High-Performance Liquid Chromatographic Analysis of Amino Acids in Ackee Fruit with Emphasis on the Toxic Amino Acid Hypoglycin A

K.D. Golden*, O.J. Williams, and **Y. Bailey-Shaw**

Department of Basic Medical Sciences (Biochemistry Section), University of the West Indies, Mona Campus, Kingston 7, Jamaica W.I.

Abstract

High-performance liquid chromatography is used to determine the amino acid content of ripe and unripe ackee fruit. Specific emphasis is placed on the level of the toxic amino acid hypoglycin A (hyp-A) in the unripe and ripe ackee fruit and seed. Unripe samples are found to contain significantly higher quantities ($P < 0.05$) of hyp-A when compared with ripe samples. Uncooked unripe fruit is found to contain 124.4 ± 6.7 mg/100 g fresh weight and uncooked ripe fruit 6.4 ± 1.1 mg/100 g fresh weight. The seed of the uncooked unripe fruit is found to contain 142.8 ± 8.8 mg/100 g fresh weight, and the seed of uncooked ripe fruit has 106.0 ± 5.4 mg/100 g fresh weight. Boiling fruit in water for approximately 30 min is efficient in removing hyp-A from the edible arilli; however, low levels of 0.54 ± 0.15 mg/200 mL are detected in the water that was used to cook the ripe fruit. The average %recovery of the amino acids was 80.34%.

Introduction

The ackee plant (*Blighia sapida*) is widespread in the island of Jamaica and its fleshy fruit (the arillus) is a staple in the Jamaican diet. The ackee plant contains two amino acids of interest, hypoglycin A (L-α-amino-β-methylene cyclopropane propionic acid) (hyp-A) and hypoglycin B (γ-glutamyl hypoglycin) (hyp-B). Hyp-A has assumed greater importance because it is a toxic amino acid that is present in the seed and flesh of the ackee fruit, and hyp-B (which is far less toxic) is found only in the seed (1).

Hyp-A is the causative agent of ackee poisoning, commonly known as Jamaican vomiting sickness (2). Jamaican vomiting sickness results from the ingestion of immature or improperly prepared ackee fruit. Patients suffering from hyp-A poisoning experience vomiting approximately 4 h after ingestion. The condition is accompanied by hypoglycemia (4), during which the patient becomes comatose and death usually occurs. Kean and Pogson (3) have shown that in the mode of action, the hypoglycin metabolite methylene cyclopropylpyruvate inhibits gluconeogenesis by inhibiting a key enzyme (pyruvate carboxylase) in gluconeogenesis. Hypoglycin has also been implicated in the inhibition of fatty acid metabolism (β-oxidation), in which there is inhibition of the acyl–CoA dehydrogenases that are active on medium and short-chain fatty acids (5). The excretion of unusual dicarboxylic acids in urine is directly related to the inhibition of the acyl–CoA dehydrogenases (6).

Although the mechanism of action of hyp-A poisoning has been elucidated, some individuals in rural Jamaica still have difficulty with ackee preparation because the water used to cook the fruit is often used as stock for soup or rice. This has been further complicated by the fact that the dose of hyp-A needed to cause fatality in humans per kilogram body weight (the LD_{50}) is unknown. Other factors of importance are the maturity of the ackee fruit at harvest and the amount of water used for cooking ackee fruit per unit fresh weight arilli.

Ackee forms a part of the Jamaican national dish (ackee and saltfish) and is consumed extensively locally. The fruit is also of commercial importance because it is widely exported to various countries worldwide. The purpose of this study was to quantitate the levels of hyp-A and other amino acids in ackee fruit and evaluate the effect of boiling on residual levels of hyp-A in cooked and uncooked ackee fruit.

Experimental

Chemicals

All chemicals were of analytical grade unless otherwise noted. Standard amino acids, phenylisothiocianate (PITC), ninhydrin, and triethanolamine (TEA) were obtained from Sigma (St. Louis, MO). Amberlite (IR-45) resin was obtained from BDH Chemicals (Poole, U.K.), and a Waters-Spherisorb reversed-phase column was obtained from Supelco (Bellefonte, PA).

* Author to whom correspondence should be written.

Plant material

Ackee fruit was obtained locally from fruit trees on the Mona campus of the University of the West Indies.

Sample preparation

Ackee fruit (100 g) was homogenized with 195 mL of 80% ethanol and strained through 2 layers of cheesecloth. The extract was then centrifuged at 2000 × *g* for 10 min in a bench centrifuge. The supernatant (205 mL) was extracted twice with 200-mL portions of toluene to remove the residual fat. The ethanol layer (200 mL) was removed and filtered through Whatman No. 4 filter paper (Whatman International Ltd., Kent, U.K.). Samples from ackee seeds were prepared similarly to samples from the arilli with the exception that the seed coat was removed before homogenization. Cooked fruit was prepared by boiling 100 g of tissue in 230 mL of water for 30 min. The water, which also contained 0.4 g of NaCl, was decanted and retained for analysis. The cooked fruit was homogenized and extracted as previously described for other samples.

Filtered extracts (200 mL) were reduced to 10 mL using a rotary evaporator Model No. 506 (Buchler Instruments, Fort Lee, NJ) and made acidic by adding 1 mL of 0.5N HCl. The mixture (10 mL) was applied to Amberlite resin IR-45 (9 × 5 cm column dimensions) and washed with water (100 mL) followed by 0.4M ammonium acetate (100 mL) at pH 9.4. Thirty-five fractions (5 mL each) were collected using an ISCO Model 328 fraction collector (ISCO, Lincoln, Nebraska). Each fraction was assayed for amino acids using 0.4% ninhydrin solution. All positive fractions were pooled (105 mL) and retained. The column was then washed with 0.4M ammonium acetate (100 mL) at pH 12.7. Positive fractions were again pooled (60 mL) and the combined-pooled fractions (165 mL) reduced to 20 mL by rotary evaporation. Hyp-A was isolated from the seeds of the ripe fruit as outlined in reference 7.

Derivatization of amino acids using PITC

A stock solution containing 2.5 µmol/mL of each standard amino acid in 0.1N HCl was prepared. A partially purified hyp-A sample (1 mL) of unknown concentration was also prepared to be used for the quantitative identification of hyp-A. From each preparation, 20 µL was pipetted into separate eppendorf tubes and 10 µL of norleucine (10 µmol/mL) was added.

The amino acid derivatization process of the standard amino acids and hypoglycin is a modification of the method of González-Castro et al. (8). After ion-exchange chromatography 100 µL of the extract of the ripe and unripe fruit was pipetted into separate eppendorf tubes and 10 µL of norleucine (10 µmol/mL) was added to each. The solutions were dried under vacuum in a Savant (Farmingdale, NY) speed vacuum at 65°C. To the residue, 60 µL of methanol–water–TEA (2:2:1, v/v) was added, and the resulting solution was evaporated under nitrogen. Sixty microliters of the derivatizing reagent, which consisted of PITC in methanol–water–TEA (7:1:1, v/v/v), was added and the tubes were left at room temperature (26°C) for 20 min. The solvents were evaporated under a stream of nitrogen and 300 µL of 0.05M ammonium acetate was added to the residue prior to analysis.

To determine the efficiency of the extraction procedure, samples were spiked with a known amount of standard amino acids (10 mg each) and carried through the extraction and derivatization processes as previously described.

High-performance liquid chromatographic apparatus

Chromatographic analysis was performed using a Beckman (System Gold-Nouveau) chromatographic system (Beckman, Fullerton, CA) consisting of a solvent module (Model 126), a UV detector (Model 168), and an autosampler (Model 508) fitted with a 20-µL injection loop. The solvent system consisted of two buffers. Buffer A was 0.05M ammonium acetate (pH 6.8) and buffer B was 0.1M ammonium acetate in acetonitrile–methanol–water (44:10:46, v/v/v) at pH 6.8. The flow rate through the column was 1 mL/min, and 20 µL of the samples were injected onto the column (Waters-Spherisorb ODS2, 5-µm film thickness, 250 × 4.6 mm). Amino acids were identified by UV detection at 254 nm. A gradient elution program was employed for analysis. Stepwise elution was used beginning with 100% A for 0.2 min and changing from 100–50% A in 35 min. Fifty percent A was changed to 25% A in 5 min, the system was held at 25% A for another 5 min and finally changed from 25% to 100% A in 10 min.

Quantitation and identification

The internal standard method was used to quantitate the amino acids in the samples according to the formula below (9):

$$K = (A_i)\,(W_x) / (A_x)\,(W_i) \qquad \text{Eq. 1}$$

where W_x is the weight of component x, K the relative response factor for the particular amino acid, A_i the area under the peak of the internal standard, A_x the area under the peak of component x, and W_i the weight of the internal standard.

The relative retention time (the ratio of the retention time of the standard amino acids to that of the internal standard) (RRT) was used to identify the amino acids that were present in the sample.

Results and Discussion

Figure 1 shows the chromatogram of the standard amino acids and the internal standard (norleucine = peak 16). Although there was some variation in the retention time of the amino acids, the RRT of the amino acids showed little variation (Tables I–III). The percent recovery of some standard amino acids is shown in Table IV, and these data were typical of all the samples analyzed (ripe fruit, unripe fruit, and cooked ripe fruit). With an average %recovery of 80.34% and with the K for each of the amino acids being unity, the efficiency of the extraction and derivatization procedures may be described as good. Based on the assessment of the percent recovery, phenylalanine, valine, and isoleucine (which like hypoglycin contain hydrophobic R-groups) gave %recoveries of 98.5%, 75%, and 63.1%, respectively. These amino acids and their representative recoveries were used to estimate the %recovery of hyp-A

because pure hyp-A is not commercially available and because a particular family of biological compounds are expected to show similar trends and properties.

Figure 2 shows the chromatogram of partially purified hypoglycin (86.4%), in which the amino acids most prominent as impurities were isoleucine (3.04%) and leucine (4.46%), which are the amino acids that usually coelute or elute close to hypoglycin (7). Alanine (2.14%), tyrosine (0.523%), valine (0.594%), phenylalanine (0.704%), and lysine (2.139%) were also detected in the hypoglycin sample. McGowan et al. (10) have also reported on the separation of isoleucine, leucine, and hypoglycin from partially purified hypoglycin samples with similar results. Chase et al. (11) used ion-exchange chromatography to determine the level of hypoglycin in canned ackee fruit; however, they were unable to separate isoleucine from methionine. The problem of isolating pure hypoglycin still remains because isoleucine, leucine, and hypoglycin have very similar chromatographic properties. To date, high-performance liquid chromatography (HPLC) is one of the few chromatographic methods that have been able to separate these three amino acids with success.

Figures 3 and 4 show typical chromatograms of the amino acids that are present in the ripe ackee fruit and the unripe ackee fruit, respectively, and the amino acid content of the ackee fruit and seed is shown in Tables I and II, respectively. The essential amino acids arginine, histidine, isoleucine, leucine, valine, lysine, phenylalanine, threonine, and tryptophan are all present in the unripe fruit and the ripe fruit (Table I). Similar results were obtained for the seed, with the exception that leucine and tryptophan were not detected in the ripe seed (Table II). Isoleucine was the only essential amino acid

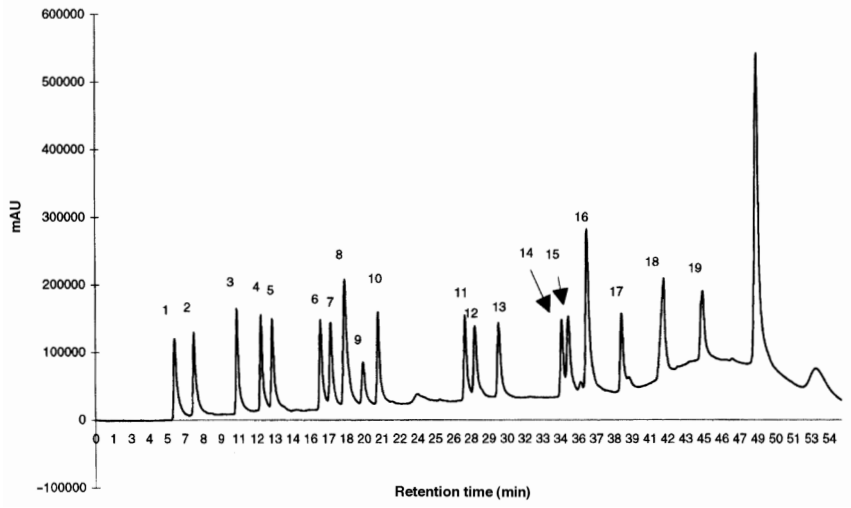

Figure 1. HPLC of the PITC derivative of the standard amino acids: aspartic acid, 1; glutamic acid, 2; hydroxy-proline, 3; serine, 4; glycine, 5; threonine, 6; alanine, 7; histidine, 8; proline, 9; arginine, 10; tyrosine, 11; valine, 12; methionine, 13; isoleucine, 14; leucine, 15; norleucine (internal standard), 16; phenylalanine, 17; tryptophan, 18; and lysine, 19.

Table I. Amino Acid Content of Ackee Fruit (Arillus)*

Amino acid	K	RRT_{AA}†	RRT_{sam}‡	Unripe fruit (Mg/100 g wwf§)	Ripe fruit (Mg/100 g wwf)
Aspartic acid	1.00	0.16 ± 0.01	0.17 ± 0.00	4.37 ± 0.24	ND**
Glutamic acid	0.99	0.20 ± 0.01	0.21 ± 0.00	80.80 ± 7.20	26.51 ± 0.35
Hydroxy-proline	1.00	0.31 ± 0.01	0.30 ± 0.01	1.64 ± 0.15	1.40 ± 0.12
Serine	1.00	0.35 ± 0.01	0.35 ± 0.01	9.58 ± 1.50	3.06 ± 0.28
Glycine	1.00	0.38 ± 0.01	0.38 ± 0.01	11.34 ± 0.48	7.13 ± 0.64
Threonine	1.00	0.45 ± 0.00	0.45 ± 0.00	26.86 ± 4.09	5.73 ± 0.44
Alanine	1.00	0.47 ± 0.00	0.47 ± 0.00	34.46 ± 2.84	14.42 ± 2.90
Histidine	1.00	0.50 ± 0.00	0.50 ± 0.00	6.38 ± 1.10	2.84 ± 0.29
Proline	1.00	0.52 ± 0.00	0.51 ± 0.01	1.45 ± 0.16	0.45 ± 0.01
Arginine	0.99	0.59 ± 0.01	0.59 ± 0.00	53.54 ± 5.99	62.88 ± 5.87
Tyrosine	1.00	0.77 ± 0.00	0.77 ± 0.00	2.02 ± 0.40	0.58 ± 0.08
Valine	1.00	0.79 ± 0.00	0.79 ± 0.00	2.52 ± 0.15	0.80 ± 0.11
Methionine	1.00	0.83 ± 0.01	0.83 ± 0.00	2.32 ± 0.21	5.67 ± 0.45
Isoleucine	1.00	0.95 ± 0.00	0.95 ± 0.00	2.47 ± 0.55	0.18 ± 0.00
Leucine	1.00	0.96 ± 0.01	0.96 ± 0.00	5.92 ± 1.04	3.77 ± 0.38
Hyp-A	1.00	0.98 ± 0.01	0.98 ± 0.00	124.4 ± 6.70	6.40 ± 1.10
Pheylalanine	1.00	1.08 ± 0.01	1.08 ± 0.00	6.03 ± 1.56	2.65 ± 0.27
Tryptophan	0.99	1.13 ± 0.00	1.12 ± 0.01	1.46 ± 0.49	0.24 ± 0.00
Lysine	1.00	1.16 ± 0.01	1.17 ± 0.01	5.68 ± 0.75	10.56 ± 1.67

* Values are mean ± standard deviation of duplicates.
† RRT of the standard amino acids.
‡ RRT of the amino acids found in the sample.
§ wwf, wet weight fruit.
** ND, not detected.

Table II. Amino Acid Content of Ackee Seed*

Amino acid	RRT_{AA}†	RRT_{sam}‡	Unripe seed (Mg/100 g wwf§)	Ripe seed (Mg/100 g wwf)
Aspartic acid	0.16 ± 0.01		ND**	ND
Glutamic acid	0.20 ± 0.01	0.21 ± 0.00	25.71 ± 3.41	29.30 ± 4.99
Hydroxy-proline	0.31 ± 0.01	0.31 ± 0.01	2.50 ± 0.29	4.98 ± 0.67
Serine	0.35 ± 0.01	0.36 ± 0.00	7.39 ± 0.95	13.56 ± 2.26
Glycine	0.38 ± 0.01	0.39 ± 0.01	6.49 ± 1.89	15.0 ± 2.51
Threonine	0.45 ± 0.01	0.46 ± 0.01	24.55 ± 3.78	23.15 ± 3.87
Alanine	0.47 ± 0.00	0.47 ± 0.00	41.04 ± 4.86	19.53 ± 3.27
Histidine	0.50 ± 0.00	0.50 ± 0.01	17.45 ± 2.35	44.46 ± 4.43
Proline	0.52 ± 0.00	0.52 ± 0.00	30.38 ± 1.95	ND
Arginine	0.59 ± 0.01	0.59 ± 0.00	23.28 ± 1.79	40.43 ± 3.52
Tyrosine	0.77 ± 0.00	0.76 ± 0.00	2.98 ± 0.13	1.11 ± 0.37
Valine	0.79 ± 0.00	0.78 ± 0.00	9.86 ± 0.87	5.92 ± 0.98
Methionine	0.83 ± 0.01	0.82 ± 0.00	2.81 ± 0.33	1.28± 0.43
Isoleucine	0.95 ± 0.00	0.95 ± 0.01	7.63 ± 2.04	4.18 ±0.40
Leucine	0.96 ± 0.01	0.97 ± 0.00	4.97 ± 0.58	ND
Hyp-B	0.98 ± 0.01	0.98 ± 0.01	142.8 ± 8.80	106.0 ± 5.40
Phenylalanine	1.08 ± 0.01	1.07 ± 0.01	4.93 ± 0.20	4.36 ± 0.46
Tryptophan	1.13 ± 0.00	1.12 ± 0.01	5.95 ± 0.56	ND
Lysine	1.16 ± 0.01	1.15 ± 0.00	15.50 ± 3.99	2.10 ± 0.70

* Values are the mean ± standard deviation of duplicates.
† RRT of the standard amino acids.
‡ RRT of the amino acids found in the sample.
§ wwf, wet weight fruit.
** ND, not detected.

Table III. Amino Acids Present in Cooked Ripe Fruit and the Water Used to Cook the Ripe Fruit*

Amino acids	RRT_{AA}†	RRT_{sam}‡	Cooked ripe fruit (Mg/100 g wwf§)	Water used to cook ripe fruit (Mg/200 mL)
Aspartic acid	0.16 ± 0.01	0.17 ± 0.01	2.23 ± 0.12	0.28 ± 0.06
Glutamic acid	0.20 ± 0.01	0.18 ± 0.00	45.15 ± 4.41	4.17 ± 1.03
Hydroxy-proline	0.31 ± 0.01	0.31 ± 0.01	2.26 ± 0.76	0.22 ± 0.04
Serine	0.35 ± 0.01	0.35 ± 0.00	11.09 ± 3.66	2.65 ± 0.46
Glycine	0.38 ± 0.01	0.39 ± 0.00	1.63 ± 0.35	1.64 ± 0.29
Threonine	0.45 ± 0.00	0.46 ± 0.01	9.62 ± 1.35	0.64 ± 0.12
Alanine	0.47 ± 0.00	0.47 ± 0.00	9.21 ± 0.99	5.33 ± 0.79
Histidine	0.50 ± 0.00	0.49 ± 0.00	7.38 ± 0.69	2.60 ± 0.24
Proline	0.52 ± 0.00	0.51 ± 0.00	8.58 ± 0.95	9.25 ± 0.76
Arginine	0.59 ± 0.01	0.59 ± 0.00	43.57 ± 4.99	11.23 ± 2.46
Tyrosine	0.77 ± 0.00	0.76 ± 0.00	1.58 ± 0.35	2.04 ± 0.39
Valine	0.79 ± 0.00	0.78 ± 0.00	3.00 ± 0.75	2.58 ± 0.46
Methionine	0.83 ± 0.01	0.82 ± 0.00	11.00 ± 1.31	1.29 ± 0.11
Isoleucine	0.95 ± 0.00	0.94 ± 0.01	ND**	1.62 ± 0.18
Leucine	0.96 ± 0.01	0.97 ± 0.01	2.25 ± 0.24	0.20 ± 0.02
Hyp-A	0.98 ± 0.01	0.98 ± 0.01	ND	0.54 ± 0.15
Hyp-A	0.99 ± 0.01	0.99 ± 0.01	(1.17 ± 0.35)	(4.96 ± 0.50)
Phenylalanine	1.08 ± 0.01	1.08 ± 0.00	5.12 ± 0.79	1.29 ± 0.10
Tryptophan	1.13 ± 0.00	1.12 ± 0.00	4.79 ± 3.47	2.20 ± 0.28
Lysine	1.16 ± 0.01	1.16 ± 0.00	2.09 ± 0.46	1.98 ± 0.24

* Values are the mean ± standard deviation of duplicates. Values in parentheses were determined from a separate sample of the cooked ripe fruit.
† RRT of the standard amino acids.
‡ RRT of the amino acids found in the sample.
§ wwf, wet weight fruit.
** ND, not detected.

that was not detected in cooked ripe fruit (Table III).

The presence of hypoglycin in the ackee fruit has been a major concern over the years. Although the level in the ripe fruit (6.38 ± 1.06 mg/100 g fresh weight) is much less than that in the unripe fruit (124.43 ± 6.73 mg/100 g fresh weight) there is still concern, because the LD_{50} level of hypoglycin for humans is unknown. The LD_{50} for rats is approximately 90–100 mg/kg body weight (12). The level of hypoglycin that was detected in the ripe fruit compared well with the value (8 mg/100 g) reported by Ellington (13). Chase et al. (11) determined that canned ackee fruit contains on an average 8.47 mg/100 g. This value compares well with the value obtained from the ripe fruit (6.38 ± 1.06 mg/100 g).

In this study, the level of hypoglycin in the cooked ripe fruit and the water that was used to cook the ripe fruit was determined. Data in Table III shows that in one set of samples, hyp-A was not detected in the cooked ripe fruit; however, it was detected in the water that was used to cook the ripe fruit, the amount being 0.54 ± 0.15 mg/200 mL water. This implies that most if not all of the hypoglycin leached from the ripe fruit into the water. It is very unlikely that boiling at 100°C could have destroyed the hypoglycin, in light of the fact that hypoglycin has a melting point of 280°C up to 284°C (14). In some instances we detected hypoglycin in the cooked ripe fruit (1.17 ± 0.35 mg/100 g fresh weight fruit), and hypoglycin at 4.96 ± 0.50 mg/200 mL was detected in the water used to cook the fruit (Table III). We can only conclude that the amount of hypoglycin present in the ripe fruit is significantly reduced when the fruit is cooked.

It is well-known that people (Jamaicans) who prepare the ripe ackee fruit for a meal discard the water in which the ripe ackee fruit is cooked, thus the likelihood of being poisoned by the ackee is significantly minimized. In recent times, with people being more aware of the danger of (*a*) cooking and eating unripe ackee and (*b*) using the pot water in which the ackee was cooked to cook rice, incidences of ackee poisoning has declined significantly over the past twenty years (1).

Based on the results presented in this study, ripe ackee fruit that is properly prepared should not be toxic, because the level of hypoglycin is much lower in the cooked ripe fruit (1.17 ± 0.35 mg/100 g wet weight fruit) (Table III) than in the uncooked ripe fruit (6.38 ± 1.06 mg/100 g wet weight fruit) (Table I).

Although the amount of each essential amino acid present in the ripe fruit is relatively small (being that the daily requirement for humans is on an average 1.46 g/day (15)), nevertheless the presence of the essential amino acids does add to the daily intake whenever the ripe fruit is consumed. Even more interesting is the fact that arginine is present in the largest quantity (62.88 ± 5.87 mg/100 g) (Table I), the irony being that although arginine is essential the precise requirement is not yet established (15). The presence of essential amino acids in the ackee fruit and the findings of Odutuga et al. (16) that the ackee fruit contains fatty acids (with linoleic, palmitic, and stearic acid being the major fatty acids in the lipids and with linoleic acid (an essential fatty acid) accounting for over 55% of the total fatty acids), then it is reasonable to conclude that the nutritional value of the ackee fruit is indeed good.

Conclusion

Ackee fruit, when adequately cooked loses significant amounts of its toxic hyp-A to the surrounding water in which it is cooked. The leaching of this toxic amino acid in this manner may be the single most important factor in the prevention of the Jamaican vomiting sickness. Although the actual

Table IV. Percent Recovery of Standard Amino Acids*

Amino acid	Observed value (mg)	Expected value (mg)	%Recovery†
Serine	2.30	3.33	69.1
Hydroxy-proline	2.74	3.33	82.3
Alanine	2.78	3.33	83.5
Threonine	2.35	3.33	70.6
Valine	2.50	3.33	75.0
Arginine	2.77	3.33	83.2
Isoleucine	2.10	3.33	63.1
Phenylalanine	3.28	3.33	98.5
Methionine	2.39	3.33	71.8
Lysine	3.54	3.33	106.3

* Data are typical for the analyses of the ripe fruit, unripe fruit, and the cooked ripe fruit. Analyses were done in duplicate.
† The average %recovery was 80.34%.

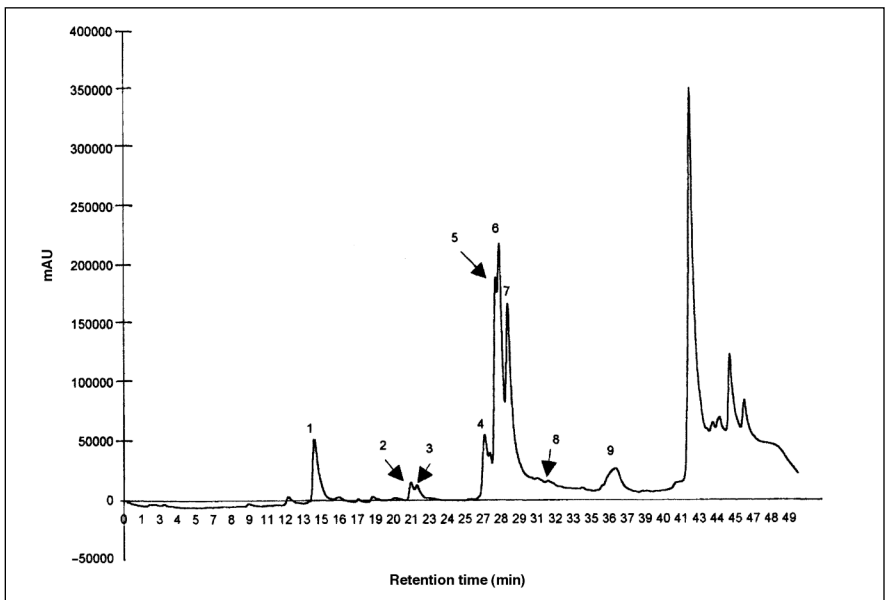

Figure 2. HPLC of the PITC derivative of amino acids in partially purified hypoglycin: alanine, 1; tyrosine, 2; valine, 3; isoleucine, 4; leucine, 5; hypoglycin, 6; norleucine (internal standard), 7; phenylalanine, 8; and lysine, 9.

amount of hyp-A that causes food poisoning is unknown, it is clear that boiling effectively reduces the residual amount of hyp-A in ripe ackee fruit, rendering it nontoxic.

Acknowledgments

This work was supported by a grant from Research and Publications (University of the West Indies, Mona). The technical assistance of Mrs. Janet Golden is also appreciated.

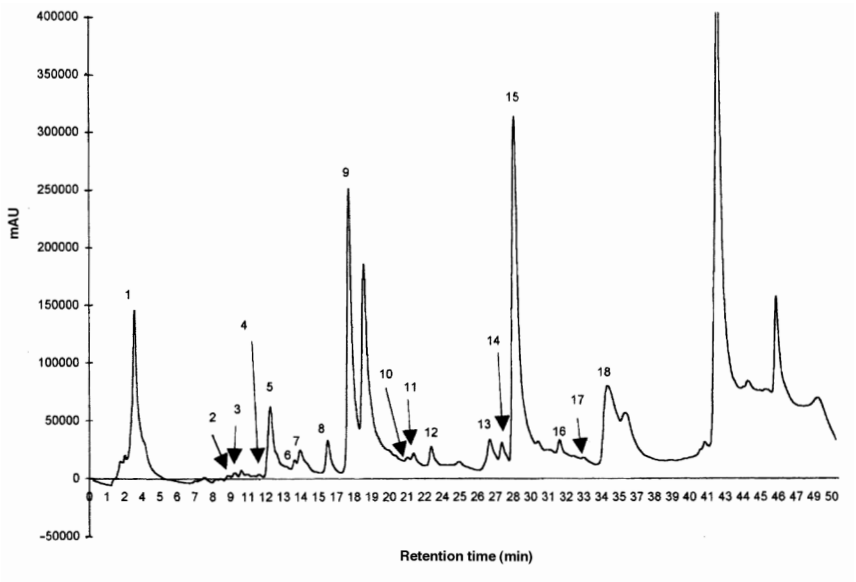

Figure 3. HPLC of the PITC derivative of amino acids in ripe ackee fruit: glutamic acid, 1; hydroxy-proline, 2; serine, 3; glycine, 4; threonine, 5; alanine, 6; histidine, 7; proline, 8; arginine, 9; tyrosine, 10; valine, 11; methionine, 12; isoleucine, 13; hypoglycin, 14; norleucine (internal standard), 15; phenylalanine, 16; tryptophan, 17; and lysine, 18. Unlabelled peaks could not be identified.

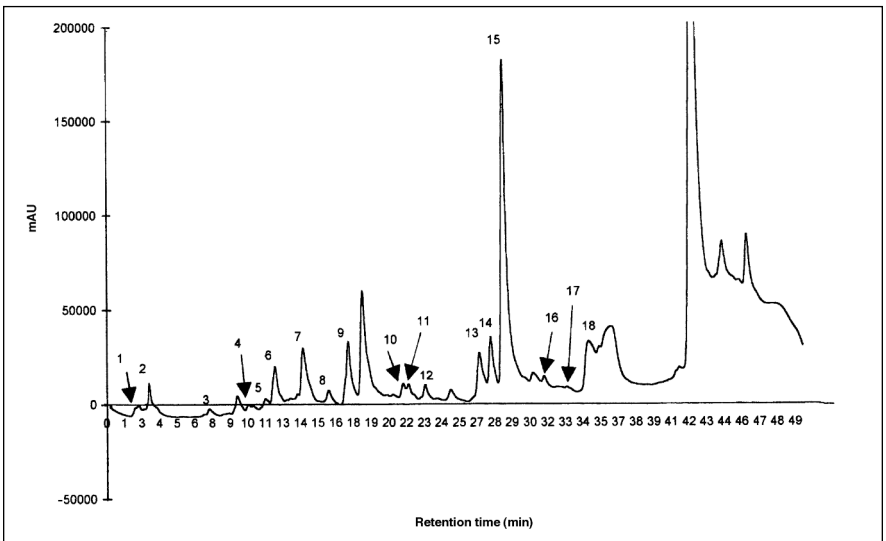

Figure 4. HPLC of the PITC derivative of amino acids in unripe ackee fruit: aspartic acid, 1; glutamic acid, 2; hydroxy-proline, 3; serine, 4; glycine, 5; threonine, 6; alanine, 7; proline, 8; arginine, 9; tyrosine, 10; valine, 11; methionine, 12; isoleucine, 13; hypoglycin, 14; norleucine (internal standard), 15; phenylalanine, 16; tryptophan, 17; and lysine, 18. Unlabelled peaks could not be identified.

References

1. E.A. Kean. *Toxicants of Plant Origin.* P.R. Cheeke, Ed. Boca Raton, FL, 1998, Chapter 10, p 229.
2. K.R. Hill. The vomiting sickness of Jamaica: a review. *West. Indian Med. J.* **1:** 243–64 (1952).
3. E.A. Kean and C.I. Pogson. Inhibition of gluconeogenesis in isolated rat liver cells by methylenecyclopropylpyruvate (ketohypoglycin). *Biochem. J.* **182:** 789–96 (1979).
4. B.D. Jelliffe and K.L. Stuart. Acute toxic hypoglycaemia occurring in the vomiting sickness of Jamaica. *Br. Med. J.* **1:** 175–77 (1954).
5. E.A. Kean. Selective inhibition of acyl-CoA dehydrogenase by a metabolite of hypoglycin. *Biochim. Biophys. Acta* **422:** 8–14 (1976).
6. K.D. Golden and E.A. Kean. The biogenesis of dicarboxylic acids in rats given hypoglycin. *Biochim. Biophys. Acta* **794:** 83–88 (1983).
7. E.A. Kean. Improved method for isolation of hypoglycin A and B from fruit of *Blighia sapida*. *J. Pharm. Pharmacol.* **26:** 639–40 (1974).
8. M.J. González-Castro, J. López-Hernández, J. Simal-Lozano, and M.J. Oruña-Concha. Determination of amino acids in green beans by derivatization with phenylisothiocianate and high-performance liquid chromatography with ultraviolet detection. *J. Chromatogr. Sci.* **35(4):** 181–85 (1997).
9. K.D. Golden and O.J. Williams. Amino acid, fatty acid, and carbohydrate content of *Artocarpus altilis* (breadfruit). *J. Chromatogr. Sci.* **39(6):** 243–50 (2001)
10. C. McGowan, V.A. Wiley, and P. Bates. Application of methodology for RP-HPLC amino acid analysis to the measurement of hypoglycin A. *Biochromatography* **4:** 161–64 (1989).
11 G.W. Chase, W.O. Landen, Jr., L.T. Gelbaum, and A.M. Soliman. Ion-exchange chromatographic determination of hypoglycin A in canned ackee fruit. *J. Assoc. Off. Anal. Chem.* **72:** 374–77 (1989).
12. P.C. Feng and S.J. Patrick. Studies of the action of hypoglycin A, an hypoglycemic substance. *Br. J. Pharmacol.* **13:** 125–30 (1958).
13. E.V. Ellington. *Hypoglycin.* E.A. Kean, Ed. Academic Press, New York, NY, 1975, p 1.
14. C.H. Hassall and K. Reyle. Hypoglycin A and B: two biologically active 'polypeptides' from *Blighia sapida*. *Biochem. J.* **60:** 334–39 (1955).
15. A.L. Lehninger. *Principles of Biochemistry*. Worth Publishers, New York, NY, 1982, pp. 762–63.
16. A.A. Odutuga, H.N. Asemota, I. Musac, K.D. Golden, and E.A. Kean. Fatty acid composition of arilli from ackee fruit (*Blighia sapida*). *Jamaican J. Sci. Tech.* **3:** 30–32 (1992).

Manuscript accepted June 14, 2002.