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Chemical Characterization of Cocoplum (*Chrysobalanus icaco,* L) Seed Oil and Seeds

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Abstract

Cocoplum (*Chrysobalanus icaco,* L) fruits contains seeds that can produce an edible oil. The goals of this study were to measure the physical chemical properties of the seeds and seed oil and to analyze the oil by ^{1}H and ^{13}C{^{1}H}-NMR to identify the types of fats, as well as the whole seeds, by inductively coupled plasma (ICP) linked to atomic emission spectroscopy (ICP-AES) to quantify the metals. In addition, the fatty acyl composition of the oil was determined by gas chromatography (GC) after hydrolyzing the triacylglycerides in the oil and esterifying the fatty acids produced. The density, refractive index, iodine index and saponification number for cocoplum seed oil were 0.9278 g•mL^{-1}, 1.508, 107 gI_2•100 g^{-1} and 180 mg KOH•g^{-1}, respectively. The seeds contained potassium, calcium, sodium, magnesium, iron, manganese, zinc and copper at concentrations of 340, 93.4, 30, 173, 2.9, 0.8, 0.8 and 0.67 mg•g^{-1}, respectively. They also contained 24.9 g•100 g^{-1} of total carbohydrates, which included 19.8 and 0.07 g•100 g^{-1} of insoluble and soluble fiber. The seed oil was found to contain triacylglycerides with a large amount of unsaturated fats, including conjugated linoleic fatty acyls. GC analysis of the hydrolyzed fats indicated the presence of palmitic, stearic, oleic and linoleic acids in the hydrolysate. These findings will give regulators several ways of determining whether or not food products labeled as containing cocoplum, abajeru or bajeru are genuine. In addition, it will help regulators decide if cocoplums should be classified as generally regarded as safe (GRAS). It will also help regulators decide what should be on the label for food products made from cocoplums.

Keywords: bajuru; *Chrysobalanus icaco,* L; NMR, fats, conjugated linoleic acid

1. Introduction

The Chrysobalanaceae family contains seventeen genera and about 525 species [6]. Traditional uses of some species in Africa and South America include treating malaria, epilepsy, diarrhea, inflammations and diabetes. *Chrysobalanus icaco*, also known as the cocoplum, abajeru and bajuru, is a medium sized shrub native to the South American coast. There, cocoplum is used in traditional medicine to treat leucorrhoea, bleeding and chronic diarrhea. It is also known for its diuretic, hypoglycemic and antiangiogenic effects. In Northern Brazil, its roots are used to treat diabetes. Previous phytochemical studies described the presence of myricetin and pomolic acid in *C. Icaco* fruit. There are also reports of its antihyperglycemic activity [6]. The leaves have been shown to normalize insulin sensitivity and blood glucose, while inhibiting weight gain in mice, in which obesity was induced by a high fat diet [15]. An essential oil was extracted from the leaves using supercritical fluid carbon dioxide [2]. The essential oil has potential anti-hypoglycemic activity and contains lupenol [2]. In another report, a hydroalcoholic extract of the leaves enriched in flavonoids was found to contain myricetin 3-O-glucuronide, quercetin and some minor myricetin derivatives [8].

The average diameter of the fruits was reported to be 2.657 cm and the mass was 9.400 g [5]. The average masses of the nuts and seeds were reported to be 3.049 and 1.558 g, respectively. According to that article, there is a large nut, in which the seeds are located. According to this nomenclature, the nuts contained 22.46% moisture, 1.19% ash, 5.93% protein, 24.92% carbohydrates, 45.50% fats and a caloric value of 533 kcal•100g^{-1} [5]. A different study reported that it was the seeds that contained 22% α-eleostearic acid (α-ESA) [13], while another indicated that there was 22.22% α-ESA in the seed oil [4]. However, these were actually fatty acyls that were part of triacylglycerides and not free fatty acids.

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Figure 1: Pictures of a Brazilian cocoplum fruit, as well as the intact stone and seed.

However, there are some inconsistencies between the studies written in Portuguese when compared to English. A report from the University of Florida indicated that the cocoplum originated in South Florida; South and Central America; the Bahamas; and western Africa [3]. The report described the fruit as being elliptic (plum-like) or nearly round; pink, whitish, or dark-purple drupe; 1.9 to 3.8 cm long; juicy and edible. There is one large brown stone and an edible white seed inside the fruit [3]. Pictures of a Brazilian cocoplum fruit, as well as the intact stone and seed inside it, are shown in Figure 1. According to this nomenclature, there are no nuts, just edible seeds, which are located in a larger stone. As a result, the oil that was produced and analyzed in this study will be called seed oil.

It has been reported that the seed oil contains the following acids (% by wt.): palmitic (4.4%), stearic (18.7%), oleic (11.2%), linoleic (6.2%), arachidic (1.0%), conjugated octadecadienoic (0.1%), α-ESA (22.22%), α-parinaric (10.62%), α-licanic (10.0%) and a previously unknown acid (18.0%), 4-oxo-octadeca-*cis*-9-*trans*-11-*trans*-13-*cis*-15-tetraenoic acid [4]. The structures of the six most abundant fatty acids in this list are shown in Figures 2 – 7. Note that two of them are omega-3 (ω-3 or n-3) fatty acids (Figures 5 and 7). That is, there is a HC=CH bond on the third carbon from the end (the omega, or ω carbon). This affects the chemical shift of the CH_3. So, the ^{1}H chemical shift of the $–CH_3$ in omega-3 fats is sufficiently different from that of the $–CH_3$ in CH_3-$(CH_2)_n$ portions of hydrocarbon tails in fatty acids and fatty acyls that are covalently bound to a glyceride backbone in triacylglycerides.

The unsaturated fats have several health benefits [14]. They are converted into anti-inflammatory lipoxins, resolvins and protectins. Lipoxin A4 plays an important role in preventing asthma and it helps regulate the activation of natural killer (NK) cells and type 2 innate lymphoid cells. Moreover, lipoxins are anti-inflammatory for neutrophils and eosinophils and may help clear inflamed tissue. Also, lipoxins promote the restoration of an injured airway epithelium by indirectly blocking the release of the pro-inflammatory cytokines [14]. At the same time, α-ESA is quickly converted to conjugated linoleic acids (CLAs), which are anti-carcinogenic and anti-atherosclerotic [13].

2. Material and Methods

2.1. Materials

Ripe cocoplum (*C. icaco* L.) fruits from the restinga (coastal forests which form on sandy, acidic, and nutrient-poor

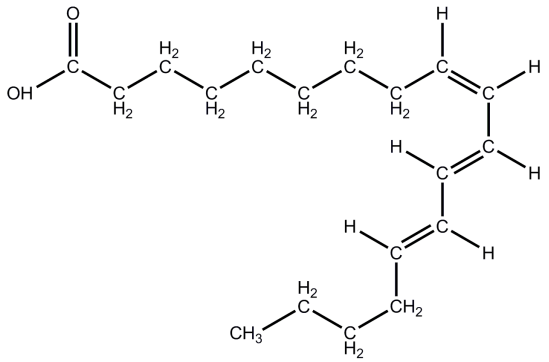

Figure 2: Structure of α-eleostearic acid, (9*Z*,11*E*,13*E*)-octadeca-9,11,13-trienoic acid, $C_{18}H_{30}O_2$, MW 278.43 g/mol.

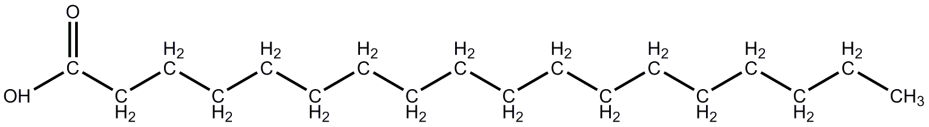

Figure 3: Structure of stearic acid, octadecanoic acid, $C_{18}H_{36}O_2$, MW 284.48 g/mol.

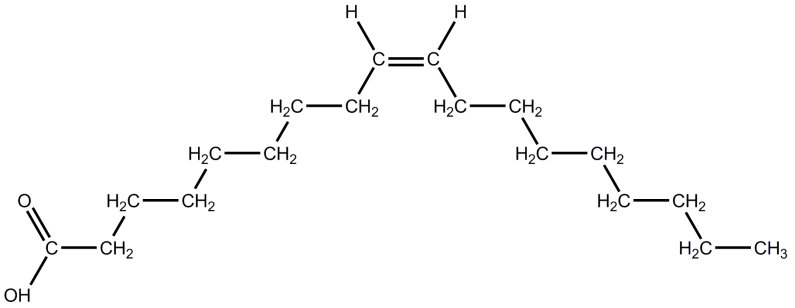

Figure 4: Structure of oleic acid, (9*E*)-octadec-9-enoic acid, $C_{18}H_{34}O_2$, MW 282.47 g/mol.

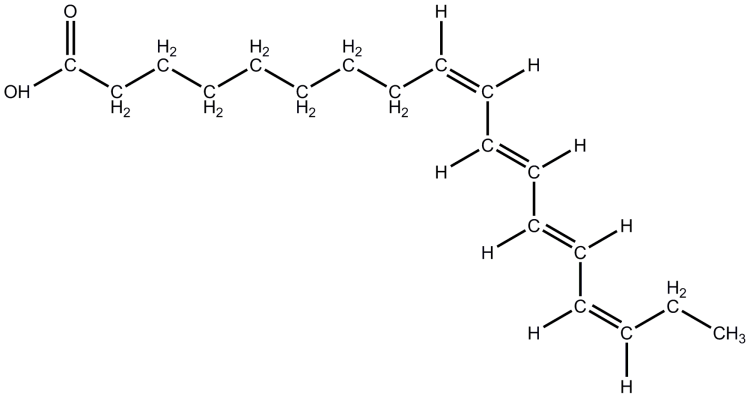

Figure 5: Structure of α-parinaric acid, (9*Z*,11*E*,13*E*,15*Z*)-octadeca-9,11,13,15-tetraenoic acid, $C_{18}H_{28}O_2$, MW 276.41 g/mol. This is an ω-3 fatty acid.

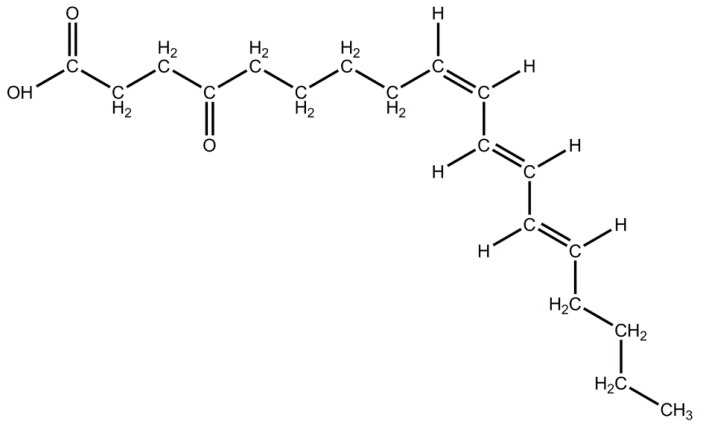

Figure 6: Structure of α-licanic acid (9*Z*,11*E*,13*E*)-4-oxooctadeca-9,11,13-trienoic acid, $C_{18}H_{28}O_3$, MW 292.41.

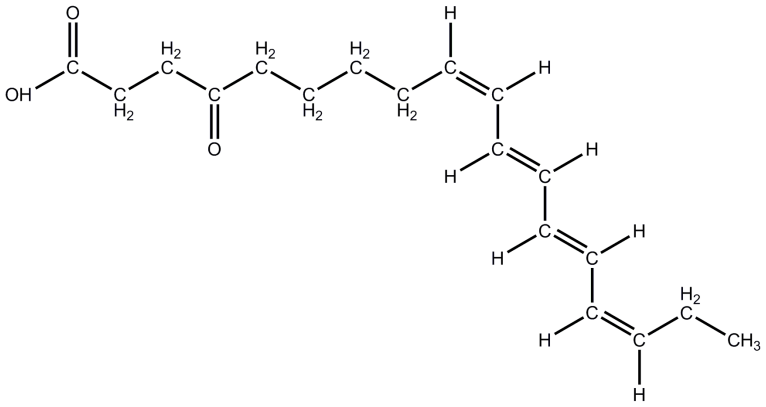

Figure 7: (9*Z*,11*E*,13*E*,15*E*)-4-oxooctadeca-9,11,13,15-tetraenoic acid, $C_{18}H_{26}O_3$, MW 290.32 g/mol. This is an ω-3 fatty acid.

Figure 8: Electric press used to make cocoplum seed oil.

Property	Cocoplum (*Chrysobalanus icaco*)	Oiticica (*Licania rigida*)	Galinha (*Couepia longipendula*)	Castanha de cutia (*C. edulis*)
Density (g•mL^{-1})	0.9278	0.960	0.9178	0.942
Refractive index at 40 °C	1.508	1.509	1.427	1.496
Iodine value (gI_2•100 g^{-1})	107	140	71	192
Saponification number (mg KOH•g^{-1})	180	194	192	188

Table 1: Physical-chemical properties of cocoplum seed oil with oils from seeds in other species of the Chrysobalanaceae family.

soils) in the Praia Seca district, a municipality of Araruama in the state of Rio de Janeiro, were harvested in November and December of 2012. They were identified based on the color of the peels (purple to black), as well as the soft, spongy texture of the white pulp. The fruits were sent to the laboratory for further processing. Shortly after being harvested, the leaves were removed from the fruits, which were then placed into plastic bags and stored in ice boxes so they could be transported to the laboratory (Laboratório de Análise e Processamento de Alimentos (LAPAL) do Instituto de Nutrição da Universidade Federal do Rio de Janeiro) and stored at –20 °C. The seeds were separated from their shells and pulverized before extracting the oil. The seed oil was obtained using an electric-powered press (Figure 8) on seeds that were previously heated to 105 °C for 30 min.

2.2. Methods

2.2.1. Physical-Chemical Properties

The density of the oil at 25 ± 0.1 °C was measured with a pycnometer in accordance with AOCS Method Cc 10a-25 [1]. The refractive index at 40 °C was measured using an Abbé refractometer in accordance with AOCS Method Cc 7-25 [1]. The peroxide index was measured by reaction with potassium iodide, followed by titration of the liberated iodine with a standard solution of sodium thiosulfate, in accordance with AOCS Method Cc 1-25 [1]. It was expressed in units of gI_2•100 g^{-1}. The saponification index was determined by titration with potassium hydroxide (KOH), in accordance with AOCS Method Cc 3-25 [1]. It was expressed in units of mg KOH•g^{-1}. Metals were quantified after dry ashing 5 g of sample at 550 °C for 30 min in a muffle furnace, dissolving the digested product in 2 M HCl and analyzing the solution by inductively coupled plasma (ICP) coupled to atomic absorption spectroscopy (ICP-AES) using a Perkin-Elmer ELAN 6000 (Waltham, MA). The total protein content was estimated by determining the nitrogen concentration using the Kjeldahl titration and a conversion factor of 5.75. The amino acid profile of the 6M HCL hydrolysate was determined using a TSM amino acid analyzer from Technicon (Oakland, CA). Total and reducing sugars, as well as amide and fiber content, were determined by standard methods [10].

2.2.2. Fatty Acyl Composition of Triacylglycerides

The triacylglycerides in the oil were hydrolyzed, producing fatty acids and glycerol. The fatty acids were reacted with BF_3•methanol to form fatty acid methyl esters, as described previously [9]. Gas chromatographic (GC) analyses were done using an Agilent 68650 Series GC System, equipped with an Agilent DB-23 (50% cyanopropyl, 50% polymethylsiloxane) capillary column, 60 m x 0.25 mm, 0.25 μm film thickness. The linear velocity of the helium carrier gas was 24 cm/s. The detector temperature was 280 °C. A temperature control program increased linearly (5 °C/min) from 110 – 215 °C The injection volume was 1.0 μL. The fatty acyl composition was determined by using fatty acid methyl ester standards (Nu-chek standard 68A).

2.2.3. NMR Analysis

NMR analyses were done using an Agilent DD2 600 MHz NMR (Santa Clara, CA). A 30° pulse width and 1 sec pulse delay were used for the ^{1}H-NMR, while a 30° pulse width and 2 sec pulse delay were used for the ^{1}H-coupled ^{13}C-NMR spectra, also known as ^{13}C{^{1}H}-NMR. Chemical shifts were referenced to the CD_3OD signals at 3.35 and 4.78 ppm (for ^{1}H) and 49.3 ppm (for ^{13}C) for the spectra of the methanolic extracts and to the $CDCl_3$ signals at 7.27 and 77.23 ppm, for ^{1}H and ^{13}C{^{1}H}-NMR, respectively. Homonuclear correlation spectroscopy (COSY), heteronuclear multiple-quantum correlation (HMQC) and heteronuclear multiple bond (HMBC) spectra were acquired using a 1.0 sec pulse delay.

3. Results and Discussion

The density, refractive index, iodine index and saponification number for cocoplum seed oil were 0.9278 g•mL^{-1}, 1.508, 107 gI_2•100 g^{-1} and 180 mg KOH•g^{-1}, respectively, as shown in Table 1. The density and refractive index are basic physical properties that can be used to help verify the identity of cocoplum seed oil. They are similar to seed oils from three other species in the Chrysobalanaceae family. The iodine value is a measure of the number of C=C double bonds in the oils. The higher the iodine value, the greater the number of C=C bonds. This is a more distinctive parameter for verifying the identity of the oil, since it is different than the values seen in other seed oils (Table 1). The saponification number is a measure of the moles of triacylglycerides in the oil. It is about the same in all four seed oils.

Metal	Concentration ($mg \cdot g^{-1}$)
Potassium	340
Calcium	93.4
Sodium	30
Magnesium	173
Iron	2.9
Manganese	0.8
Zinc	0.8
Boron	1.1
Vanadium	<0.001
Nickel	0.09
Chromium	0.037
Molybdenum	0.002
Selenium	0.020
Copper	0.670
Iodine	0.100
Aluminum	0.02
Rubidium	0.52
Arsenic	0.002
Lithium	0.04
Scandium	<0.04
Barium	0.004
Titanium	0.34
Strontium	0.22
Zirconium	0.002
Mercury	<0.01
Lead	0.002

Table 2: Metals in cocoplum seeds.

Amino Acid	Concentration ($mg \cdot g^{-1}$)
Histidine	0.05
Leucine	0.12
Isoleucine	0.05
Phenylalanine	0.05
Lysine	0.05
Threonine	<0.01
Tyrosine	0.05
Valine	0.27
Tryptophan	<0.01
Serine	0.18
Glycine	0.12
Proline	0.06
Alanine	0.12
Asparic acid	0.18
Glutamic acid	0.12

Table 3: Amino acid composition of cocoplum seeds.

The concentrations of potassium, calcium, sodium, magnesium, iron, manganese, zinc, copper and other metals in cocoplum seeds are listed in Table 2, which demonstrates that these seeds can provide some important mineral nutrients and have no detectable mercury in them.

The amino acid composition of the proteins in cocoplum seed oil are shown in Table 3. Like the other physical and chemical properties, these can be used to help verify the identity of cocoplum seed oil.

Cocoplum seed oil also has some carbohydrates, including

Type of Carbohydrate	Concentration g•100 g^{-1}
Reducing sugars	1.58±0.07
Non-reducing sugars	1.76±0.06
Amides	1.75±0.07
Insoluble fiber	19.76±0.12
Soluble fiber	0.07±0.01
Total carbohydrates	24.92±0.05

Table 4: Carbohydrates in cocoplum seeds.

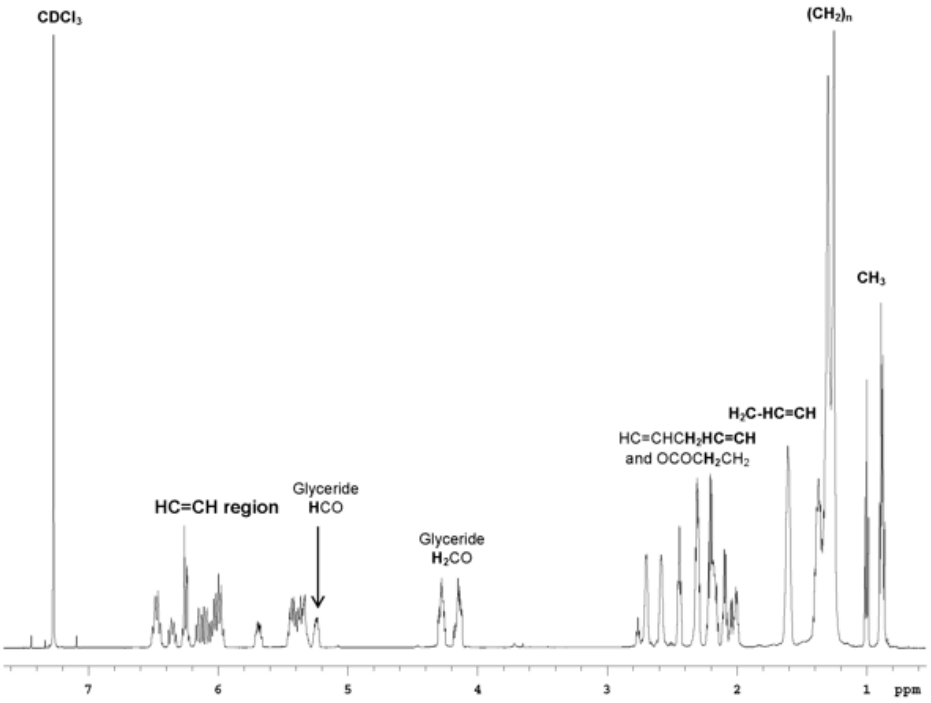

Figure 9: ^{1}H-NMR of bajuru seed oil, in $CDCl_3$.

reducing and non-reducing sugars, carbohydrates with amides attached, as well as soluble and insoluble fiber, as shown in Table 4. As a result, the seeds could be a good source of dietary fiber, while being relatively low in sugars.

The ^{1}H-NMR spectrum of cocoplum seed oil is shown in Figure 9. The signals that are due to methyls ($–CH_3$) on the end of hydrocarbon chains, as well as $–CH_2$- and HC=CH groups in the chains were seen, along with $–H_2CO–$ and –HCO– groups in the glyceride backbone of triacylglycerides are identified. This is similar to those seen in olive oil and the lipid portion of açaí (*Euterpis oleracea* Mart), which contain primarily a mixture of saturated and monounsaturated fats, such as stearic (18:0) and oleic acids (18:1), with lower concentrations of 18:2 and 18:3 [7, 11]. As in açaí and olive oil, $–CH^3$ groups that are attached to at least two $–CH_2–$ groups produced signals from about 0.80 – 0.95 ppm. However, the $–CH_3$ group in ω-3 fats produces chemical shifts from about 0.95 – 1.01 ppm [7, 11]. The spectrum was expanded from 0 – 4.5 ppm in Figure 10. The signals due to the $–CH_3$ groups are labeled. The integrals (peak areas) of the two different signals (peaks) due to $–CH_3$ groups in this region can be used to calculate the relative molar concentrations of ω-3 fats, as described by equation (1), where A and B are the integrals of the signals from 0.80 – 0.95 and 0.95 – 1.01 ppm, respectively.

$$\text{Eq(1)\% } \omega\text{-3 fats} = 100\left(\frac{B}{A+B}\right)$$

Based on this equation, the mole percent of ω-3 fats in the cocoplum seed oil was 31%. The signals due to $–(CH_2–)_n$ from 1.2 - 1.4 ppm, the CH_2CH_2OCO of triacylglycerides at 1.6 ppm, the $-CH_2CH=CH$ (labeled C), the CH_2CH_2OCO of triacylglycerides (labeled D), $HC=CHCH_2CH=CH$ (labeled E) and the H_2CO of triacylglycerides are apparent. The labels A, B, C, D and E are consistent with nomenclature for ^{1}H-NMR spectra of açaí and olive oil [7, 11]. The unlabeled signals were due to conjugated HC=CH in unsaturated fatty acyls that are not in açaí and olive oil. These signals were not seen in olive oil or açaí [7, 11].

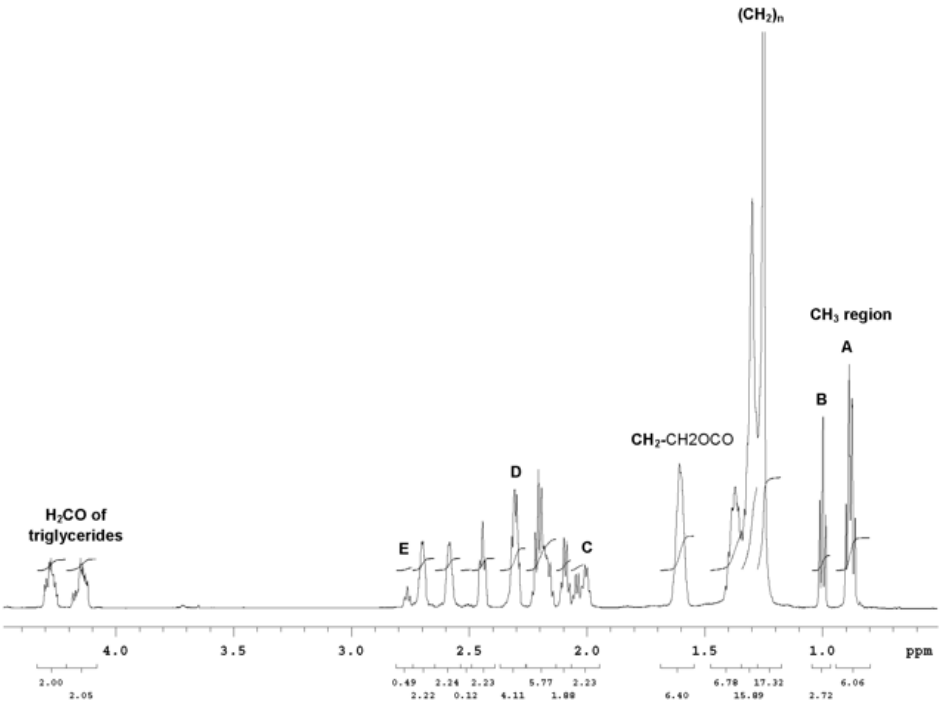

Figure 10: ^{1}H-NMR of bajuru seed oil, in $CDCl_3$, expanded about the region from 0 – 4.5 ppm, where signals due to two types of $–CH_3$ groups (A and B), as well as $–(CH_2)_n$, $–CH_2CH_2OCO$ of triacylglycerides, $–CH_2CH=CH$ (labeled C), $–CH_2CH_2OCO$ of triacylglycerides (labeled D), $HC=CHCH_2CH=CH$ (labeled E) and the H_2CO of triacylglycerides appear. The labels A, B, C, D and E are consistent with nomenclature for ^{1}H-NMR spectra of açaí and olive oil [7, 11]. The unlabeled signals were due to conjugated HC=CH in unsaturated fatty acyls that are not in açaí and olive oil.

Fatty Acid	Abbreviation	Percent by Mass
Myristic	C14:0	0.06%
Palmitic	C16:0	5.95%
Margaric	C17:0	0.07%
Stearic	C18:0	25.2%
Oleic	C18:1	20.4%
trans-linoleic	C18:2	1.38%
Linoleic	C18:2	13.3%
Linolenic	C18:3	0.07%
Arachidic	C20:0	1.17%
Eicosanoic	C20:1	0.45%
Not identified		32.0%

Table 5: Fatty acid composition of hydrolyzed triacylglycerides in cocoplum seed oil.

Stearic, oleic, linoleic and palmitic acids were the four most abundant fatty acids in the hydrolyzed triacylglycerides in cocoplum seed oil, as shown in Table 5.

The ^{1}H chemical shifts of all the signals in the complete spectrum are listed in Table 6. Due to the extra signals that are in the spectrum of the cocoplum seed oil, the percent saturated, 18:1, 18:2 and 18:3 fats could not be calculated accurately, as they were in açaí and olive oil [7, 11]. There were also additional signals in the region where signals due to HC=CH bonds appeared, as shown in Figure 11. The isolated HC=CH bonds in oleoyl groups produced signals from 5.31 – 5.35 ppm, as they did in açaí and olive oil [7, 11]. However, they overlap with signals from 5.35 – 5.48. So, they could not be integrated acuurately. There are also signals from 5.65 – 5.73 and 5.95 – 6.55 due to other conjugated HC=CH groups. Based on published NMR spectra of conjugated linoleic acids, the chemical

Signal	ppm	Assignment	Area
1	0.87	-C**H**$_3$ (all others)	6.67
2	0.99	-C**H**$_3$ (ω-3)	3
3	1.2-1.4	-(C**H**$_2$)$_n$-	36.42
4	1.60	OCO-CH2-**CH**$_2$-	6.99
5	2.0	-C**H**$_2$-CH=CH-	4.50
6	2.12-2.22	-C**H**$_2$-CH=CH-	6.38
7	2.3	ROOC-**CH**$_2$-CH$_2$-	4.5
8	2.42	O=C-**CH**$_2$-CH$_2$-	2.41
9	2.57	O=C-**CH**$_2$-CH$_2$-	2.32
10	2.69	CH=CH**CH**$_2$CH=CH	2.37
11	2.75	CH=CH**CH**$_2$CH=CH	0.52
12	4.14	Glyceride –C**H**$_2$O-	2.26
13	4.28	Glyceride –C**H**$_2$O-	2.17
14	5.26	Glyceride –C**H**O-	1.03
15	5.3-5.45	C**H**=C**H** isolated	4.6
16	5.66-5.69	C**H**=C**H** conjugated	0.92
17	5.94-6.16	C**H**=C**H** conjugated	5
18	6.22-6.26	C**H**=C**H** conjugated	2.03
19	6.32-6.38	C**H**=C**H** conjugated	0.97
20	6.42-6.5	C**H**=C**H** conjugated	2.08

Table 6: ^{1}H-NMR chemical shifts, assignments and peak areas (integrals) of cocoplum oil, in $CDCl_3$.

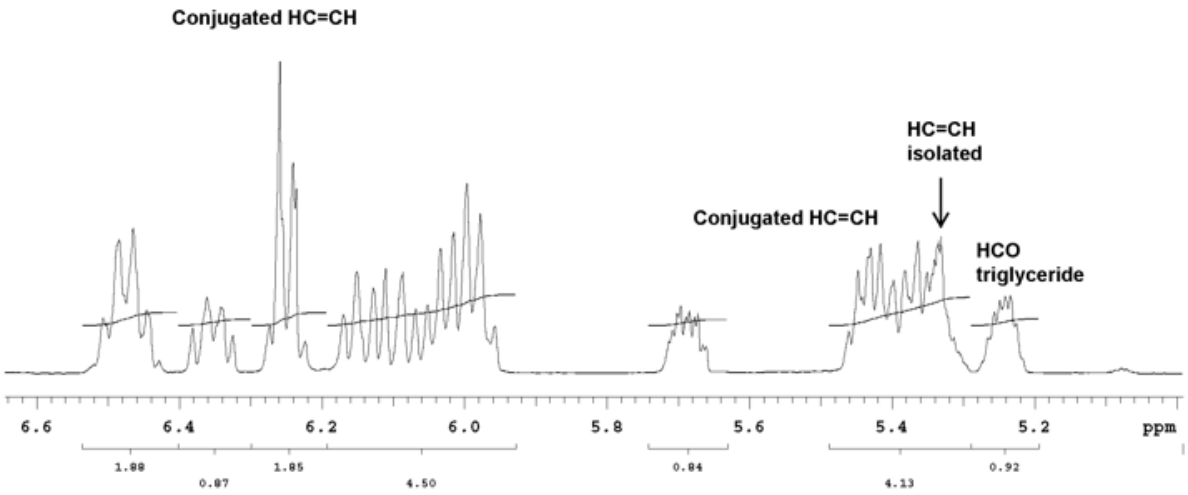

Figure 11: ^{1}H-NMR of bajuru seed oil, in $CDCl_3$, expanded about the region from 5.0 – 6.8 ppm, where signals due to the HCO of triacylglycerides from 5.22 – 5.29 ppm, isolated HC=CH from 5.31 – 5.35 ppm and conjugated HC=CH from 5.35 – 5.48, 5.65 – 5.73 and 5.95 – 6.55 ppm.

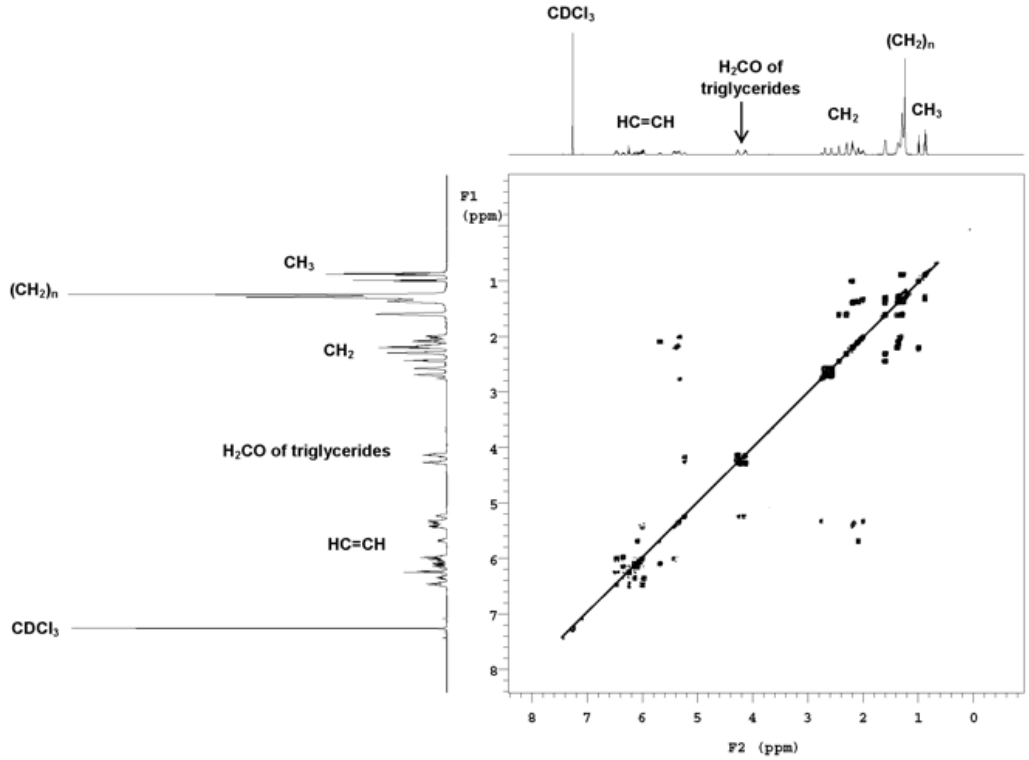

Figure 12: COSY NMR spectrum of bajuru seed oil, in $CDCl_3$. The signals that are not on the diagonal line show which ^1H are linked to each other.

shifts depend on whether the HC=CH bond is in the Z (*cis*) or E (*trans*) configuration [4, 12]. It is important to note that conjugated linoleic acids are relatively unstable when heated in the presence of oxygen [16], so cocoplum seed oil should not be used for cooking. It is more suited for use in salad dressings and sorbets that are not heated. It can also be consumed by itself or put on other foods after they have been cooked, such as pasta.

The COSY spectrum (Figure 12) shows which hydrogens (^1H) are linked to each other. To interpret the spectrum, an imaginary line can be drawn diagonally, from the bottom left to upper right. Each of the signals in the ^1H-NMR spectra produced signals on this diagonal. Linkages to other hydrogens appear off the diagonal. Squares can be drawn using the off diagonal peaks as corners of the square. For example, the signals due to $–CH_3$ at about 0.8 – 1.0 ppm form one corner of the square, while signals due to $–(CH_2)_n–$ at about 1.2 – 1.4 ppm form the other corner. Similar squares appear upon examination of the spectrum. They show linkages between HC=CH and $–(CH_2)_n–$ groups, as well as the $–(CH_2)_n–$ and H_2CO and HCO of triglycerides. Similarly, the links between adjacent HC=CH groups in conjugated linoleic acids are apparent. This shows that the ^1H and COSY NMR spectra are consistent with the presence of triacylglycerides, with a mixture of saturated and unsaturated fatty acyls attached. This includes the fatty acyl versions of α-ESA and the other fatty acids shown in Figures 2 - 7.

Further evidence supporting the presence of triacylglycerides with fatty acyls (but not free fatty acids) comes from the $^{13}C\{^1H\}$-NMR, shown in Figure 13. Many of the signals seen in this spectrum also appeared in the spectra of açaí and olive oil [7, 11]. As in the ^1H spectrum, there were additional signals in the $^{13}C\{^1H\}$-NMR spectrum. This includes signals from 208.45 – 208.63 ppm due to ketone carbonyls (C=O), such as the ones in the oxo acids shown in Figures 5 and 7. There are no signals in the region of 179 – 180 ppm, indicating the lack of detectable free fatty acids. Instead, there are signals from 172.08 – 172.91 ppm due to ester carbonyls that are in triacylglycerols. There are several signals due to HC=CH groups, as shown when the spectrum is expanded about the region from 125 – 136 ppm, as shown in Figure 14. The signals from 129.89 – 129.30 ppm and from 130.13 – 130.31 ppm were due to isolated HC=CH bonds, like the ones in oleoyl and linoleoyl fatty acyls [7, 11]. There were many other signals that were due to conjugated HC=CH bonds, like the ones in conjugated linoleic acids.

None of the signals in the carbonyl region are due to aldehydes, since none of them showed single bond coupling in the HMQC spectrum (Figure 15). Coupling between $–CH_3$ groups and signals due to $–CH_2–$ and between individual $–CH_2–$ groups are apparent in the region from 14 – 45 ppm on the vertical (^{13}C) axis and 0.8 – 1.4 ppm on the horizontal (^1H) axis. This is more obvious when the spectrum is expanded around these regions, as seen in Figure 16.

Multiple bond couplings are shown in the HMBC spectrum

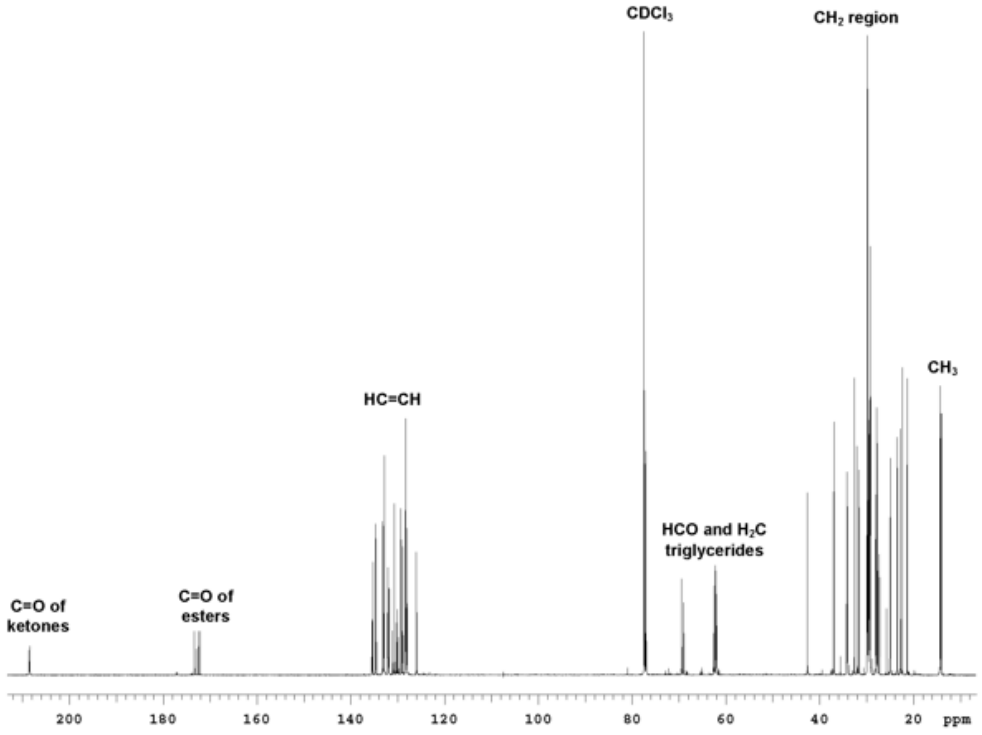

Figure 13: $^{13}C\{^{1}H\}$-NMR of bajuru seed oil, in $CDCl_3$.

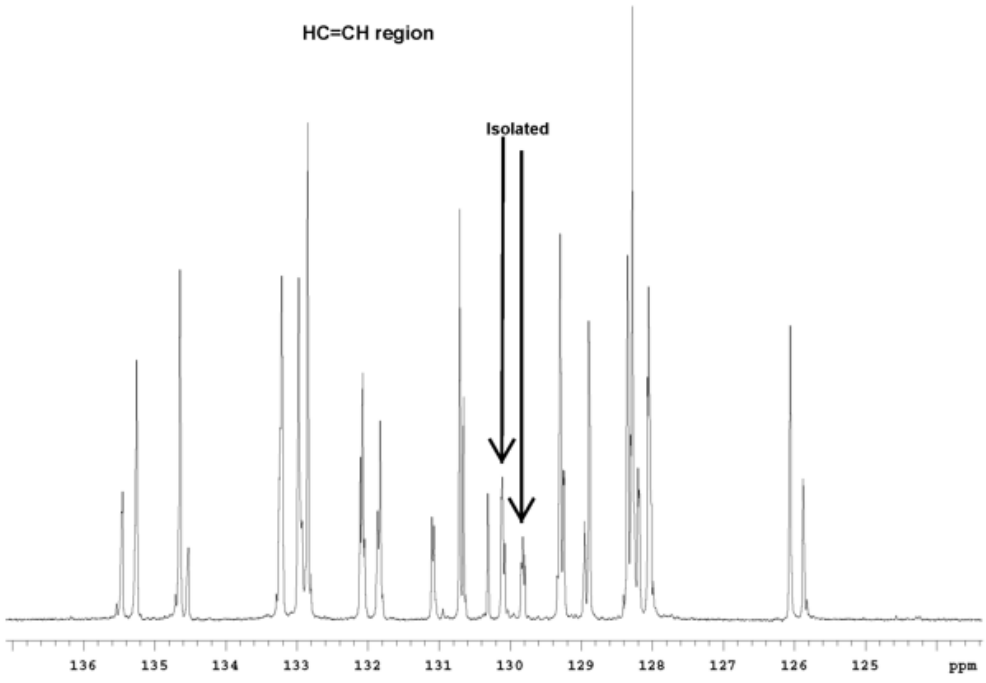

Figure 14: $^{13}C\{^{1}H\}$-NMR of bajuru seed oil, in $CDCl_3$. The signals due to isolated HC=CH bonds (as in oleoyl and linoleoyl fatty acyls) are marked. All other signals are due to conjugated HC=CH bonds (as in conjugated linoleic acids).

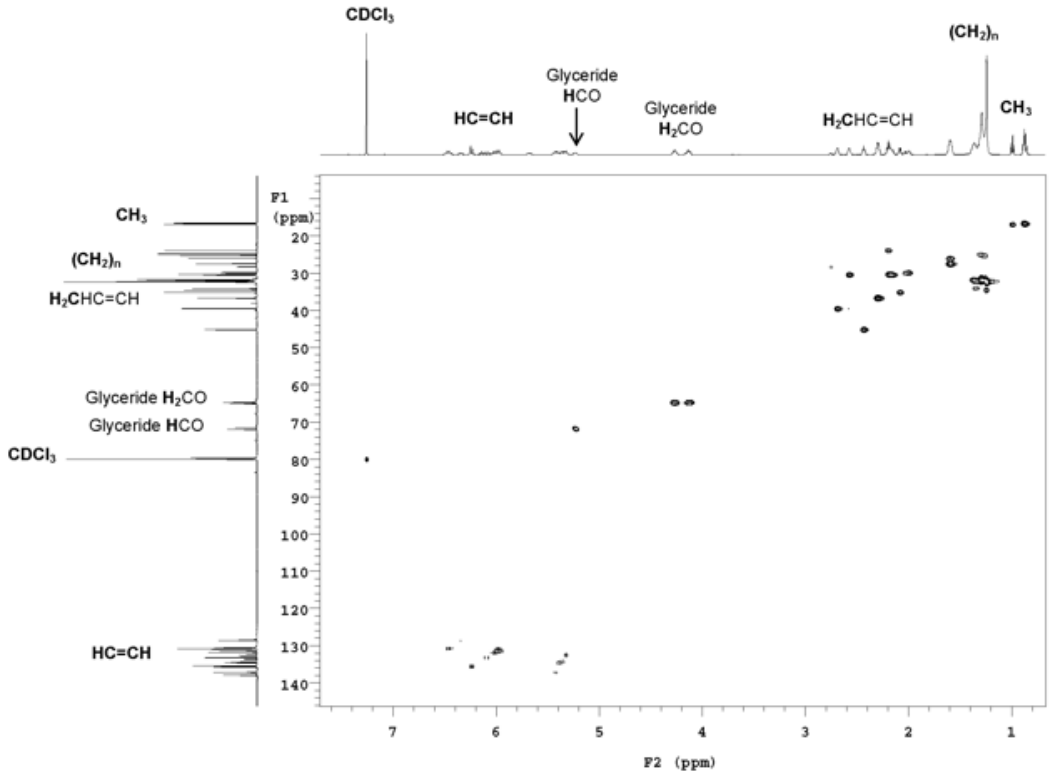

Figure 15: HMQC-NMR of bajuru seed oil, in $CDCl_3$.

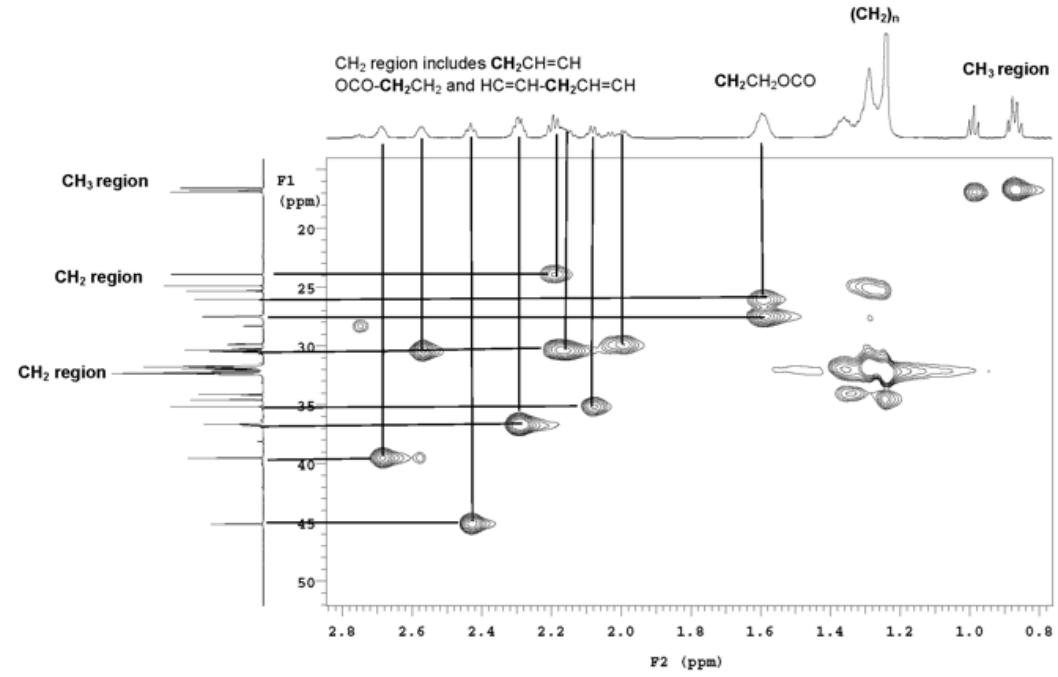

Figure 16: HMQC-NMR of bajuru seed oil, in $CDCl_3$, expanded about the region along the vertical axis (^{13}C) from 15 – 50 ppm and the horizontal axis (^{1}H) from 0.8 – 2.8 ppm. Lines show the connections between signals due to $CH_2CH=CH$, $OCO\text{-}CH_2CH_2$ and $HC=CH\text{-}CH_2CH=CH$ groups.

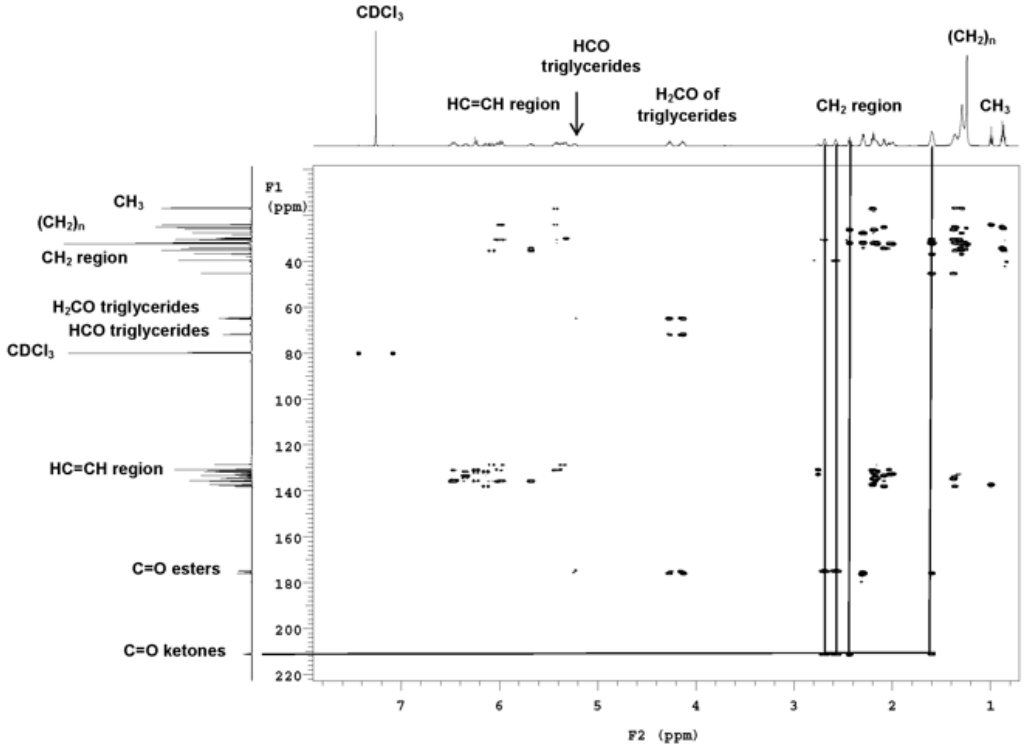

Figure 17: HMBC-NMR of bajuru seed oil, in $CDCl_3$. The lines show the links between signals due to the C=O of ketones and CH_2 groups through multiple bond coupling.

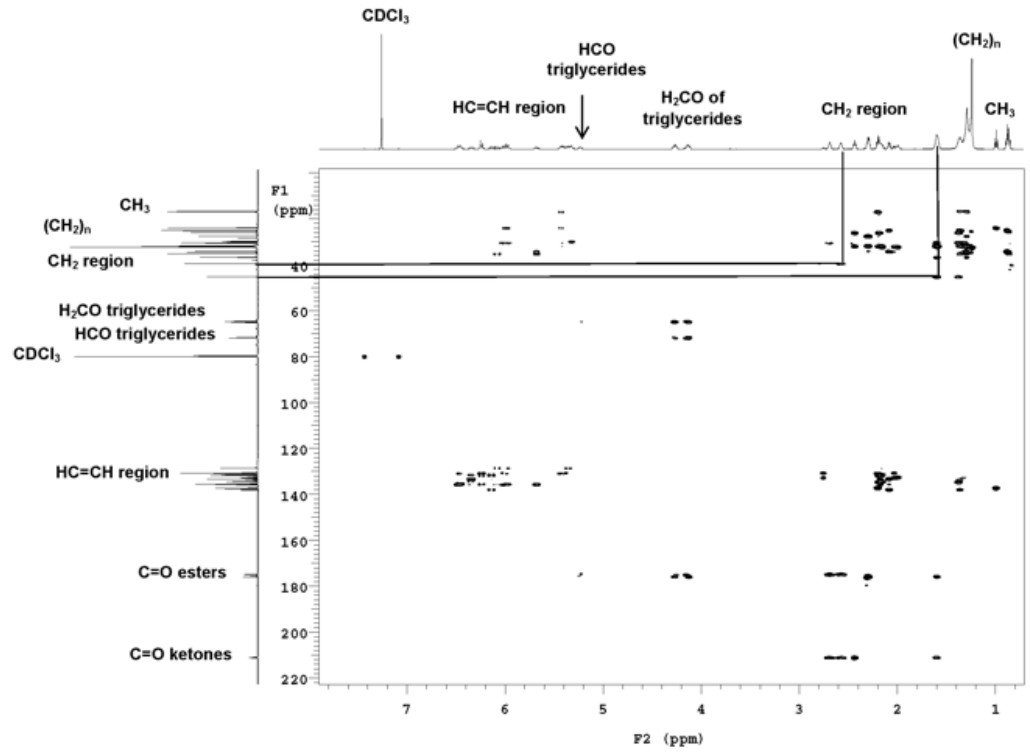

Figure 18: HMBC-NMR of bajuru seed oil, in $CDCl_3$. The lines show the links between signals at due to the CH_2 groups through multiple bond coupling.

in Figure 17. Many of them can be expected from the structures and moieties that were apparent in the other NMR spectra. However, the HMBC (Figure 18) spectrum is ideal for showing that signals at 37.00 and 42.61 ppm are due to $–CH_2$ groups that are bound to the ketone C=O, as in the oxo acids shown in Figures 5 and 7. The lines show the links between signals that are due to the CH_2 groups through multiple bond coupling.

4. Conclusion

Cocoplum seeds and seed oil may be a nutritious dietary source of conjugated linoleic acids, unsaturated keto acids, and oleic acid. They are present as fatty acyls that are attached to triacylglycerides. Cocoplum seed oil should not be used for cooking, because conjugated linoleic acids are thermally unstable and potential pro-oxidants [16]. It is more suited for use in salad dressings and sorbets that are not heated. It can also be consumed by itself or put on other foods after they have been cooked, such as pasta. The seeds also contain dietary fiber, protein and important minerals. These findings will give regulators several ways of determining whether or not food products labeled as containing cocoplum, abajeru or bajeru are genuine. In addition, it will help regulators decide if cocoplums should be classified as generally regarded as safe (GRAS). It will also help regulators decide what should be on the label for food products made from cocoplums.

5. Declaration of Conflicting Interest

The authors declare that there is no conflict of interest.

6. Disclaimer

This work should not be taken as having an impact on FDA policy or regulations.

7. Acknowledgments

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8. Article Information

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