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Bioactive micro-constituents of ackee arilli (*Blighia sapida* K.D. Koenig)

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Abstract: Ackee (*Blighia sapida* K. D. Koenig) is an exotic fruit widely consumed in the Caribbean countries. While there is extensive research on the presence of hypoglycin A, other bioactive compounds have not been studied. We identified and quantified the changes in bioactive molecules (total phenol, ascorbic acid, hypoglycin A, squalene, D: A-Friedooleanan-7-ol, (7.alpha.), and oleic acid), antioxidant potential, and volatile compounds during two stages of ripe. A clear reduction in hypoglycin A, ascorbic acid, and total polyphenols during the maturation process were observed. On the contrary, oleic acid, squalene, and D: A-Friedooleanan-7-ol, (7.alpha.) contents increased about 12, 12, and 13 times, respectively with advancing maturity. These bioactive molecules were positively correlated with radical scavenging (DDPH and ABTS). Solid phase microextraction (SPME) and gas chromatography coupled mass spectrometry (GC/MS) analysis revealed more than 50 compounds with 3-penten-2-one and hexanal as the major compounds in the fully ripe stage. The results suggested that ripe ackee arilli could serve as an appreciable source of natural bioactive micro-constituents.

Key words: Antioxidants, Friedooleanan, Hypoglycin, Polyphenols, Squalene.

INTRODUCTION

Currently, there is a worldwide interest to generate innovative products with high nutritional and functional properties from exotic fruits. Recent studies have shown that frequent consumption of fruits and vegetables is associated with a reduced risk of chronic diseases because of the large amounts of antioxidant compounds they contain including phenolic compounds, carotenoids, anthocyanins and tocopherols (Contreras et al. 2011). In particular, phenols are related to the reduction of different deteriorating processes in the human body through their ability to diminish free radical formation after their consumption (Villa-Rodríguez et al. 2011).

The ackee fruit (*Blighia sapida* K.) was introduced from West Africa to Central America in the eighteenth century, and, in Jamaica, it

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became an essential part of Jamaican cuisine and eventually gained status as the national fruit of the country (Atolani et al. 2009). The fruit has a significant role in the local and even regional and international diets and economies (Benkeblia and López 2015). In fact, its popularity as a culinary delicacy has increased internationally; the fruit in brine is mainly exported to Canada, the UK, and the USA, generating high revenues ($ 13.971 M US dollars in 2015) for the agricultural sector of the country (STATIN 2017). In some other countries of the region, especially the north side of Colombia, it is used for ornamental purposes showing good adaptation to the edaphic and climate conditions.

The fruit is pear-shaped and opens spontaneously in three fragments when ripe. The arilli are the cream coloured edible part with a large black seed attached to the end of each piece. Traditional medicine has shown supposed medicinal properties of the ackee arilli which can be used to cure or relieve symptoms like fever, constipation, skin infections, and dysentery (Ekué et al. 2010, Olusegum and Olutomi 2013). Nevertheless, the ackee fruit has some disadvantages since it contains two toxic molecules depending on the maturation stage, hypoglycin A (L-α-amino-β-methylene cyclopropyl propionic acid) and hypoglycin B (γ-L-glutamyl-α-amino-β-methylene cyclopropyl propionic acid). Both are present in the seeds of the fruit, but only hypoglycin A occurs in the arilli (Bowen-Forbes and Minott 2011, Gaillard et al. 2011). The concentration of hypoglycin A is high in the green unripe fruit, but declines as the ripening process advance making it edible. Ingestion of unripe fruit can lead to a toxic condition called "Jamaican vomiting disease" (Blake et al. 2006).

On the other hand, it has been suggested that a low residual concentration of hypoglycin in ackees may be useful in the development of new therapies in people with specific diseases. The following administration (four weeks) from 100 to 400 mg/kg bodyweight of methanolic extract of *Blighia sapida* leaves reduced blood glucose level at all doses used and prevented oxidative stress and dyslipidemia in alloxan-induced diabetic rats (Oloyede et al. 2014). A great part of the research on the ackee fruit has been focused on the study of hypoglycin A (HGA) and B (HGB), but studies regarding the presence of other phytochemical groups are scarce (Benkeblia and López 2015, Garg and Mitra 1967, Antwi et al. 2009, Dossou et al. 2014). Additionally, little is known about the chemical changes occurring during the ripening process of the fruit. Therefore, the present study focused on the identification of some bioactive micro-constituents in ackee arilli from Colombia and their variations during two different ripening stages.

MATERIALS AND METHODS

PLANT MATERIAL

The ackee fruits were randomly collected from trees in Los Farallones National Park (Valle del Cauca, Colombia) and classified into two stages of maturity according to the scale described by Bowen-Forbes and Minott (2011). The fruits were selected according to their appearance, discarding bruised or rotten fruit. Finally, 10 fruits per tree were selected from 20 different trees (Fig.1). The arilli portion was separated from the seeds and lyophilised.

ANTIOXIDANT ACTIVITY

The extraction procedure described by Pérez et al. (2008) was followed with some modifications: four hundred milligrams (400 mg) of lyophilised ackee arilli were mixed in a vial containing 16 mL of methanol-water (1:1 v/v) with constant stirring for 1 hour. The tubes were centrifuged (2241 × g for 15 min at 25°C), and the supernatant was recovered and filtered (Whatman No. 1 filter). A volume of 16 mL of an acetone-water solution (7:3 v/v) was added to the residue and was then stirred

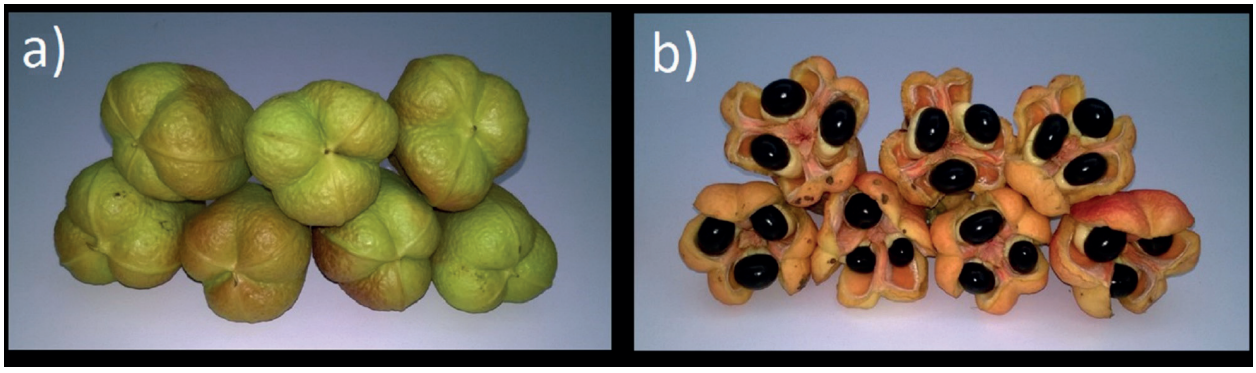

Figure 1 - Ackee fruit at different ripening stages. **a**) Maturation Stage 1 closed fruit, green pod, and green seed and **b**) Maturation Stage 5, ripe fruit with exposed arilli, open pods, orange-red, and black seeds.

and centrifuged under the same conditions. The supernatant was recovered again.

ABTS assay

The radical scavenging activity was measured by the ABTS radical cation discolouration assay described by Re et al. (1999), as µmol of Trolox equivalents per gram of fresh sample and was calculated by the ratio of the correlation coefficient of the dose-response curve of the sample and the correlation coefficient of the dose-response curve of the Trolox standard.

DPPH radical scavenging activity assay

The radical scavenging activity in the different extracts of ackee arilli was measured using the stable free radical DPPH (Brand-Williams et al. 1995). The procedure described by Villa et al. (2011), was followed with some modifications. The radical solution was prepared by mixing 2.5 mg of DPPH radical with 100 mL of pure methanol and was adjusted to 0.70 ± 0.02 absorbance at a wavelength of 515 nm (Genesys 10uv spectrophotometer). Next, 2.0 mL of the radical solution was placed in a test tube and 100 µL of the diluted extract was added. The mixture was shaken in a vortex and kept in the dark for 30 min until the measurement. The results were expressed in EC_{50} in mg/mL (concentration of antioxidant required to reduce the absorbance of the radical by 50%).

POLYPHENOL EXTRACTION AND QUANTIFICATION

The extraction to determine the polyphenol content (unconjugated + conjugated) in ackee arilli was performed by the method described by Vinson et al. (1998) with some modifications. In brief, 4 mL of 70% acetone with 1% hydrochloric acid were added to 0.2 g of the sample and the solution was shaken at 200 rpm for 3 h at room temperature. The mixture was centrifuged at 1089 *g* for 30 min and the supernatant was filled up to 5 mL with extraction solution. Free polyphenols (unconjugated) were extracted by the same procedure but without hydrochloric acid.

Four millilitres of the supernatants were loaded onto a Strata C18-U cartridge (1g, 6 mL) connected to a vacuum system (Phenomenex Inc., Torrance, CA), previously conditioned with 10 mL of methanol and followed by 25 mL of water. After the complete absorption of the sample, the hydrophilic compounds were eluted twice with 4 mL of 0.1 N sulphuric acid and discharged. The amphiphilic extracts containing polyphenols were eluted twice with 4 mL of 70% acetone and their polyphenol content was quantified after the reaction with Folin-Ciocalteu's reagent according to Singleton

and Rossi (1965). Extracts (1 mL) were diluted in 50 mL of bi-distilled water in 100 mL flasks and mixed with 5.0 mL of Folin-Ciocalteau's reagent (Sigma, Steinheim, DE) and, after 5 min, with 20 mL of sodium carbonate (15%). The flasks were mixed and allowed to sit in the dark for 30 min. The absorbance of the solution was measured at 750 nm on a Lambda Bio 20 UV/VIS spectrophotometer (Perkin Elmer, Boston, MA) and the results were expressed as mg of gallic acid equivalents (GAE) per gram of dry weight.

The analysis of individual polyphenols in the extracts was carried out by HPLC using the method described by Schieber et al. (2001). The separation of phenolic compounds was carried out using a 1200 Agilent Series HPLC (Agilent Technologies, Milano, Italy) equipped with a G1322 degasser, a G1311A quaternary pump, a G136A Column thermostat, a thermo-autosampler injection system, a column oven, and a diode array detector. The system was controlled with Agilent ChemStation for Windows (Agilent Technologies). An Aqua 5 µm C_{18} (250·4.6 mm I.D.) from Phenomenex (Torrance, CA, USA) and a security guard C ODS (433.0 mm I.D.) were used. The column temperature was regulated at 25°C. The mobile phase consisted of 2% (v/v) acetic acid in water (eluent A) and 0.5% acetic acid in water and acetonitrile (1:1, v/v; eluent B). The gradient program was: 10% B to 55% B (50 min), 55% B to 100% B (10 min), 100% B to 10% B (5 min). The injection volume for all samples was 10 mL. Simultaneous monitoring was performed at 280 nm, 320 nm and 370 nm at a flow-rate of 1 mL min^{-1}. Spectra were recorded from 200 to 600 nm.

ORGANIC ACIDS AND SUGAR CONTENT

Lipids were previously removed from the ackee arilli samples in Soxhlet equipment with ether and the samples were homogenised with 25 mL of H_2SO_4 5 mM in an Ultra-Turrax at 15.000 rpm for 30 seconds. The samples were then centrifuged at 4500 × g for 10 min at 4°C and the supernatants were filtered through a 0.45 µm Millipore filter (Millipore Corporation). Organic acids and sugars were analysed with HPLC equipment (Elite Lachrom, Hitachi) coupled with refractive index detector L-2400. Twenty microlitres of the sample were injected in a column (Agilent Hi-Plex H, 300 mm × 6.5 mm, 8 µm) using H_2SO_4 5 mM as mobile phase with an operating flow rate of 0.4 mL/min and 65°C.

DETERMINATION OF THE LIPID FRACTION COMPOSITION

The lipid Fraction was extracted from lyophilised ackee arilli samples with petroleum ether (bp 60−80°C, reflux, two hours) in a Soxhlet apparatus and concentrated in a vacuum. The chemical composition of the lipid fraction was determined by Gas Chromatography coupled to Mass Spectrometry (GC-MS) in a Shimadzu model GCMS QP2010 Ultra system, operated in full scan mode (range 35-500 m/z) with a scan speed of 1000 scans s^{-1}, operated in electron impact (EI) mode at 70 eV. Separation was carried out on a Restek (Bellefont, PA, U.S.A.) chemically bonded Rtx-5MS fused-silica capillary column (30 m×0.25 mm i.d.×0.25 µm film thickness) with temperature program as follows: beginning at 40°C for two minutes, increasing linearly up to 280°C in 20 min, and placed on hold for 20 min. The injector was operated at 280°C in split mode at 1:20 split ratio. Helium was used as the carrier gas at a flow rate of 1.01 mL min^{-1} and the flow was controlled by the linear velocity at 36.2 cm/s. Samples (1.0 µL) were injected with an AOC-20i+s auto-sampler. The identification of the compounds was established based on their retention index and mass spectra using the NIST11 and WR10 library.

HYPOGLYCIN A

Determination of hypoglycin A in the fruit was performed by the method described by Dundee and Minott, using an internal standard calibration method (Dundee and Minott, 2012). The determination was carried out using an HPLC equipment (Elite Lachrom, Hitachi), equipped with a UV detector (254 nm), injected on to a Chromolit RP-18 column (100 × 4.6 mm; Guard cartridge: Chromolit RP-18, 5 × 4.6 mm Merck, Germany). Hypoglycin A in ackee arilli samples was identified by the comparison of the retention times of its phenylisothiocyanate (PITC) derivative with the derivative of the homologue standard (L-Leucine) and was quantified using the respective calibration curve established for the PITC derivative of the homologue standard.

VOLATILE COMPOUNDS

The extraction of volatile compounds was carried out by the procedure indicated by Chaves-López et al. (2015) with some modifications: 10 g of pulp obtained by hand separation from the peel was added to 30 mL of distilled water and mixed, 10 mL of this sample was mixed with 2.5 g of NaCl and placed in a 50 mL vial containing a micro-stirring bar (Chaves-López et al. 2015). Samples were equilibrated for 40 min at 30°C and a solid-phase microextraction (SPME) manual device equipped with 50/30 divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, Bellefonte, PA) was used to extract free volatile compounds from the ackee arilli juice. The fibre was conditioned in a GC injector port at 270°C for 1 h before use.

The isolation and identification of the volatile compounds were performed on a Varian (Palo Alto, CA) CP3800 gas chromatograph equipped with a 60 m 0.25 mm i.d. DB-1 (df = 0.25 lm; J&W Scientific, Folsom, CA) fused silica capillary column. Analyses were carried out using helium as carrier gas at a column flow of 0.6 mL/min in a split ratio of 1:5 with the following program: (i) 80°C for 0 min, (ii) temperature ramp rate of 3.0°C/ min from 80 to 210°C and hold for 1 min followed by, (iii) a temperature ramp rate of 25°C/min from 210 to 300°C and hold for 3 min. The temperatures of the injector and detector were 230 and 300°C, respectively. Several compounds were identified by 3 different analytical methods: (i) retention index (KI), (ii) GC–MS retention times (authentic chemicals), and (iii) mass spectra (authentic chemicals and NIST05 spectral library collection). Identification was considered tentative based only on mass spectrometry data.

STATISTICAL ANALYSIS

Two batches of 10 samples of unripe and ripe fruits were analysed. The analyses were run in triplicate. Data were expressed as means ± SD and statistically analysed by the determination of the least significant difference (LSD at $p<0.05$) using SAS (2007) program, version 9.2.

RESULTS AND DISCUSSION

HYPOGLYCIN A

Hypoglycin A is an amino acid with the ability to induce hypoglycaemia by inhibiting gluconeogenesis cofactors (CoA and carnitine) that are essential for long-chain fatty acid oxidation. This toxin undergoes dramatic changes in the fruit as described by Gordon and Jackson-Malete (2015). In fact, the content of hypoglycin A in the ackee arilli decreased from 286.0 ± 31.6 mg/100 g in the unripe fruit to 43.1 ± 4.9 mg/100 g in the ripe fruit. Although some researchers suggested that the geographical location of the trees does not significantly influence the HGA content of the fruits ($p>0.05$), there is evidence of significant differences in HGA content (Table I shows data regarding the presence of HGA in ackee from different countries) because the physiological

TABLE I
Hypoglycin A content in ackee arilli (*Blighia sapida*) fruits.

Source	Unripe Ackee fruit (mg/100 g sample)	Ripe Ackee fruit (mg/100g sample)	Reference
Colombia	286.0±31.6*	43.1±4.9*	This study
West India	124.4±6.7	6.4±1.1	(Golden et al. 2002)
Jamaica	7939±509	596±77	(Bowen-Forbes and Minott 2011)
South Florida	111±N.S	10±N.S	(Brown et al. 1992)
Paramaribo (Suriname)	510±10	N.S	(Gaillard et al. 2011)
Burkina Faso	810±10	N.S	(Gaillard et al. 2011)
Jamaica	920±10	N.S	(Gaillard et al. 2011)

*Expressed as means ± standard deviations of ten samples.
N.S: Not Specified.

conditions of the trees vary according to the place of origin of the fruit and the harvest season (winter or summer) (Bowen-Forbes and Minott 2011, Gaillard et al. 2011, Gordon and Jackson-Malete 2015). However, it is important to highlight that mature ackee arilli on regular-to-medium sized seeds typically contain lower levels of hypoglycin A than mature arilli on very small seeds (Dundee and Minott 2012). It is suggested that lower residual hypoglycin concentrations in ackees may be useful to develop new therapies in people with specific diseases (Oloyede et al. 2014).

SUGAR AND ORGANIC ACID CONTENT

Ripening of climacteric fruit usually results in the breakdown of starch to sugars to promote sweetness, a decrease the amount of organic acids and phenols to minimise bitterness and astringency and an increase volatile compounds to produce characteristic flavours and aromas. As evidenced by our results, the sugars (fructose, glucose, and sucrose) and organic acids found in the ackee arilli decreased with the maturation state (Table II). These results are in accordance with researchers which reported that total sugars increased during the first four stages of the ackee ripening process and decreased during the last ripening stage (Emanuel and Benkeblia 2011). In this context, fruits with low sugar content (lower than 1%) are fruits with low carbohydrate content and therefore less sweet flavour. In addition to the changes in sugar concentration during the ackee ripening stages, researchers reported changes in three short-chain fructooligosaccharides identified as 1-kestose (1F-b-D-fructofuranosyl sucrose), nystose (1F(1-b-D-fructofuranosyl)2 sucrose) and DP5 (1F(1-b-D-fructo- furanosyl)3 sucrose) (Benkeblia and López 2015).

The organic acid content found in the fruit corresponds to the presence of ascorbic, citric and succinic acid. Ascorbic acid or vitamin C is an important water-soluble vitamin present in foods. In particular, the values of ascorbic acid found in the ackee arilli were 128.1 mg A.A/100g. Although this parameter is difficult to compare because the contents are highly variable, depending on parameters such as the state of maturity, harvesting period, and variety, the ackee arilli presented higher values compared to those found in different exotic fruits like purple passion fruit (36.3 mg A.A/100g), guava (65.8 mg A.A/100g), or the sweet gold pineapple cultivar (61.0 mg A.A/100g), and lower when compared with fruits such as the Cortibel guava fruit cultivar (168.36 mg A.A/100g) (Valente et al. 2011, Soares et al. 2007).

TABLE II
Organic acid and sugar content in Ackee arilli (*Blighia sapida*) fruits.

	Sugar (g/100g sample)			Organic acid (mg/100 g sample)		
	Saccharose	**Glucose**	**Fructose**	**Citric acid**	**Succinic acid**	**Ascorbic acid**
Unripe	1.66±0.04a	0.90±0.05a	1.33±0.07a	135.0±8.1a	107.7±4.5a	128.1±4.2a
Ripe	0.64±0.02b	0.40±0.03b	0.52±0.03b	50.9±3.2b	52.3±2.5b	65.6±3.8b

Results are expressed as means ± standard deviations of ten samples. Data expressed as fresh weight. Different letters in the same column indicate significant differences ($p < 0.05$).

LIPIDS

The fatty acid profile of the fruit is important because it has implications in health and in the stability of the oil. The total lipids in the ackee arilli increased during the ripening process. In fact, while unripe fruits showed only 2.20% d.w, the ripe ones showed values of 33.51% d.w content. These values are higher than those previously reported 28.34% and 20.02% (Machel et al. 2013, Oladiji et al. 2009), but lower than those reported 55.8% and 46.2% (Goldson et al. 2014, Mitchikpe 2007). These differences could be attributed to the variable ecological conditions including temperature, humidity, soil, etc., as well as the harvest time and ackee varieties.

The fatty acid percentages present in ackee arilli are shown in Table III. The major oil components in the ackee arilli sampled in this study corresponded to oleic acid (C18:1) (63.45%) followed by palmitic acid (16:0) (21.35%) and stearic acid (18:0) (3.51%) in oil. Studies conducted in ripe ackee arilli from Jamaica reported that palmitic, stearic, and linoleic (18:1) acids were the predominant fatty acids (Odutuga et al. 1992). The same authors suggested that the presence of sunlight during the opening of the fruits increased the lipid content. Also, other researchers noted that ackee oil of Nigeria is rich in behenic, palmitoleic, oleic, gadoleic, erucic, and 9, 12-eicosanoic acids (Oladiji et al. 2009). On the other hand, it was reported that the major acid in the arilli from Jamaica was oleic acid (Δ9-*cis*-oleic acid, an omega n-9) (Emanuel et al. 2013). A diet with high contents of oleic acid (polyunsaturated fatty acid) has been estimated to reduce the risk of suffering coronary heart disease, and to reduce the risk factors for cardiovascular disease such as those related to thrombogenesis, *in vitro* LDL oxidative susceptibility, and insulin sensitivity (Lopez-Huertas 2010).

It is important to acknowledge the presence of two important terpenes in the lipid fraction of Colombian ackee arilli: D:A-Friedooleanan-7-ol, (7.alpha.), and squalene (317 ± 29 and 448 ± 28 mg/100 g Ackee, respectively). To our knowledge, this is the first time that these two compounds are reported in ackee arilli. The squalene, a functional lipid, is highly unsaturated hydrocarbon with antioxidant activity that contributes to the reduction of cholesterol, triglyceride levels in serum, and protects against a variety of cancers (Xiao et al. 2016). In addition, squalene has many physiological functions, such as the promotion of superoxide dismutase activity *in vivo*, enhancement of immune responses and membrane stabilising properties (Bhattacharjee and Singhal 2003, Ko et al. 2002). However, since squalene is an intermediate in endogenous cholesterol synthesis, it has been suggested that it can lead to an increase in cholesterol, which translates to a greater risk for the development of atherosclerosis (Salvo et al. 2017).

Squalene is produced by both animals and plants as a biochemical intermediate. In particular, it is present in high quantities in *Amaranthus cruentus* (6000-8000 mg/100 g) (Popa et al. 2015), in the brazil nut (13.8 mg/100 g oil) (Ryan et al. 2009), in *Pistacia vera* L. (up to 21.8 mg/100g)

TABLE III
Contents lipids in Ackee arilli (*Blighia sapida*) fruits.

Name	Unripe	Ripe
Fatty acid	g/100 g	g/100 g
Palmitic acid (C16:0)	0.108 ± 0.005b	1.397 ± 0.043a
Oleic acid (C18:1)	0.344 ± 0.015b	4.152 ± 0.164a
Stearic acid (C18:0)	0.021 ± 0.002b	0.230 ± 0.025a
Triterpenes	mg/100 g	mg/100 g
D:A-Friedooleanan-7-ol, (7.alpha.)-	24 ± 3b	317 ± 29a
Squalene	36 ± 5b	448 ± 28a

Results are expressed as means ± standard deviations of ten samples. Data expressed as fresh weight of ackee arilli. Different letters in the same row indicate significant differences ($p < 0.05$).

and in *Prunus armeniaca* L. kernel oils (12.6–43.9 mg/100 g of oil) (Salvo et al. 2017, Rudzińska et al. 2017). Several studies suggested that the content of these compounds is significantly affected by the variety among the species and different climate conditions (Salvo et al. 2017, Rudzińska et al. 2017). On the other hand, researchers found that squalene can be used to treat *S. aureus* infections, but we found no evidence of inhibitory activity against *S. aureus* of ackee extracts *in vitro* (Sri-Charan-Bindu et al. 2015).

D:A-Friedooleanan-7-ol, (7.alpha.) is poorly known, however, it is reported that friedelane-type triterpenes might have potent anti-diabetic activity as suppressors of hepatic glucose production in insulin-resistant states (Ardiles et al. 2012). To our knowledge, this is the first report of the presence of D:A-Friedooleanan-7-ol, (7.alpha.) and squalene in ackee fruit.

PHENOLIC CONTENT

The phenolic content of fully ripe fruits was about 6-fold lower than the unripe with 1.5 mg GAE $g^{-1}_{d.w.}$. and 2.1 mg GAE $g^{-1}_{d.w.}$ of free and total polyphenols, respectively (Table IV). These values are slightly lower than those reported for apples using the same extraction solvent (Sacchetti et al. 2008). Since ripe ackee shows lower moisture content than that of apples (68.1 g $100g^{-1}_{f.w.}$) when expressed as fresh weight, the total polyphenol content is higher than that of apples (0.67 mg GAE $g^{-1}_{f.w.}$), but this data only allows ackee to be classified as a fruit with low polyphenol content (Vinson et al. 2001).

When calculated based on total polyphenols data, the percentage of conjugation of polyphenols is about 30% and is lower than that of apples, which is about 50% (Vinson et al. 2001). Previous research reported that the level of total phenolic compounds is somewhat higher in the arilli of unripe ackee fruits, but decreases by 30% during the ripening process. The level of total phenolic compounds ranged from 10.59 mg g^{-1} fresh weight at the unripe stage 1 to 7.38 mg g^{-1} fresh weight at the ripe stage 5 (Emanuel and Benkeblia 2011).

Polyphenols in Ackee were also determined by HPLC analysis, and the most abundant ones (in terms of peak intensity) were catechin, epicatechin, and phloridzin which was found in trace amounts. Upon ripening, the abundance of catechin and epicatechin decreased, whilst the content of phloridzin increased. The percentage of conjugation of catechins in ripe ackee arilli is about 30%, similar to that of apples, whilst phloridzin shows a percentage of conjugation of 46%.

Catechin and epicatechin are polyphenols commonly found in tropical fruits (such as cacao) and other common fruits such as apple, plum, and grapevine. On the contrary, phloridzin

TABLE IV
Total polyphenol and single polyphenol content of unripe and fully ripe ackee fruits.

		Polyphenols					
		Unripe			Ripe		
Method		Free	Conjugated	Total	Free	Conjugated	Total
Singleton and Rossi (1965)	GAE	8.50±0.09a	3.27±0.03a	11.78±0.09a	1.46±0.01b	0.65±0.01b	2.11±0.02b
	%_Conjugation		27.8%			30.8%	
Schieber et al. (2001)	(+) catechin	12.8±0.1a	3.77±0.04a	16.6±0.1a	0.150±0.002b	0.075±0.001b	0.225±0.003b
	(-) epicatechin	3.60±0.04a	1.32±0.01a	4.92±0.05a	0.351±0.004b	0.174±0.002b	0.525±0.005b
	Phloridzin	0.084±0.001b	0.058±0.001b	0.142±0.003b	0.125±0.001a	0.105±0.001a	0.230±0.001a
	Total	16.5±0.1a	5.15±0.01a	21.7±0.1a	0.63±0.01b	0.35±0.01b	0.98±0.03b
	%_Conjugation		23.8%			36.1%	

GAE: gallic acid equivalent. Data expressed as mg/g dry weight. Different letters in the same row and parameter indicate significant differences ($p < 0.05$).

is not a common polyphenol since it is found primarily in apples; closely related species from the Rosaceae family do not contain phloridzin and only trace amounts were reported in strawberry. The abundance of catechin and epicatechin in ackee fruit is interesting from the nutritional point of view since these polyphenols are bioavailable and abundant antioxidants in foods with widely recognised functional properties, such as cacao, chocolate, and tea (Crozier et al. 2012).

ANTIOXIDANT ACTIVITY

The antioxidant capacity of the ackee arilli measured by ABTS and DPPH methods increased during the ripe process, as reported in Fig. 2, and the values were higher than those reported for other exotic fruits from South America (Contreras et al. 2011, Vasco et al. 2008). This corroborates the findings of Hamzah et al. (2013) who stated that this fruit could be a potential source of natural antioxidants. Previous research has reported that the increase of the antioxidant activity is associated to physiological factors such as maturity, as well as technological factors like storage conditions and processing (Lindley 1998, Heyles and Lugasi 2006). Thus, we can hypothesize that ripe Ackee arilli could serve as a considerable source of natural antioxidants.

During ripe, fruit suffers physiological and biochemical changes, including the biosynthesis and accumulation of pigments, lipids, vitamins, and antioxidants, among others (Villa-Rodríguez et al. 2011, Goulao and Oliveira 2008). There are also other factors that are present in the physiology such as exposure to light and air, and the presence of pathogens, stress and environmental changes. At these stages, the plant raises physical and chemical barriers, such as the development of secondary metabolites or other types of molecules, where the fruit is protected by a defensive reaction that increases the concentration of antioxidant compounds. In this study, we observed that ackee arilli showed considerable amounts of squalene that probably contributed to the antioxidant activity.

VOLATILE COMPOUNDS

It is well known that the aroma of fruits comes from some components formed during the fruit

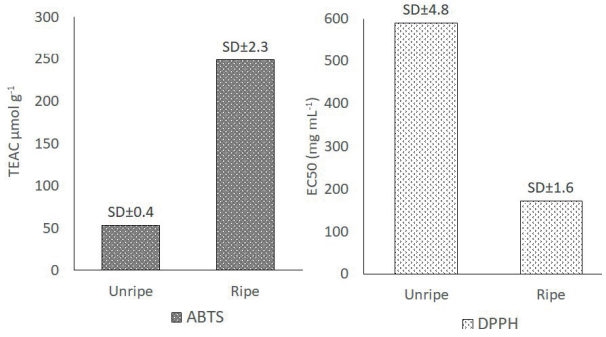

Figure 2 - Antioxidant capacity of the ackee arilli (*Blighia sapida*) fruit in two maturation stages. ABTS expressed in TEAC (Trolox equivalent antioxidant capacity) as µmol of Trolox equivalents per gram of fresh sample. DPPH expressed in EC50 (concentration of ackee extract required to reduce the absorbance of the radical by 50%) in mg/mL.

ripening process with a low aroma threshold value and high relative content. Many factors affect the volatile composition of fruit, including the degree of maturity and ripening stages, environmental conditions, postharvest handling and storage conditions, as well as the genetic composition of the plant. To the best of our knowledge, information related to the volatile compounds content that contributes to the aroma of the ackee fruit has not been reported yet. The volatile fraction present in the two maturation stages of ackee fruit is reported in Table V. For all the compounds, identification was based on chromatographic peak RI and similarity index (SI) higher than 90%.

The successful identification of 54 compounds during the two stages of maturation yielded different chemical classes of volatile compounds like aldehydes (15), esters (15), carboxylic acids (7), alcohols (6), ketones (4), monoterpenes (3) and alkanes (4). The composition of the volatile fraction changed throughout the ripening stages as evidenced during the present research, where several volatile compounds showed an increase in the relative peak area during ripeness. In particular, there was an increase in ketones, esters, and alkanes. On the contrary, aldehyde and alcohol content decreased during the ripening process.

Among the volatile compounds detected in the unripe ackee arilli fruit, aldehydes correspond to 40% of the volatile content, followed by alcohols (19%), acids (16%), and esters (10%). In this stage of maturation, hexanal, acetic acid, and benzaldehyde were the most abundant compounds, representing nearly 19%, 14% and 13% of the volatile fraction, respectively. The contribution of 1-octen 3-ol, dodecane, spiropentanoic acid methyl ester, and 1-hexanol, was also pronounced and ranged between 5% and 9.6% of the relative area. Aldehydes, alcohols, and esters may result from enzymatic reactions during fruit ripening. In particular, the presence of C6 aldehydes, alcohols, and esters suggests that the lipoxygenase pathway may be activated during the ripening process (Sansone-Land et al. 2014). Hexanal was found in both maturation stages with a high area percentage. Other C6 compounds were found at different concentrations such as 1-hexanol, 3-hexen-ol, 2-hexenal (E), hexanoic acid-2-penthylethyl ester and hexanoic acid methyl ester. In addition, other products derived from the enzymatic degradation of fatty acids like heptanal, octanal, 3-penten-2-one and 2-heptanone were present.

In the ripe stage, aldehydes were the most abundant compounds with 25%, followed by ketones, acids, and esters with 20%, 17%, and 15%, respectively. The volatile fraction was dominated by 3-penten-2-one (14.95%), which is usually described to have a sharp, acetone-like and fruity odor, hexanal (13.51%), that could be correlated with the characteristic odour of unripe fruits, dodecane (7.1%) and n-hexadecanoic acid (6%), that confer waxy notes, acetophenone (5.6%) which provides aromatic notes described as sweet flowers, and nonaldehyde (5%).

The compounds 3-penten-2-one, n-propyl acetate, 2-butanone 4hydroxy-3-methyl, 2-butenal-2-methyl (E), 2-heptanal and gamma terpinene were present only in the fully ripe stage of fruits, contributing to the overall aroma of ripe ackee

TABLE V
Volatile fraction composition in two maturation stages of ackee fruits (expressed as peak area %).

Compound	KI (Exp)	Unripe	Ripe
Alcohols			
pentanol	2088	3.94±0.21a	0.40±0.03b
3-hexen-ol	847	0.67±0.04	n.d
1-hexanol	841	9.20±0.70a	2.59±0.52b
1-heptanol	951	0.10±0.03a	0.07±0.03a
1-3-octen-ol	963	5.08±0.18a	0.28±0.04b
1-octanol	1052	0.31±0.02a	1.44±0.25b
Esters			
formic acid allyl ester	586	0.03±0.002	n.d
Butyl acetate	804.9	0.32±0.03	n.d
n-propyl acetate	686	n.d	0.43±0.05
spiropentanoic acid methyl ester	842	6.98±0.61	n.d
butanoic acid-3-methyl ester	1041	0.75±0.04a	0.19±0.03b
2-propenoic acid, 3-phenyl methyl ester (E)	1350	0.01±0.002a	1.72±0.56b
octanoic acid methyl buthyl ester	1430	0.03±0.004a	4.16±1.02b
oxalic acid hexadecyl 2-phenyl ester		0.03±0.001a	0.19±0.01b
ictanoic acid hexyl ester	1571	0.04±0.002a	0.23±0.11b
diethyl phalato	1543	0.31±0.03a	1.12±0.13b
hexanoic acid-2-penthylethyl ester	1616	0.22±0.03a	1.23±0.08b
isopropyl miristate	1836	0.06±0.01	n.d
octanoic acid-2-pentyl ester	1814	0.84±0.04a	3.18±0.31b
hexanoic acid methyl ester	1909	0.49±0.03a	2.84±0.54b
Acids			
formic acid	543	1.17±0.17a	2.35±0.73b
acetic acid	660	13.80±1.54a	3.33±0.87b
nonanoic acid	1263	0.36±0.08a	1.28±0.11b
dodecanoic acid	1554	0.12±0.04a	0.36±0.06b
tetradecanoic acid	1772	0.38±0.02a	0.96±0.13b
n-hexadecanioic acid	1972	0.20±0.07a	6.05±0.71b
octadecanoic acid	2174	0.91±0.10a	3.04±0.14b
Ketones			
3-penten-2-one	662	n.d	14.95±1.06
2-butanone, 4 hydroxy-3-methyl	832	n.d	0.06±0.02
5-hepten-2-one-6-methyl	960	1.16±0.14a	0.18±0.02b
Acetofenone	1052	2.20±0.45a	5.64±0.74b
Aldehydes			
butanal-3-methyl	628	0.09±0.04	n.d
2-butenal-2-methyl (E)	748	n.d	0.69±0.02

TABLE V (continuation)

Compound	KI (Exp)	Unripe	Ripe
Hexanal	771	19.86±1.51a	13.52±1.54b
2-hexenal (E)	838	0.66±0.06	n.d
Heptanal	879	0.26±0.03a	0.38±0.07b
2-heptenal	927	n.d	0.21±0.05
Benzaldehyde	928	11.83±1.18a	2.10±0.35b
Octanal	981	2.17±0.32a	0.94±0.20b
Benzeneacetaldehyde	1012	1.06±0.19a	0.08±0.01a
2-octenal (E)		0.11±0.03	n.d
furandicarboxy aldehyde	1996	0.10±0.03a	0.25±0.06b
1-nonanalaldehyde	1128	1.80±0.02a	5.96±0.76b
Decanal	1184	0.49±0.26a	0.93±0.18b
2-undecenal	1191	0.28±0.04a	0.11±0.02b
Dodecanal		0.25±0.05	0.41±0.05b
Monoterpens			
o-cynene	1051	1.03±0.14a	1.22±0.30a
Limonene		1.06±0.19a	2.15±0.51b
gamma terpinene		n.d	0.05±0.01
Alkans			
Decane		0.59±0.13a	1.15±0.37b
Dodecane		5.14±1.10a	7.19±0.26b
Tetradecane		1.12±0.20a	2.03±0.68a
Hexadecane		0.08±0.02	n.d

Results are expressed as mean value ± standard deviation of five samples.
Different letters in the same row indicate significant differences between unripe and ripe stage (*t*-test, $p < 0.05$).
n.d: Not detected.
R.T: Retention time.

fruit. However, it is important to underline that no single compound or a simple combination of these compounds has the typical smell of ripe fruit.

CONCLUSION

The present study represents a contribution to the chemical and functional characterisation of ackee fruit. Although polyphenols are reduced during the ripe stage, ackee arilli could serve as an appreciable source of natural antioxidants like citric acid, squalene, and oleic acid. Thus, ripened ackee fruit can be categorised as a functional food that could compete with other tropical fruits how banana, mango, papaya, passion fruit, or pineapple. In this work, for the first time, we identified and quantified the squalene and D: A-Friedooleanan-7-ol, (7.alpha.). In addition, the volatile profile of unripe and ripe arilli was identified.

AUTHOR CONTRIBUTIONS

Carlos D. Grande-Tovar, Antioxidant activity analysis, research, conceptualisation, writing-original draft preparation, and writing-review & editing. Johannes Delgado-Ospina, Bromatological analysis and hypoglycin content analysis by HPLC,

research, conceptualisation, writing-original draft preparation, and writing-review & editing.

Luisa F. Puerta, Antioxidant activity analysis, research, and writing-original draft preparation. Gloria C. Rodríguez, Hypoglycin content analysis by HPLC, conceptualisation

Giampiero Sacchetti, Polyphenol characterisation and quantification. Antonello Paparella GC-MS volatile analysis, writing-review and editing. Clemencia Chaves-López. GC-MS volatile analysis, writing-review, and editing.

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