XII-AAZ-
6.404

PROGRESS REPORT NO 4

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

Professor Montri Chulavatnatol, Ph.D.

Project Leader

Department of Biochemistry

Faculty of Science

Mahidol University

Rama 6 Road, Bangkok 10400

THAILAND

Rec'd in SCI MAY 2 1 1988

9

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, 1987 - April 30, 1988 (6 months)

Budget Allocation for This Period: 471,510 Baht

Contents

1. Background/Introduction:

During this period, no change was made on the personnal. A few applicants for the vacent position of technical assistant were interviewed. However, none was found suitable and more candidates were being sought. Two graduate students, Miss Panomporn Panutat and Miss Nuntaporn Pitiguagool, undertook an advanced biochemistry project on the study of peroxidase during cassava root deterioration. The project lasted for 4 months, December 1987-March 1988. Since then, one student, Miss Panomporn Panutat, started her M.Sc. thesis work on the use of cassava tissue culture to study factors affecting cyanogenic glucoside synthesis and accumulation. In addition, the principle investigator went on a study tour for 3 weeks (April 12-30, 1988) to two institutions: Professor E.E. Conn's laboratory in the Department of Biochemistry and Biophysics, University of California at Davis, California, U.S.A. and Dr. R.D. Cooke's laboratory at the Overseas Development and Natural Resources Institute in London, England.

2. Objectives:

2.1 Characterization of cassava linamarase

2.2 Characterization of cassava glucosyltransferase

2.3 Peroxidase during cassava root determination

2.4 Cassava tissue culture

2.5 Tour of laboratories

3. Results/Discussion

3.1 Cassava linamarase was being studied in terms of structure and catalysis. Antibody was raised in rabbit against the purified enzyme. This was used to probe the structural differences among the isozymes. Proteolytic fragmentation of the structure was performed and the fragments would be analyzed to compare the three isozymes. The catalytic property was abolished by modification of lysine residues. These studies were being performed by Miss Pennapa Kisamanonta.

3.2 Cassava glucosyltransferase was being purified using fast-protein liquid chromatography (FPLC). Its instability was the major hindrance in its purification. The partially purified enzyme was characterized for its catalytic and structural properties. These studies were being written up as an M.Sc. thesis by Miss Panpaka Chalermisrachai.

3.3 Cassava peroxidase was studied in cassava root parenchyma and cortex. Physiological deterioration of the roots stored at room temperature was followed for 7 days. During this period, peroxidase activity of the parenchyma decreased more than that of cortex. The cortex enzyme was found to be heat-stable while that of the parenchyma was heat-labile. The parenchyma enzyme moved faster than the cortex enzyme during electrophoresis under non-denaturing condition. Both, however, showed similar subunit molecular weight by electrophoresis in the presence of sodium dodecyl sulfate. Thus peroxidase changes corresponded with the browning response during the root deterioration. The study was carried out by Miss Panomporn Panutat and Miss Nantaporn Pitiguagool.

3.4 Cassava tissue culture facility was improved by the addition of a laminar flow hood in the plant tissue culture room. More plantlets were produced and they were found to possess both linamarin and linamarase. So the plantlets produced under the sterile condition of the tissue culture bottles offerred an ideal opportunity to study environmental effects on the biosynthesis of the cyanogenic glucosides. Miss Thidarat Eksittikul was in charge of the operation of the plant tissue culture room.

3.5 Tour of laboratories had two major objectives: to learn more about other cyanogenic glycosides and to make known our studies on cassava cyanogenic glucosides. The P.I. learned much more about other plant cyanogenic glycosides in Professor E.E. Conn's laboratory which was a strong center where the work in this area had been carried out for over three decades. In Dr. R.D. Cooke's institute, much was learned about the deterioration of cassava roots and other tropical root crops. Also there was a major effect on the biotechnology of cassava food processing. Our work was made known when the P.I. gave seminars in both laboratories. As a result, the P.I. was contacted by scientists from England and Denmark to do collaborative research with the aim to develop cyanide-free cassava.

4. Conclusion/Remarks:

We have made further progress in understanding the cassava linamarase, glucosyl-transferase, peroxidase and cassava root deterioration. Cassava tissue culture has been set up and is ready for more definitive study.

5. Workplan for the Next Period:

5.1 Biotransformation of linamarin using plant tissue culture.

5.2 Biochemical changes during cassava root deterioration.

6. Attached document:

6.1 Montri Chulavatnatol, Somsak Ruchirawat, Thidarat Eksittikul, Uma Prawat, Panpaka Chalermisrachai, Pissopa Kitjaharn and Hansa Prawat (1987) Biotransformation of cyanogenic glucosides in cassava (*Manihot esculenta* Crantz). Abstract presented in the First Princess Chulabhorn Science Congress, Bangkok, 10-13 December 1987.

BC-27

BIOTRANSFORMATION OF CYANOGENIC GLUCOSIDES IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Montri Chutavatnatol*, Somsak Ruchirawat**, Thidarat Eksittikul*, Uma Prawat**, Panpaka Chalermisrachai*, Pissopa Kitjaharn* and Hansa Prawat**

Departments of Biochemistry and Chemistry**, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

Storage roots of cassava are the main source of carbohydrates for 500 million poor people in 30 tropical countries. In 1986, the world cassava production was 143 million tons with Thailand and Brazil as the top producers, each producing 20 million tons. In the same year, Thailand earned 19,000 million baht from exporting 1/3 of her cassava products. Hidden in this agro-economic profile is a health problem. Cassava possesses two known cyanogenic glucosides: linamarin and lotaustralin, with the former being the majority. These glucosides are toxic because of HCN liberated upon their hydrolysis. With the aim to lessen the toxicity, biosynthesis and degradation of linamarin in cassava were studied. Valine was converted by plant microsome into acetone cyanohydrin. Then the latter was conjugated with glucose to form linamarin in the reaction catalyzed by a glucosyltransferase. The glucosyltransferase was found in young cassava leaves. Its native molecular weight was 46,000. Uridine 5'-diphosphate-glucose was the preferred donor of the glucosyl moiety and acetone cyanohydrin was the best glucose-acceptor. The hydrolysis of linamarin, catalyzed by linamarase, yielded glucose and acetone cyanohydrin. The latter broke down spontaneously into acetone and HCN. In addition to cassava root parenchyma as the known source of linamarase, cassava petiole, stem and root cortex were found to be richer sources of the enzyme. Thus, the enzyme was purified from these sources. It was an oligomeric enzyme, exhibiting a native molecular weight of 600,000 and the subunit molecular weight of 63,000. It was separated into three isozymes by chromatofocusing. It was specific for the hydrolysis of a β-glycosidic bond between glucose or fucose and an aglycone. An aliphatic aglycone was preferred over an aromatic one. From the root cortex, both linamarin and lotaustralin were isolated by hot ethanol extraction, followed by a silica gel column chromatography. Both linamarin and linamarase were essential for the cyanide determination in cassava trade. *(Supported by USAID/PSTC 936-5542-G-00-6028-00.)*

15