

Caribbean Poison Information Network
(CARPIN)

First Scientific Conference

Terra Nova All-Suite Hotel

Saturday June 3, 2006

HYPOGLYCIN : A Toxic Amino Acid of the Ackee plant

PRESENTER K D GOLDEN

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Unripe Ackee
(Scientific name: *Blighia sapida*)

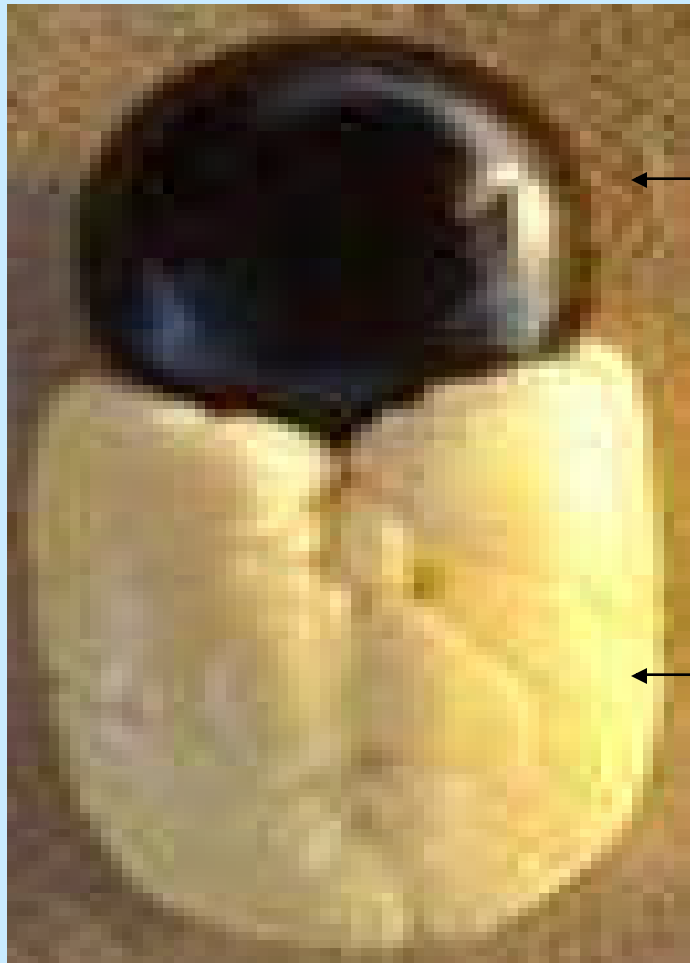


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Ripe Ackee



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Seed

Aril

Conference June 3-4, 2000

OBJECTIVE

- **TO DETERMINE THE HYPOGLYCIN CONTENT OF :-**
 - a) THE RIPE AND UNRIPE FRUIT**
 - b) THE COOKED RIPE FRUIT**
 - c) THE WATER USED TO COOK THE RIPE FRUIT**

History of ackee research at UWI Mona

- **1950's , 60's and 70's**
- **Ingestion of the of unripe ackee fruit linked to Jamaican vomiting sickness (P. C. Feng)**
- **Structure of hypoglycin A elucidated (E. V. Ellington / C. Hassall / K. Reyle)**
- **Effects of hypoglycin A on metabolism (E. A. Kean / C. Von Holt)**
- **Assay methodology for hypoglycin developed (E. A. Kean)**
- **Mode of action of hypoglycin A on the pyruvate carboxylase and Acyl CoA dehydrogenase (E. A. Kean / E. Y. Morrison)**

History of research cont'd

- **1980's**
- **Production of dicarboxylic acid in rats given hypoglycin A.(E A. Kean / K. Golden)**
- **Proposed mechanism of hypoglycin A synthesis in ackee.(E. A..Kean / C. Lewis)**
- -----
- **2002**
- **Hypoglycin content of cooked ackee (K Golden)**

INTRODUCTION

- **JAMAICAN VOMITING SICKNESS(ACKEE POISONING): KNOWN TO OCCUR IN JAMAICA AS EARLY AS 1875**
- **AFFECTS MOSTLY CHILDREN (2-10 YEARS OLD)**
- **THE ACKEE IS INDIGENOUS TO WEST AFRICA WHERE IT IS CALLED **ANKYE** OR **ISHIN****
- **BELIEVED TO BE FIRST BROUGHT TO JAMAICA BY **Dr THOMAS CLARKE**, ALTHOUGH SOME SCIENTIST BELIEVED IS WAS INTRODUCED TO JAMAICA BY **CAPTAIN BLIGH** OF HMS BOUNTY. IT HAS BEEN CALLED *Blighia sapida* SINCE **KOEING** NAMED IT IN 1806.**

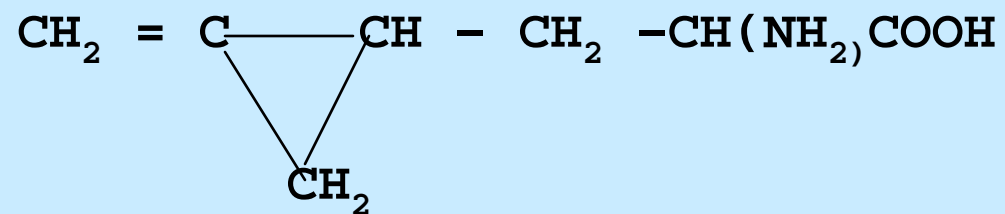
INTRODUCTION CONT'D

- The ackee plant contains two amino acids of interest, **Hypoglycin A** and **Hypoglycin B** (γ -glutamyl hypoglycin). Hypoglycin A (L- α -amino - β -methylenecyclopropyl propionic acid) is a toxic amino acid that is present in the ackee fruit and the seed of the fruit; while hypoglycin B which is far less toxic is found in greater quantity in the seed. In general hypoglycin A is usually written as hypoglycin.

JAMAICAN VOMITING SICKNESS

- **CAUSATIVE AGENT IS HYPOGYLCIN**
- **CLINICAL FEATURES OF JAMAICAN VOMITING SICKNESS:-**
 - **(a) Hypoglycemia**
 - **(b) Vomiting**
 - **(c) Patient becomes comatose and death may follow.**

STRUCTURE OF HYPOGLYCIN(Ellington et al 1958)

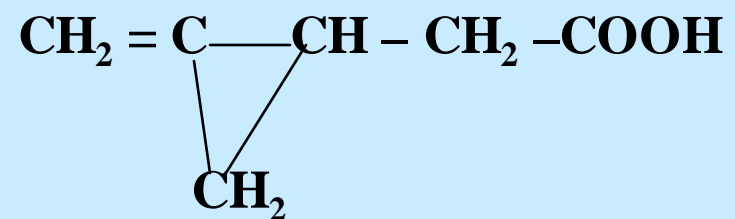


METABOLISM OF HYPOGLYCIN

- **HYPOGLYCIN IS METABOLISED LIKE THE BRANCHED-CHAIN AMINO ACIDS (Val, Leu and Ileu)**
- **WHERE THERE IS DEAMINATION FOLLOWED BY DECARBOXYLATION:-**

- **HYPOGLYCIN(A) $\xrightarrow{1}$ α KETO ACID $\xrightarrow{2}$ MCPA-CoA**
- **MCPA=METHYLENECYCLOPROPYL ACETIC ACID**
- **1= AMINO ACID TRANSFERASE**
- **2= α -KETO ACID DECARBOXYLASE**
- **MCPA-CoA ESTER IS THE TOXIC METABOLITE OF HYPOGLYCIN.**

MCPA:STRUCTURE



MCPA-CoA MODE OF ACTION

- **MCPACoA IS A POWERFUL INHIBITOR OF FATTY ACID METABOLISM (β -OXIDATION). IT INHIBITS MEDIUM AND SHORT CHAIN **ACYL - CoA DEHYDROHROGENASE**. (KEAN, E.A., 1976).**
- **AMINO ACID METABOLISM IS ALSO AFFECTED, WHERE ISOVALERYL CoA, GLUTARYL CoA AND α -METHYL-BUTYRYL CoA DEHYDROGENASES ARE INHIBITED.**(TANAKA ET AL 1971).
- **MCPACoA IS ALSO AN INHIBITOR OF GLUCONEOGENESIS (HENCE THE REASON FOR HYPOGLYCEMIA); IT ALLOSTERICALLY INHIBITS **PYRUVATE CARBOXYLASE**, A KEY ENZYME IN GLUCONEOGENESIS (TANAKA, K. ET AL, 1976).**

How safe is ripe ackee?

- The incidence of ackee poisoning has decline significantly over the years, as people are more aware of the danger of consuming unripe ackee.
- Ripe ackee has hypoglycin , but when prepared properly is nontoxic. WHY?

**TO ANSWER THE QUESTION WE DECIDED TO
LOOK AT THE HYPOGLYCIN CONTENT OF:**

- a) THE UNCOOKED RIPE ACKEE**
- b) THE COOKED RIPE ACKEE**
- c) THE WATER USED TO COOK THE RIPE ACKEE.**

METHODS

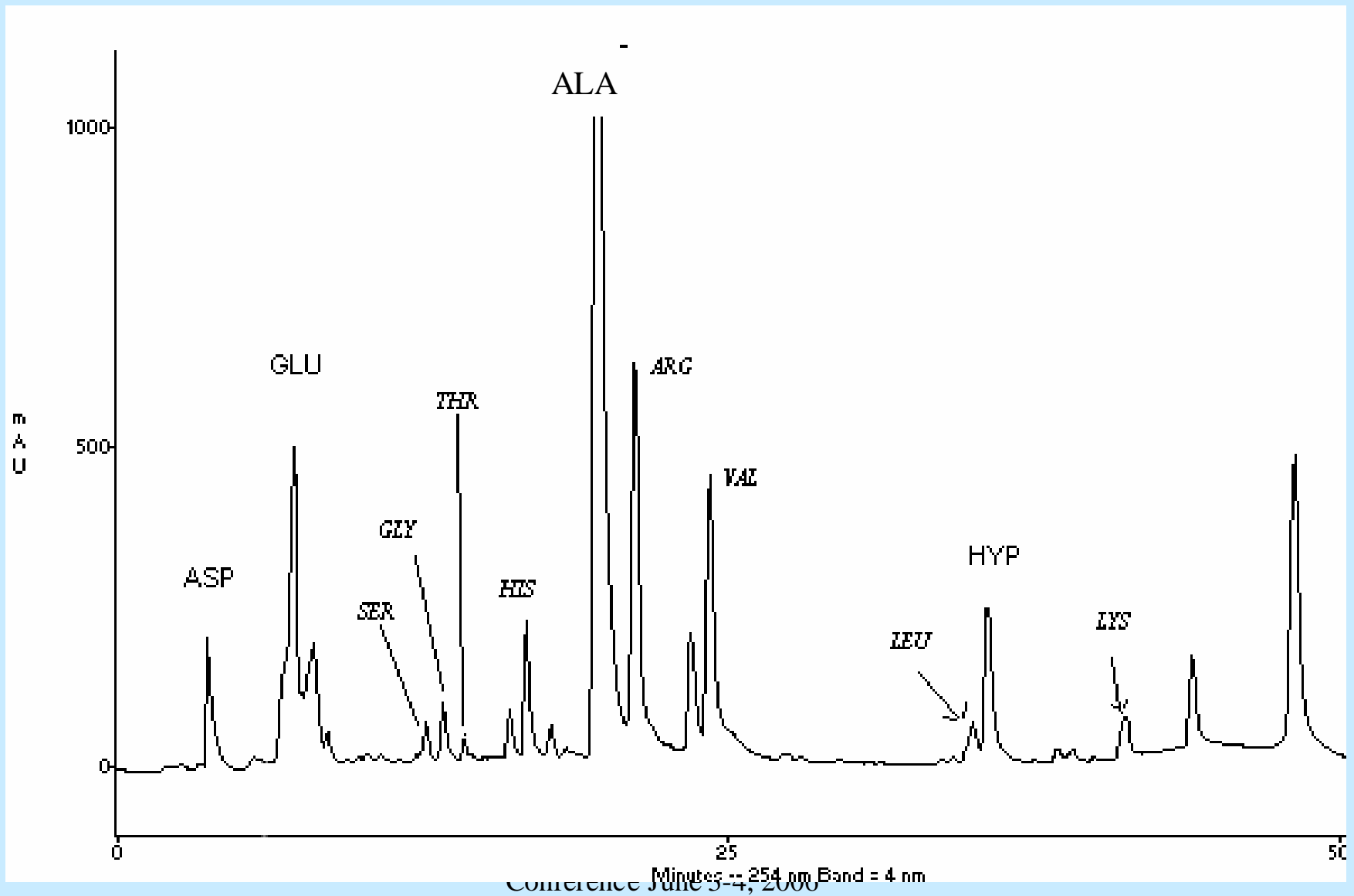
- **Extraction of amino acids from ackee fruit**
- **Derivatization of the amino acids using the Edman reagent (Phenyl isothiocyanate)**
- **Analysis of the derivatized samples using High Performance Liquid Chromatography (HPLC)**

METHODS CONT'D

- 1. Extract ackee fruit(1g) with 80% ethanol, using a blender.**
- 2. Filter and centrifuge slurry at 5 000 rpm for 5 minutes.**
- 3. From aliquots of the supernatant obtained use Edman reagent to make phenylisothiocyanate derivatives of the amino acids present.**
- 4. HPLC parameters: Flowrate 1ml/ml , gradient elution using buffered solvents. Detection at 254 nm.**

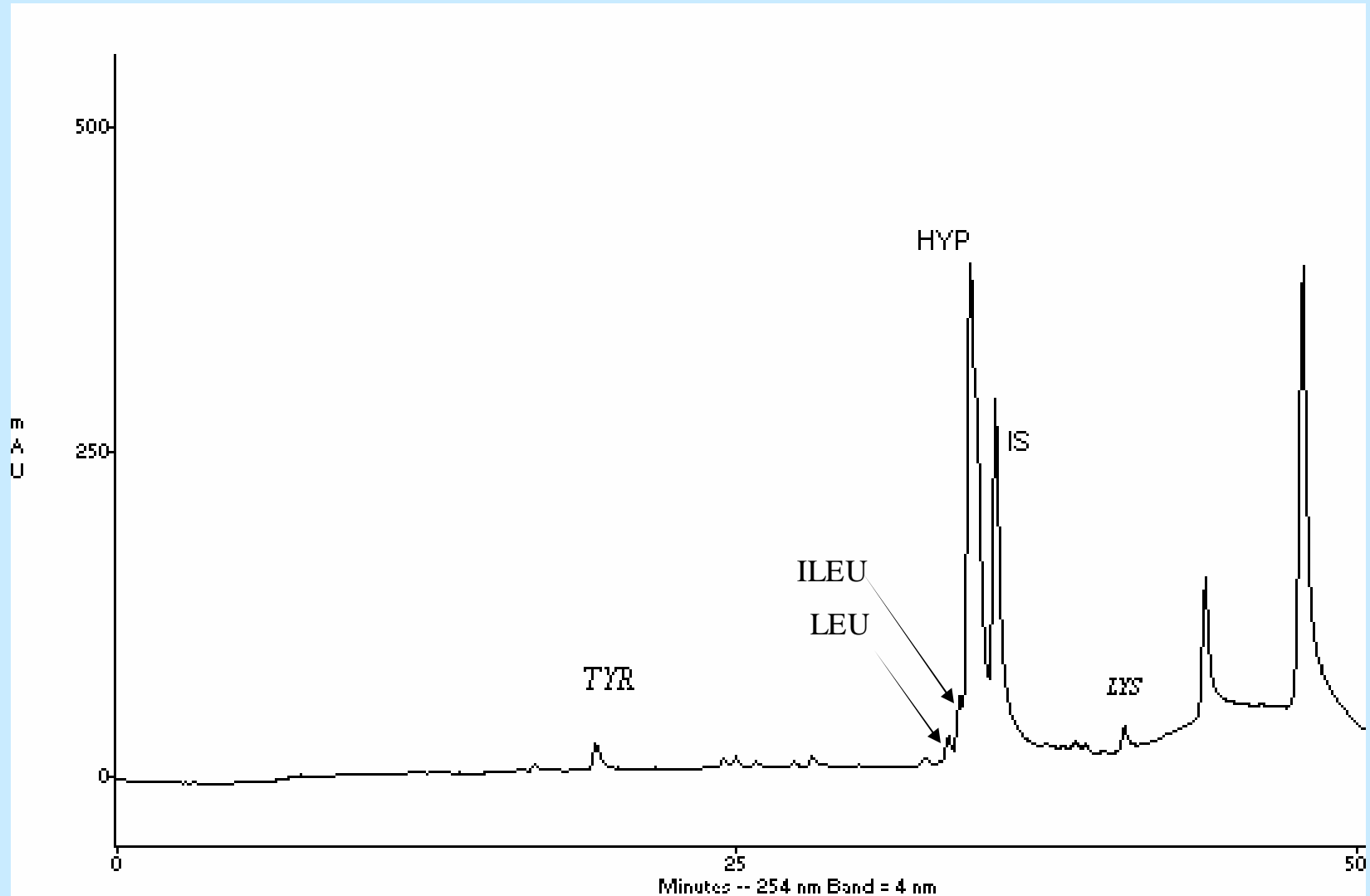
RESULTS

TYPICAL CHROMATOGRAM OF AMINO ACIDS FROM RIPE ACKEE



RESULTS

TYPICAL CHROMATOGRAM OF PARTIALLY PURIFIED HYPOGLYCIN



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RESULTS

TABLE 1. HYPOGLYCIN CONTENT OF ACKEE FRUIT

AMINO ACID	UNRIPE FRUIT Mg/100g WWF	RIPE FRUIT Mg/100g WWF ⁺
HYPOGLYCIN	124.4±6.70	6.40 ±1.10*

+ WET WEIGHT FRUIT

* MEAN \pm STANDARD DEVIATION OF DUPLICATES.

RESULTS

TABLE 2. HYPOGLYCIN CONTENT OF COOKED FRUIT AND WATER

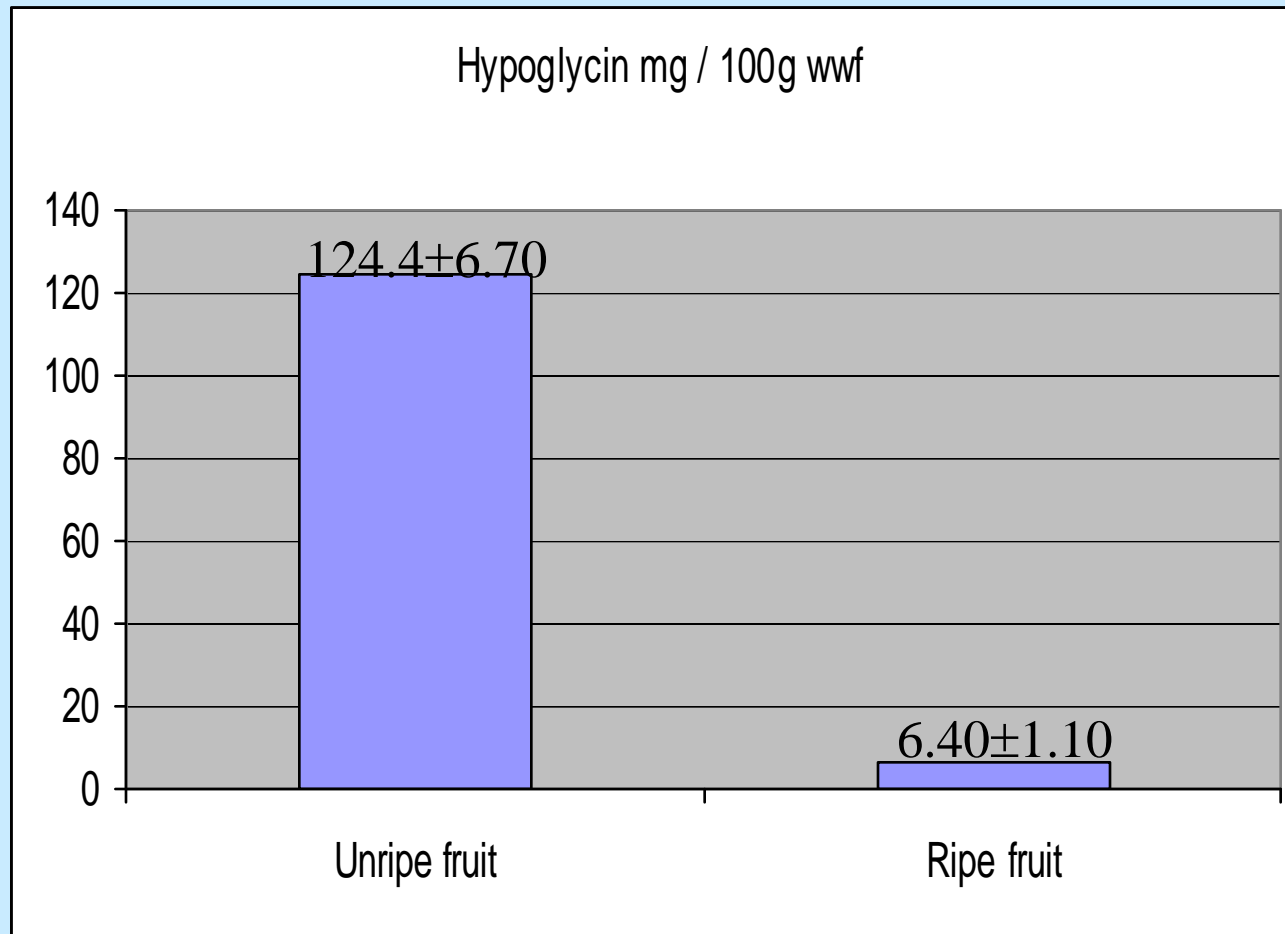
AMINO ACID	<u>Cooked ripe fruit</u> Mg / 100g wwf	<u>Water used to cook ripe fruit</u> Mg / 200 ml
HYPOGLYCIN(S1)	Nd+	0.54±0.15*
HYPOGLYCIN(S2)	1.17 ±0.35	4.96 ±0.50

S1 = sample 1; S2 = sample 2.

+ NOT DETECTED. *MEAN ± STANDARD DEVIATION OF DUPLICATES

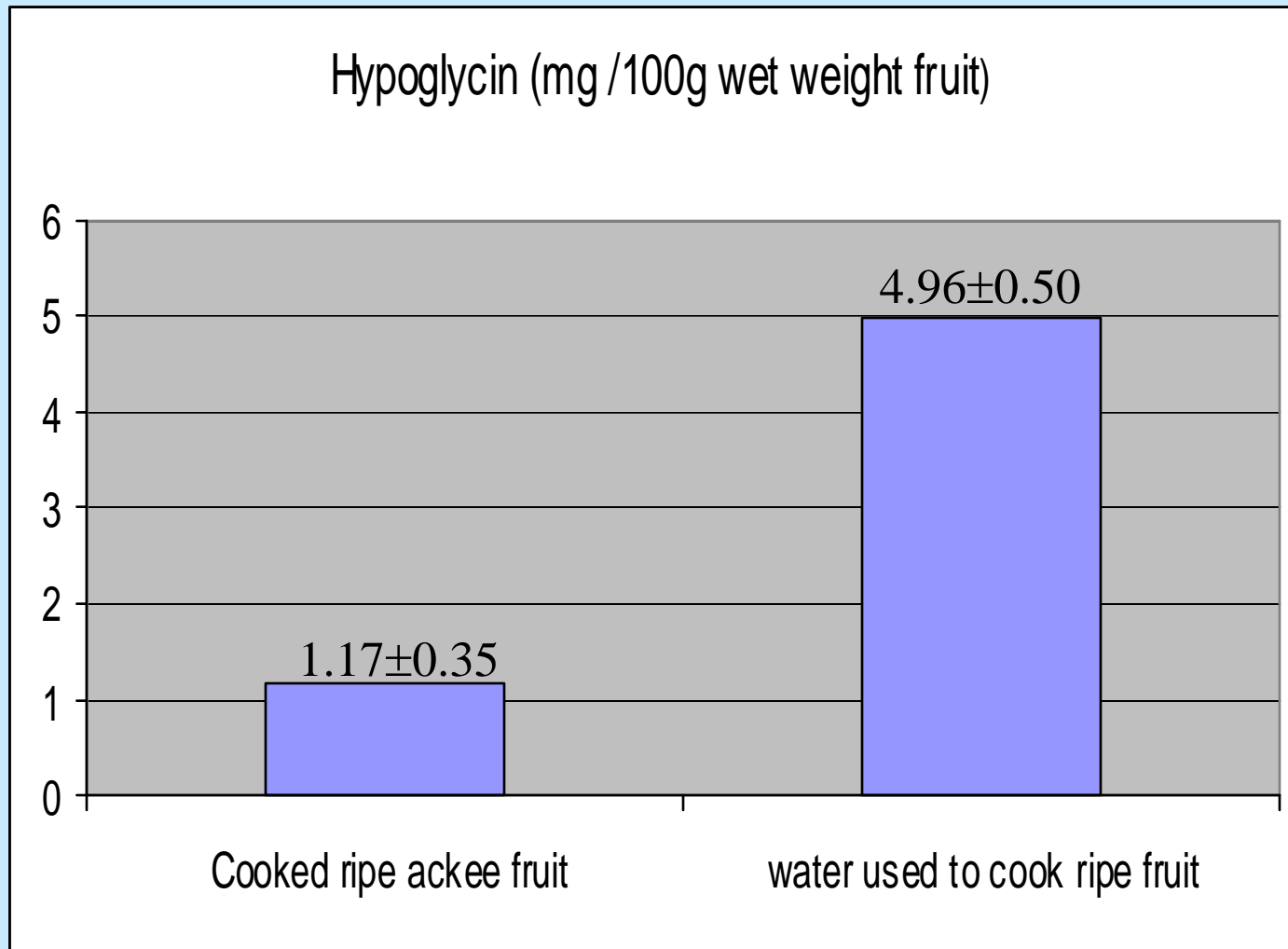
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CANNED ACKEE : 3.22 Mg/ 100g

HYPOGLYCIN CONTENT OF RIPE & UNRIPE FRUIT



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HYPOGLYCNIN CONTENT OF COOKED FRUIT



Current research

Isomers of hypoglycin: D. Minott and Camille Bowes (Chemistry Department).

Discussion

- **THE PRESENCE OF HYPOGLYCIN IN THE ACKEE FRUIT HAS BEEN A MAJOR CONCERN OVER THE YEARS.**
- **ALTHOUGH THE LEVEL OF HYPOGLYCIN IN THE RIPE FRUIT IS ONLY 6.38 ± 1.06 Mg / 100g FRESH WEIGHT OF THE FRUIT, THE LD₅₀ LEVEL OF HYPOGLYCIN FOR HUMANS IS UNKNOWN.**
- **THE LD₅₀ FOR RATS IS 90 – 100 Mg/ kg BODY WEIGHT, WHILE FOR RABBITS AND MONKEYS IT'S 10 –20 Mg/ kg BODY WEIGHT.**
- **IN THIS STUY WE FOUND THAT A SIGNIFICANT AMOUNT OF THE HYPOGLYCIN WAS EXTRACTED FROM THE RIPE FRUIT UPON COOKING; ON AN AVERAGE 90.5%.**

DISCUSSION

- **THE PREPARATION OF ACKEE BEFORE CONSUMPTION IS OF VITAL IMPORTANCE.**
- **THE WATER USED TO COOK THE ACKEE MUST BE DISCARDED AND NOT USED TO COOK RICE. WHY?. RICE COOKED IN THIS WAY WILL BE COATED WITH HYPOGLYCIN.**

CONCLUSION

**IT IS CLEAR THAT BOILING EFFECTIVELY
REDUCES THE AMOUNT OF HYPOGLYCIN IN
THE RIPE FRUIT, RENDERING IT NONTOXIC.**

Future Work.

1. The biochemist and the molecular biologist must work together and develop a transgenic ackee plant that will produce ackee that is free of hypoglycin.
2. This would undoubtedly solve the problem where the exportation of ackee to the USA is concerned and allow the country to earn the much needed foreign exchange.

REFERENCES

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