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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 6/11/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6.4 of
rec'd 6/11/87
Cyanogenic Glycosides

Attachment

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PROGRESS REPORT NO 2

PROJECT TITLE:

Changes in Cyanogenic Glycosides and Related Enzymes in Cassava Roots During Storage and Processing

A RESEARCH PROJECT

USAID/PSTC PROGRAM

Grant No. 936-5542-G-00-6028-00

SUBMITTED BY

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THAILAND

Rec'd in SCI JUN 11 1987

Project Profile

Country:	Thailand
Grant no.:	936-5542-G-00-6028-00
Program:	Program on Science and Technology Cooperation
Project Title:	Changes in Cyanogenic Glycosides and Related Enzymes in Cassava Roots During Storage and Processing
Project Leader:	Professor Montri Chulavatnatol, Ph.D.
Organization:	Biochemistry Department, Faculty of Science, Mahidol University, Bangkok 10400
Co-investigator:	Assoc. Professor Somsak Ruchirawat, Ph.D.
Project Consultant:	None
Authorized Officer:	Professor Natth Bhamarapravati, M.D., D.Sc.
Total Project Budget:	US$129,670
Project Duration:	May 7, 1986-December 31, 1989
Reporting Period:	November 1, 1986 - April 30, 1987 (6 months)

Budget Allocation for This Period: 1,108,900 Baht

Contents

1. Background/Introduction:

During the past 6 months, November 1, 1986 to April 30, 1987, equipments and supplies on order began to arrive in the laboratory. Among these, two major items were a set of fast-protein-liquid chromatography (FPLC) and a set of microcomputer. In addition, another technical assistant, Miss Hansa Prawat, B.Sc. (Prince of Songkla University) was employed starting January 1, 1987 to help in organic chemistry work in Dr. Somsak Ruchirawat's laboratory. Supplies of cassava roots, stems, leaves and seeds from local sources were also reaching the laboratory.

2. Objectives:

2.1 To prepare linamarin from cassava root cortex

2.2 To prepare linamarase from cassava stems, petioles and root cortex

2.3 To determine change in linamarin in cassava roots during storage

3. Materials and Methods: See Progress Report No. 1

4. Results/Discussion:

4.1 Crude syrup containing cyanogenic glucosides was prepared from cassava root cortex in the biochemistry laboratory. The crude syrup was further fractionated in the organic chemistry by solvent extraction and thin-layer chromatography (TLC). Gram quantity of linamarin was obtained with high purity as verified by NMR spectroscopy. Thus, we achieved our objective of isolating linamarin from cassava for use in enzyme study. In future, we may be able to supply this expensive cyanogenic glucoside for use as standard in cyanide assay performed in cassava trade. In addition to the pure linamarin, we also found other fractions containing potentially new glucosides in the crude syrup from

cassava root cortex.

4.2 Linamarase was purified from the cassava stems, petioles and root cortex. Since these tissues were rich in the enzyme, the purification was not very complicated. The preparations from these tissues were characterized and shown to be similar. See further details in the attached copy of ICSU Short Report 6, 380-381 (1986).

4.3 Harvested cassava roots were kept at room temperature for several days. External signs of deterioration can be easily noted. After 3 days of storage, the tissue became softened. By 6 days, fungal growth on the surface was quite evident. When the roots were cut open, browning of tissue can be observed after 3 days of storage and became quite marked after 6 days. See attached photographs. After 3 days of storage, in the root cortex, linamarase, free cyanide and protein content dropped while linamarin level rose (Table 1). The result confirmed the findings of Kojima *et al* (1983) and suggested a possible linamarin synthesis in cassava root during storage and deterioration.

Table 1 Changes in linamarin, free cyanide, linamarase and protein in cassava root cortex during storage at room temperature.

Parameter (n = 8)	Section*	Day 0	Day 3
Linamarin (μmol/g wet wt.)	1	1.13 ± 0.82	1.40 ± 1.01
	2	0.82 ± 0.92	1.22 ± 0.79
	3	1.40 ± 1.05	1.82 ± 0.68
Free cyanide (μmol/g wet wt.)	1	0.37 ± 0.36	0.21 ± 0.16
	2	1.12 ± 0.73	0.79 ± 0.77
	3	0.82 ± 0.65	0.36 ± 0.50
Linamarase (μmol/min/mg)	1	0.95 ± 1.65	0.31 ± 0.66
	2	0.70 ± 1.19	0.23 ± 0.60
	3	0.27 ± 0.49	0.15 ± 0.39
Protein (mg/g wet wt.)	1	0.07 ± 0.02	0.09 ± 0.03
	2	0.05 ± 0.04	0.07 ± 0.03
	3	0.05 ± 0.01	0.08 ± 0.02

*Section of the root: 1 = head; 2 = middle; 3 = tip

5. Conclusion/Remarks:

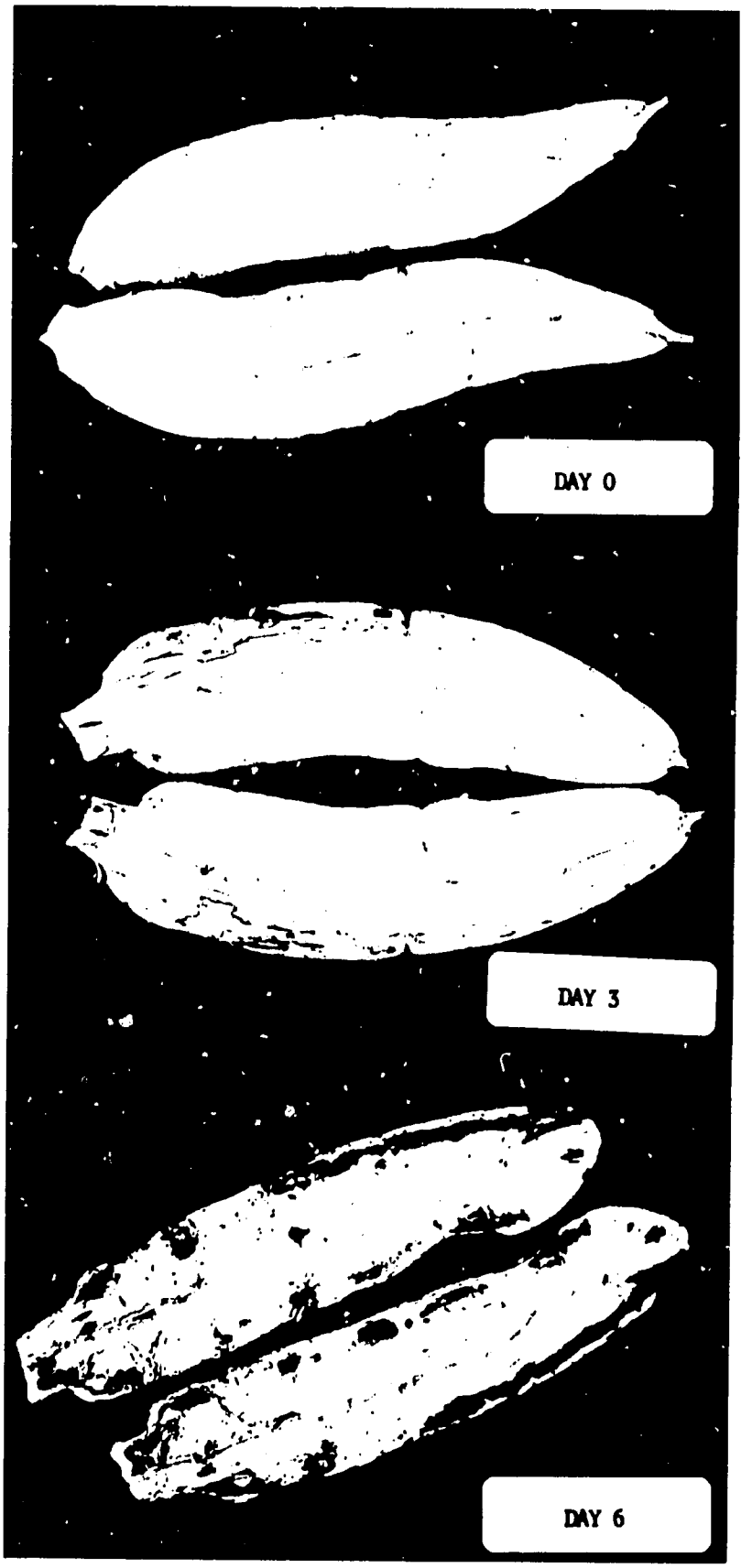
We could prepare pure linamarin and linamarase from cassava tissues for use in our further study. The change in the linamarin in deteriorating roots was confirmed.

6. Workplan for the Next Period:

We shall isolate and identify the unknown glucosides from the cassava roots. Analogues of linamarin will be synthesized and used to study the purified linamarase. Further characterization of the linamarase will be performed. Effects of gases and chemical treatment on the storage of cassava roots will be investigated.

7. Reference:

Kojima, M., Iwatsuki, N., Data, E.S., Villegas, D.V. and Uritani, I. (1983) Plant Physiol. 72, 186-189.


DAY 0
DAY 3
DAY 6

ICSU Short Report 6, 380-381 (1986).

COMPARISON OF CASSAVA LINAMARASES FROM PETIOLE, STEM AND ROOT CORTEX

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Roots of cassava (*Manihot esculenta* Crantz) are an important staple food in the tropics. However, cassava roots also contain two toxic cyanogenic glucosides, linamarin and lotaustralin, and their degrading enzyme, linamarase (or linamarin β-D-glucoside glycohydrolase; EC 3.2.1.21). The enzyme has been purified from root parenchyma (1) and used for linamarin assay in cassava trading and research. Being the second largest cassava-producer in the world (after Brazil), Thailand has a great abundance of stems, leaves and root cortex as by-products from cassava plantation and industry. So we decided to explore these by-products as possible new sources of linamarase.

Cassava tissue pieces were chopped and homogenized in ice-cold 0.1 M acetate buffer, pH 5.5, containing 0.1 mM phenylmethylsulfonyl fluoride (PMSF) in a Waring blender. After passing through a Miracloth to remove insoluble material, the homogenate was centrifuged at 10,000 g for 20 min at 4°C. The supernatant fluid was brought to 65% saturation by adding solid ammonium sulfate. Initial experiments showed that most of the enzymic activity (90%) was precipitated at this salt concentration. The precipitate was recovered by centrifugation at 10,000 g for 50 min at 4°C, redissolved in 10 mM phosphate buffer, pH 5.5 and dialyzed against the buffer (3 x 50 vol) overnight at 4°C. Linamarin was prepared from cassava root cortex as described previously (2).

Among the tissues extracted, petiole, stem and root cortex were found to have high specific activities of the enzyme (Table 1). Thus they were used in further study. By passing the ammonium sulfate preparation through a column of Sepharose 6B, the

Table 1 Distribution of linamarase in cassava tissues (mean ±SD)

Ammonium sulfate fraction from	Preparation (n)	Protein/fresh weight (mg/gm)	Specific activity (μmol/min/mg prot.)
Leaf	4	0.32 ± 0.23	0
Petiole	5	0.92 ± 0.65	2.85 ± 2.02
Stem	4	1.25 ± 0.27	1.76 ± 1.10
Root cortex	3	0.28 ± 0.14	1.56 ± 1.53
Root parenchyma	3	0.39 ± 0.01	0.05 ± 0.04

enzyme was separated from other proteins and found to have a native M_r of 600,000 (for stem and petiole enzymes). By this technique, the cortex enzyme was partially resolved into two peaks with M_r of 570,000 and 400,000. The active fractions were pooled, applied onto a chromatofocusing column and eluted with Polybuffer having pH range of 7 to 4. The enzyme from each source was separated into two isozymes: one with pI of 4.1 and another with pI <4 which was eluted with 1 M NaCl. By SDS-PAGE, all fractions contained a major band with M_r of 63,000 which was the subunit of linamarase. After the gel filtration step, most other proteins were eliminated and the enzyme preparation was about 90% pure. Using the enzyme preparation from the Sepharose column, the K_m and V_{max} were estimated (Table 2). On the basis of these values, linamarases from petiole and stem were similar but different from that of the root cortex. The findings suggested that the petiole, stem and root cortex were possible new sources of the enzyme.

Table 2 Properties of cassava linamarases

Enzyme source	Native M_r($\times10^{-3}$)	Subunit M_r($\times10^{-3}$)	pI value	K_m ($M\times10^4$)	V_{max} (nmol/min/mg)	Optimal temp (°C)
Petiole	600	63	4.1; <4	5.6	3.14	65
Stem	600	63	4.1; <4	5.0	3.33	55
Root cortex	400;570	63	4.1; <4	2.1	1.40	60

Supported by a grant from USAID/PSTC 936-5542-G-00-6028-00. T.E. received a student grant from MOSTE.

REFERENCES

(1) Cooke, R.D., Blake, G.G. and Battershill, J.M. (1978) Phytochemistry 17, 381-383.
(2) Kojima, M., Iwatsuki, N., Data, E.S., Villegas, C.D.V. and Uritani, I. (1983) Plant Physiol. 72, 186-189.

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