in Humans of dietary intake of cyanogenic glucosides from roots of sweet cassava consumed in Cuba. *Natural Toxins* 1995;3:114-7.

Hetzel BS. The story of iodine deficiency disorders. An international challenge in nutrition. New York: Oxford University Press, 1989.

Howlett WP, Brubaker GR, Mlingi N, Rosling H. Konzo, an epidemic upper motor neuron disease studied in Tanzania. *Brain* 1990;113:223-35.

Howlett WP, Brubaker G, Mlingi N, Rosling H. A geographical cluster of konzo in Tanzania. *J Trop Geograph Neurol* 1992;2:102-8.

Imungi JK. Cassava in Kenya: Consumption, cyanide content and toxicity. Proceedings of the Inter-disciplinary Coference on Natural Toxicants in Food; 1986 Oct 15-18th; Zurich. Zurich: EURO FOOD TOX II.

Jones WO. Manioc in Africa. Stanford: University Press, 1959.

Jonsson U. Potential role of cassava as a weaning food. IITA/ UNICEF Meeting on Cassava. Mar 1986; Ibadan. Dar-es-Salaam, Tanzania: UNICEF, 1989.

Joseph A. Influence de la technologie traditionelle du manioc sur les teneurs en elements mineraux et en phosphore phyitique. *Ann Nutr Aliment* 1973;27:127-39.

Kachondham Y. Rapid rural appraisal and rapid assessment Procedures: A Comparison. In: Scrimshaw NS, Gleason GR, eds. Rapid assessment procedures - qualitative methodologies for planning and evaluation of health related programmes. Boston (MA): International Nutrition Foundation for Developing Countries (INFDC), 1992.

Kavishe FP. Malnutrition in Tanzania: A situational analysis in 1990. Dar-es-Salaam, Tanzania: TFNC, Report No. 1322, 1990.

Kingsley M. Travels in West Africa (1893-1895). 5th ed. London: Macmillan & Co., 1982.

Koch MB, Sibbesen O, Swain E, Kahn RA, Liangcheng D, Bak S, Ann B, Moller BL. Possible use of a biotechnological approach to optimize and regulate the content and distribution of cyanogenic glucosides in cassava to increase food safety. *Acta Horticulturae* 1994;**375**:45-60.

Kojima M, Iwatsuki N, Data ES, Villegas CDV, Uritani I. Changes of cyanide content and linamarase activity in wounded cassava roots. *Plant Physiol* 1983;**72**:186-9.

Kumar K. Conducting key informant interviews in developing countries. AID programme design and evaluation methodology. Report No 13. Washington D.C.: Agency for International Development, 1989.

Kumar K. Rapid, Low-Cost data collection methods. AID programme design and evaluation methodology. Report No 10. Washington D.C.: Agency for International Development, 1987.

Labianca DA. On the nature of cyanide poisoning. *J Chem Educ* 1979; **56**:788-91.

Lancaster PA, Brooks JE. Cassava leaves as human food. *Econ Botany 1983;37:331-48.*

Lancaster PA, Ingram JS, Lim MY, Coursey DG. Traditional cassava based foods: Survey of processing techniques. *Econ Botany* 1982; **36**:12-45.

Ljungqvist BG, Mellander O, Svanberg U. Dietary bulk as a limiting factor for nutrient intake in pre-school children. 1. A Problem description. *J Trop Pediatr* 1981;**27**:481-91.

Lorri WSM. Nutritional and microbiological evaluation of fermented cereal weaning foods [Doctoral thesis]. Göteborg, Sweden: Chalmers University of Technology, 1993.

Lundquist P. Determination of cyanide and thiocyanate in humans[Doctoral thesis]. Linköping, Sweden: Linköping University, 1992.

Lundquist P, Rosling H, Sörbo B. Determination of cyanide in whole blood, erythroccytes, and plasma. *Clin Chem* 1985;**31**:591-5.

Lundquist P, Sörbo B. Rapid determination of toxic cyanide concentrations in blood. *Clin Chem* 1989;**35**:617-19.

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

Lundquist P, Mårtensson J, Sörbo B, Öhman S. The adsorption of thiocyanate by anion exchange resins and its analytical application. *Clin Chem* 1983;**29**:403.

Lundquist P, Mårtensson J, Sörbo B, Öhman S. Turbidimetry of inorganic sulfate, ester sulfate and total sulfur in urine. *Clin Chem* 1980;**26**:1178-81.

Lundquist P, Kågedal B, Nilsson L. An improved method for determination of thiocyanate in plasma and urine. *Eur J Clin Chem Clin Biochem* 1995;**33**:343 9.

MacLennan R, Gaitan E. Measurement of thyroid size in epidemiological surveys. Report of the IV meeting of the PAHO Technical Group on endemic goitre and cretinism. PAHO, 1974: 195-7.

Madeley J. Make way for super cassava. *Ceres* 1993;(140): 2-6.

Mahungu NM, Yamaguchi Y, Almazan AM, Hahn SK. Reduction of cyanide during processing of cassava into some traditional African foods. *J Sci Food Agric* 1987;**1**:11-5.

Makene WJ, Wilson J. Biochemical studies in Tanzanian patients with ataxic neuropathy. *J Neurol Neurosurg Psychiatry* 1972;53: 31-3.

Maletnlema TN. Tanzania: Nutrition and government policy - socialism as a solution. In: Winikoff B, ed. Nutrition and national policy. Cambridge (MA), USA: The MIT Press, 1978.

Ministry of Health, Mozambique. Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in acassava staple area in Mozambique. 1. Epidemiology and clinical laboratory findings in patients. *Bull WHO* 1984;62:477-84.

Mlingi NLV. Nutritional evaluation of fermented cassava flour with special reference to protein quality, iron availability and bulk properties [Master thesis].Uppsala, Sweden: Uppsala University, 1984.

Monekosso GL, Wilson J., Plasma thiocyanate and vitamin B12 in Nigerian patients with degenerative neurological disease. *Lancet* 1966;i:1062-4.

Mosha AC, Svanberg U. Preparation of weaning foods with high nutrient density using flour from germinated cereals. *Food Nutr Bull* 1983;5:10-4.

Nahrstedt A. Cyanogenesis and the role of cyanogenic compounds in insects. In: Evered D, Harnett S, eds. Proceedings of the Ciba Foundation Symposium 140 on Cyanide compounds in biology; 1988 Nar 15-17, London. Chichester, UK: John Wiley & Sons Ltd., 1988: 131-50.

Nambisan B, Sundaresan S. Effect of processing on the cyanoglucosides in cassava. *J Sci Food Agric* 1985;36: 1197-1203.

Nartey F. Biosynthesis of cyanogenic glucosides in cassava. In: Chronic Cassava Toxicity: Proceedings of an interdisciplinary workshop: 1973 Jan 29-30; London. Ottawa: IDRC-010e:73-87.

Nelson M. Assessment of Food consumption and nutrient intake. In: Margetts B, Nelson M, eds. Design concepts in nutritional epidemiology. Oxford: Oxford Medical Publications, 1991.

Nicholls L, Sinclair HM, Jelliffe DB. Tropical Nutrition and Dietetics, p 375. London: Bailliere, Tindall and Cox, 1960.

Nwapa F. Cassava Song. Enugu, Nigeria: Tana Press, 1986.

Nweke F. Collaborative study of cassava in Africa - project description. COSCA Working Paper Series No. 1. Ibadan, Nigeria: IITA, 1988.

Nweke F. Processing potential for cassava production growth in Africa. COSCA working Paper No 11. Ibadan, Nigeria: IITA, 1992.

O'Brien GM, Taylor AJ, Poulter NH. Improved enzymic assay for cyanogens in fresh and processed cassava. *J Sci Food Agric* 1991; 56:277-89.

Oke OL. Eliminating cyanogens from cassava through processing: Technology and Tradition. *Acta Horticulturae* 1994;375:163-74.

Oke OL. Cassava as food in Nigeria. *World Rev Nutr Diet* 1968;9: 227-50.

Okigbo BN. Nutritional implications of projects giving high priority to the production of staples of low nutritive quality. The case of cassava (Manihot esculenta, Crantz) in the humid tropics of West Africa. *Food Nutr Bull* 1980;2:1-10.

Osuntokun BO. Cassava diet, chronic cyanide intoxication and neuropatthy in Nigerian Africans. *World Rev Nutr Diet* 1981; 36:141-73.

Oyenuga VA. Nigeria's foods and feeding stuffs. Their chemistry and nutritive value. 3rd edition. Ibadan, Nigeria: Ibadan University Press, 1968.

Parke TH. My personal experiences in Equatorial Africa as a medical officer of the Emin Pasha Relief Expedition. London: Sampsonlow, Marston & Co., 1891.

Patel MN, Yim GKW, Isom GE. Blockade of N-methyl aspartate receptors prevents cyanide-induced neuronal injury in primary hippocampal cultures. *Toxicol Appl Pharmalcol* 1992;**115**:124-29.

Patton MQ. Qualitative Evaluation and Research Methods 2nd edition. London: Sage Publications, 1990.

Pelto PJ, Pelto GH. Anthropological Research: The structure of Inquiry. New York: Cambridge University Press, 1978.

Pereira JF, Seigler DS, Splittstoesser W. Cyanogenesis in sweet and bitter cultivars of cassava. *Hort Science* 1981;**16**:776-7.

Perez C, Scrimshaw NS, Munoz JA. Technique of endemic goitre surveys. In: Endemic goitre, World Health Organization, Monograph series No 44. Geneva: WHO, 1960: 369-83.

Pettersen J, Cohen S. The effects of cyanide on brain mitochondrial cytochrome oxidase and respiratory activities. *J Appl Toxicol* 1993;**13**:9-14.

Power J. Cassava: Poor man's cinderella. International Herald Tribune 1986 May 7th.

Pré J, Vassy R. Urine thiocyanate: Creatinine ratio as a reliable indicator of cigarette smoking. *Clin Chim Acta* 1991; **204**:87-94.

Rosling H. Measuring effects in humans of dietary cyanide exposure from cassava. *Acta Horticulturae* 1994;**375**:271-83.

Ruddell WSI, Blendis LM, Walters CL. Nitrite and thiocyanate in the fasting and secreting stomach and in saliva. *Gut* 1977;*18*:73-7.

Rutishauser IHE. Factors affecting the intake of energy and protein by Ugandan pre-school children. *Ecol Food Nutr* 1974; **3**:213-22.

Räsänen L. Validity and reliability of recall methods. In: Hautvast JGAJ, Klaver W, eds. The diet factor in epidemiological research. Report 1. Wageningen, The Netherlands: EURO-NUT, 1982.

Sabry ZI, Shadarevian SB, Cowan JW, Campbell JA. Relationship of dietary intake of sulphur amino acids to urinary excretion of inorganic sulphate in man. *Nature* 1965;**206**:931-3.

Sandell EB, Kolthoff IM. Micro-determination of iodine by a catalytic method. *Mikrochim Acta* 1937;1:9-25.

Sauti RFN, Saka JOK, Kunsiya EG. Preliminary communication in research note on composition and nutritional value of cassava - maize composite flour. Paper presented at the Third East and Southern Africa Root Crops Workshop 1987 Dec; Mzuzu, Malawi.

Schulz V. Clinical pharmacokinetics of nitroprusside, cyanide, thiosulphate and thiocyanate. *Clin Pharmacokinet* 1984;**9**:239-51.

Scrimshaw SCM. Adaptation of anthropological methodologies to rapid assessment of nutrition and primary health care. In: Scrimshaw NS, Gleason GR, eds. Rapid Assessment Procedures - qualitative methodologies for planning and evaluation of health related programmes. Boston (MA): International Nutrition Foundation for Developing Countries (INFDC), 1992: 25-37.

Scrimshaw SCM, Hurtado E. Rapid assessment procedures for nutrition and primary health care. Anthropological approaches to improving programme effectiveness. Los Angeles: University of California, 1987;II. UCLA Latin American Centre Publications.

Selmar D. Translocation of cyanogenic glucosides in cassava. *Acta Horticulturae* 1994;375:61-7.

Sennappa M, Mlingi N. Household food security and the role of cassava. A case study from Tanzania. In: Proceedings of the Third Africa Food and Nutrition Congress; 1988 Sep 5-8; Harare, Zimbabwe. Nutrition and Food Security, Vol 2:734-63.

Silvestre P, Arraudeau M. Le Manioc. Paris, France: Maisonneuve et Larose & Agence de cooperation culturelle et technique, 1983.

Singh HB, Wasi N. Detection and determination of cyanide - A review. *Int J Environ Anal Chem* 1986;26:115-36.

Sundaresan S, Nambisan B, Easwari-Amna CS. Bitterness in cassava in relation to cyanoglucoside content. *Indian J Agricult Sci* 1987;57:37-40.

Svanberg U. Dietary bulk in weaning foods [Doctoral thesis]. Göteborg, Sweden: Chalmers University of Technology, 1987.

Swai ABM, McLarty DG, Mtinangi BL, Tatala S, Kitange HM, Mlingi N, Rosling H, Howlett WP, Brubaker GR, Alberti KGMM. Diabetes is not caused by cassava toxicity: a study in a Tanzanian community. *Diabetes Care* 1992;15:1378-85.

Sörbo B. Crystalline rhodanese. *Acta Chem Scand* 1953;7:238-9.

Tanzania Food and Nutrition Centre (TFNC 1992b). The food and nutrition situation in Tanzania: Country paper for the International Conference on Nutrition; 1992, Dec; Rome. TFNC Report No 1445.

Terra GJA. The significance of leaf vegetables especially of cassava in Tropical nutrition. *Trop Geogr Med* 1964;2:97-108.

Trolli G, 1938. Paraplégie spastique épidémique, "Konzo" des indigènes du Kwango. In: Trolli G, ed. Résumé des observations réunies, au Kwango, au sujet de deux affections d'origine indéterminée. Brussels: Fonds Reine Elisabeth, 1938: 1-36.

Tylleskär T, Banea M, Bikangi N, Cooke R, Poulter N, Rosling H. Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. *Lancet* 1992;339:208-11.

Tylleskär T. The causation of konzo: Studies on a paralytic disease in Africa [Doctoral thesis]. Uppsala, Sweden: Uppsala University, 1994.

Tylleskär T, Légué FD, Petersson S, Kpizingui E, Stecker P. Konzo in the Central African Republic. *Neurology* 1994;44:959-61.

van Heijst ANP, Maes RAA, Mtanda AT, Chuwa LMM, Rwiza HT, Moshi NH. Chronic cyanide poisoning in relation to blindness and tropical neuropathy. *Clin Toxicol* 1994;32: 549-56.

Vasconcelos AT, Twiddy DR, Westby A, Reilly PJA. Detoxification of cassava during gari preparation. *Int J Food Sci Technol* 1990;25:198-203.

Watson JD. Riboflavin content of selected plant foods of Ghana. *Ghana J Agric Sci* 1979;9:71-3.

Willet WC, Sampson L, Stampfer MJ. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985;122:51-65.

Willet W. Nutritional epidemiology. Oxford: Oxford University Press, 1990;15:69-91.

Virion A, Deme D, Pommier J et al. Opposite effects of thiocyanate on tyrosine iodination and thyroid hormone synthesis. *Eur J Biochem* 1980;112:1-7.

Vis HL, Vuye A, Hennart P. Serum levels of free amino acids in mothers at delivery, newborns, and adult males. In: Delange F, Iteke FB, Ermans AM. Nutritional factors involved in the goitrogenic action of cassava. Ottawa: IDRC, 1982: 70-3.

von Queisser H. Tödliche Blausäurevergiftungen durch Genuß sußer cassava (Manihot aipi). *Deutsche Gesundheitwesen* 1966; **21**: 726-8.

Young L, Maw GA. The metabolism of sulphur compounds. London: Methuem 1958.