Assessment and profiling of the fatty acids in two ackee fruit (*Blighia sapida* Koenig) varieties during different ripening stages

Machel A Emanuel, a Fabiola Gutierrez-Orozco, b Elhadi M Yahia b and Noureddine Benkeblia a

Abstract

BACKGROUND: The ripening of fruits is characterized by physical, chemical and biochemical compositional changes such as color, sugars and phenolic compounds. Ackee fruit is famous in Jamaica and the Caribbean. This study aimed to assess the variation of fatty acids in two varieties (cheese and butter) ackee (*Blighia sapida*) fruits during five different ripening stages.

RESULTS: The total fatty acid content of ackee fruit was much higher in arils and ranged from 283.4 to 465.1 g kg$^{-1}$ dry weight (DW), while in husk they ranged from 235.2 to 465.1 g kg$^{-1}$ DW in both varieties. Total fatty acid content declined in the arils and the husks as the fruit ripened. Five major fatty acids were found: palmitic acid (C16:0) and stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). In the arils, oleic acid was found at the highest concentration, followed by palmitic, stearic, linoleic and last linolenic acid. The unsaturated:saturated ratio of fatty acids varied from 1.23 to 3.26 in the arils of both varieties, and from 1.03 to 5.05 in the husk. Monounsaturated:polyunsaturated fatty acids ranged from 8.56 to 25.19 in the arils and from 0.62 to 2.33 in the husk.

CONCLUSION: The results show that ackee arils contain much higher levels of fats than the husk and the major fatty acid in the arils was oleic acid (Δ9-cis-oleic acid, an omega n-9), while in the husk unsaturated fatty acids were higher than the saturated ones. Oleic acid was the major fatty acid in both varieties, and aril fatty acid content was 10–20 times higher than in the husk. In both varieties, unsaturated fatty acids were relatively higher than saturated ones; however, total fatty acids showed a decline with ripening for arils and husk tissues.

Keywords: fatty acids; arils; husk; ripening; *Blighia sapida*

INTRODUCTION

The ackee fruit, *Blighia sapida* Koenig, belongs to the Sapindaceae and is a native plant of West Africa and was introduced to Jamaica in the 18th century. The ackee tree is a tropical evergreen that grows about 10 m tall, with yellow-red fruit that is about 10 cm wide and weighs about 100–200 g. As the ackee fruit ripens, the color of the fruit changes from green to yellow, to yellow-red, and then to fully red when the fruit is ripe. When ripe, the fruit splits longitudinally into three sections to reveal glassy black seeds in each section, surrounded by a thick yellow oily fleshy portion (the aril), which is edible and has a nutty flavor.1 Ackee trees are found throughout all Jamaican parishes; however, the major part grows in Clarendon and St Elisabeth parishes. In Jamaica, two types of ackee are recognized: ‘cheese’ or hard and ‘butter’ or soft. The cheese ackee aril is hard, cream colored and retains its shape when cooked, while the butter ackee aril is soft and yellow, losing its shape easily during cooking.

The ripe fleshy ackee aril is widely consumed by Jamaicans; it is one of the national dishes and is considered one of the national symbols in Jamaica.

The unripe fruit contain a water-soluble toxin, hypoglycin A (L-aminomethylenecyclopropylpropionic acid) and the less toxic hypoglycin B. The latter compound is the γ-glutamyl conjugate of hypoglycin A. Unripe ackee fruit also contain glutamate analogs that are carboxycyclopropylglycine compounds.2 To prevent toxicity, the seeds and husk of the ackee fruit must be carefully removed and the aril thoroughly washed and cooked before consumption. However, cooking arils of unripe ackee fruit does not destroy the toxins, whereas cooking arils of ripe fruit effectively eliminates the toxicity by leaching hypoglycin A.3,4

Extensive literature exists on the toxicity of hypoglycin A,5–8 however, there is no referenced data reporting the profiling and the composition of the fatty acids ofackee fruit except for those reported by Odutuga et al.9 on fats of arils ofackee fruit at different opening pericarp stages (closed, open in dark, open in sun and
open on tree), and they noted that linoleic acid was the major fatty acid (>55% of total fatty acids), followed by palmitic acid (>14%). This would be considered as a major gap due to the high content of fats in the arils of the fruit.

The aim of this study was to profile the fatty acids of both arils and husk of two different varieties of ackee fruit during different ripening stages.

EXPERIMENTAL

Plant material

Samples of ackee fruits were collected from two different locations. The cheese (hard) ackee was harvested from trees growing in the Botanical Garden lands, Department of Life Sciences (18° N, 76° W), University of the West Indies, Mona Campus, while the butter (soft) ackee fruits were harvested from trees grown in the parish of Clarendon (17° N, 77° W). The fruits were sampled during the different ripening stages as defined below. Although the Bureau of Standards of Jamaica (http://www.jbs.org.jm/) scales the ackee into eight growing and ripening stages depending on the size, color of fruits, which varies from early green (stages 1–5) to red (stages 6–8), and opening of the husk, which start from the ripe stage (stage 5) to the fully ripe and edible stage (stage 8), only the five last stages (stages 4–8) were sampled for our investigation because arils during the first three stages (stages 1–3) are quasi-absent or too small to be sampled properly. On the other hand, and for toxicity considerations, only the last stage (stage 8) is considered as ‘Safely Edible’ due to the very low level of hypoglycin A. The ackee fruits were harvested from the same trees in 2009 and 2010 (two fruits from different trees growing closely in the same row). Immediately after the fruits were harvested, the seeds were discarded and samples of ackee fruits were separated into arils and husk.

Lipid extraction

Lipids from the samples were extracted following the method described by Folch et al. Samples of 0.5 g of either dry arils or husk were homogenized in 10 mL of a mixture of chloroform–methanol (2:1, v/v). After dispersion, the mixture was sonicated for 5 min and centrifuged at 10 000 rpm for 10 min at room temperature. The homogenate was filtered to recover the liquid phase, to which 1.5 mL NaCl (9 g L⁻¹) was added. After centrifugation at 2000 rpm for 5 min, the top layer was removed and the lower phase was roto-evaporated and resuspended in 1 mL hexane. Lipid extraction was performed in triplicate for each fruit (three arils were used to extract the three samples).

Fatty acid methylation

The fatty acids were methylated as described by Christie. For preparation of fatty acid methyl esters, a sample of 100 µL of the lipid extract was mixed with 3 mL of a 3.75 mol L⁻¹ NaOH–methanol solution and heated to 100 °C for 25 min in a water bath. Then 6 mL of 3.25 mol L⁻¹ HCl–methanol was added and the mixture was heated to 80 °C for 10 min. After that, 3.75 mL of a 1:1 mixture of hexane and methyl tert-butyl ether was added and the bottom layer was discarded. Finally, 9 mL of 0.3 mol L⁻¹ NaOH–water was added and the top layer was transferred for injection into the gas chromatograph. Fatty acid methylation was done in triplicate (as mentioned above).

Pure standards of fatty acids (lauric (12:0), myristic (14:0), palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3)) were purchased from Sigma-Aldrich and were subjected to the same methylation procedure described above.

Gas chromatographic analysis of the fatty acids

An HP 5890 Series II gas chromatograph equipped with a flame ionization detector (FID) was used for analysis. The gas chromatographic separation was done using an HP-INNOWax 30m × 0.25 mm × 0.25 µm column. The injection volume was 0.5 µL and the split ratio was 20:1. The temperature settings were 250 °C for the injector and 270 °C for the detector. The temperature program was 180 °C for 1 min, then to 220 °C at 1.5 °C min⁻¹ and maintained at 220 °C for 1 min. Helium was used as the carrier gas (head pressure, 25 psi) at a flow rate of 1.8 mL min⁻¹. The auxiliary gas was helium with a flow rate of 23.2 mL min⁻¹. For the FID, hydrogen and air flows were 30 and 400 mL min⁻¹, respectively. For quantification, standards of the individual fatty acids were used, and all samples were evaluated in triplicate.

Statistical analysis

All the analyses were carried out in triplicate (each analysis was run on three arils of the same fruit) and experimental work was duplicated (during two harvesting seasons: 2009 and 2010). Data were expressed as the means ± SD and analyzed statistically by determination of least significant difference (LSD at P ≤ 0.01) using GraphPad Prism 4.03 (GraphPad Software, Inc., La Jolla, CA, USA).

RESULTS AND DISCUSSION

Total fats content

The total fats content of ackee fruit was much higher in the arils than in the husk (Fig. 1). In arils, total fats ranged from 283.4 mg g⁻¹ DW (dry weight) (stage 5) to 465.1 mg g⁻¹ DW (stage 2) in the cheese variety, and from 235.2 mg g⁻¹ DW (stage 5) to 409.3 mg g⁻¹ DW (stage 2) in the butter variety. We noticed that fat content decreased during ripening (Fig. 1A). In husk, total fat was 10- to 20-fold lower than that observed in arils, and ranged between 13.62 mg g⁻¹ DW (stage 4) and 24.01 mg g⁻¹ DW (stage 1), and from 10.80 mg g⁻¹ DW (stage 5) to 22.70 mg g⁻¹ DW (stage 1) in cheese and butter varieties respectively, and similarly a decrease was noticed during the ripening stages (Fig. 1B).

Although a few studies have reported fat content of ackee fruit husk, very few studies reported their content in arils. First, Morton reported that total lipids of raw arils were 187.8 g kg⁻¹ of fresh weight (FW). Later, Singh et al. reported that dried arils contain 264.0 g kg⁻¹ DW of oils, while Ouattara et al. reported 453.2 g kg⁻¹ DW. These discrepancies of data on total fats of arils are most likely due to the different extraction methods since different solvents were used, and also to quantification methods. Odutuga et al. also reported a different fat content of arils, varying between 36.01 and 580.0 g kg⁻¹ DW, and this content was related to the opening stage. However, this study was carried out on ackee at different stages that was opened artificially except for one stage.

Fatty acid profile

Analysis of fats revealed five major fatty acids including two saturated acids, namely palmitic (C16:0) and stearic (C18:0), and three unsaturated fatty acids, namely oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) (Tables 1 and 2).

In the arils of both cheese and butter varieties, oleic acid was found at the highest concentration, followed by palmitic, stearic, linoleic acid, and lastly linolenic acid (Table 1). The average
concentration during the five ripening stages of the cheese variety was 237.5, 80.7, 32.2, 13.4 and 6.74 mg g\(^{-1}\) DW for oleic, palmitic, stearic, linolenic and linoleic acids, respectively, and 6.2, 2.9, 2.9, 2.7 and 2.2 mg g\(^{-1}\) DW for oleic, palmitic, stearic, linoleic and linolenic acids, respectively.

To date, there are no referenced data reporting the different fatty acids in husk of ackee fruit except those of Odutuga et al.\(^5\) showing the variation of the different fatty acids of arils of ackee that was opened artificially, except for one stage opened on the tree. In addition to the fatty acids reported in our study, these authors reported four other saturated fatty acids, namely myristic (C14:0), arachidic (C20:0), behenic (C22:0) and ligniceric acid (C24:0), and one unsaturated fatty acid – palmitoleic acid (C16:1) – although the levels of these fatty acids were very low compared to the main acids found. While most of the fatty acids reported by these authors are well known, the presence of palmitoleic acid is not common. This rare 16-carbon monounsaturated fatty acid, also known as omega-7, is found in only one other known plant (macadamia nut, *Macadamia integrifolia*). Palmitoleic acid is believed to have a potent antiviral, antibacterial and healing effect in humans. It is also similar to lipids naturally occurring in skin of sebum, is believed to provide important healing and anti-aging benefits for skin problems, and its consumption is reported to impart health benefits to humans.\(^15\)

### Unsaturated:saturated fatty acid ratio

The ratio of unsaturated:saturated fatty acids was less in arils than in husk (Fig. 2). In arils, the ratio varied from 1.23 (stage 5) to 3.26 (stage 3) and averaged 2.30 in the cheese variety, and from 1.60 (stage 1) to 2.64 (stage 2) and averaged 2.15 in the butter variety (Fig. 2A). In the husk, the ratio varied from 1.44 (stage 2) to 5.02 (stage 5) and averaged 3.51 in the cheese variety, and from 1.03 (stage 2) to 2.86 (stage 5) and averaged 2.11 in the butter variety (Fig. 2B).

The ratio of unsaturated:saturated fatty acids in arils varied regularly, indicating that a balanced level of the different fatty acids was maintained during ripening of the fruit. In the husk, an imbalance was noted during the last three ripening stages, where higher levels of unsaturated fatty acids were noted, indicating that the husk tissues likely use the fatty acids as a source of metabolites and energy instead of sugars, which are very low in the husks (unpublished data). Based on the fatty acids reported by Odutuga et al.\(^5\) we estimated the unsaturated:saturated ratio and found that it ranged from 1.3 to 1.9, indicating that the

| Table 1. Fatty acid content (mg g\(^{-1}\) DW) of the arils of cheese and butter varieties of ackee fruit during the different ripening stages |
|-----------------|----------------|----------------|-----------------|-------------------|----------------|
| Maturity stage | Palmitic acid (16:0) | Stearic acid (18:0) | Oleic acid (18:1) | Linoleic acid (18:2) | Linolenic acid (18:3) |
| Cheese variety | 85.2 ±10.4a | 23.8 ± 2.8ab | 248.7 ±29.3ab | 16.5 ± 4.5a | 2.4 ± 0.3a |
| 1 | 92.2 ± 5.5a | 33.9 ± 3.5ab | 324.1 ± 15.0a | 18.9 ± 7.6a | 2.2 ± 0.8a |
| 2 | 67.0 ± 16.5ab | 19.7 ± 7.8b | 272.1 ± 50.1ab | 10.8 ± 2.7a | 1.7 ± 0.2a |
| 3 | 78.7 ± 31.6ab | 36.8 ± 13.9ab | 198.6 ± 67.6a | 9.6 ± 1.5a | 1.2 ± 0.27a |
| 4 | 80.2 ± 15.6a | 47.0 ± 9.7a | 143.5 ± 41.3b | 11.4 ± 2.8a | 1.7 ± 0.4a |
| 5 | 68.8 ± 12.9a | 42.7 ± 6.7a | 169.5 ± 41.4ab | 4.8 ± 1.4b | 3.9 ± 0.9b |
| Butter variety | 73.7 ± 14.1a | 36.0 ± 6.3a | 275.2 ± 3.2a | 5.5 ± 0.9ab | 8.4 ± 1.4ab |
| 1 | 46.1 ± 23.1a | 41.2 ± 7.8a | 189.7 ± 5.9ab | 6.4 ± 1.1ab | 6.8 ± 1.2ab |
| 2 | 42.1 ± 19.9a | 40.3 ± 3.9a | 158.9 ± 4.3ab | 9.8 ± 1.04a | 6.9 ± 1.8ab |
| 3 | 42.2 ± 9.0a | 36.7 ± 9.4a | 146.2 ± 4.3b | 7.2 ± 1.7ab | 9.9 ± 2.3a |

Values with different letters in the same column are significantly different at \(P \leq 0.05\).
Table 2. Fatty acid content (mg g\(^{-1}\) DW) of the husk of cheese and butter varieties of ackee fruit during the different ripening stages

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Palmitic acid (16:0)</th>
<th>Stearic acid (18:0)</th>
<th>Oleic acid (18:1)</th>
<th>Linoleic acid (18:2)</th>
<th>Linolenic acid (18:3)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cheese variety</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.37 ± 0.27a</td>
<td>4.64 ± 0.15b</td>
<td>6.9 ± 2.13a</td>
<td>5.5 ± 1.52a</td>
<td>5.6 ± 1.3ab</td>
</tr>
<tr>
<td>2</td>
<td>3.31 ± 0.56a</td>
<td>3.41 ± 0.78ab</td>
<td>4.1 ± 0.91a</td>
<td>5.6 ± 1.03a</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>3.71 ± 0.27a</td>
<td>n.d.</td>
<td>2.9 ± 0.86a</td>
<td>5.1 ± .86a</td>
<td>7.3 ± 1.4b</td>
</tr>
<tr>
<td>4</td>
<td>2.72 ± 0.11a</td>
<td>n.d.</td>
<td>6.2 ± 1.25a</td>
<td>4.7 ± 0.39a</td>
<td>n.d.</td>
</tr>
<tr>
<td>5</td>
<td>1.38 ± 0.09a</td>
<td>1.19 ± 0.06a</td>
<td>5.9 ± 1.36a</td>
<td>4.2 ± 0.41a</td>
<td>2.81 ± 0.20a</td>
</tr>
<tr>
<td><strong>Butter variety</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>4.6 ± 1.09a</td>
<td>2.5 ± 0.41ab</td>
<td>8.8 ± 1.53b</td>
<td>3.5 ± 0.56</td>
<td>2.3 ± 0.41</td>
</tr>
<tr>
<td>2</td>
<td>3.9 ± 0.42a</td>
<td>6.6 ± 1.2a</td>
<td>6.2 ± 1.2ab</td>
<td>2.7 ± 0.37</td>
<td>1.9 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>2.2 ± 0.35a</td>
<td>2.7 ± 0.2ab</td>
<td>5.7 ± 0.67ab</td>
<td>2.8 ± 0.18</td>
<td>2.3 ± 0.26</td>
</tr>
<tr>
<td>4</td>
<td>2.1 ± 0.31a</td>
<td>1.6 ± 0.42ab</td>
<td>4.7 ± 1.31a</td>
<td>1.2 ± 0.32</td>
<td>2.3 ± 0.34</td>
</tr>
<tr>
<td>5</td>
<td>1.7 ± 0.23a</td>
<td>1.1 ± 0.09b</td>
<td>5.6 ± 1.02ab</td>
<td>2.4 ± 0.43</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Values with different letters in the same column are significantly different at \( P \leq 0.05 \).

n.d., not detected.

Figure 2. Variation of unsaturated:saturated ratio of fatty acids in (A) arils and (B) husk of cheese and butter varieties of ackee fruit at different ripening stages.

Figure 3. Variation of monounsaturated fatty acids (MUFA) to polyunsaturated fatty acids (PUFA) in (A) arils and (B) husk of cheese and butter varieties of ackee fruit at different ripening stages.

level of unsaturated fatty acids is higher than that of saturated ones.

**Monounsaturated fatty acid (MUFA):polyunsaturated fatty acid (PUFA) ratio**

We estimated the MUFA:PUFA ratios and noted that significant differences were observed between the arils and husk (Fig. 3). In the arils, MUFA:PUFA ranged from 10.95 (stage 5) to 25.19 (stage 3) and averaged 16.62 in cheese variety, while in butter variety this ratio ranged from 8.56 (stage 5) to 19.80 (stage 2) and averaged 14.34 (Fig. 3A). In the husk, MUFA:PUFA ranged from 0.62 (stage 1) to 1.32 (stage 4) and averaged 0.75 in cheese variety, while in butter variety this ratio ranged from 1.12 (stage 3) to 2.33 (stage 5) and averaged 1.47 (Fig. 3B).

As for the previous ratios, we also estimated MUFA:PUFA ratio based on the fatty acids reported by Odutuga et al.\(^9\) and we noted that MUFA:PUFA ratio was very low (less than 0.02) because these authors reported that linoleic acid was the major fatty acid, with more than 55 % of the total fatty acids determined. This discrepancy could be attributed to the fact that these authors used ripened fruits and opening was done artificially, except for one sample which opened ‘on tree’, while in our investigation...
we harvested fresh ackee fruits with natural full opening at stage 5.

CONCLUSION
The results show that ackee arils contain much higher levels of fats than the husk and the major fatty acid in the arils was oleic acid, whereas in the husk unsaturated fatty acids were higher than saturated ones. We also noted that unsaturated:saturated ratio was much higher in the arils compared to the husk, and MUFA:PUFA ratio showed a similar pattern and was much higher in the arils than in the husk. Results showed also that ackee arils are a good source of omega n-9. However, the level of omega n-6 and omega n-3 fatty acids is lower (5 – 10% of the total unsaturated fatty acids), and these are well known for their beneficial impacts on health.

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REFERENCES