ORIGINAL ARTICLES

Fat Profile of Jamaican Ackees, Oleic Acid Content and Possible Health Implications

A Goldson, D Bremmer, K Nelson, DA Minott

ABSTRACT

Objective: *To re-investigate the composition of ackee oil and unequivocally determine its principal fatty acid components.*
Methods: *Oil was extracted from the edible portion of ackees harvested in three different studies (I – III) by several analysts; studies I and II utilized composite samples from several trees while study III consisted of ackees from seven separate trees. The oils were either saponified and methylated or trans-methylated and the fatty acid methyl ester content analysed by gas chromatography-mass spectrometry (GC-MS). Relative fatty acid composition was quantified based on chromatographic peak areas while fatty acids were identified by mass spectrometry. The degree of unsaturation of the ackee oils was characterized by determination of the iodine value.*
Results: *Gas chromatography-mass spectrometry data from the three studies were assessed. Relative fatty acid composition for the ackee oils was consistent across the three studies. The major fatty acid components were oleic acid (55.44%), palmitic acid (25.57%) and stearic acid (12.59%); linoleic acid was present in minor to undetectable amounts. An iodine value of 49 was determined which is consistent with the high oleic acid content of the ackee oil.*
Conclusion: *The ackee samples analysed were rich in the monounsaturated fatty acid (MUFA) oleic acid. Consideration should be given to potential protective health effects of diets which include ackee.*

Keywords: Ackee (*Blighia sapida)*, linoleic acid, prostate cancer, oleic acid

Perfil de Grasas del Seso Vegetal Jamaicano, Contenido de Ácido Oleico, y Posibles Implicaciones para la Salud

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RESUMEN

Objetivo: *Re-investigar la composición del aceite del seso vegetal (ackee) y determinar de manera inequívoca sus componentes principales de ácidos grasos.*
Métodos: *Se extrajo el aceite de la porción comestible de sesos vegetales de la* Blighia sapida *(ackee), cosechados como parte de tres estudios diferentes (I – III) por varios analistas. Los estudios I y II utilizaron muestras combinadas de varios árboles de* Blighia sapida, *en tanto que el estudio III estuvo formado por muestras de siete árboles de* Blighia sapida *por separado. Los aceites fueron saponificados y metilados o transmetilados, y el contenido de éster metílico de ácidos grasos fue analizado mediante técnicas de cromatografía de gases y espectrometría de masas (GC-MS). La composición relativa de ácidos grasos se cuantificó sobre la base de las áreas cromatográficas pico, mientras que los ácidos grasos se identificaron mediante espectrometría de masas. El grado de insaturación de los aceites de seso vegetal fue caracterizado mediante la determinación del valor de yodo.*
Resultados: *Se evaluaron los datos de la cromatografía de gases acoplada con espectrometría de masas de los tres estudios. La composición relativa de ácido grasos de los aceites de seso vegetal, fue constante*

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a través de los tres estudios. Los componentes principales del ácido graso fueron el ácido oleico (55.44%), el ácido palmítico (25.57%), y el ácido esteárico (12.59%). El ácido linoleico estuvo presente en cantidades que fluctuaron de menores a indetectables. Se determinó un valor de yodo de 49, el cual está en correspondencia con el alto contenido de ácido oleico en el seso vegetal.

Conclusión: *Las muestras analizadas de sesos vegetales de la* Blighia sapida *(ackee) eran ricas en ácidos grasos monoinsaturados (MUFA por sus siglas en inglés) y ácidos oleicos. Debe prestarse atención a los posibles efectos protectores de la salud de dietas que incluyen sesos vegetales.*

Palabras claves: Seso vegetal (*Blighia sapida*), ácido linoleico, ácido oleico, cáncer de próstata

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INTRODUCTION

Although ackees (*Blighia sapida*) are eaten in several countries, its consumption has been integrally linked with Jamaica for centuries. A popular dish, ackee is enjoyed by all age groups and socio-economic classes in Jamaica (1). Ingestion of immature ackees results in Jamaican Vomiting Sickness (JVS), attributable to consumption of high levels of the non-proteinogenic amino acid, hypoglycin A, found in the edible portion of the ackees (2, 3). Over the past six decades, much work has been done on ackee, its toxic metabolite, hypoglycin A, and biological activity, periodically stimulated by isolated outbreaks of vomiting sickness (4−9). The fruit's notoriety has led to strict regulatory controls being imposed on the hypoglycin A levels of the product in commerce (10).

In recent years, attention has been re-focussed on ackees with the controversy surrounding a possible causal relationship with the alleged high incidence of prostate cancer in Jamaican men. It had been noted that the rate of prostate cancer in Jamaica ranks amongst the world's highest (11), although subsequent papers have shown that the age-standardized rates for prostate cancer are significantly lower than that previously reported (12−14). Notwithstanding these findings, recently released global statistics indicate that in the Caribbean, the mortality rate from prostate cancer is unsurpassed by any other region (12).

It is theorized that in many cancers, diet plays an integral role in promoting (15, 16), and conversely, in controlling the disease (17−19). Several studies have demonstrated a link between the consumption of fatty foods and cancer in general, with foods high in linoleic acid being of concern in relation to prostate cancer (16), although some researchers have disputed the association (19, 20). Ritch *et al* demonstrated a positive correlation between dietary linoleic acid, prostate-specific antigen (PSA) and prostate cancer in Jamaican men (21, 22). Ackee has been indirectly implicated in efforts to determine dietary factors unique to Jamaica that can contribute to the alleged high levels of prostate cancer. A high-fat food, it had been reported by Odutuga and co-workers to contain linoleic acid levels in excess of 55% of the crude lipid fraction (23). A subsequent report by Wellington and associates (24) was not consistent with these findings, indicating not only that the linoleic acid concentration in ackee was low, but that the principal fatty acid was instead stearic acid.

The inference to ackee as a possible causal agent for prostate cancer in Jamaican males had been based exclusively on its presumed high linoleic acid content and its anecdotal substantial consumption by the Jamaican populace (22). With conflicting information in the literature, it became necessary to re-examine the fatty acid composition of ackee. This report outlines the content analysis on the ackee fat conducted in separate studies (I – III) by several analysts in our research laboratory over a prolonged interval. An evaluation of the fat profile in terms of its fatty acid composition is provided with a comparison to and subsequent validation by the iodine value (IV) of the ackee oil.

METHODS

Ackee fruits (stage 7: mature, half-open, seeds and aril clearly visible) were harvested from several randomly selected trees located on the grounds of The University of the West Indies (Kingston, Jamaica), in three separate studies, I (October-November 1998), II (February–March 2006) and III (February–March 2008). Arilli were separated from the refuse (pods, placenta and seeds), oven dried to constant weight (55 ^{o}C for six days; Grieve Laboratory Oven LW-201C, Round Lake, IL), and milled (30 seconds, 25 ^{o}C; Ika-Werke M20 Analytical Mill, Staufen, Germany). Oil was extracted from the dried, milled ackee arilli either with dichloromethane, methanol (1:1 v/v, rt, 24 hours) (I) or with petroleum ether (bp 80−100 ^{o}C, reflux, two hours) in a soxhlet apparatus (II and III) and concentrated *in vacuo*. A single composite sample was used in each of study I and II, whereas in study III, seven samples consisting of fruits ($n \geq 12$) collected from seven ackee trees (representing the two main ackee varieties, 'Butter' and 'Cheese') were separately extracted and analysed. Per cent crude fat (dry weight basis) was determined in duplicate by the ratio of mass of oil to mass of aril (study III). Ackee arilli oil samples from study III (0.12 g) were analysed in duplicate using Hubl's method (25) and the IV expressed as grams iodine absorbed per 100 g oil.

Ackee oil (I, 1.0497 g), dissolved in propan-2-ol (150 cm^3) was treated with sodium hydroxide (0.408 g in 2 cm^3 water, rt). The saponified fat (0.8044 g in 50 cm^3 methanol, 4 ^{o}C) was methylated with diazomethane (26). On completion of the reaction, the mixture was concentrated *in vacuo* and the resulting fatty acid methyl esters (FAMEs) examined by gas chromatography-mass spectrometry (GC-MS). Alternatively, soxhlet extracted ackee oil (II or III, 0.003 g) was trans-methylated with methanol/acetyl chloride solution [HPLC grade] (27).

Methylated ackee oil in hexane (1.0 µL) was chromatographed on an HP6890 series gas chromatograph equipped with a flame ionization detector (FID) interfaced with an HP5973 mass selective detector. Constituent FAMEs were eluted in helium carrier gas (flow rate 1 cm^3/minute) through a DB-VRX column (20 m x 0.18 mm id x 1.0 µm film thickness; Agilent, Santa Clara, CA) in an oven programmed at 60 °C for three minutes and increased at a ramp rate of 10 °C/minute up to 250 °C for 15 minutes. Samples were injected at 230 °C while the detector was maintained at 250 °C. Constituents were identified by comparison with authentic standards (Sigma, St Louis, MO) and with the National Institute of Standards and Technology (NIST) library of mass spectra and subsets (match quality > 80%). Percentage relative amounts of individual FAMEs are based on peak areas without detector response factor correction.

RESULTS

Soxhlet extraction of the crude fat from the ackee arilli in study III (yield 55.8 ± 3.1% dry weight basis) confirmed the high-fat content previously reported in the literature (23, 24). Methylation of each of the oils in the three studies (I – III) and subsequent GC-MS analysis of the FAMEs from the ackee oils gave the percentage fatty acid composition; fatty acids present in the oil at a concentration of 0.1% or above are given in Table 1. The major oil components were found to be oleic acid (55.44%), palmitic acid (25.57%) and stearic acid (12.59%). Minor fatty acids were gondoic (0.99%) and arachidic (1.35%) acids with linoleic acid being found at very low (0.23–0.67%) to undetectable levels.

Table 1: Percentage fatty acid composition in ackee oils from studies I, II, and III

FAME[a]	% fatty acid of ackee oil			
	I	II	III	Average
Myristic (C14:0)	0.19	0.24	0.15 ± 0.05[b]	0.19
Palmitoleic (C16:1)	0.41	0.59	0.50 ± 0.13	0.51
Palmitic (C16:0)	21.93	26.08	28.7 ± 4.3	25.57
Margaric (C17:0)	0.48	0.15	0.10 ± 0.02	0.24
Oleic (C18:1)	54.22	56.28	55.8 ± 3.1	55.44
Stearic (C18:0)	13.04	13.36	11.4 ± 2.6	12.59
Linoleic (C18:2)	nd[c]	0.67	0.23 ± 0.09	0.30
Gondoic (C20:1)	2.08	0.54	0.36 ± 0.16	0.99
Arachidic (C20:0)	2.68	0.81	0.57 ± 0.13	1.35

[a]FAME – fatty acid methyl ester present in the oil at a concentration of ≥ 0.1% are reported, [b]Mean ± standard deviation (n = 14), [c]nd – not detected

The fatty acid composition, shown in Table 1, was used to predict the IV of the ackee oils (Table 2), that is, how much iodine is absorbed by the mono-, di- and tri-unsaturated fatty acids in 100 g of oil. The IV had not been determined experimentally for oils I and II originating from composite arilli samples. Ackee oils extracted from seven trees in study III had an average IV of 49 ± 13 (Table 2), in agreement with the predicted IV.

Table 2: Iodine value (IV) for ackee oils in studies I, II and III

Ackee oil study	Predicted IV[a]	Measured IV[b]
I	50.8	–
II	52.8	–
III	51.3	49 ± 13[c]

[a]Estimated amount of iodine absorbed by unsaturated fats, [b]Experimentally determined iodine value, [c]Mean ± standard deviation (n = 12)

DISCUSSION

In contrast to previous reports which had identified the predominant fatty acid as linoleic acid [55.2–58.3%] (23) and stearic acid [65.93–68.49%] (24), we have demonstrated across three studies that the major fatty acid in ackees is oleic acid. Only palmitic acid (21.93 – 28.71%) was recorded at levels in agreement with composition data previously published for ackees grown in Jamaica by Odutuga and associates (23.6–27.1%) and Wellington *et al* [28.5–31.0%] (23, 24). Analysis of oils (III) extracted in duplicate from fruits of seven different ackee trees showed that for the ackees sampled, the fatty acid profile was consistent irrespective of the analyst (studies I – III) or the method of extraction and methylation (I *versus* II and III) of the fatty acids or the specific tree from which the fruit was obtained (study III). No differences in composition were observed between oils from the 'butter' or 'cheese' ackee varieties. Thus, the fatty acid profile of the ackees sampled has been unequivocally established.

Iodine value characterizes the extent of unsaturation in oils, and although its relevance has been questioned (28), IVs are still employed as a standard in the industry. An IV of 49 is characteristic of oils that are high in monounsaturated and saturated fats. Agreement of the actual and predicted IVs provided corroboration of the fatty acid composition. Predicted IVs corresponding to ackee oil compositions proposed by Odutuga *et al* [high linoleic acid levels] (23) and Wellington *et al* [high stearic acid content] (24) were 107.0 and 5.6, respectively. It is clear that the reported fatty acid profiles are incompatible with the experimentally determined chemical data (IV 49). Jamaican ackees are seemingly consistent in unsaturated fat content as assessed by IV. Barnett measured an IV of 49 on cold-pressed ackee oil (29), while Wellington also recorded an IV of 49 for oil extracted by acetone at room temperature from cooked ackees which had been purchased in a market located in St Andrew (24). In Barnett's report, the source of the fruits was not stated.

Interestingly, diets high in oleic acid, typified by the Mediterranean diet, are associated with low risks of prostate cancer and, in some instances, oleic acid has been shown to inhibit the growth of prostate cancer cells (30–32). Avocado, with an oleic acid content similar to ackee, has demonstrated health beneficial properties (33). Studies conducted by Jackson and co-workers (34) have shown an inverse relationship between dietary monounsaturated fatty acids (MUFAs) and the risk of prostate cancer in Jamaican males. Additionally, no association between ackee consumption and prostate cancer risk was established, while higher avocado intake coincided with lower incidences of prostate cancer.

In conclusion, oil extracted from the edible portion of the ackee fruit was shown to be high in oleic acid and not linoleic acid or stearic acid as previously reported. If the composition data reported herein holds true for fruits grown across the country, then ackee cannot be considered a significant dietary source of linoleic acid in Jamaica. As the debate continues on the causal role of fatty acids in the promotion of prostate cancer, it is clear that if linoleic acid is indeed a contributing factor then investigators would have to look beyond ackee to explain the alleged unusually high incidence of prostate cancer in Jamaican men. Indeed, it may be speculated that ackee could provide a protective effect against prostate cancer given its high MUFA content which is similar to that of avocado.

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