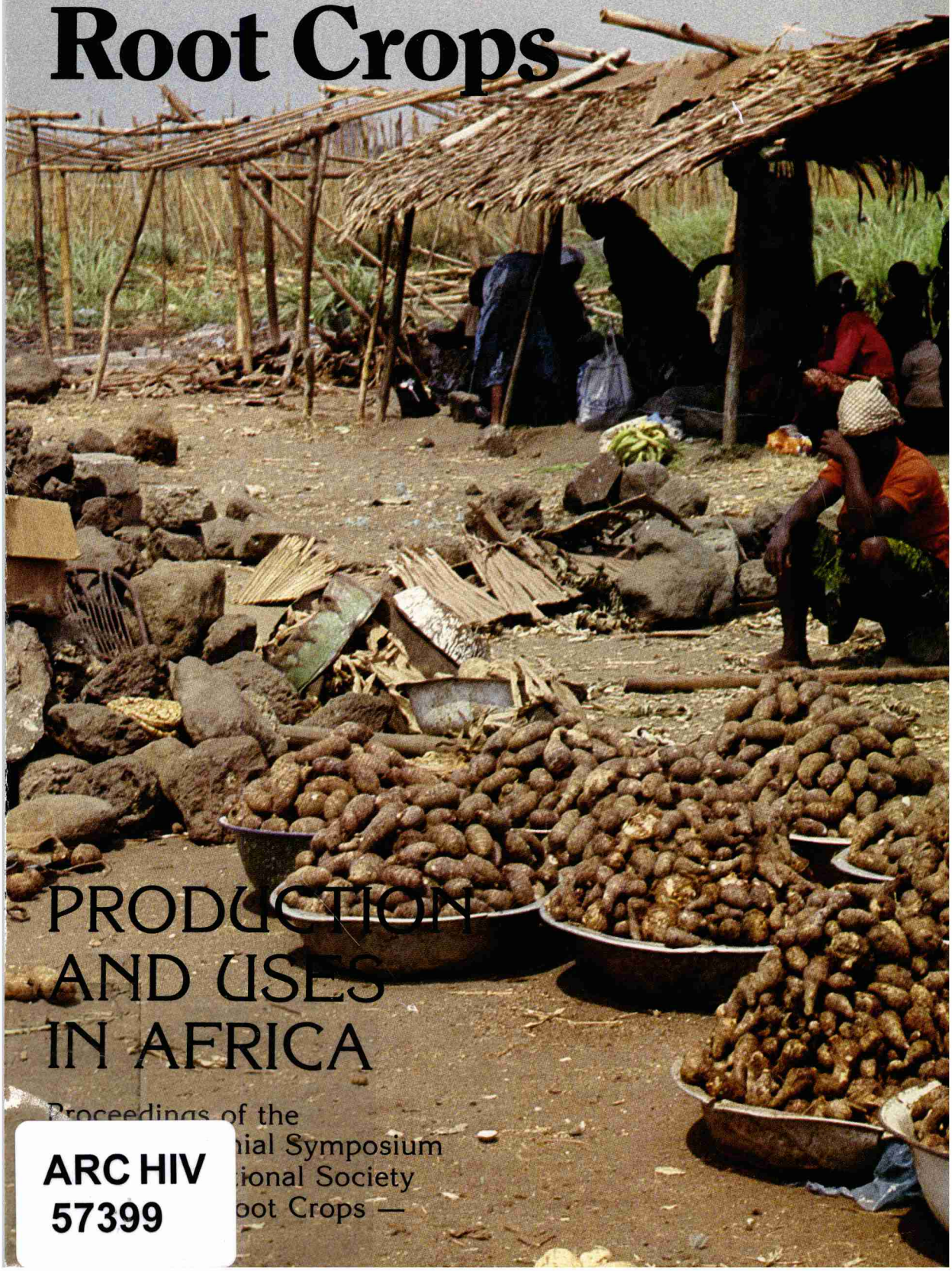
Tropical Root Crops



PRODUCTION AND USES IN AFRICA

Proceedings of the
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The International Society for Tropical Root Crops — Africa Branch was created in 1978 to stimulate research, production, and utilization of root and tuber crops in Africa and the adjacent islands. The activities include encouragement of training and extension, organization of workshops and symposia, exchange of genetic materials, and facilitation of contacts between personnel working with root and tuber crops. The Society's headquarters are at the International Institute of Tropical Agriculture in Ibadan, Nigeria, but its executive council comprises eminent root and tuber researchers from national programs throughout the continent.

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IDRC-221e

Tropical root crops: production and uses in Africa : Proceedings of the second Triennial Symposium of the International Society for Tropical Root Crops — Africa Branch held in Douala, Cameroon, 14–19 August 1983. Ottawa, Ont., IDRC, 1984. 231 p.: ill.

/Cassava/, /root crops/, /plant production/, /Africa/ — /genetic improvement/, /planting/, /plant diseases/, /pests of plants/, /intercropping/, /fertilizers/, /crop yield/, /sweet potatoes/, /plantains/, /agriproduct processing/, /nutritive value/, /food enrichment/, /feed/, /agricultural research/, /conference report/, /list of participants/.

UDC: 633.68 ISBN: 0-88936-409-5

Microfiche edition available.
Il existe également une édition française de cette publication.

TROPICAL ROOT CROPS: PRODUCTION AND USES IN AFRICA

Abstract

A mixture of original research, updates on procedures, literature reviews, and survey reports, this document resulted from the second symposium of the International Society for Tropical Root Crops — Africa Branch, with 77 participants from 16 countries. The focus was cassava, yams, cocoyams, and sweet potatoes, from the perspectives of breeders, agronomists, soil specialists, plant pathologists, entomologists, nutritionists, food technologists, etc. Learning from past successes and failures, many of the researchers directed their efforts toward problems obstructing progress in reaching improved production and use of root crops and attempted to view, realistically, the context in which their results would be applied.

Résumé

Résultats de recherches récentes, mises à jour sur les méthodes de recherche, revues de publications et rapports de sondages sont contenus dans ce document issu du Deuxième symposium de la Société internationale pour les plantes-racines tropicales — Direction Afrique, qui a réuni 77 participants de 16 pays. Des communications sur le manioc, le taro, le yam et la patate douce ont été présentées par des phytosélectionneurs, des agronomes, des pédologues, des phytopathologistes, des entomologistes et des spécialistes de la nutrition et des aliments, entre autres. Tirant leçon de leurs succès et de leurs échecs, beaucoup de ces chercheurs ont dirigé leurs efforts vers la solution des problèmes qui entravent l'augmentation de la production et de la consommation des plantes-racines et ont tenté de considérer d'un œil réaliste le contexte qui sera celui de l'application de leurs recherches.

Resumen

Una mezcla de investigaciones originales, actualizaciones de procedimientos, reseñas de literatura e informes de encuestas, este documento es el resultado del segundo simposio de la Sociedad Internacional de Raíces Tropicales, Filial Africana, que contó con 77 participantes de 16 países. El simposio se centró en la yuca, el ñame, el cocoñame y las batatas, desde la perspectiva de los fitomejoradores, los agrónomos, los especialistas en suelos, los patólogos vegetales, los entomólogos, los nutricionistas, los tecnólogos alimenticios, etc. A partir de los éxitos y fracasos anteriores, muchos de los investigadores encaminaron sus esfuerzos hacia los problemas que obstaculizan el avance para lograr una producción y un uso mejorados de las raíces y trataron de obtener una visión realista del contexto en que los resultados pueden ser aplicados.

IDRC-221e

TROPICAL ROOT CROPS:
PRODUCTION AND USES IN AFRICA

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PROCEEDINGS OF THE SECOND TRIENNIAL SYMPOSIUM OF THE INTERNATIONAL SOCIETY FOR TROPICAL ROOT CROPS — AFRICA BRANCH HELD IN DOUALA, CAMEROON, 14 – 19 AUGUST 1983

CONTENTS

MICROBIAL BREAKDOWN OF LINAMARIN IN FERMENTING CASSAVA PULP

M.A.N. EJIOFOR[1] AND NDUKA OKAFOR[2]

Linamarin-hydrolyzing microorganisms were inoculated into fresh cassava pulp. The bacteria and yeasts grew well: generation times were 59, 58, 78, and 74 minutes for *Alcaligenes faecalis*, *Leuconostoc mesenteroides*, *Saccharomyces cerevisiae*, and *Rhodotorula minuta*, respectively. They hydrolyzed cassava linamarin, releasing 0.090, 0.094, 0.102, and 0.100 mg HCN/g of pulp, respectively. Alone, the linamarase endogenous to the cassava released only 0.045 mg HCN/g of pulp. We believe, therefore, that the four microorganisms can be used in the commercial detoxification of cassava pulp during the production of gari or other fermented cassava-based meals.

Earlier, we (1981) suggested that microbial means be used to reduce the cyanide level of cassava. We had found that the cyanide level was drastically reduced during the natural fermentation of cassava and felt that the reduction was caused partially by microbial enzymatic activity. As far as we know, no other researchers have investigated the potential for microbial hydrolysis, but we have identified several microorganisms capable of breaking down linamarin in purified form: *Alcaligenes faecalis, Leuconostoc mesenteroides, Saccharomyces cerevisiae, Rhodotorula minuta, Fusarium oxysporium, Aspergillus niger,* and *A. flavus*. Of these, *F. oxysporium, A. niger,* and *A. flavus* have been reported to be sources of food poisoning and diseases (Frazier 1971; Banwart 1979). Also, fungal forms that cause spoilage in foods, i.e., those with hyphal strands and spores that readily destroy the colour and texture of the products, should be eliminated from further work. We tested the abilities of four of the organisms, individually and collectively, to grow in and detoxify cassava pulp. A safe and cheap method of preserving the organisms for supply to the farmer has not yet been worked out.

MATERIALS AND METHODS

A fresh root harvested from 1-year-old cassava (Nwugo variety) was washed, peeled, and rewashed. The peeled root was immersed completely in 70% ethanol for 1 minute and flamed. The sterile outer tissue was charred. We removed a cylinder of tissue (1.2 cm in diameter) aseptically, using a sterile cork borer. We cut 1 cm of the tissue from both ends, aseptically transferring and crushing the remaining 10.5 cm. The pulp was tested and was found to be sterile.

We then placed 10-g portions of pulp into 250-mL volumetric flasks. A sterile 10-mL bottle without stopper was gently placed upright in the centre of the bottom of each flask, and 2 mL of 0.1 M NaOH was pipetted into the bottle. One flask was set aside as a control. The pulp in the other flasks was inoculated with five loopfuls of organism. All the flasks were firmly stoppered with sterile cotton wool and incubated in a Gallenkamp orbital incubator (rotating at 100 rpm for 144 h at 28°C). The hydrocyanic acid released was trapped in the NaOH solution, whereas the glucose liberated was left in the pulp medium. All gases released were tested for ammonia. The pulp was sampled for HCN at 24-h intervals by the methods of Aldridge (1944) and Epstein (1947) as modified by Jorgensen. Different combinations of the organisms were also used, and the rate at which they broke down linamarin was assayed. The results were statistically analyzed, and changes in the populations and generation times were determined. The pH of growth in the pulp was monitored.

The effect of HCN on the survival and growth of the microorganisms was also studied. A 0.1% solution of sodium cyanide was incorporated in glucose yeast agar (GYA) and broth and was dispensed in 50-mL amounts into 250-mL flasks. The pH was adjusted to 6.5 and 5.0 for the

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Table 1. Mean HCN (mg) released from 1 g of pulp by the microorganisms.

Time (h)	Pulp only	*A. faecalis*	*L. mesenteroides*	*S. cerevisiae*	*R. minuta*
0	0.008	0.008	0.006	0.009	0.008
24	0.020	0.062	0.036	0.026	0.032
48	0.032	0.082	0.088	0.042	0.070
72	0.045	0.090	0.100	0.102	0.094
96	0.051	0.078	0.094	0.105	0.108
120	0.058	0.060	0.082	0.086	0.092
144	0.065	0.038	0.046	0.052	0.045

bacteria and yeasts, respectively. Eight separate flasks were prepared, two for each organism. Thereafter, five loopfuls of each organism were used to inoculate the broth in each flask. Immediately, microbial cell counts were estimated from 0.025 mL of the broth placed on dried GYA plates, evenly spread on the surface of the agar, and allowed to dry before being incubated at 28°C overnight.

Sodium cyanide crystals (1 g) were dissolved in distilled water, the final volume being 1 L. The solution was dispensed in two equal portions, one for the GYA plates and the other for the broth. Both were seeded with the organisms and contained 0.1% cyanide. Other plates and tubes contained 0.2%, 0.3%, and 0.4%. The mouths of the tubes containing the broth were stoppered with filter paper previously wetted with Nessler reagent. After incubation (72 h) at 28°C, the tubes were warmed to 50°C in a water bath and observed for colour change (Collins and Lyne 1976). The agar plates were observed for appearance of colonies.

In studies of the preservation of the microorganisms for supply, we peeled 1 kg of fresh cassava roots. The cortex was washed, hand grated, and oven-dried to about 50% moisture. We then treated one-half of the substance with $(NH_4)_2SO_4$, 0.1%; KH_2PO_4, 0.05%; K_2HPO_4, 0.05%; $MgSO_4$, 0.02%, $CaCl_2$, 0.02%, and NaCl 0.03% to bring the water activity of the pulp to about 0.70. This substance was to be used for the bacteria. The other half was treated with K_2HPO_4, 0.87%; $(NH_4)_2SO_4$, 0.4%; $MgSO_4 \cdot 7H_2O$, 0.05%; $CaCl_2$, 0.03%, were incorporated, the water activity being about 0.67. This substance was to be tested as a medium for maintaining the yeast. Treating the pulp with these salt concentrations ensured the water activity was maintained at the desired level and prevented further microbial growth. The pH of the pulp mixtures was maintained at 5.0 for the bacteria and 3.0 for the yeasts. The mixtures were divided into 10-g portions and placed in dry, sterile universal bottles along with 10 loopfuls of pure culture of one organism. Each bottle was made airtight and stored at 4°C. Later, fresh pulp was inoculated with the culture and allowed to ferment at room temperature for 24 h before being pressed for assessments of the juice.

Results

The HCN and glucose released when the organisms were seeded on fresh pulp increased with time (Table 1) up to a maximum at 72 h for the bacteria — *A. faecalis* and *L. mesenteroides* — and at 96 h for the yeasts — *S. cerevisiae* and *R. minuta*. The control maintained a steady, although gradual, increase in the amount of HCN released up to 144 h (Table 1).

A highly significant ($P < 0.01$) difference existed between the levels of HCN released by cassava linamarase when acting alone and those released when it was acting in combination with the microorganisms.

The ability of the organisms to grow on the linamarin was shown by the growth patterns obtained when viable cell counts were taken. In the cassava pulp, the population of each organism was appreciably affected by the increase in time of fermentation. The bacteria started off with a rapid increase in number until they had tripled at about 72 h. Thereafter, there was a steady fall in the number of viable cells. The yeasts, on the other hand, took longer (48 h) to show appreciable increases in population. Subsequently, rapid increases in growth were observed and were maintained until after 96 h when the number of viable cells was more than five times the initial amount and *S. cerevisiae* had reached a plateau. *Rhodotorula minuta* continued to increase. The generation times for *A. faecalis, L. mesenteroides, S. cerevisiae,* and *R. minuta* were derived as 59, 58, 78, and 74 minutes, respectively. The pH of the pulp medium was also affected by the fermentation. The acidity was observed to increase gradually, although this increase be-

came less pronounced after 96 h. The yeasts produced a greater drop in pH than did the bacteria.

In-vitro studies showed that *S. cerevisiae* could grow without detectable inhibition when 1% solution of sodium cyanide was incorporated in its growth medium. Ammonia was liberated.

When combinations of the organisms were used to inoculate fresh sterile pulp, we observed very rapid breakdown of linamarin when all the organisms were seeded together. Changes in pH were most pronounced when all four organisms were used.

The organisms preserved in the pulp medium were viable after 6 months. Dehydrating the pulp by oven-drying and by addition of salts reduced its water activity to the level required to control growth of the organisms and kept them viable at minimal cost.

Discussion

The four organisms are able to break down linamarin and to grow and multiply in fresh cassava pulp. This means they can grow effectively in the presence of low levels of cyanide. They probably survive either by inducing enzymes that degrade and detoxify liberated HCN or by forming cyanide-resistant enzymes. Knowles (1976) reported many microorganisms that degrade cyanide to nontoxic products, and bacteria have been reported to convert cyanide to CO_2 and ammonia, nitrite, or nitrate (Knowles 1976). The cyanide can also be detoxified by conversion of β-cyanoalanine or other products that are intermediate products in cyanide assimilation (Nartey 1981). This conversion has been widely reported and probably explains why, in our studies after 72 and 96 h, the levels of HCN declined in the pulp inoculated with microorganisms: some of the organisms metabolized and incorporated the cyanide. Detoxification by the bacteria and yeasts was most effective during the first 72 h and 96 h respectively. Most of the glucosides in the pulp are broken down and the HCN in bound and free forms is liberated, leaving only small quantities.

The cyanide content of fresh cassava roots, as reported by various workers, varies from 0.6 mg to 160 mg/100 g (Osuntokun 1970; Montaldo 1977). However, by the method of microbial hydrolysis, values as low as 3.8, 4.6, 5.2, and 4.5 mg/100 g were obtained — values considered to be innocuous.

Any subsequent processing (drying, washing, cooking, soaking, or pulverizing) should further reduce residual cyanide.

We wish to thank the departments of microbiology and biochemistry, University of Nigeria, Nsukka, and the National Root Crops Research Institute, Umudike, for most of the material and equipment used in this work.