



Thermal Stability, α -Tocopherol and β -Carotene Content of Ackee (*Blighia sapida*) Aril Oil

Zoie Aimey,^{1*} Coniel Roye,² and Andrea Goldson-Barnaby,^{1*}

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Author's Affiliations

¹Department of Chemistry,
The University of the West Indies,
Mona, Kingston 7, Jamaica

²Department of Chemistry,
University of Technology,
Kingston 6, Jamaica

Corresponding Authors

Zoie Aimey &
Andrea Goldson-Barnaby

znaimy@gmail.com
&andrea.goldson03@uwimona.edu.jm

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Abstract

The ackee, *Blighia sapida*, a tropical fruit, is processed as canned ackee in brine. A significant quantity of waste is generated during processing which can be transformed into an edible oil. The thermal stability of ackee aril oil, its smoke point, acid value, free fatty acid content, free radical scavenging activity, α -tocopherol, β -carotene, and total carotenoid content were determined. Ackee aril oil exhibited high free radical scavenging activity ($98.5 \pm 0.7\%$) and contains carotenoids (44.3 ± 0.6 ppm), β -carotene (4.79 ± 0.12 ppm) and α -tocopherol (1.70 mg/kg). After heating there was a significant increase in β -carotene and a significant decline in α -tocopherol ($p < 0.05$). 1,3-Dioleoylpalmitin was identified as the main triacylglycerol in the oil. The oil had a smoke point of 232°C . Ackee aril oil appears to be thermally stable and can be utilized commercially in food applications.

Keywords

Ackee oil; Smoke point; Carotenoids; β -carotene; Tocopherol

Introduction

The ackee, *Blighia sapida* K.D. Koenig, was introduced to the island of Jamaica, from West Africa. The ripe fruit is easily recognizable based on its red and yellow colouration (Figure 1). At full maturity the fruit weighs between 96-102 g. Inside the red exterior of the fruit is the fleshy edible part referred to as the aril. This portion of the fruit typically has three thick, oily pegs with a pale, yellow hue which surrounds black glossy seeds. The evergreen tree from which this fruit is borne, bears fruit year-round with two major ripening seasons in spring (January to March) and summer (June to August).^{1,2}



Figure 1: *Blighia sapida* fruits

Ackee while known for its unique flavor is potentially toxic. Arilli from the unripe fruit contains a high concentration of the toxin, hypoglycin A (> 1000 ppm) which translocates to the seed as the fruit matures making the arilli safe for consumption. At full maturity the concentration of hypoglycin A declines to <0.1 ppm.³ Hypoglycin A is a water-soluble amino acid. Arilli are typically blanched prior to consumption which aids in the leaching of any residual toxin that may be present in the fruit. Sinmisola *et al.*, (2019) has suggested that heat reduces the concentration of hypoglycin A in ackees, making the raw sun-dried arilli a safe snack that is consumed in Ivory Coast.¹ This is however debatable as the toxin is heat stable. In Africa, the arilli is fried, roasted and incorporated into sauces and soups.¹

Currently the most common processing method for ackee in Jamaica is canning. Initially, the presence of the toxin made processing and exportation difficult. Strict processes and standards were however established to facilitate exportation of the canned product. Mature arilli are harvested and inspected, ensuring they meet the guidelines specified in the Ackee Maturity Index Chart compiled by the Bureau of Standards Jamaica. The seed and raphe are removed from the arilli of the fruit. The arilli is subsequently washed in glacial acetic acid solution (0.1%) and potable water. Arilli are drained, blanched and canned with hot brine. The final process is the seaming and retorting of the filled cans. The canned ackees are considered commercially sterile at 35°C and 55°C. The upper limit of hypoglycin A in canned ackees is <150 ppm for Canadian and European markets and <100 ppm for the United States of America.²

A significant amount of ackee arilli waste generated from the canning process can be considered for use in the production of ackee aril oil. Vegetable oils are the primary source of edible fats, comprising upwards of 75% of the total lipids consumed in the world.⁴ Fruit oils are however becoming more prevalent due to the increased need for nutritive fats which provide fatty acids, some of which are deemed essential. Popular fruit oils include coconut oil, olive oil, palm oil and avocado oil. Fruit oils are usually extracted from the flesh of the fruit (mesocarp) or the seed kernel. Edible oils provide energy and essential fatty acids. In the body they are carriers of fat-soluble vitamins, maintain normal body temperature and protect body tissue.^{4,5} They may be used as salad oils, baking or frying fats, margarine and cooking oil.

A review article written by Wray *et al.* (2020) summarizes several research articles detailing the characterization of ackee aril oil.² The identity of the primary fatty acid present within the oil was however uncertain with some researchers reporting linoleic acid as the primary fatty acid and others oleic acid.² Subsequent work conducted by Goldson *et al.*, (2002) utilizing various chromatographic and spectroscopic techniques including gas chromatography and mass spectrometry, identified oleic acid as the primary fatty acid in ackee aril oil (55%), followed by palmitic acid (25%) and stearic acid (12%) from fruits grown in Jamaica.⁶ Ackee arilli from fruits grown in Nigeria had a different profile, consisting of 12-eicosanoic acid (21%), behenic acid (15%), gadoleic acid (12%) and oleic acid (7%).⁷ The fatty acid profile of the fruit may be influenced by its geographical location. Oladiji *et al.* (2009) also investigated the smoke, fire and flash points of ackee aril oil from Nigeria.⁷ Differences in the chemical composition of the ackee may influence the thermal stability of oil.

Research on ackee aril oil continues to extend beyond its chemical composition. There are no known reports on the thermal stability of Jamaican ackee aril oil. The objectives of this study were to evaluate the thermal stability of ackee aril oil and further characterize the oil. The acid value, free fatty acid value, carotenoid, β -carotene and α -tocopherol content of the oil were determined before and after heating. This is the first report on the α -tocopherol content of the oil. The oil was also characterized utilizing nuclear magnetic resonance spectroscopy.

Materials and Methods

1,1-Diphenyl-2-picrylhydrazyl (DPPH), α -tocopherol, β -carotene and deuterated chloroform were purchased from Sigma-Aldrich, St Louis, USA. Solvents utilized for High Performance Liquid Chromatography (HPLC) were HPLC grade.

Equipment

Blender, beakers, watch glasses, burette, clamps, volumetric flasks, round-bottom flasks, filter paper, glass funnels, test tubes, balance, thermometer, Corning PC-420D hot plate, water bath, vortex, blender, centrifuge, GCA Corporation Gravity Convection Incubator, BUCHI Rotavapour R-124, HI96801 refractometer (Hanna instrument), Thomas Scientific TLC plates, ultraviolet lamp, Genesys 10S UV-Vis, Thermo Fisher Scientific spectrophotometer, Beckman System Gold HPLC, BrukerBioSpin 500 MHz.

Reagents and solutions

The developing solvent for thin layer chromatography was hexane:ethyl acetate (90%:10% v/v).

Phosphomolybdic spray reagent consisted of phosphomolybdic acid (10 g), ceric sulphate (1.25 g), conc. sulphuric acid (12 mL) and water (238 mL).

A β -carotene (Sigma-Aldrich, $\geq 95\%$) stock solution (50 ppm) was prepared using hexane. From this stock solution, standards (10, 20, 30, and 40 ppm) were prepared.

A stock solution (10 $\mu\text{g/mL}$) of α -tocopherol was prepared with mobile phase (25:22:3 (v/v/v) methanol/acetonitrile/methylene chloride) and α -tocopherol standard (Sigma-Aldrich, $\geq 96\%$). From this stock solution, standards of concentrations 2, 4, 6 and 8 $\mu\text{g/mL}$ were prepared. A standard blank was also prepared.

Harvesting and dehydration of ackee fruits

Mature ackees were harvested from trees located at the Department of Chemistry, The University of the West Indies, Mona Campus, Jamaica. Arilli were separated from the raphe, husk and seeds of the fruit. The arilli were dehydrated in a GCA Corporation Gravity Convection Incubator (25°C, 5 days) whereas the raphe, husk and seeds were discarded.

Lipid extraction

Dried arilli were ground and extracted with hexane (20 g/100mL, 96 h). The resulting extract was concentrated *in vacuo* (BUCHI Rotavapour R-124) and the percentage yield of oil determined gravimetrically.

Smoke point determination

Ackee aril oil was heated and its smoke point determined based on the American Oil Chemists' Society (AOCS) Method Cc 9a-48.⁸ At the smoke point, a thin and continuous bluish smoke is observed.⁹ Temperatures at which there were significant physical changes (frothing, boiling and colour changes) were also noted.

Index of refraction

The index of refraction of ackee aril oil at various stages of thermal degradation was measured utilizing a HI96801 refractometer (Hanna instrument).

Thin layer chromatography

Ackee aril oil at various stages of thermal degradation was analyzed utilizing thin layer chromatography (TLC) plates (Thomas Scientific) coated on polyester with silica gel containing 254 nm

fluorescent indicator. Chromatoplates were visualized under an ultraviolet lamp and after spraying with phosphomolybdic spray reagent followed by heating.

Acid value and free fatty acid (FFA) determination

Ackee aril oil (1 g) was dissolved in hot neutralized ethanol (10 mL) and titrated against NaOH (0.1 M). Phenolphthalein was utilized as indicator. The acid value and FFA were determined utilizing the following equations:¹⁰

$$\text{Acid Value} = \frac{\text{mL of 0.1 M NaOH} * 5.61}{\text{mass of sample}}$$

$$\text{FFA \%} = \frac{\text{mL of 0.1 M NaOH} * 10^{-4} * M * 100}{\text{mass of sample}}$$

M (282.47 g) is the molecular weight of oleic acid, the predominant fatty acid in ackee aril oil.

Free radical scavenging activity

The free radical scavenging activity of ackee aril oil was determined utilizing the DPPH assay.¹¹ Ackee aril oil (200 mg) was added to ethanol (2 mL, 80%, v/v) containing HCl (1%, v/v). To the resulting solution (0.5 mL, aliquots), DPPH (0.5 mM, 0.3 mL) and ethanol (3 mL) were added. Samples were incubated in the dark (30 min). The absorbance of the samples was measured at 517 nm using a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific). Percentage free radical scavenging activity was determined utilizing the following equation:

$$\% \text{ Free radical scavenging} = (1 - A_1/A_0) \times 100$$

Where:

A_1 = Absorbance of sample

A_0 = Absorbance of control

Carotenoid content determination

Ackee aril oil (0.5g) was transferred to a volumetric flask (25 mL) and hexane (25mL) added. Samples were analysed at 446 nm using a spectrophotometer (Genesys 10S UV-Vis, Thermo Fisher Scientific).¹² The carotenoid content was determined using the following equation:¹³

$$\text{Carotenoid content (ppm)} = \frac{V \times 383 \times (A_s - A_b)}{100 \times W}$$

Where:

V = 25 mL

383 = Extinction coefficient for carotenoid

A_s = Absorbance of sample
 A_b = Absorbance of blank (Cuvette error)
 W = Weight of sample

A calibration curve was generated, and the equation of the line ($y = 0.424x + 0.0306$) used to determine the concentration of β -carotene ($R^2 = 0.99$).

α - Tocopherol content

The α -tocopherol content of ackee aril oil was determined via High Performance Liquid Chromatography (HPLC). To each test tube, ackee aril oil (0.1g), ascorbic acid (0.05 g), ethanol (90.2% v/v, 5 mL) and 80% (w/v) NaOH (0.5 mL) were added.¹⁴ Air was removed from the tubes *in vacuo*. The tubes were placed in a water bath (70 °C, 30 min) and vortexed periodically, before placing in an ice bath (5 min.). Deionized water and hexane were added to the tubes and vortexed (30 s). The tubes were placed in a centrifuge (10 min) and the hexane layer decanted. Hexane (5 mL) was added to the residual layer for re-extraction. The resulting extract was concentrated *in vacuo*, the mobile phase added and the mixture vortexed (30 s).

The HPLC system used was the Beckman System Gold HPLC. The system was flushed with the mobile phase at a flow rate of 1.5mL/min for 10 min and the column equilibrated. Standards (30 μ L) were injected into the column and analysed at a flow rate of 1.0 mL/min. The method blank (30 μ L) and samples (30 μ L) were then injected and analysed at the same flow rate. A standard curve of peak area versus injection quantity was generated. The concentration of α -tocopherol was calculated using the following equations:

Injection quantity of standard (μ g) = concentration of standard (μ g/mL) \times injection volume (mL)

Concentration (μ g/g) =

$$\frac{\frac{\text{quantity of injected sample } (\mu\text{g})}{\text{injection volume (ml)}} \times \text{final vol of sample (mL)}}{\text{sample weight (g)}}$$

Nuclear Magnetic Resonance Spectroscopy

^1H and ^{13}C NMR characterization were performed on the ackee aril oil, utilizing a Bruker Avance DRX-500 MHz spectrometer (Bruker BioSpin GmbH, Silberstreifen, Germany), equipped with a 5-mm broadband inverse probe and a 5-mm dual probe, employing deuteriochloroform (CDCl_3) as a solvent and referenced to tetramethylsilane (TMS) as internal standard. ^{13}C spectral editing were

obtained by DEPT experiments. Chemical shifts (δ) were expressed in ppm and coupling constants in Hz and the following terms were used: singlet (s), doublet of doublet (dd), triplet (t), multiplet (m).

Statistical analysis

Samples were analysed in triplicate. The mean and standard deviation of the data were reported.

The Student t-test was conducted to determine any differences between the means at the significant level of $p < 0.05$ (Microsoft Office Excel 2019).

Results and Discussion

Ackee arilli is a rich source of lipids with unsaturated fatty acids accounting for the major fraction of the oil.⁶ The oil is comparable to olive oil in that oleic acid is the main fatty acid present.^{6,15} Prior reports have investigated oil extraction from the arilli via Soxhlet extraction with the use of petroleum ether.¹⁶ In the current study direct solvent extraction with hexane was utilized. This resulted in a yield of $45.6 \pm 3.7\%$ ackee aril oil which is higher than that reported by Anderson-Foster et al., (2012) who obtained a yield of $37.0 \pm 4.9\%$.¹⁶ One advantage of direct solvent extraction is that it is conducted in the absence of heat which yields a higher quality oil. There is also a greater retention of antioxidants and heat labile components. The resulting oil extract was bright yellow in colour (Figure 2) with a distinctive odour of roasted ackee. Currently ackee aril oil is not available commercially but may be considered for utilization as an edible oil in culinary applications.



Figure 2: Ackee aril oil

Thermal stability

During heating, oils are subject to degradation. High temperatures utilized during frying (170°C - 200°C) can induce oxidation, hydrolysis, isomerization and polymerization.¹⁷ Along with frying temperature, these reactions are also dependent on oil composition, commercial application, oil turnover rate, frying time, antioxidants, ingredients and properties of the fried food.¹⁷ Oil degradation impacts

nutritive value, food safety, oil and food quality.¹⁸ Physical changes brought about by the insoluble non-volatile products of oxidation and hydrolysis reactions include darkening of oil colour, increased foaming and a decrease in the smoke point.¹⁹ Upon heating, ackee aril oil changed colour from yellow to brown. This colour change may be due to the development of non-volatile decomposition products or pigments and α -, β -unsaturated carbonyl compounds, diffusion of products from the thermal decomposition of fatty acids into the oil, caramelized carotenoids, oxidation of phenolic antioxidants and Maillard reaction. Phenolic compounds can also polymerize forming brown-coloured macromolecules. Ultimately, the darkening of the oil suggests significant degradation.¹⁹ Colour change, specifically the darkening of oil, is a rapid test used in the food industry to judge the quality of frying oil.

During heating, foaming was observed at the surface of the ackee aril oil in the form of yellow froth. This appeared at approximately 60°C and persisted until 185°C. The colour of the froth darkened at 100°C. There was no observable change in the consistency of the oil during heating. The index of refraction of the oil increased from 68.7° to 69.4° and finally 70.5° at the smoke point (Table I). Based on these observations we posit that the index of refraction may be used to monitor oil degradation.

The smoke point is the temperature at which an oil begins to produce “a continuous wisp of smoke”.²⁰ It is indicative of the decomposition of the oil to glycerol and its free fatty acids and corresponds to reduced organoleptic and nutritional quality. It also varies depending on the source of the oil as well as the quality of refinement it has undergone.²¹ The smoke point provides information on whether an oil is fit for frying. Regulations specify a smoke point above 200°C. This value decreases when triacylglycerols are hydrolysed producing free fatty acid and glycerol. The vapour pressure and boiling point of fatty acids are lower than triacylglycerols.²² Oils with high smoke points are good for deep-fat frying.²¹ Ackee aril oil had a smoke point of 232°C which is comparable to sesame oil (242°C) and higher than coconut oil (193°C).²³⁻²⁴ This suggests that ackee aril oil could be considered for frying. There was no observable difference in the TLC of unheated and heated ackee oil (Figure 3). Evidence of degradation was however observed at the smoke point of the oil.

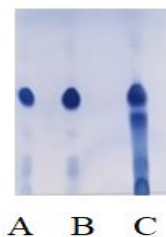


Figure 3: TLC of unheated (A), heated (B) and ackee aril oil at its smoke point (C)

Acid value and free fatty acid value

The acid value (AV) is another parameter utilized to determine oil quality and measures the extent of lipid oxidation thereby showing the extent of rancidity.¹⁸ The acid value for ackee aril oil was 2.38 ± 0.03 mg KOH/g. This is comparable to the value of 1.83 reported by Anderson-Foster et al. (2012).¹⁶ After frying the value increased to 2.76 ± 0.05 mg KOH/g (Table 1). Hydrolysis of oils at high temperatures such as that utilized for deep frying, can occur in the presence of moisture and air, forming free fatty acids (FFA). The FFA value is therefore used to monitor oil quality during frying and by extension the quality of fried food. It is the number of milligrams of KOH required to neutralise FFAs in 1 g of oil. This value increases with an increase in frying time and the number of frying cycles.¹⁹ The extent of the degradation is measured using the FFA value. The FFA value for ackee aril oil was $1.20 \pm 0.02\%$. The relationship between FFA content and the smoke point is inversely proportional. Oils which are lighter and more refined have higher smoke points.²¹

Triacylglycerols degrade when heated and in the presence of moisture producing free fatty acids, monoacylglycerols, diacylglycerols and glycerol.²⁵ After heating there was a significant increase ($p < 0.05$) in the FFA value and AV (16.7% and 16.4% respectively) which indicates that some degree of degradation occurred which can introduce off flavours and odours. Volatile compounds such as saturated and unsaturated aldehydes, ketones, hydrocarbons, lactones, alcohols, acids, esters, furans, and aromatic compounds, are produced.²⁵ The increase in FFA can also decrease the smoke point. Notably, acid values for both heated and unheated oils are within the upper limits (4.0 mg KOH/g) required for vegetable oils.²⁶ It can be inferred that even at frying temperatures of 180°C, there are few fatty acids and the oil is less susceptible to rancidity. This is a positive sign for the nutrition and quality of the oil, as the toxic compounds released when rancid can cause neurological disorders, heart diseases and cancer.²⁷ It should be noted however that FFAs

quickly evaporate during frying and therefore an accurate representation of the FFAs may not be captured during analysis.²⁵

Free radical scavenging activity

Edible oils are good sources of antioxidants such as carotenes and tocopherols which lend to their oxidative stability. These compounds also enhance the nutritive quality of oils as carotenes and tocopherols are precursors of vitamins A and E.^{4,18} Carotenes and tocopherols enhance the oxidative stability of oils as well as their nutritive value. Based on the literature, oils containing carotenoids and tocopherols are less susceptible to thermal oxidation, however their efficacy reduces as storage or processing temperatures increase.¹⁹ Ahmed Alyas et al. (2018) found an inversely proportional relationship between temperature and β -carotene content.¹⁹ Higher temperatures also induced a more significant loss. Ackee aril oil exhibited high free radical scavenging activity ($98.5 \pm 0.7\%$) indicating that the oil is rich in antioxidants.

Carotenoids

Carotenoids are organic pigments responsible for the yellow, orange and red colours of fruits and vegetables. They can potentially prevent chronic diseases, cancers, diabetes and cardiovascular diseases and are precursors of vitamin A. There are two classes of carotenoids: carotenes and xanthophylls. β -carotene has one of the highest pro vitamin A activity in comparison to other carotenes and can serve as a fat radical scavenger and singlet oxygen quencher (antioxidant properties). Vitamin A consumption is important, as insufficient amounts in the body can lead to night blindness and compromised growth and immune functions.⁴

Ackee aril oil has an inherent yellow colour which is due to the presence of carotenoids. Ackee aril oil had a carotenoid content of 44.3 ± 0.6 ppm. β -carotene only comprises 10% of the total carotenoids in ackee aril oil (4.79 ± 0.12 ppm) suggesting that other components within the arilli contributes to its antioxidant activity. Based on its carotenoid content we can infer that ackee aril oil is a good source of vitamin A. The carotenoids present contributes to the oil's oxidative stability. Interestingly, upon heating, there was a significant increase in the carotenoid content and β -carotene content of the oil by 107% and 125% respectively ($p < 0.05$). Hashemi *et al.* (2019) reported that high temperatures can induce an increase or decrease in carotenoid content with the effect being dependent on temperature, food matrix, pH and targeted carotenoid, among other factors.²⁸ Carotenoid content may

increase to a certain temperature after which its concentration may decline due to a reduction in the activity of enzymes involved in carotenoid synthesis.²⁸ Higher temperatures and longer heating times can therefore induce more significant losses in β -carotene.¹⁸

α -Tocopherol

Tocopherols are found in high concentrations in unsaturated oils.²⁹ There are four different types of tocopherols (α , β , γ and δ) which are named based on differences in structure, metabolism, and biological activity.⁴ Tocopherols exhibit antioxidant properties.²⁵ Alpha-tocopherol has the highest hydrogen-donating power and is the most capable quencher of peroxy free radicals, but has the least relative antioxidant potency. More α -tocopherol is required to induce antioxidant activity as compared to the other tocopherols.^{4, 30}

This is the first report on the α -tocopherol content of ackee aril oil. The level of α -tocopherol in ackee oil is low (1.70mg/kg) as compared to other sources of vegetable oil. Levels ranging from 31.74 to 1410.55 mg total tocopherols/kg of extract, have been reported for soybean oil based on the method of extraction.³¹ Based on the low levels of α -tocopherol within the ackee oil, it does not appear to contribute significantly to the oxidative stability of the oil. It should however be noted that tocopherols appear to be most effective at low concentrations in vegetable oils.²⁵ Upon heating, there was a significant decrease in α -tocopherol by 60% ($p < 0.05$). Al-attar (2013) found that as temperature increased (70°C to 130°C), tocopherol content decreased.³² Tocopherol degradation was also dependent on the oil source.³² Table 1 is a summary of the physicochemical properties of ackee aril oil.

Table 1. Physicochemical properties of ackee aril oil

Parameter	Original	Heated
Acid value (mg KOH/g)	2.38 ± 0.03	2.76 ± 0.05
FFA (%)	1.20 ± 0.02	1.395 ± 0.021
Index of refraction	68.7	69.4
β -carotene (ppm)	4.79 ± 0.12	10.81 ± 0.53
Carotenoids (ppm)	44.3 ± 0.6	91.8 ± 4.9
α -Tocopherol (mg/kg)	1.70	0.68
% Free radical scavenging	98.5 ± 0.7	89.6 ± 0.4

NMR Spectroscopy

Nuclear Magnetic Resonance (NMR) Spectroscopy may be utilized as a tool to determine the chemical composition of oils. This method of analysis requires minimum sample preparation, is rapid and non-destructive. It may also be utilized to

detect adulteration and has been used in this capacity for olive oil and sunflower oil.³³

In the current study, NMR spectroscopy was utilized to track changes in the composition of the ackee oil before and after heating. There was no major observable difference in the NMR spectra of unheated and heated ackee aril oil samples (Figures 4 and 5). The NMR spectral data of ackee aril oil was consistent (Tables 2 and 3) with that reported by Goldson-Barnaby *et al.* (2018).³⁴ The ¹H NMR revealed a peak resonating at δ 4.68 for the heated oil sample which was absent from the unheated sample. This may be due to oil degradation and the formation of an alcohol such as glycerol. The oil is monounsaturated as evident from a peak resonating at δ 5.22. Additionally, the methine proton at δ 4.18 integrated to be 0.25 while the peak at δ 5.22 integrated to be 1.00 indicating the presence of 4 protons in the double bond region. There was no evidence of the presence of a bis allylic group which indicates the absence of linoleic acid. We can infer that the two double bonds within the sample are not in the same fatty acid chain, as evident from the absence of linoleic acid which suggests that the triacylglycerol within ackee oil consists of 2 unsaturated fatty acid chains and 1 saturated fatty acid chain.

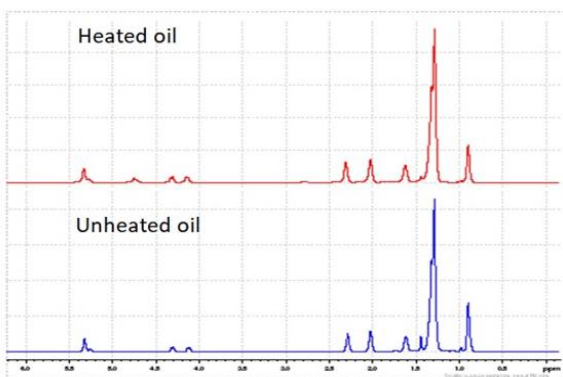


Figure 4: ¹H NMR spectra of heated vs unheated ackee aril oil

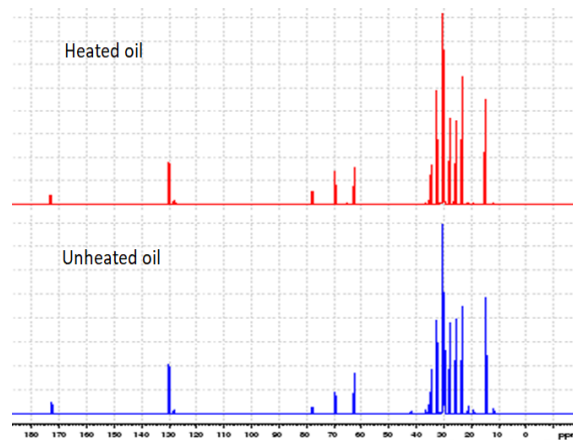


Figure 5: ¹³C NMR spectra of heated vs unheated ackee aril oil

Table 2. ¹H NMR spectroscopy data for unheated ackee aril oil

Proton	Functionality	Ackee Oil δ (ppm)
R-CH ₃	Terminal methyl	0.79 (t); 0.87 (d)
CH ₂	Methylene	1.20 (s)
CH ₂ -CH ₂ -COO	Acyl Chains	1.50 (s)
CH ₂ -CH=CH	All saturated fatty acids	1.92 (s)
CH ₂ -COO	All acyl chains	2.18 (t)
CH ₂ O(α)	Glycerol	4.01 (dd);
	(triacylglycerol)	4.22 (dd)
CH ₂ O(β)	Glycerol	5.14 (m)
	(triacylglycerol)	
CH=CH	Olefinic protons	5.22 (m)

Table 3: ¹³C NMR spectroscopy data for unheated ackee aril oil

Carbon	Assignment	Ackee Oil δ (ppm)
α -CH ₃	Acyl chains	13.55; 14.06
β -CH ₃	Acyl chains	22.59; 22.48
C3	Acyl chains	25.11; 25.46;
		26.79
C8-C11 (oleyl)	Allylic	27.09
C8-C14 (linoleyl)	Allylic	-
CH _{2n}	Acyl chains	29.04-29.68
C16	Linoleyl	31.44; 31.53;
		31.73; 31.87
α -C2	Acyl chains	33.87; 33.70
β -C2	Acyl chains	34.41; 34.53
α -CH ₂ O	Glycerol moiety	61.80
β -CH ₂ O	Glycerol moiety	68.84
β -C9	Oleyl	127.74; 127.90
β -C10	Oleyl	129.31;
		129.52; 129.64
α -C1	Glycerol moiety	171.96; 172.33
β -C1	Glycerol moiety	

This data is supported by research conducted by Goldson (2002) in which the major triacylglycerol within the oil was purified and analysed by Gas Chromatography/Mass Spectroscopy and Mass Spectrometry.³⁵ Oleic acid (2) and palmitic acid (1) were identified as the fatty acid side chains within the triacylglycerol. The position of the oleic acid within the triacylglycerol was however unclear. It was originally postulated that oleic acid occupied positions 1 and 2. A closer look at the mass spectral data however revealed that oleic acid was actually present at positions 1 and 3 which was further confirmed in the present study through the use of Heteronuclear Multiple Bond Correlation (HMBC) and Correlation Spectroscopy (COSY). Extensive HMBC and COSY correlations revealed that carbon peaks resonating at δ 4.17 which are on positions 1 and 3 of the glycerol backbone are HMBC to a carbon in the unsaturated fatty acid chain resonating at δ 171. The main triacylglycerol within ackee arilli oil was identified as 1,3 dioleoylpalmitin.

Conclusions

Ackee aril oil is rich in antioxidants. β carotene and α -tocopherol contribute to the antioxidant properties of the oil. The oil is monounsaturated. Oleic acid is the main fatty acid present. TLC analysis and NMR spectral data suggests that the oil is thermally stable and may be commercialized for use as an edible oil. It may also be considered for frying applications. The waste generated from the processing of canned ackee in brine can be channeled into making value added products and may be included in various food formulations.

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Conflict of interest

The authors declare that there are no conflicts of interest to the present work.

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