JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Tracking Hypoglycins A and B over Different Maturity Stages: Implications for Detoxification of Ackee (*Blighia sapida* K.D. Koenig) Fruits

Camille S. Bowen-Forbes and Donna A. Minott*

Department of Chemistry, The University of the West Indies, 2 Plymouth Crescent, Mona, Kingston 7, Jamaica

Supporting Information

ABSTRACT: Consumption of improperly ripened ackee (*Blighia sapida* K.D. Koenig) often results in fatalities. The causal toxin, hypoglycin A, decreases in the edible arilli upon maturity; regulation of hypoglycin A in the arilli is thus critical. Hypoglycin B, also toxic, is confined to the seeds. Hypoglycins A and B were tracked in ackees grown in Jamaica over different maturity stages using RP-HPLC. Studies on the 'Butter' and 'Cheese' ackee varieties and across two different harvest seasons were conducted. In 'Cheese' ackees, hypoglycin A decreased from about 8000 mg/kg in the green arilli and seeds to 271 and 1451 mg/kg, respectively, in the ripe fruit whereas hypoglycin B levels in the seeds increased from 1629 to 11774 mg/kg. The strong inverse relationship demonstrated that hypoglycin B in the seeds serves as a sink for hypoglycin A from the ripening arilli and is thereby involved in the detoxification mechanism of the fruit.

KEYWORDS: *Blighia sapida*, ackee, hypoglycin A, hypoglycin B, detoxification, Jamaica

INTRODUCTION

The ackee fruit (*Blighia sapida* K.D. Koenig) is significant to the Jamaican culinary heritage, being honored as the national fruit and having been consumed by locals for centuries, primarily in the dish ackee and cod fish.[1] Ackee plays a major role in Jamaica's agricultural sector, with processed ackees yielding an export value in excess of U.S.$ 13.5 million in 2009.[2] The plant, a member of the Sapindaceae family,[3] is not endemic to Jamaica, but was introduced from West Africa during the 18th century.[4] It is known worldwide as much for its toxicity as for its culinary use. The fruit contains two toxins, 2-amino-3-(methylenecyclopropyl)-propionic acid (hypoglycin A, **1**), present in the arilli (edible portion) and seeds, and hypoglycin B (**2**), its γ-glutamyl conjugate, found only in the seeds[5−7] (Figure 1). Hypoglycin A occurs naturally in ackees as a diastereomeric mixture of the (2*S*,4*R*) and (2*S*,4*S*) forms. The former is the dominant isomer in the arilli.[5]

Ingestion of arilli from fruits that have not sufficiently matured may result in a disorder called Jamaican vomiting sickness (JVS).[8−10] Several decades ago, JVS occurred epidemically in Jamaica on an annual basis. It was established in 1916 that JVS resulted from the ingestion of the unripe ackee fruit,[11] and four decades later, the two toxic principles were isolated and identified.[12] The condition is characterized by severe hypoglycemia, depletion of hepatic glycogen, neurotoxicity, disturbances of carbohydrate and lipid metabolism, and organic aciduria. These symptoms have been attributed to the transaminated, oxidatively decarboxylated metabolite of hypoglycin A, methylene cyclopropylacetic acid coenzyme A, which inhibits the β-oxidation of fatty acids and interferes with gluconeogenesis.[13,14] Persons having this disorder usually experience vomiting, convulsion, and, in extreme cases, coma, leading to death.[9,11,14,15] Despite the rarity of the occurrence of the condition in the past few decades, JVS, more appropriately termed toxic hypoglycemic syndrome, continued to generate interest, perhaps fueled by the 2000−2001 outbreak in Haiti.[16,17]

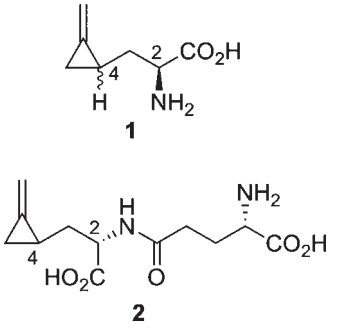

Figure 1. Structures of hypoglycins A (**1**) and B (**2**).

With improved public education campaigns, there had been only a limited number of cases per year in Jamaica, with few associated deaths;[18] however, within the period from December to February 2011, there was an unusual outbreak of suspected ackee poisonings resulting in several confirmed fatalities. Previous research demonstrated that although ackee was eaten by individuals across the economic spectrum in Jamaica, the main consumers were persons in the lower socioeconomic bracket in the rural areas of Jamaica. On a body weight basis children had the highest dietary exposure to ackee, and hence hypoglycin A, and were among the most vulnerable section of the population. In this most recent outbreak the majority of poisoning victims

Received: December 1, 2010
Revised: March 16, 2011
Accepted: March 17, 2011
Published: March 17, 2011

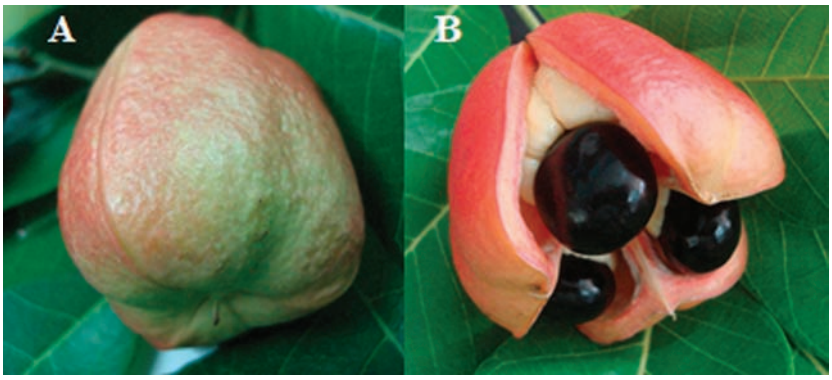

Figure 2. Ackee at different stages of maturity: (A) full-sized immature fruit with pod closed; (B) mature, tree-ripened fruit with open pod revealing arilli (edible) and seeds.

were adult males in rural Jamaica. Typically, ackee diets contain hypoglycin A in the range of 1.21−89.28 μg/g ackee.[19] Given that the maximum tolerated dose of hypoglycin A was found to be 231 ± 63 mg/kg body weight for male Sprague−Dawley rats and 216 ± 63 mg/kg body weight for female rats,[20] it is expected that a typical diet containing mature ackees should pose no food safety concerns to the average consumer. It is being hypothesized that delayed fruit ripening could have encouraged individuals to forcefully open the immature ackee pods (Figure 2A) before natural maturation (Figure 2B). Notably, no suspected case of poisoning was ascribed to consumption of the canned product. In 1973, the U.S. Food and Drug Administration (FDA) placed a worldwide import ban on ackee to reduce the possibility of unripe fruits with high levels of hypoglycin A entering the United States.[21] The ban, which lasted for 27 years, was partially lifted in 2000.

Whereas hypoglycin A is present at high levels in the immature arilli, the mature arilli possess appreciably lower quantities,[8,22] rendering the fruit fit for consumption. Very little is known about the mechanism whereby this reduction occurs, but it has been hypothesized that hypoglycin A is translocated to the seeds as the fruit ripens.[23] Detoxification of the arilli would be facilitated with subsequent metabolization of **1** to **2**. This hypothesis is supported by the presence of a γ-glutamyl transpeptidase in the ackee seeds.[24] Hypoglycin B has been shown to be less toxic than hypoglycin A.[25,26] Although the profile of the changes in hypoglycin A levels in relation to fruit maturity has been examined, no such study on hypoglycin B has been reported. To evaluate the hypothesis linking hypoglycin A decline in ackee with an increase in hypoglycin B concentration, ackees were analyzed in our laboratory.

According to previous papers, when hypoglycin A is isolated from ackees, leucine and isoleucine are invariably present as impurities. This mixed isolate was typically employed as the standard for quantification of hypoglycin A in ackees. Hypoglycin A in unprocessed and processed ackees was previously analyzed using a RP-HPLC method involving precolumn derivatization with *o*-phthaldialdehyde (OPA) in the presence of 2-mercaptoethanol.[8,27] Chromatography yielded three closely resolved peaks, which were attributed to isoleucine, hypoglycin A, and leucine, in that order of elution. Subsequently, a more efficient method, which involved precolumn derivatization with phenylisothiocyanate (PITC), was developed[28] and utilized in the analysis of hypoglycin A in canned ackees. This method was modified in our laboratory and used in the tracking of **1** and **2** in ackees over six maturity stages (see the Supporting Information). Currently, PITC derivatization is the official method used for the analysis of hypoglycin A in ackee products.[21]

Forty-eight varieties of ackees, grouped into two main types, 'Butter' and 'Cheese', had been previously identified in Jamaica on the basis of ease of rooting from stem cuttings as well as canning properties.[29] The first type describes fruits yielding soft, light-yellow arilli and the latter, fruits yielding harder, cream-colored arilli.[30] 'Cheese' ackees are usually preferred for canning due to their firmer texture. Some consumers are, however, of the opinion that 'Butter' ackees have a better flavor. HPLC studies were conducted on the two colloquial varieties of ackee, 'Butter' and 'Cheese', and on fruits harvested during the two main seasons of the fruit, over six maturity stages. This is the first report of hypoglycin A in ackee relative to season and variety. Although there is a lone report of the levels of hypoglycin B in ripe and unripe ackee seeds (3.3 and 0.4 mg/g), the values reported were said to be based on a personal communication.[24] This is therefore the first substantiated report of the analysis of hypoglycin B in ackee.

MATERIALS AND METHODS

General Experimental Procedures. NMR spectra were obtained on a Bruker Avance DRX 500 (Bruker Biospin, Rheinstetten, Germany), in D_2O with 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) as the internal standard. HPLC analyses of OPA-derivatized amino acids were performed on a Phillips PU 4001 chromatograph (Pye Unicam, Ltd., Cambridge, U.K.) with a PU 4024 fluorescence detector (excitation filters at 360 and 325 nm, emission filter at 440 nm). The column used was a 150 mm × 4.6 mm i.d., 5 μm, Pecosphere 5 RP-18, with a 4 mm × 4 mm i.d. guard column of the same material (Perkin-Elmer, Waltham, MA). The chromatographic system employed for the PITC derivatives consisted of a Waters 600 instrument with a photodiode array detector (Waters 996). Separation was achieved on a C_{18} Pico-Tag amino acid analytical column (150 mm × 3.9 mm i.d., 4 μm) (Waters Chromatography Division, Milford, MA).

Reagents. HPLC grade solvents, phenylisothiocyanate, OPA solution (1 mg of *o*-phthaldialdehyde/mL solution with 2-mercaptoethanol as the sulfhydryl moiety), L-leucine, and L-isoleucine were obtained from Sigma-Aldrich Co., St. Louis, MO. Nanopure water (Nanopure Infinity Ultrapure Water System, Bransted, Dubuque, IA) was used for chromatography.

Plant Material. *B. sapida* K.D. Koenig (family Sapindaceae) fruits were collected from six trees, three each of the 'Butter' and 'Cheese' ackee varieties grown on the Mona campus of the University of the West Indies, Kingston, Jamaica. The trees were in close proximity to each

Table 1. Maturity Scale of Ackee Fruits Based on Visual Appearance of the Component Parts

	color		
stage	pods	seeds	arilli
1	totally green to yellow-green or orange-green to dull red	green; base may have a small burgundy tinge	yellow-green
2	yellow-green or orange-red	light burgundy and green throughout	yellow
3	red with yellow spotting to orange-yellow; may have some green coloration	burgundy	yellow
4	yellow-green with some red or orange-yellow	black	yellow
5	dull and slightly shriveled; orange and yellow, orange-yellow, orange-red, or red-yellow (pods open)	black	yellow
6	very dull and shriveled; mostly red, yellow, or orange (pods open)	black	yellow

other, over an approximate area of 150 m^2. These trees are representative of those from which ackee is typically harvested, that is, naturally propagated plants in isolated stands, rather than in cultivated orchards. Voucher specimens of the respective varieties ('Butter' 35445 and 'Cheese' 35447) were deposited at the University of the West Indies Herbarium, Mona. Ackees were sorted into six stages of maturity (Table 1). A minimum of six fruits were analyzed for each stage, representing at least two fruits from each tree. In a few instances, fruits at a particular stage were unavailable for a given tree. Composite ackee samples at each stage were composed of at least one fruit from each 'Butter' and Cheese' tree, in most cases, that is, at least six fruits per stage.

Isolation of Hypoglycin A and Hypoglycin B Standards. Hypoglycins A and B were isolated from stage 4 ackee seeds (obtained from an undifferentiated mix of 'Butter' and 'Cheese' varieties), harvested from the same locale, using a modified ion exchange chromatographic method.[31] It involved the use of cation exchange, followed by anion exchange, resins (Dowex 50 and Dowex 1, respectively). The resins were obtained from Sigma-Aldrich Co. Hypoglycin A (98% purity) was present as a diastereomeric mixture, with the (2*S*,4*S*) isomer accounting for 10% diastereomeric excess over its (2*S*,4*R*) counterpart. Hypoglycin B (95% purity), also obtained as diastereomers, had its (2*S*,4*R*) isomer in 40% excess relative to the (2*S*,4*S*) isomer. The method used for isolation, along with the NMR characterization of hypoglycins A and B, are detailed in previous papers.[32,33]

Sample Preparation. For the seasonality studies, ackee fruits were harvested during two bearing seasons: March 2003 (season 1, S1) and July−September (season 2, S2) of the same year. Fruits were sorted into six maturity stages on the basis of visual examination of fruit color and degree of lobe separation of mature fruits (stages 5 and 6), fruit dimensions (mass, width, and height), instrumental color analysis, and textural analysis.[32] The seeds and arilli were detached from the pods, and the raphe was removed. The arilli were cut into small pieces (about 5 mm), and duplicate 3 g samples were placed in 50 mL centrifuge tubes and stored at −80 °C until required for analysis. The samples were thawed prior to extraction with 80% aqueous EtOH (15 mL for 45 s at 13500 rpm and then 10 mL for 30 s at the same speed) using an Ultra-Turrax T25 basic homogenizer (IKA Works, Wilmington, NC). Samples were centrifuged after each extraction for 15 min at 1500 rpm. The combined extracts were made up to 50.0 mL and filtered (0.2 μm pore). Forty-microliter aliquots of the filtered extracts were transferred to 1 mL Pyrex vials and evaporated to dryness under nitrogen at approximately 35 °C. For the varietal studies, the same procedure was carried out separately on 'Butter' and 'Cheese' ackees. Raphe samples were obtained from fruits harvested in March 2004. Duplicate 2 g raphe samples were treated in the same manner as the seed and arilli samples.

OPA Derivatization of Amino Acids. Hypoclycin A, L-leucine, and L-isoleucine were derivatized with OPA using a previously described method[27] and were subjected to RP-HPLC. The hypoglycin A standard was spiked separately with isoleucine and leucine.

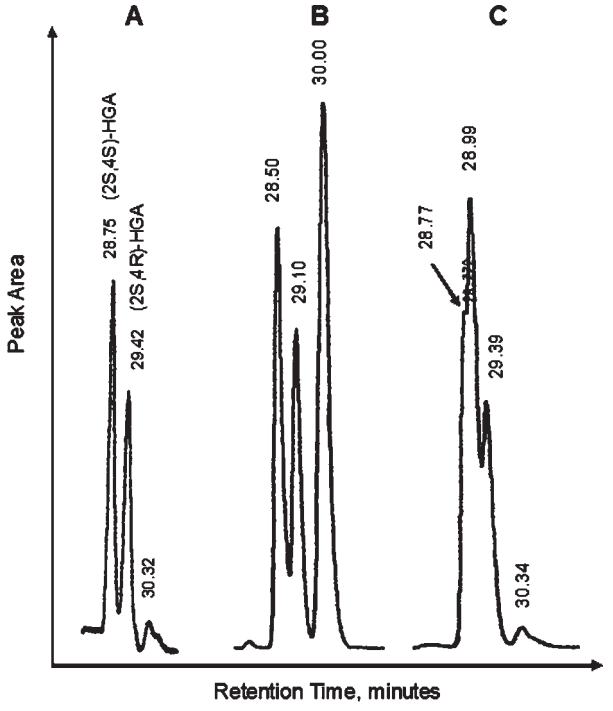

Figure 3. Chromatogram of OPA-derivatized hypoglycin A standard obtained from *B. sapida* seeds: (A) hypoglycin A (HGA) showing two diastereomers; (B) hypoglycin A spiked with leucine (leucine at t_R 30.00 min); (C) hypoglycin A spiked with isoleucine. The peak at t_R 28.77 min represents (2*S*,4*S*)-hypoglycin A coeluted with isoleucine (t_R 28.99 min).

PITC Derivatization. One hundred microliters of the derivatizing solution (methanol, water, triethylamine, PITC, 7:1:1:1) were added to the dried ackee extracts, which were covered with parafilm, vortexed, and allowed to stand for 20 min at room temperature. Evaporation of excess reagent under nitrogen (40 °C) completed the process. The derivatized samples were dissolved in 500 μL of sodium acetate buffer solution (eluant A) prior to the injection of 20 μL aliquots for HPLC analysis.

Hypoglycins A and B were similarly derivatized to give calibration standards in the ranges of 2−40 and 9−56 mg/kg, respectively.

HPLC Analysis. The eluting solvents were A, 0.14 M sodium acetate containing 0.05% triethylamine and 6% acetonitrile, pH 6.2, adjusted with acetic acid; and B, 75% acetonitrile/water. The samples were eluted according to the following gradient: 0−3.6 min, 85% A at 0.8 mL/min;

3.6−8 min, 85% A at 1.5 mL/min; 8.0−12 min, 0% A at 1.5 mL/min; 12−17 min, 85% A at 1.5 mL/min. The eluent was monitored at 254 nm.

Statistical Analysis. Duplicate samples were analyzed. Data were differentiated at a significance level of $p < 0.05$ using Student's t test in the two-sided mode. Pearson's product moment correlation coefficient was calculated using the standard statistical package in Microsoft Office Excel 2007.

Meteorological Data. Rainfall data for Mona, Jamaica, located within the vicinity of the collection site, and temperature data recorded at the Norman Manley International Airport, Kingston, Jamaica, were provided by the National Meteorological Centre, Climate Branch, Kingston, Jamaica. Monthly rainfall (in millimeters) was recorded as the cumulative sum of daily rainfall amounts measured with a standard meteorological rain gauge, the daily period being defined as from 7:00 a.m. to 6:59 a.m. the following day.

RESULTS AND DISCUSSION

Hypoglycin A and B Analyses. The purity of the isolated hypoglycin A (98%) to be used as the standard was determined by HPLC (OPA derivatization) and NMR spectroscopy. ^{13}C NMR of the hypoglycin A standard indicated the presence of the two hypoglycin A diastereomers and a trace of leucine; there was no spectroscopic evidence of isoleucine.[33] The chromatogram of the OPA-derivatized hypoglycin A revealed two major peaks at t_R 28.75 and 29.42 min, respectively, representing the (2*S*,4*S*) and (2*S*,4*R*) diastereomers of **1**. This is not surprising because it is known that reversed phase HPLC analysis of OPA-derivatized amino acids can result in resolution of enantiomers due to the diastereomeric isoindoles produced in the derivatization process.[34] The unnormalized chromatograms obtained from the analogue output are shown in Figure 3. A minor peak eluted at t_R 30.32 min. This impurity (Figure 3A) was presumed to be the OPA derivative of leucine corresponding to the minor impurity identified in the hypoglycin A standard by NMR. Its identity was confirmed by spiking with an authentic sample of L-leucine (Figure 3B). Spiking with L-isoleucine resulted in coelution of the OPA derivatives of isoleucine and the (2*S*,4*S*) isomer of hypoglycin A (Figure 3C). Because the NMR spectrum had shown no presence of isoleucine, it became apparent that in the past, analysis of hypoglycin A in ackees utilizing the OPA method had yielded misleading results, as one of the hypoglycin A diastereomers was being erroneously identified as isoleucine.[8,27] This is an important finding for research and regulatory purposes because it has direct consequences for the levels of hypoglycin A previously reported in ackees. This error was probably derived through inadequate characterization of the hypoglycin A reference materials used. Since 2000, HPLC analysis of hypoglycin A has been routinely carried out using PITC derivatization. With this method the hypoglycin A diastereomers are not resolved and are typically displayed as a single peak or as minimally resolved peaks; additionally, there is no coelution with isoleucine.[28] The purity of the hypoglycin B reference material (95%) was ascertained on the basis of NMR analysis, as well as HPLC analysis of its PITC derivative. Similar to hypoglycin A, the ^{13}C NMR spectrum displayed several sets of paired peaks indicating that hypoglycin B also exists in nature as a pair of diastereomers.[33] The hypoglycin B diastereomers were not resolved in the HPLC chromatograms.

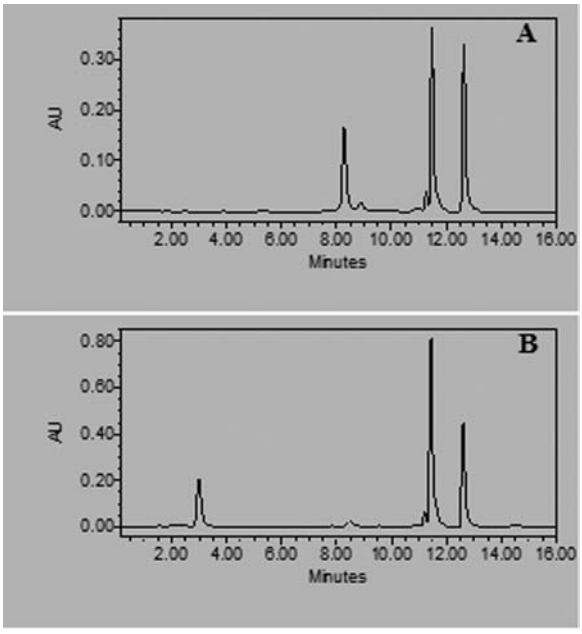

Figure 4. Chromatograms of (A) PITC-derivatized hypoglycin A, t_R 8.3 min, and (B) hypoglycin B, t_R 3.0 min, obtained from *B. sapida* seeds. The peaks occurring after 8.5 min are due to the reagents.

Linear calibration responses were observed for both **1** (2−40 μg/mL) and **2** (9−56 μg/mL), with respective correlation coefficients of 0.9998 and 0.9994. The PITC derivatives eluted in the order hypoglycin B, isoleucine, leucine, and hypoglycin A. The retention times of hypoglycin B, leucine, and hypoglycin A were 3.0, 7.7, and 8.3 min, respectively, in an HPLC run of 17 min duration (Figure 4). Baseline resolution was obtained between the PITC derivatives of isoleucine and hypoglycin A,[28] unlike that

Table 2. Variation in Hypoglycins A and B in 'Cheese' and 'Butter' Ackee Fruits with Maturity[a]

	hypoglycin A (mg/kg)				hypoglycin B[b] (mg/kg)	
	arilli		seeds		seeds	
stage	'Cheese'	'Butter'	'Cheese'	'Butter'	'Cheese'	'Butter'
1	7939 ± 509	6730 ± 620	8246 ± 750	8432 ± 435	1629 ± 191	3102 ± 623
2	7382 ± 250	6163 ± 139	6758 ± 48	5140 ± 82	4702 ± 271	5393 ± 508
3	5232 ± 614	4426 ± 810	1903 ± 188	4489 ± 80	7029 ± 403	10575 ± 1297
4	694 ± 58	842 ± 145	893 ± 65	1091 ± 62	11491 ± 952	11914 ± 2228
5	595 ± 77	412 ± 204	1097 ± 17	2579 ± 4	12408 ± 1290	19317 ± 1741
6	271 ± 58	551 ± 101	1451 ± 79	2629 ± 69	11773 ± 171	12619 ± 1206

[a] Values represent the mean ± SD for $n = 2$ values. [b] Hypoglycin B was not detected in the arilli.

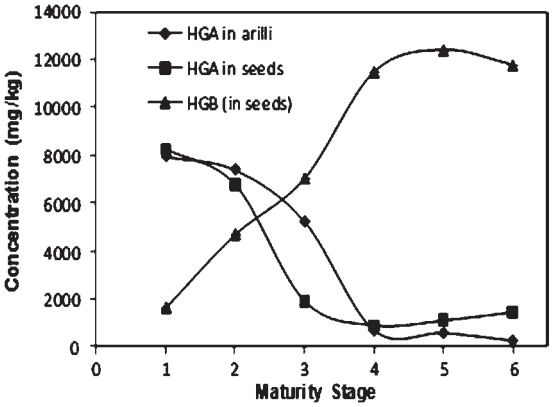

Figure 5. Hypoglycin A (HGA) and B (HGB) relationship in ackee arilli and seeds of the 'Cheese' variety relative to maturity.

Table 3. Variation in Hypoglycin A in Ackee Raphe with Maturity[a]

stage	hypoglycin A[b] (mg/kg)
1	11400 ± 235
2	3090 ± 69
3	2570 ± 155
4	2240 ± 320
5	581 ± 86
6	598 ± 9

[a] Composite ackee arilli were harvested in March 2004. [b] Values represent the mean ± SD for $n = 2$ values.

found with the corresponding OPA derivatives. Leucine−PITC, which eluted close to hypoglycin A−PITC, was also sufficiently resolved from it.

Trends in Hypoglycins A and B with Maturity. There was no evidence of **2** in the arilli or raphe at any of the maturity stages evaluated. In keeping with previous studies, a sigmoidal decline in **1** was observed for the ackee arilli with increasing stages of maturation.[8,27] A similar trend was observed for the hypoglycin A in the raphe and seeds. Conversely, as the ackee matured an increase in the hypoglycin B content of the ackee seeds was evident (Table 2; Figure 5). There was a strong inverse correlation between the hypoglycin A concentration in the arilli and hypoglycin B concentration in the corresponding seeds ('Cheese' ackee Pearson correlation value = −0.978) over the period that the fruit matured. Changes of hypoglycin A and B concentrations in the seeds with maturity showed an analogous relationship ('Cheese' ackee correlation value = −0.931). These results are in contrast to statements in the literature which intimate that hypoglycin A and B levels in the seeds are independent of the ripeness of the arilli.[21] The levels of hypoglycin A also declined in the raphe with increased maturity (Table 3). In a study conducted by Sarwar and Botting,[28] the hypoglycin A levels in ackee pods (416 mg/kg) did not vary with maturity. The pods were therefore not expected to contribute to variations in the hypoglycin levels and were not included in this investigation.

Varietal Studies. There were no differences in the general trend of hypoglycin A and B levels between the 'Cheese' and 'Butter' ackee varieties. The hypoglycin A concentration in the 'Cheese' arilli fell to an average level of 433 mg/kg in mature fruits (stages 5 and 6). A comparable mean level of 481 mg/kg was found in mature 'Butter' arilli (Table 2). In general, greater quantities of hypoglycin B were observed in seeds from the 'Butter' ackee. Factors that contribute to the observed variations in hypoglycin content in ackees have not been fully explored; however, it is thought that there are varieties which are naturally low in hypoglycin. This is the subject of ongoing investigations. In addition, variations in climatic conditions may affect the amount of hypoglycin produced in the plants from year to year.

Table 4. Hypoglycin A Variations in Ackee Fruits Harvested in Different Seasons

	hypoglycin A[a] (mg/kg)			
stage	arilli, S1[b]	arilli, S2[c]	seeds, S1	seeds, S2
1	5626 ± 148	6173 ± 444	5413 ± 444	8184 ± 818
2	3773 ± 235	4330 ± 947	5171 ± 632	6070 ± 339
3	3168 ± 86	3610 ± 120	3577 ± 358	3119 ± 81
4	1152 ± 100	808 ± 121	787 ± 83	1097 ± 79
5	122 ± 7	176 ± 11	763 ± 14	1097 ± 69
6	103 ± 2	87 ± 12	619 ± 74	1128 ± 22

[a] Values represent the mean ± SD for $n = 2$ values. [b] S1, March 2003. [c] S2, July−Sept 2003.

Seasonal Variations. When composite samples of fruits (comprising 'Butter' and 'Cheese' varieties) harvested over the two main bearing seasons were analyzed, the hypoglycin content of the mature opened ackees was found to be lower than previously obtained (Table 4), lending support to the existence of low-hypoglycin varieties. With the exception of stage 6, the arilli of ackees harvested during season 2 possessed greater hypoglycin A levels than those of their counterparts (Table 4). The difference was significant for stage 5, which is the stage critical to ackee processors. At this stage, S1 arilli were found to possess 122 mg/kg hypoglycin A, whereas those of S2 had 176 mg/kg. In general, the seeds harvested during S2 possessed higher hypoglycin A levels compared with S1 seeds, with the only exception being at stage 3, when S2 seeds recorded a slightly lower hypoglycin A concentration. There was, however, no significant difference at the 0.05% limit.

The concentration of nutrients and antinutrients in many food crops is known to be influenced by environmental factors.[35−37] During S1, 48 mm of rainfall was experienced for the month of collection in the area of harvest (Mona, Jamaica). There was no rainfall recorded in the preceding month. For the second harvest period S2, there was an average of 72 mm of rainfall each month, with 29 mm documented in the preceding month. March recorded an average low temperature of 23 °C and a high of 30 °C, whereas the corresponding temperatures for July−September were 26 and 33 °C. Season 2, which gave rise to higher hypoglycin A concentrations in the arilli, was therefore characterized by greater rainfall and higher temperatures relative to season 1. Although there is no scientific corroboration, reports in government archives indicate a historic association between incidences of "vomiting sickness" and the damper months and damper locations in Jamaica. More recently (2005), a temporary ban on ackee exports was imposed following detection of elevated hypoglycin A levels,[38] which occurred after the island felt the impact of several successive major hurricanes with attendant increase in incident rainfall.[39] These results suggest that the role of meteorological conditions on adverse hypoglycin A levels in the ackee fruit warrants further studies.

Implications for the Ackee Industry. Mature ackees with just-opened pods are used for processing, as well as for domestic use. In Jamaica, commercial processing of ackees is done mainly via

canning in brine. The frozen product is also marketed locally and internationally on a smaller scale. Canned ackees are available in the domestic market and are exported to several countries including several Caribbean islands, Japan, The Netherlands, and Australia. The principal export markets are, however, the Uniteds States, Canada, and the United Kingdom.[2] Regulatory limits for hypoglycin A in canned ackees have been established at 100 mg/kg for the Unites States and 150 mg/kg in Canada and the United Kingdom. Research conducted by the U.S. Department of Agriculture, the U. S. Food and Drug Administration, and others showed that the levels of hypoglycin A found in canned ackees ranged from 2.77 to 252.58 mg/kg, with the majority (>77%) of samples possessing <100 mg/kg.[21] Ackee arilli soften with maturity and become less suited for processing; therefore, stage 5 ackees are more commonly processed. Consequently, the results obtained for this stage are of particular relevance to ackee processors.

Our seasonal studies revealed that stage 5 ackee arilli harvested during the wetter season 2 had a significantly greater hypoglycin A content (54 mg/kg difference) compared to those harvested in the dryer season 1 (March). Although the hypoglycin A content of S2 arilli was greater, the level was not alarming. Hypoglycin A is a water-soluble amino acid, which is partially leached from the fruit during canning, resulting in reduced amounts in the end product. Whereas the 'Cheese' arilli contained 83 mg/kg more hypoglycin A than 'Butter' arilli at stage 5, this difference should not significantly affect the levels present in the drained, canned arilli, due to the leaching phenomenon. In a year in which differences in rainfall between the seasons was evident but not that remarkable, variety seemed to have had a greater influence on the overall hypoglycin A content.

Detoxification Mechanism of Ackee Fruit. It has been proposed that γ-glutamyl transpeptidase is involved in the catalysis of γ-glutamyl dipeptides, formed and accumulated in storage tissues such as bulbs and seeds during the ripening of fruits.[40] Coupled with the fact that γ-glutamyl transpeptidase is present in ackee seeds, our findings strongly support the hypothesis that hypoglycin A is translocated from the arilli (and the raphe) to the seeds, where hypoglycin B serves as a sink. Hypoglycin B is thus involved in the detoxification mechanism of the fruit. In this study, hypoglycin A isolated from the seeds had the (2*S*,4*S*) diastereomer in excess. It would be plausible to expect that synthesis of **2** from **1**, originating in the seeds, would lead to an excess of the (2*S*,4*S*) isomer. Indications are that the major diastereomer of hypoglycin B[31,41] has the (2*S*,4*R*) stereochemistry, as does hypoglycin A from the arilli,[5] lending support to the hypothesis that hypoglycin A from the arilli is also incorporated in hypoglycin B in the seeds.

■ ASSOCIATED CONTENT

Supporting Information. Maturity scale consisting of six stages devised for ackee fruits to facilitate study of the effect of maturity on levels of hypoglycins A and B; maturity classification based on visual observation of component parts of fruit color. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: 876-927-1910. Fax: 876-977-1835. E-mail: donna.minott@uwimona.edu.jm.

Funding Sources

Financial support for this project was provided by the Office of Graduate Studies and Research, UWI, Mona. We gratefully acknowledge the Caribbean Regional Development Programme for Economic Competitiveness (CPEC), from which we obtained funding for HPLC training.

■ DISCLOSURE

This material was partially presented as a poster at the Institute of Food Technologists' Annual General Meeting, Orlando, FL, June 2006. None of the artwork or tables presented here were incorporated in the poster.

■ ACKNOWLEDGMENT

Appreciation is extended to the Veterinary Services Division, Ministry of Agriculture, Jamaica, in particular E. Dakin, and Bureau of Standards, Jamaica, in particular J. Kerr, for facilitating HPLC analysis.

■ REFERENCES

(1) Higman, B. W. Fruits. In *Jamaican Food: History, Biology, Culture*; University of the West Indies Press: Kingston, Jamaica, 2008; pp 151−158.

(2) Statistical Institute of Jamaica (STATIN). *External Trade Statistical Bulletin* **2010**, *2*, 9.

(3) Adams, C. D. Description of families. In *Flowering Plants of Jamaica*; University of the West Indies, Mona, Jamaica (distributed by Robert MacLehose & Co. Ltd.: Glasgow, 1972; p 441.

(4) Lewis, C. B. The ackee: a brief historical note. *Bull. Sci. Res. Counc. Jam.* **1962**, *1*, 12.

(5) Baldwin, J. E.; Adlington, R. M.; Bebbington, D.; Russell, A. T. Asymmetric total synthesis of the individual diastereoisomers of hypoglycin A. *Tetrahedron* **1994**, *50*, 12015–12028.

(6) Fowden, L.; Smith, A. Peptides from *Blighia sapida* seed. *Phytochemistry* **1969**, *8*, 1043–1045.

(7) Kean, E. A.; Lewis, C. E. Biosynthesis of L-β-(methylene cyclopropyl)alanine (hypoglycin) in *Blighia sapida*. *Phytochemistry* **1981**, *9*, 2161–2164.

(8) Brown, M.; Bates, R. P.; McGowan, C.; Cornell, J. A. Influence of fruit maturity on the hypoglycin A level in ackee (*Blighia sapida*). *J. Food Saf.* **1992**, *12*, 167–177.

(9) Glasgow, A. M. Hypoglycin toxicity: studies on ammonia metabolism. *Biochem. Pharmacol.* **1983**, *32*, 746–748.

(10) Mills, J.; Melville, G. N.; Bennett, C.; West, M.; Castro, A. Effect of hypoglycin A on insulin release. *Biochem. Pharmacol.* **1987**, *36*, 495–497.

(11) Borison, H. L.; Pendleton, J., Jr.; McCarthy, L. E. Central vs. systemic neurotoxicity of hypoglycin (ackee toxin) and related substances in the cat. *J. Pharmacol. Exp. Ther.* **1974**, *190*, 327–333.

(12) Hassall, C. H.; Reyle, K. The toxicity of the ackee (*Blighia sapida*) and its relationship to the vomiting sickness of Jamaica; a review. *West Indian Med. J.* **1955**, *4*, 83–90.

(13) Tanaka, K.; Kean, E. A.; Johnson, B. Jamaican vomiting sickness − biochemical investigation of two cases. *New Engl. J. Med.* **1976**, *295*, 461–467.

(14) Kean, E. A. Hypoglycin. In *Toxicants of Plant Origin*; Cheeke, P. R., Ed.; CRC Press: Boca Raton, FL, 1989; Vol. III, pp 229−262.

(15) Billington, D.; Osmunden, H.; Sherratt, S. A. Mechanisms of the metabolic disturbances caused by hypoglycin and by pent-4-enoic acid. In vitro studies. *Biochem. Pharmacol.* **1978**, *27*, 2879–2890.

(16) Barceloux, D. G. Akee fruit and Jamaican vomiting sickness (*Blighia sapida* Koenig). *Disease-a-Month* **2009**, *55*, 318–326.

(17) Joskow, R.; Belson, M.; Vesper, H.; Backer, L.; Rubin, C. Ackee fruit poisoning: an outbreak investigation in Haiti 2000−2001, and review of the literature. *Clin. Toxicol.* **2006**, *44*, 267–273.

(18) Toxic hypoglyceamic syndrome Jamaica 1989−1991. *CAREC Surveillance Rep.* **1992**, *18*, 39−41.

(19) Blake, O. A.; Jackson, J. C.; Jackson, M. A.; Gordon, C. L. A. Assessment of dietary exposure to the natural toxin hypoglycin in ackee (*Blighia sapida*) by Jamaican consumers. *Food Res. Int.* **2004**, *37*, 833–838.

(20) Blake, O. A.; Bennink, M. R.; Jackson, J. C. Ackee (*Blighia sapida*) hypoglycin A toxicity: dose response assessment in laboratory rats. *Food Chem. Toxicol.* **2006**, *44*, 207–213.

(21) Whitaker, T. B.; Saltsman, J. J.; Ware, G. M.; Slate, A. B. Evaluating the performance of sampling plans to detect hypoglycin A in ackee fruit shipments imported into the United States. *J. AOAC Int.* **2007**, *90*, 1060–1072.

(22) Chase, G. W.; Landen, W. O.; Soliman, A. G. M. Hypoglycin A content in the aril, seeds, and husks of ackee fruit at various stages of ripeness. *J. Assoc. Off. Anal. Chem.* **1990**, *73*, 318–319.

(23) Fowden, L. Hypoglycins and related compounds: occurrence, isolation and biosynthesis. In *Hypoglycin, Proceedings of a Symposium*; Kean, E. A., Ed.; Academic Press: New York, 1975; pp 11−19.

(24) Kean, E. A.; Hare, E. R. γ-Glutamyl transpeptidase of the ackee plant. *Phytochemistry* **1980**, *19*, 199–203.

(25) Baldwin, J. E.; Ostrander, R. L.; Simon, C. D.; Widdison, W. C. Stereospecific inactivation of the general Acyl-CoA dehydrogenase from pig kidney by (*R*)-(−)-(methylenecyclopropyl)acetyl-CoA and (*S*)-(+)-(methylenecyclopropyl)acetyl-CoA. *J. Am. Chem. Soc.* **1990**, *112*, 2021–2022.

(26) Hassall, C.; Reyle, K.; Feng, P. Hypoglycin A, B: biologically active polypeptides from *Blighia sapida*. *Nature* **1954**, *173*, 356–357.

(27) McGowan, C.; Wiley, V. A.; Bates, R. P. Application of methodology for RP-HPLC amino acid analysis to the measurement of hypoglycin A. *BioChromatography* **1989**, *4*, 161–164.

(28) Sarwar, G.; Botting, H. G. Reversed-phase liquid chromatographic determination of hypoglycin A (HG-A) in canned ackee fruit samples. *J. Assoc. Off. Anal. Chem.* **1994**, *77*, 1175–1179.

(29) Webster, S. A.; Mitchell, S. A.; Reid, W. A.; Ahmad, M. H. Somatic embryogenesis from leaf and zygotic embryo explants of *Blighia sapida* 'cheese' ackee. *In Vitro Cell. Dev. Biol.—Plant* **2006**, *42*, 467–472.

(30) Johnson, N. Jamaica ackee industry poised for growth. *Agriculturist* **2000**, *11*, 10–12.

(31) Kean, E. A. Improved method for isolation of hypoglycins A and B from fruit of *Blighia sapida*. *J. Pharm. Pharmacol.* **1974**, *26*, 639–640.

(32) Bowen, C. S. Evaluation of hypoglycins A and B content and the phytochemistry of *Blighia sapida*. Ph.D. Thesis, Department of Chemistry, The University of the West Indies, Mona Campus, Kingston, Jamaica, 2006.

(33) Bowen-Forbes, C. S.; Minott, D. A. Structural characterization of hypoglycin B, a diastereomeric dipeptide from the ackee fruit (*Blighia sapida* Koenig) by NMR experiments. *Magn. Reson. Chem.* **2009**, *47*, 1004–1006.

(34) Koller, M.; Echert, H. Derivatization of peptides for their determination by chromatographic methods. *Anal. Chim. Acta* **1997**, *352*, 31–59.

(35) Roberts, M.; Minott, D. A.; Tennant, P. F.; Jackson, J. C. Assessment of compositional changes during ripening of transgenic papaya modified for protection against *Papaya Ringspot Virus*. *J. Sci. Food Agric.* **2008**, *88*, 1911–1920.

(36) Kallio, H.; Yang, B.; Peippo, P. Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. *J. Agric. Food Chem.* **2002**, *50*, 6136–6142.

(37) Mpofu, A.; Sapirstein, H. D.; Beta, T. Genotype and environmental variation in phenolic content, phenolic acid composition, and antioxidant activity of hard spring wheat. *J. Agric. Food Chem.* **2006**, *54*, 1265–1270.

(38) Dolan, L. C.; Matulka, R. A.; Burdock, G. A. Naturally occurring food toxins. *Toxins* **2010**, *2*, 2289–2332.

(39) Climate Branch. *Preliminary Monthly Rainfall Summary for September 2010*; Meteorological Service Jamaica: Kingston, Jamaica, 2010; pp 6.

(40) Storozhenko, S.; Belles-Boix, E.; Babiychuk, E.; Hérouart, D.; Davey, M. W.; Slooten, L.; Montagu, M. V.; Inzé, D.; Kushnir, S. γ-Glutamyl transpeptidase in transgenic tobacco plants. Cellular localization, processing, and biochemical properties. *Plant Physiol.* **2002**, *128*, 1109–1119.

(41) Blake, O. A. Chemical and toxicological characterisation of selected components of the ackee (*Blighia sapida*) fruit. M.Philos. Thesis, Department of Chemistry, The University of the West Indies, Mona Campus, Kingston, Jamaica, 2002.