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CASSAVA SAFETY: LESSONS FROM AN INTERDISCIPLINARY WORKSHOP

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Abstract

To review the state of knowledge on cyanide issues in cassava, the International Workshop on Cassava Safety was held at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, during 1-4 March 1994. The meeting resolved that the biochemical pathway for the synthesis of cyanogenic glucosides in cassava was well understood, but that the physiological processes controlling their accumulation in various tissues were still unknown.

To efficiently control cyanogenesis over the short term, cassava must be adequately processed. The dynamics of cyanogen removal and the factors involved are now known, thanks to the development of a new analytical method for determining glucosides and their breakdown products. Advances in the molecular biology of cyanogenesis, combined with conventional plant breeding, will now make possible the optimization of cyanogenic glucoside levels and distribution in cassava.

Agronomic research has shown that environmental factors can be as important as genetic factors in determining the levels of cyanogenic glucosides in cassava roots. The understanding of causal relationships between cassava cyanogenesis and associated human diseases has improved, particularly in relation to outbreaks of paralytic diseases, and acute poisoning. These outbreaks occurred in socio-economically deprived communities who have traditionally relied on cassava as their staple food and who, because of food shortages, war, or poverty, make short cuts in their traditional processing methods.

New socio-economic findings emphasized the importance of cassava processing—not only for safety's sake, but also for expanding cassava production by improving shelf-life, facilitating transport, and introducing consumer-specific tastes and textures into cassava products.

Introduction

For thousands of years, cassava has been a diet item for people living in the Amazon Basin, despite its potential toxicity. Since its introduction about 400 years ago, cassava has spread

throughout Africa and is now a major tropical crop, varying in significance, from Senegal, eastwards to Mozambique and Ethiopia, and southwards to Angola. The importance of cassava as a staple is now well established.

Throughout the tropical world, cassava ranks fourth in importance after rice, sugar cane, and maize. The presence of varying amounts of cyanogenic glucosides and their breakdown products (cyanohydrins and hydrogen cyanide) in cassava food products has been a cause of concern because of their potential effect on health. The relationship between cassava consumption and health—particularly the thyroid function—has been reviewed in two workshops sponsored by the International Development and Research Centre (IDRC) of Canada in 1973 (Nestel and MacIntyre 1973) and in 1982 (Delange and Ahluwalia 1983).

Since the second workshop, many advances have been made in:

(1) Understanding cyanogen removal during processing;

(2) Improving analytical methods;

(3) Understanding the causal relationships between cassava cyanogenesis and human ill health, especially the factors underlying toxicity;

(4) Elucidating the genetic basis of the synthesis of cyanogenic glucosides; and

(5) Understanding the socio-economic mechanisms influencing cassava production.

The reasons why the 1994 International Workshop on Cassava Safety was held were:

(1) The increased importance of cassava in agricultural and economic development and in food security, particularly in Africa;
(2) Reports of outbreaks of a new paralytic disease (*konzo*) and acute poisoning (*ofa*)—both of which were attributed to cyanide exposure from insufficiently processed cassava roots;
(3) The need to understand the biological and social role of cyanogenesis in cassava and to consider how recent advances in various scientific disciplines could be used to expand cassava production; and
(4) The need to better understand safety issues when developing and promoting new cultivars in communities under economic or ecological stress.

At a meeting of the ISTRC's Africa Branch in Kampala, Uganda, 1992, the Working

Group on Cassava Safety (WOCAS) was formed. The Group's aims are to make recommendations for promoting safe cassava, based on current knowledge; identify research needs and develop research strategies; and identify people working in this field and facilitate exchange of information and experiences.

Considering that reported toxic effects of cassava are relatively rare in relation to its wide use as a staple, the Group decided that cassava *safety* was a better working concept than cassava *toxicity*. Hence, the name of the working group and, subsequently, of the Workshop.

The Workshop aimed to take stock of the present state of knowledge on safety issues related to cyanogenesis in cassava and to disseminate this information more widely among researchers in the field. It was organized around seven main themes: biology of cyanogenesis, analytical methods, agronomic research, cassava processing and cyanogen removal, livestock feeds, human health and nutrition, and socio-economic considerations. Leading researchers in these fields were invited to prepare discussion papers. Summaries and recommendations from all sessions were debated in a final plenary session. After the Workshop, WOCAS members met to crystallize the ideas that emanated from the final plenary session into the set of recommendations reproduced here. Every effort was made to respect the spirit of the debates.

Workshop Summary and Recommendations

Biology of cyanogenesis

To develop cassava cultivars (with low or high cyanogenic potential) that satisfy user needs and preferences in diverse socio-economic and agro-ecological conditions, conventional plant breeding can be complemented effectively by biotechnological approaches. Current knowledge on synthesis, degradation, transport, and regulation of cyanogenic glucosides in the cassava plant provides possibilities of developing new approaches and tools for optimizing the content and distribution of cyanogenic glucosides and associated enzymes in cassava.

Three genes, coding for key enzymes that control the biosynthesis and degradation of cyanogenic glucosides, have already been isolated and cloned:

(1) The gene for cytochrome P450, an enzyme that catalyses the rate-limiting conversion of the parent amino acid to the corresponding oxime in the biosynthetic pathway;
(2) the gene for linamarase, which catalyses the degradation of linamarin to acetone cyanohydrin; and

(3) The gene for hydroxynitrile lyase, which catalyses the degradation of acetone cyanohydrin to hydrogen cyanide and acetone.

Important genes that have yet to be isolated include those coding for glucosyltransferase, which converts linamarin to its transport form linustatin; simultaneous di-glucosidase, which splits linustatin in the first step of its metabolism to a non-cyanogenic compound; and root- and leaf-specific promoters effective in cassava.

An efficient transformation system for cassava is urgently needed to introduce relevant genes already available and those to be developed. Progress in cassava transformation and regeneration (not reviewed at this meeting) appears likely to permit transgenic cassava plants, using currently available genes and others to be ready for controlled testing within 2 to 3 years.

The currently observed demand among cassava farmers, processors, and consumers for cultivars with a high cyanogenic glucoside content and/or with bitter taste may reflect a tight genetic coupling of the cyanogenic character and bitterness to other beneficial characteristics. Despite this tight coupling, these associations may be broken by continued traditional breeding that use more accurate analytical methods (e.g., antibodies and cDNA probes) for selecting desired cultivars.

Cyanogenesis can be controlled by:

(1) Transforming cassava by introducing an anti-sense construct of cytochrome P450 under the control of a strong constitutive promoter to produce acyanogenic plants.

(2) Inserting tissue-specific promoters or developmentally controlled promoters in front of the cytochrome P450 gene to limit production of linamarin to certain tissues and at specific periods of plant growth.

(3) Introducing a strong promoter in front of the linamarase gene to increase the breakdown of linamarin during processing.

(4) Preventing linamarin conversion to the transport metabolite linustatin.

(5) Increasing the conversion of linustatin to asparagine instead of its conversion back to linamarin, which may result in the accumulation of protein nitrogen in the roots.

Plants so obtained would constitute ideal research material for specifically testing the relationship, if any, between cyanogenic glucoside content and desired properties such as starch quality, insect resistance, and bitterness. If any models or experimental approaches prove to be useful, plant breeding can be used to transfer the desired cyanogen metabolism into appropriate cultivars. When genetic transformation of cassava has been extended to a wider range of genotypes, conferring the desired cyanogenesis phenotype on varieties improved for quantitative traits (i.e., more difficult to define genetically) may become possible by transforming elite selections. This option would relieve the plant breeder of a set of selection objectives for cyanogenesis, thereby permitting faster progress for quantitative traits such as yield and environmental adaptation.

Molecular biology research may produce experimental data within 5 years to answer some of the questions that cannot now be answered. However, results will probably not be transferred to cassava farmers in the next 10 years. These efforts must therefore be combined with continued efforts to expand our knowledge of effective, practical processing techniques to reduce cyanogen levels in cassava products.

Over the long term, molecular biology can offer more than mechanisms for removing cyanogenic glucosides. Combined with plant breeding and other disciplines, it provides a new, potentially powerful approach to circumvent the loss of desirable functions found in association with cyanogens by introducing nutritionally less problematical factors, including plant protective agents and quality factors.

Similar to the considerable resources being spent for research on temperate crops by industrialized countries, basic research on cassava must be intensified on such aspects as nutritive value, productivity, and tolerance of biotic and abiotic stresses, if this crop is to provide economic resources and food security to tropical countries.

Analytical methods

Within itself, a tissue of a cassava plant may contain widely varying levels of cyanogenic glucosides and linamarase activity. In addition, these levels may vary between different organs of the same plant, between plants of the same variety, whether in apparently similar or different environments, and between different varieties. Sampling procedures are therefore critical for statistically validating results, no matter the chemical methods used. Sampling protocols should be standardized, depending on the kind of material and purpose of measuring. Researchers should be aware that handling and storing fresh and processed cassava collected for analysis, as well as extracts obtained from these, must be standardized and validated, as considerable losses can occur. Simple mobile equipment for homogenization and extraction needs to be developed.

Linamarin and, to a lesser extent, lotaustralin are the cyanogenic glucosides found in the cassava plant. Only when tissues are damaged, mainly by chemical or microbial actions, do the cyanogenic glucosides decompose to cyanohydrins that may further hydrolyse to the poisonous hydrogen cyanide. At harvest, then, intact cassava tissues contain only cyanogenic glucosides, not hydrogen cyanide. Processed products may, however, contain varying amounts of cyanogenic glucosides, cyanohydrins, and hydrogen cyanide. The development of analytical methods for the separate determination of these three types of cyanogenic compounds has advanced the understanding of the dynamics of cyanogen removal during processing. (The simplistic references to cyanide or total cyanide content in cassava continue to hamper this understanding and should be avoided.)

Different chemical assay methods are needed as no one technique serves all requirements. The method should be chosen according to resources available and to the objectives of the analysis. Many developing country laboratories, which typically have limited resources, require robust, low-cost, and simple methodologies. Simple, specific, and relatively sensitive methods for use in field surveys are much needed. Of importance for both qualitative and quantitative techniques is reproducibility. Particularly important in enzyme-mediated methods is the standardization of pH and thus the use of effective buffer systems. Autolytic methods, which rely on endogenous enzyme for glucoside hydrolysis, are unreliable for processed products in which the endogenous enzyme may have been inactivated. The use of exogenous linamarase is recommended but is currently constrained by high costs.

Alternative sources of low-cost but effective enzymes should be explored. One alternative is to produce crude linamarase preparations from cassava leaves or root cortex. Another is to immobilize linamarase to allow its repeated use. HPLC (high-performance liquid chromatography) techniques allow the separate measurement of cyanogenic glucosides and cyanohydrins but are costly and complicated. Potentiometric methods that use cyanide electrodes have limited sensitivity and reproducibility.

The safe handling of reagents used in estimating cyanogens needs to be taken into account. The pyrazalone/pyridine reagent used as a colour reagent is highly toxic and volatile, and must be prepared daily. Its use requires appropriate safety equipment, which may not be readily available in developing countries. A better alternative as a colour reagent is the combination of isonicotinate and 1,3-dimethyl barbiturate. Picrate and tetra base can be used in qualitative and semi-quantitative assays, although their potential health hazards should also be investigated.

Interfering compounds—which occur in samples rich in oils, fats, proteins, and phenolic compounds—provide significant problems in extraction, recovery, and colourmetric

estimation. These problems need to be solved, given the many prepared foods that may contain high levels of added oils and proteins.

Agronomic research

Cassava varieties show a very wide range of cyanogenic glucoside levels in storage roots. New findings in health and food sciences now call for a revision of the "safe levels" established more than 40 years ago.

Although cyanogenic potential is inherited, progress in the conventional breeding for this trait has been slow because of the polygenic and recessive nature of its inheritance and, until recently, inadequate sampling strategies. The environment exercises a significant influence on the expression of cyanogenic potential in cassava. A thorough review of existing knowledge and a focus on identifying genotypes with greater stability across environments and at key developmental stages are essential. The contribution of field cultural practices in modulating cyanogenesis also needs to be addressed.

Some cassava-growing communities prefer bitter or potentially toxic varieties. Observational studies indicate that bitterness or toxicity may significantly discourage animals from feeding on the roots and thus damaging the plant. Current evidence suggests correlation between cyanogenic potential and bitterness, but some varieties with extreme expression of either trait do not follow the general trend. The compound responsible for bitterness needs to be identified and the reasons for preferring bitter toxic varieties in some farming communities need to be established. Results of these studies will determine the value of developing varieties with, for example, a low cyanogenic potential but bitter taste, should the latter be the factor that deters animals from feeding on the plant.

Researchers still need to establish whether acyanogenesis is a viable option for addressing cassava safety. A recent study, which needs confirmation by further research, suggested that cyanogenic glucosides play a role in pest resistance. The possibility of partitioning cyanogenic glucosides into inedible plant parts, while maintaining pest resistance, also needs to be explored.

Available data on relationships between cyanogenic potential and morphological and/or agronomic traits show many inconsistencies. Molecular markers for detecting genotypes with low or high cyanogenic potential are needed to accelerate breeding efforts to control cyanogenesis.

Cassava processing and cyanogen removal

Processing can reduce the cyanogenic content of roots and leaves of even the most potentially

toxic varieties to safe levels. A myriad of processing methods, however, exists and not all are equally effective in reducing cyanogens. The effectiveness of these techniques needs to be verified for different cultivars.

Most of the principles of cyanogen removal during processing are now understood. Current knowledge indicates that plant cells contain cyanogenic glucosides, mainly linamarin. Disintegration of the cells brings linamarin into contact with the endogenous enzyme linamarase, resulting in the hydrolysis of linamarin into glucose and acetone cyanohydrin. Acetone cyanohydrin, in turn, breaks down into acetone and hydrogen cyanide (HCN), either by action of the enzyme hydroxynitrile-lyase or spontaneously at increased rates at higher pH. The latter pathway appears to be the principal one. The volatile HCN (boiling point 25.7 ^{o}C) escapes into the air.

Effective cyanogen reduction is achieved in two steps: first, by disintegrating the cells (which brings about glucoside hydrolysis) through grating, crushing, microbial fermentation, enzymic action, or any combination of these. Second, by causing the spontaneous breakdown of cyanohydrin under conditions of high pH, higher temperatures, and reduced moisture content (MC) during drying. The factors determining cyanohydrin stability need better understanding.

Processing methods that involve effective disintegration, followed by heating or drying, result in the highest removal of cyanogens. Examples of these methods include mechanical grating, followed by roasting, as in the production of *gari* and *farinha*; and microbial fermentation, followed by drying or steaming, as in the production of *lafun* and *chickwangue*. Incomplete disintegration will result in residual cyanogens, particularly linamarin; and incomplete drying or heating may result in residual cyanohydrin. Whether reduced linamarase activity is a limiting factor in removing cyanogens in some cultivars is not yet known. Similarly, the role played by hydroxynitrile-lyase needs further research.

Direct sun-drying of whole fresh roots achieves only partial removal of glucosides. Slower drying extends the effect of linamarase activity but simultaneously allows microbial growth. Chipping fresh roots, which involves extensive mechanical tissue damage, will facilitate glucoside breakdown, but slicing roots with minimal tissue damage followed by rapid drying will result in a high retention of glucosides. In sun-dried cassava pieces, an inverse relationship seems to exist between cyanogen and microbial content. Possibilities for optimizing cyanogen removal while minimizing microbial contamination should be explored further. The issue of mycotoxin contamination in sun-dried cassava, as well as in cassava deliberately made mouldy, needs to be addressed. Studies have identified mycotoxins in some sun-dried root products, but not in deliberately moulded cassava.

The ineffective processing methods found in some communities can lead to cassava products with high levels of residual cyanogens and, thus, cases of poisoning. The simple introduction of improved processing methods is a powerful way of reducing levels to safe limits in such communities.

As commercial cassava processing intensifies and the scale of cassava operations increases, safety issues become more critically important, for example, inhalation of hydrogen cyanide vapours from roasting cassava should be minimized by good ventilation. Disposal of processing effluents is also expected to become an increasing problem because of high biological oxygen demands (BOD) resulting from the effluents' high solid contents.

Few details are known about why people process cassava the way they do and the factors leading to it. Recommendations regarding processing should therefore take into account the quality characteristics of the raw materials and the end products as they relate to the broader socio-economic and cultural environment. The relationships between sensory characteristics, bitterness, and cyanogenic content as they relate to cassava processing need further research.

Cassava as livestock feed

Effective processing techniques for removing cyanogens exist for preparing dried cassava chips for animal feed. A total cyanogen level of <100 mg HCN equivalence per kilogram of dried cassava for inclusion in balanced compound animal feed is economically acceptable in intensive livestock production systems. Cyanogens in feeds may increase requirements for sulphur compounds, iodine, zinc, copper, and selenium. Optimal levels of these compounds per unit of cyanogen need to be determined for the various livestock species.

Cassava roots, leaves, and wastes are often used as components of livestock feed in rural farming communities. Sporadic deaths attributed to cyanogens in cassava have been reported for various livestock production systems. These claims should be substantiated and safe-handling strategies developed for incorporating cassava into livestock feed, particularly in smallholder systems. Problems of cassava toxicity in livestock also appear to stem from microbial contamination as a result of poor handling and humid climates. Efforts to improve the safety of cassava-based feed should therefore also address microbial quality.

Human health and nutrition

Hydrogen cyanide is rapidly lost during processing and, as a result, probably does not constitute the main source of dietary cyanide exposure from insufficiently processed cassava. The main sources may be residual linamarin and acetone cyanohydrin, which are broken down

in varying degrees to cyanide in the human body. A substantial proportion of ingested linamarin is absorbed from the gut and excreted unchanged in the urine; thus, the dietary cyanide exposure can be considerably lower than expected from the total amount of cyanogens ingested. Cyanide release from ingested linamarin may depend on the presence of active ß-glucosidases from cassava, other foods, or microflora in the gut.

Although few published reports exist, dietary cyanide exposure from insufficiently processed cassava is believed to cause acute poisonings when food shortages and social instability induce short cuts in established processing methods. Cases of poisoning may also occur when varieties with high glucoside levels are introduced rapidly into communities who lack efficient processing methods. Hospitals who receive such cases should be provided with rapid analytical methods and cyanide antidotes, thus saving patients and verifying the cause of poisoning. Unnecessary sensationalism can also be avoided. The importance of *gari* in West Africa and the attribution of acute poisonings to short cuts in *gari* processing in Nigeria highly justify studying whether these short cuts result in products with dangerous cyanogen levels.

The thiocyanate load, resulting from dietary cyanide exposure, can aggravate iodine deficiency disorders (IDD), especially goiter and cretinism, in populations with low iodine intake. This dietary effect, however, is of secondary importance to the global problems of IDD. Iodine supplements, which receive high international priority, can counteract the effect of thiocyanate from cassava on the thyroid gland.

Strong, but inconclusive, evidence exists of a causal role for cyanide exposure from cassava in the paralytic diseases *konzo* and tropical ataxic neuropathy (TAN). Although the pathogenic mechanisms are still unknown, these diseases occur only in populations with severe socio-economic problems, monotonous diet, and food insecurity. The acute onset of *konzo* is attributed to several weeks of high cyanide exposure, resulting from short cuts in cassava processing and concomitant low protein intake that reduces the rate of cyanide-to-thiocyanate conversion. The gradual onset of TAN is linked to several years of moderate cyanide exposure, combined with low intake of protein and some constituents of the vitamin B complex.

The supposed association between dietary cyanide exposure and malnutrition-related diabetes, as well as tropical pancreatitis, remains speculative as no epidemiological data yet support such an association. The suggested aggravating role of cyanide exposure from cassava in protein-energy malnutrition also still lacks supporting data.

To advance the understanding of safety limits for cyanogens in the diet, the following studies should be carried out:

(1) Research on animal models, which can help further explain the mechanisms

involved and clarify causal factors of diseases associated with cyanide exposure from cassava;

(2) Long-term follow-up studies of populations known to have had high dietary cyanide exposure in combination with various dietary deficiencies can provide new information on safe cyanogen levels in cassava products;

(3) The cyanogenic potential of cassava cultivars—together with residual levels of cyanogenic compounds in their products, potential linamarin intake, and potential cyanide exposure—should be studied in cassava-eating communities where no related diseases are found.

Such studies can be facilitated by the new, sensitive, specific, and rapid analytical methods that have recently been developed for testing, in blood or urine, the levels of linamarin, cyanide, thiocyanate, and the alternative cyanide metabolites, amino-thiazoline-carboxylic acid and cyanate.

Given its ability to produce on marginal soils and in drought, cassava is crucial for the food security of those areas where toxic effects are reported. Affected populations state that bitter and potentially toxic varieties provide the better food security. Given the constraints to agriculture in such areas, these varieties may paradoxically have an overall positive effect on human survival. Positive ways of preventing toxicity are introducing new varieties and promoting effective processing, rather than banning certain varieties. Most of the 400 million people who consume cassava on a daily basis are not at risk from the diseases described above. From a public health perspective, the linkages between cassava and these toxico-nutritional diseases are similar to those between monotonous rice diets and the nutritional disease beriberi or between monotonous maize diets and pellagra. The major concern with cassava-related diseases is that their underlying cause—severe social instability, agro-ecological crises, and food insecurity—are becoming commoner in many parts of Africa.

Human diseases linked to cassava cyanogenesis are entirely preventable. Preventive actions include promoting effective processing, iodine supplementation, and dietary improvements. The diseases can also be prevented by measures against underlying causes, such as food shortages, socio-economic deterioration, and market distortions. Introducing high-yielding cassava cultivars with low glucoside levels may be a long-term preventive measure for farming and food systems where cassava varieties with high glucoside levels are not indispensable for food security. Such cultivars, however, should be promoted only when they are proven to perform well under stress in the local farming systems.

A cyanogen level of 300 mg HCN equiv. per kilogram of dry weight (10 mg/100 g wet wt) has been used as the upper limit for ‘low cyanide’ in breeding programmes since 1994.

This level is 30 times higher than the 10 mg HCN equiv. dry wt defined by FAO and WHO as a safe level for cassava products in the *Codex Alimentarius*. These levels should be revised according to the new knowledge currently available from several disciplines. Estimates should be based on cyanide detoxification rates in humans, necessary safety margins for natural toxins, degree of cyanide release from ingested cyanogens, expected daily consumption, and degree of cyanogen removal during processing. Theoretical levels should be compared with empirical measurements of the content of cyanogenic compounds in processed and fresh products consumed without effect by human populations according to general principles for natural substances in food.

Socio-economic considerations

For most cassava consumers, cyanide intoxication is not a concern. In some communities—particularly those facing nutritional deficiency and economic hardship—long-term exposure to dietary cyanide from cassava is an aggravating factor for diseases attributed to chronic cyanide intoxication. In situations of war, social distress, drought, or economic instability, populations may be forced to survive for extended periods on cassava as the sole food that remains. Food shortages may lead to short cuts in processing to obtain food more quickly. Such short cuts result in high residual cyanogen levels, which cause acute poisoning in consumers. In communities with cases of cassava-related poisonings, intervention strategies should recognize social, cultural, and economic peculiarities to find the appropriate approaches for effective implementation.

Problems of cassava poisoning are linked to situations of economic deprivation. Enhancing local economies may be one strategy of intervention. Transnational and multiregional markets need to be explored and developed. But the ability of cassava products to enter new markets will depend on product quality with respect to convenience, performance, and safety. Building rural infrastructure and amplifying trade relationships between and among various indigenous communities would be part of developing the cassava market.

Currently, cassava varietal dissemination is largely a local, farmer-initiated event. We therefore need to understand the local rationale behind farmers' adoption of new cultivars. The rate of introducing improved cultivars with characteristics desired by local farmers should be increased. Cassava cultivars with higher levels of cyanogenic glucosides than those already used should never be introduced without a simultaneous and vigorous promotion of appropriate processing methods. New cultivars with very low levels of cyanogenic glucosides and which are likely to perform well in areas affected by cassava toxicity should be introduced immediately to those areas as a matter of priority. Varietal characteristics should be linked with particular processing methods.

Diverse cassava-processing techniques that are ecologically, socio-culturally, and technologically appropriate should be evaluated for a range of socio-economic and ecological settings, and disseminated. Because many traditional forms of cassava processing are gender skewed (i.e., carried out only by women and children), labour-saving technologies should be promoted to reduce women's workload and increase productivity without compromising their access to income.

The development and consumption of supplementary foods—both indigenous and introduced—in conjunction with various cassava food products should be promoted. We recommend exploring new uses of cassava to improve the economy of cassava-growing communities.

Cassava safety can best be improved by distributing desirable varieties, promoting effective processing techniques, and diversifying markets for this root crop.

Conclusions

Several topics discussed at the Workshop could not be settled and require further study. The reasons remain unclear for the use of bitter and toxic cassava cultivars by communities where the risk of poisoning is great. The levels of cyanogenic glucosides in fresh cassava roots currently used as a criterion by plant breeders for developing genotypes with low cyanogenic potential do not agree with the understanding of safety limits for cyanogens in cassava. A proposal to revise the safe levels of cyanogenic glucosides takes into account variables such as the toxic cyanide exposure rate in humans, cyanide uptake from the gut, level of daily cyanogen ingestion, and cyanogen content of consumed products. It also recognizes the factors controlling these variables.

Long-term exposure to subclinical amounts of cyanogens from cassava-based diets may influence human biological fitness and micro-evolution. Evidence to support or reject this hypothesis is currently limited.

The relationship between the bitterness of fresh cassava roots and their total cyanogen content needs further clarification. Although the correlation coefficient between the two is high, whether a cause-effect relationship exists needs to be established.

Although cyanogenic glucosides are believed to play a role in pest resistance, irrevocable proof has not yet been obtained. Proof may come when genetic engineering techniques can silence, in cyanogenic varieties, the gene(s) coding only for the biosynthesis of cyanogenic glucosides. Such silencing may demonstrate that a pest-resistant variety becomes susceptible when it can no longer produce cyanogenic glucosides.

The terminology used in scientific literature to report the concentration of various cyanogenic compounds found in cassava is highly diverse, often confusing, and sometimes misleading. Because an agreement could not be reached during the meeting, advice was sought afterward from the International Union of Pure and Applied Chemistry (IUPAC). Their suggestions, together with the current state of knowledge and the need to foster a better understanding of safety issues in cassava, contributed towards the following recommendations on terminology:

(1) It should be recognized that intact and fresh cassava tissues contain mainly the cyanogenic glucosides linamarin and lotaustralin.

(2) Processed or damaged tissues may contain varying amounts of cyanogenic glucosides, cyanohydrins, and hydrogen cyanide. The recommended analytical procedure is to determine the total amount of all three compounds

(Fraction A), the total amount of cyanohydrins and hydrogen cyanide (Fraction B), and the amount of hydrogen cyanide (Fraction C). Fraction A should be referred to as 'total cyanogen content', Fraction B as 'non-glucosidic cyanogen content', and Fraction C as 'hydrogen cyanide content'. The 'cyanogenic glucoside content' is obtained by subtracting Fraction B from Fraction A, while the 'cyanohydrin content' is obtained by subtracting Fraction C from Fraction B. The recommended unit to be used is 'mg HCN equiv. Kg'.

(3) Authors should indicate whether their data are calculated on a fresh or dry matter basis.

(4) The potential for a sample to produce HCN, expressed as the'total amount of HCN equivalent weight of sample' has been called the 'HCN potential', 'HCN-releasing potential', 'cyanide potential', or 'cyanogenic potential'. The last term is to be preferred. Abbreviations such as *HCNp*, *CNp*, or *CNP* are to be discouraged.

References

Bokanga M; Essers AJA; Poulter N; Rosling H; Tewe O, eds. 1994. International Workshop on Cassava Safety. Acta Hortic (Wageningen) 375.

Delange F; Ahluwalia R, eds. 1983. Cassava toxicity and thyroid: research and public health issues. Proc. Workshop; 31 May-2 June 1982, Ottawa. IDRC-207e. International Development Research Centre, Ottawa.

FAO and WHO. Codex Alimentarius.

Nestel B; MacIntyre R, eds. 1973. Chronic cassava toxicity. Proc. Interdisciplinary Workshop; 29-30 Jan, London. IDRC-010e. International Development Research Centre, Ottawa.