FRACTIONATION AND EVALUATION OF *CARAPA GUIANENSIS* SEED OIL

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Abstract

Fractionation and Evaluation of *Carapa guianensis* Seed Oil

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The seed oil of *Carapa guianensis*, known as Crabwood oil (CWO) and its fractionation was studied, focussing on chemical composition and physicochemical functionality. Compounds never reported before in CWO were identified, including limonoids such as Trichillin, ketostearic and hydroxyoleic acids. Yield of olein and stearin fractions correlated strongly with solvent polarity. Important bioactives were partitioned as a function of solvent polarity in the olein vs. stearin fractions. For example, up to 35% more of Gedunin were present in the liquid fractions. Solid fat content, crystallization and melting temperature and microstructure were significantly affected by fractionation and shown to be strongly correlated with solvent polarity. Fractionation was demonstrated to be a powerful tool to extend the physical functionality of CWO and concentrate its bioactives.

Keywords: *Carapa guianensis*, Crabwood Oil, Guyanese Crabwood Oil, Fractionation, Dry and Solvent fractionation, Liquid fraction, Solid fraction, Physical characteristics, Chemical characteristics

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"At times, our own light goes out and is rekindled by a spark from another person. Each of us has cause to think with deep gratitude of those who have lighted the flame within us." - Albert Schweitzer.

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Layout of the Thesis

Chapter 1 provides an introduction of the thesis and its objectives and related hypotheses. The literature review of the subject is divided in seven subsections that describe the Carapa Aublet (Crabwood) tree, the techniques used to extract the oil from the seeds and its fractionation. The Chemical characterization and the physical properties of the Crabwood Oil (CWO) are also described as well as its traditional and current uses and its activity against plant and human bacterial and fungal pathogens.

In the second chapter, **Section 2.1** describes the materials used. **Section** Error! Reference source n ot found. details the fractionation methods and presents the nomenclature used to specify the fractions throughout the thesis. Approaches utilized for molecular analyses are described in **Section 2.3** and measurements of physical properties are described in **Section 2.4**. Finally, the statistical analysis methods employed are described in **Section 2.5**.

Chapter 3 presents and discusses the results from the experimental work. **Section 3.1** details the fractionation results. **Section 3.2** discusses the molecular profile of CWO and its fractions, primarily focusing on potentially anti-microbial and anti-fungal unsaponifiable compounds. The physical description of Crabwood oil and fractions are discussed and compared to Cocoa and Shea butter. **Section 3.3** discusses the samples' subcell structure and **Section 3.4**, their microstructures. The thermal transition behavior is presented in **Section 3.5 and t**he thermal degradation behavior in Section 3.6.Finally, the solid fat content data is presented and discussed in **Section 3.7**.

Chapter 4 provides a conclusion and final remarks, identifying the findings of the research and discusses the challenges and opportunities for the crabwood.

CHAPTER 1

1 Chapter 1: Introduction

Guyana covers an area of 215,000 square kilometers, approximately 85% of which is covered by old growth standing forests. The country's floral diversity includes over 8,000 species, with approximately 6,500 identified species [1]. Guyana's mostly intact biodiversity has been, for generations, part of the country's social, health, environmental and economic fabric [2, 3]. The *Carapa* Aublet species, commonly referred to as Crabwood and Andiroba, from the *Meliaceae* family of flowering plants, comprise 51 genera and about 575 species of small to large trees and (rarely) shrubs [4] that receive special attention because of their high economic value [5, 6]. Some species of the *Meliaceae* family such as *Cedrela oderata* (Spanish cedar) and *Swietenia mahagoni* (American mahogany) are prized for their timber [7]. Others such as *Azadirachta indica* (Neem) are utilised for the medicinal properties of their oils, resins and other extracts [7, 8]. The Crabwood tree is valued for both its timber and the medicinal properties of its seed oil and the extracts from its leaves and bark [9].

Crabwood trees are native to tropical and subtropical regions and thrive in rich soils found in swamps, alluvial flats, marshes, and uplands of South and Central America, the Caribbean and tropical Africa [10-12]. Three species of Crabwood are found in Guyana: *Carapa guianensis*, the most predominant, *Carapa akuri* and *Carapa surinamensis* (also referred to as *Carapa procera)* [5, 13, 14].

CWO would be interesting to fractionate because of its peculiar saturation levels, significant amounts of active components and seasonal variability. Unlike the stearin of other tropical oils

which have high separation temperatures and therefore high solid fat content, the stearin part of CWO separate from the olein at relatively low temperature (~4 °C) [15] suggesting low solid fat content. The reported chemical composition of CWO generally indicates a stearin part dominated by palmitic acid which would be effective in topical applications, alleviating skin dehydration and facilitating the absorption of bioactive compounds and an olein fraction dominated by oleic acid which can be used in emulsion formulations, allowing for both topical and oral administration of the oil [16-18].

Although the industrial and domestic usefulness of Crabwood Oil (CWO) is well documented, its fractionation and characteristics and potential uses of its stearin and olein fractions need to be studied to provide insight into the utility of such fractionation to yield specialty oils and fats. The present study utilizes established methods of phytochemical and physicochemical functionality analysis to compare composition and functionality of a *Carapa guianensis* seed oil sourced from Guyana, and its solid (stearin) and liquid (olein) fractions obtained with different fractionation methods, namely dry fractionation, and fractionation with three solvents with varying polarity: ethanol, acetone and ethyl acetate.

The objectives of the thesis are to:

1. Obtain liquid and solid fractions of the Crabwood Oil using dry fractionation and solvent.
2. Evaluate the chemical and physical properties of Crabwood Oil and its fractions.
3. Evaluate the partitioning of bioactive components in the solid and liquid fractions, as a function of solvent polarity.

The following hypotheses are stated:

1. Native Crabwood Oil can be fractionated by both dry and solvent-mediated fractionation methods to yield solid and liquid fractions at room temperature.
    a. The yield of solid and liquid fractions would be dependent on the fractionation method.
    b. The yield of solid and liquid fractions would be correlated to the polarity of the solvents.

These fractions will demonstrate:

2. Measurable differences in abundance of specific bioactive phytochemicals
    a. Between solid and liquid fractions especially in solvent-mediated fractions.
    b. Depending on the polarity of the solvent.
3. Measurable differences in the lipid profiles
    a. Between solid and liquid fractions especially in solvent-mediated fractions.
    b. Depending on the polarity of the solvent.
4. Measurable and predictable differences in physical properties including thermal degradation, phase transformation behavior and solid fat content between the Crabwood Oil and its fractions, and between the solid and liquid fractions.

A description of the crabwood tree, its taxonomy, ecology, and economic value are presented in **Section 1.1**. The methods used to extract the oil from the seeds of crabwood (traditional, cold press/ industrial and solvent extraction techniques are presented in **Section 1.2.** The principle of fractionation, its utility in the food and cosmetic industries is related to its potential utility to Crabwood oil is presented in **Section 1.3**. The chemical profile including lipids and bioactive components of CWO is detailed in **Section 1.4**, and its physical properties that are related to its quality control and application are presented in **Section 1.5**. The traditional and modern uses of

CWO are presented in **Section 1.6**. Finally, the bioactivity of CWO against phytopathogenic fungi and microbes of public health importance are briefly discussed in **Section 1.7**.

1.1 Overview of Crabwood

1.1.1 Botany of Crabwood

Carapa guianensis typically grows to heights of 25-35 m, sometimes reaching up to 55 m [11, 19] and has an oval crown and slightly buttressed base [12]. Pictures of the Crabwood tree, flowers, fruit and seeds are provided in **Figure 1**.

Figure 1. picture of Carapa guianensis taken in Region 10 Guyana. Pictures of the flowers, fruits and seeds of crabwood are sourced from iNaturalist database (iNaturist.org) which allows free sharing, adapting and distribution.

Its bark may be smooth or flaky, ranging from grey to grey-brown while its inner bark is fibrous red to pink-brown [20]. The leaves are large, alternate and paripinnate compound leaves with a prominent central vein on the underside. Its flowers are small (no greater than 8 mm) white, predominantly 4-mere flowers [11, 19]. The *Carapa guianensis* fruit is a 4-compartment woody dehiscent capsule, with two to four smooth, pale brown oil-rich seeds in each compartment. Each lobe contains 2-4 seeds, weighing 20–40 g each. The flowering of *Carapa guianensis* in Guyana is annual, and occurs in November-February, during the dry season, and fruiting mainly from April-July in the wet season which maximizes pollination and seed dispersal [10]. There is a shorter rainy season from November-January [21], which allows for a smaller, secondary fruiting period that typically occurs in November [14]. *Carapa guianensis* is a masting species characterized by synchronized and highly variable seed production [22]. Rough estimates of seed production range from 50 to 200 kg of seeds per tree, for a typical and masting year, respectively [23-25].

The fruit cracks open when ripe, shedding the seeds into the environment. The harvesting period of seeds is critical for oil production because they are an important food source for various animals, including armadillos, peccaries, pacas, deer and agoutis [10]. These animals compete for the seeds with the indigenous populations but are also a crucial part in their subsistence [26]. The rodents play a particularly important role in spreading the seeds on the forest floor [23]. Typically, the rodents collect and bury the seeds before consumption [27]. This allows to more easily crack the hard shells and soften the biter taste of tannins that deter their immediate consumption [28].

1.1.2 Economic Value

Crabwood is a valuable commodity for the quality of its wood and medicinal properties of its parts [11, 29]. Its timber is frequently logged for its mahogany-like qualities [30, 31]. Its flowers, leaves

and bark are traditionally used by indigenous peoples for medicinal purposes [10, 32] and is currently frequently logged for its mahogany-like timber quality [5, 6]. The extracts from its leaves, bark and seeds are recognized in traditional medicine to improve wound healing and treat various conditions such as fungal infections [33], malaria [34], and fever [35]. It is believed that the biological activity of the different parts of the plant are attributed to the presence of unsaturated fatty acids, limonoids and phenolic compounds. Appreciable amounts of bioactive compounds were identified in the bark and leaves of Carapa genus. Hydroalcoholic extractions of the phytochemicals from *Carapa guianensis* bark [36] and leaf [35] were screened for saponins, triterpenes, sterols, alkaloids and flavonoids and evaluated for wound healing activity. Methanolic extracts of *Carapa procera* bark were screened for the same compounds and for tannins and glycosides for potential antimicrobial activity [37].

The oil extracted from its seeds, Crabwood Oil (CWO), is especially distinguished for its traditional medicinal applications. [10] It is used as itch relieving, insect-repelling [38, 39], anti-inflammatory [40], anti-malarial [41, 42] and skin protectant medicine [43, 44]. The literature reports extensively on its medicinal uses. CWO has been reported to exhibit highly efficient analgesic, antipyretic, anti-bacterial, anti-inflammatory, anti-cancerous, anti-tumour, anti-fungal, and anti-allergic properties [45, 46]. Additionally, by-products from seed production are attracting a growing interest its natural abrasiveness and potential bioactivity [31, 47].

Crabwood Oil has been used to make medicinal products such as soaps to alleviate skin diseases, preservatives and repellent candles [48]. Less traditional formulations include shampoos, creams, and other emulsified cosmetics [49-51]. Local pharmacies in the capital city of Georgetown process the oil industrially into soap, candles and insecticidal washes [6]. The pharmaceutical and homeopathic industries sell the oil in capsules to treat diabetes and rheumatism [41]. Crabwood

Oil is also mentioned in patents as an ingredient in formulations for treating dermatological conditions like psoriasis, cellulite, and in antiallergic medicine [52-54]. Crabwood Oil was even included in patented insect-repelling paint formulations [55], a first in potentially emerging larger industrial applications of the oil.

The medicinal properties of *Carapa guianensis* are attributed to its limonoids, the highly oxygenated tetranortriterpenoids, at the origin of a diverse range of biological functions [56]. These bioactive components are predominantly present in the seeds, but also in the other parts of the tree such as leaves and bark [57]. The species of the Meliaceae family are known to comprise abundant and structurally diversified limonoids [58]. *Azadirachta indica* (Neem), for example, is a species of the family comprising limonoids and shows medicinal properties like *Carapa guianensis*, including anti-inflammatory and anti-allergic properties [45, 59, 60].

Indigenous Peoples in Guyana and elsewhere are still extracting the seed oil using ancestral methods and commercially trading it as medicine on a small scale [61]. The difference in quality and hence pricing between the traded Crabwood Oils is mostly based on visual differences, mainly color, which varies from pale yellow to amber. The trade also concerns the separated liquid and solid parts sold for different prices; the liquid being more prized than the solid part. One liter of Crabwood Oil sold in Guyana for US$ 3.50 in 2000 [6], and currently is sold for approximately US$13; the increase reflecting the increased demand for the item locally, as its medicinal properties have become more widely known. Currently, the collection of the seeds is done by the indigenous people of Guyana, but commercial production is considered in the forest concessions.

1.2 Extraction of Crabwood Seed Oil

Beside the traditional methods typically carried out by the indigenous people, which are the current primary methods of extraction [32], several techniques involving industrial equipment are used to extract the oil from the Crabwood seeds [62].

The Crabwood Oil extraction activity occurs around the seed production periods because the seeds can only be stored for a short time in the tropical climates dominated by heat and humidity and also because of the very high unsaturation of the seeds oil [41]. The timing of the harvest is also critical for the quality and yield because the Crabwood seeds are subject to numerous insect predators, including grubs of *Meliaceae*, particularly *Hypsipyla ferrealis* and *Hypsipyla grandella* (Lepidoptera: Pyralidae), which attack the fruit and damage the seeds [23, 63]. Traditionally, the seeds are stored in water to prevent the beetle attacks [3].

The presence and yields of the oil's constituents may be affected by interspecies variations, environmental conditions and extraction methods employed [3, 64, 65]. However, these variations are not always necessarily manifested visibly [29, 66]. The seeds are fermented to destroy the seed endocarp and kill the seed germ. Although not excluded, biotransformation might not be the initial purpose of the fermentation stage because the oil is not used for human consumption. Fermentation as used in cocoa beans normally develop flavors and allow the characteristic bitter taste of chocolate [67]. The oil's characteristics, such as chemical composition, physical properties and bio-efficacy, may vary depending on, environmental factors, seed collection timing, physiological state and extraction method [29, 66, 68-70].

1.2.1 Artisanal Methods

The indigenous populations of the Amazon region, including Guyana [16, 32, 71, 72], and populations of Africa [68, 70] use similar harvest and oil extraction methods. They all involve similar steps: seed collection, boiling, fermentation (2-4 weeks), drying, and oil collection. There are variations from region to region, with specific methods affecting the efficacy of the extraction and the appearance of the oil obtained [66]. The yields of crabwood oil from artisanal methods are affected by several process factors including harvest period, storage, fermentation and cooking times. Although acknowledging that they are not very accurate, [71, 73] reported yields which vary from as low as 2% to as high as 22%. Nascimento et al., [74] using hexane and Soxhlet method reported 56 ± 2% oil content in the seeds of *Carapa guianensis* and 47 ± 3%, in and *Carapa vasquezii* which are significantly higher than those obtained with the traditional methods.

Typically, the seeds are collected in the forest from the ground underneath the Crabwood trees and washed in clean water. The seeds are sometimes stored in water to remove the larvae of *Hypsipyla sp.* [71]. The seeds are then cooked in boiling water and left to ferment in the shade for 2 to 4 weeks before they are shelled, and the kernel is removed, hand-kneaded and stored in a semi-porous fibre or plastic bag to drain the excess water. The kernel mass is then spread out over trays and exposed to direct sunlight to dry and gradually release the oil or, in some cases, placed in porous sacks and squeezed. The process is repeated until the mass is dry [11, 16, 32, 72].

An alternative method involves drying, peeling and then boiling the seeds to obtain a fat that is then scooped off and packaged [75]. This method produces a more translucent oil than any other traditional method.

1.2.2 Cold Press Extraction

The cold pressing method is most commonly used in the commercial production of Crabwood Oil. It is typically done by mechanical pressing of milled and dried seeds [65]. The seeds may be milled, or knife lacerated and dried at 40 to 70℃ to achieve the desired moisture content and then mechanically cold-pressed using hydraulic presses [72]. It is the technique of choice for the pharmaceutical and cosmetics industries because of a balance between efficiency, economics, and preservation of bioactive components [65, 72].

There is a wide variation in the reported yields of Crabwood Oil from the commercial production processes varying from 15 to 23% [6, 71]. One parameter that significantly affects the yields obtained is the drying temperature of the seeds [32]. For example, Araujo-Lima et al. [76] evaluated the effect of thermal treatment of crabwood seed harvested at the same period from identified trees at the same location on oil yields. They reported that previously frozen seeds were dried at 60 °C then pressed at room temperature and yielding 15% w/w of oil. Another set of these seeds were autoclaved at 121 °C for 15 mins, then subjected to drying at 60 °C then pressed at room temperature, this process yielded 20% more oil indicating that autoclaving before drying improves oil yields [76].

The industrial processing of Crabwood seeds generates a significant amount of by-product, reported to be around 2/3 w/w. It is reported to include phenolic structures in amounts potentially capable of biomass valorization which would potentially have numerous and varied technological application with important commercial value [77]. The Crabwood seed bagasse, with its natural abrasiveness, has been shown to be a potential excellent exfoliating agent [78]. CWO was also researched to produce biodiesel [79, 80]. The value of the Crabwood oil, however, would be greatly undermined with such large volume and low value products.

1.2.3 Solvent Extraction

Solvents are extensively used for the extraction of natural products. The choice of a solvent for extraction depends on polarity, which affects solubility and selectivity. Energy requirements for solvent recovery and safety are also considered, particularly in the context of industrial operations. Alcohols, such as ethanol and methanol, are commonly used in solvent extraction of phytochemicals [81]. Lesoul et al. reported that ethanol and acetone are the best solvents for flavonoid extractions from macerated medicinal plants [82]. Water extracts would be useful for the extraction of the water-soluble bioactive components (soluble limonoids) but not those insoluble in water. The water-insoluble and water-soluble limonoids were extracted simultaneously using hydrotropes which are highly water-soluble organic salts [83]. There is no agreement on the best solvent extraction methods for Crabwood Oil [72, 76]. The relative polarity, boiling point (BP) and heat of vaporization of select solvents used for oil extraction are listed in **Error! Reference source not found.** [84-86].

Table 1-1: Solvent polarities, boiling points (BP) and heat of vaporization of common solvents in order of increasing relative polarity.

Solvent	Molar mass (g/mol)	Relative Polarity	BP (°C)	Heat of vaporization	
				(kJ/kg)	(kJ/mol)
Hexane	86.18	0.009	68.9	365	31.5
Diethyl ether	74.12	0.117	34.6	391	29.0
Ethyl acetate*	88.11	0.228	77.1	404	35.6
Chloroform	119.38	0.259	61.2	246	29.2
Dichloromethane	84.93	0.309	40.0	330	28.0
Acetone*	56.2	0.355	56.1	534	31.0
1-butanol	74.12	0.586	117.7	556	41.2
Ethanol*	46.07	0.654	78.4	924	42.6
Methanol	32.04	0.762	64.6	1165	37.3
Water	18.02	1.000	100	2260	40.6

* Solvents used in the fractionation of CWO.

The solvent extraction methods are the most efficient and are preferred when higher yields are sought. Nardi et al. [71] estimated that in a very efficient solvent process, an average yield of about 17% w/w would be obtained from fresh seeds. However, with non-polar solvents, the yields of limonoids and other bioactive compounds are typically low. In that regard, non-solvent extraction methods such as supercritical carbon dioxide can be used to efficiently extract the limonoids and other compounds and nutrients like phenolic compounds and proteins [87].

The extraction of CWO is typically carried out via continuous methods, such as Soxhlet extraction, with petroleum ether or hexane [69, 76, 88]. Several studies have reported on extracting Crabwood Oil with a mix of solvents of varying polarities and other extraction techniques, evaluating the oil yields and molecular profiles [65, 85, 89]. These studies show that adjusting the extraction parameters can optimize the yield and phytochemical content. For example, ultrasound-assisted solvent extraction increased the concentration of sterols and limonoids in the oil [65]. For similar or higher extraction yields, pressurized n-butane extraction of Crabwood Oil presents the advantages of being more sustainable than solvent extraction and lower operational cost than supercritical extraction [89].

1.3 Fractionation of Crabwood Oil

Fractionation refers to the crystallization-based separation of a range of melting points. The process is controlled to induce a partial, or ‘fractional’ crystallization. The fractionation is typically performed using simple crystallization from the melt without any additives (so-called dry fractionation) or crystallization in the presence of a solvent (solvent fractionation), followed by filtration of the crystals. Plant oil fractionation is done to recover fractions with narrower composition ranges and altered physical properties which can find specific uses; for example, the

replacement of hydrogenated fats or cooking oils with saturated fractions of vegetable oils with improved nutritional values [90-95]. Similarly, Crabwood Oil can be sustainably processed using fractionation, to optimize its physical characteristics and extend its utility.

The fractionation of the Crabwood Oils is an area of great importance that has attracted very little interest. Like other tropical oils like palm oil [96, 97], Coconut Oil and Shea Butter [90] which are commonly fractionated to make novel and valuable materials such as chocolate substitutes [98], Crabwood Oil can be fractionated into solid (stearin) and liquid (olein) fractions [99, 100]. CWO fractions would be industrially significant because of the sizable amounts of their potent bioactive components which would enable creating specialty fats and oils of medicinal and cosmetic interest. CWO, which noticeably appears more solid/liquid depending on the season seeds are collected is even more interesting to fractionate because of the significant difference in saturation levels that such variability entails.

1.4 Chemical Composition of Crabwood Oil

CWO is typically composed of two main groups, lipids and non-saponifiable phytochemical compounds. The lipid portion of the oil represents 95-98% of the oil's composition [101]. The remaining 2-5% group are secondary metabolites comprising terpenes, sterols, and phenolic compounds [76, 77].

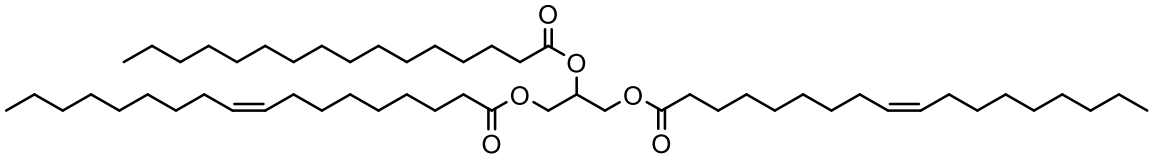
1.4.1 Lipid Profile of Crabwood Oil

The lipid component of CWO comprises primarily triacylglycerols (TAGs) with high levels of unsaturated fatty acids [2, 29, 40, 102] and other minor components including free fatty acids (FAs), diacylglycerols (DAGs), monoacylglycerols (MAGs) and phospholipids [3, 34, 40, 43, 101,

103]. The reported lipid fraction consists of up to 67% TAG, 10% DAG, 20% MAG and free fatty acids (FFA). The level of unsaturation can reach up to 60% [101, 104].

1.4.1.1 TAGs

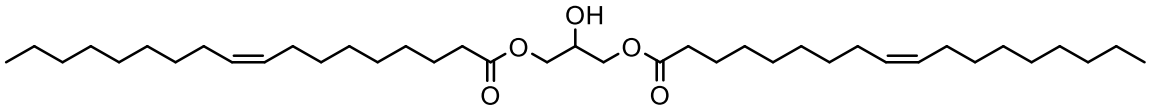
The TAG profile of CWO shows that 1,2-dioleoyl-3-palmitoyl-glycerol (POO) is the most abundant TAG, accounting for approximately 20% of all the TAGs present. The structure of POO is represented in **Scheme 1-1**. Other reported TAGs include 1-palmitoyl-2-palmitoyl-3-oleoyl-glycerol (PPO), 1-palmitoyl-2-stearoyl-3-oleoyl-glycerol (POS), and triolein (OOO). These together make up less than half the content of POO in CWO [101, 104-106].



Scheme 1-1: Structural representation of POO.

1.4.1.2 DAGs

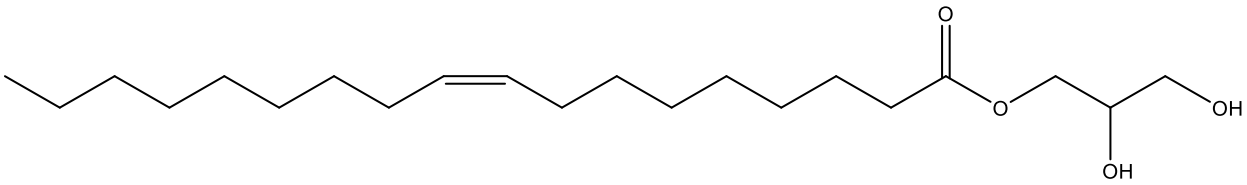
DAGs are immediate precursors for TAG synthesis [107] and can also be formed if TAGs undergo lipolysis or hydrolysis, so that their presence in the oil reflects the types of TAGs present. The structure of diolein, the DAG made up of two oleic acids and glycerol, is shown in **Scheme 1-2**.



Scheme 1-2: Structural representation of diolein.

1.4.1.3 MAGs

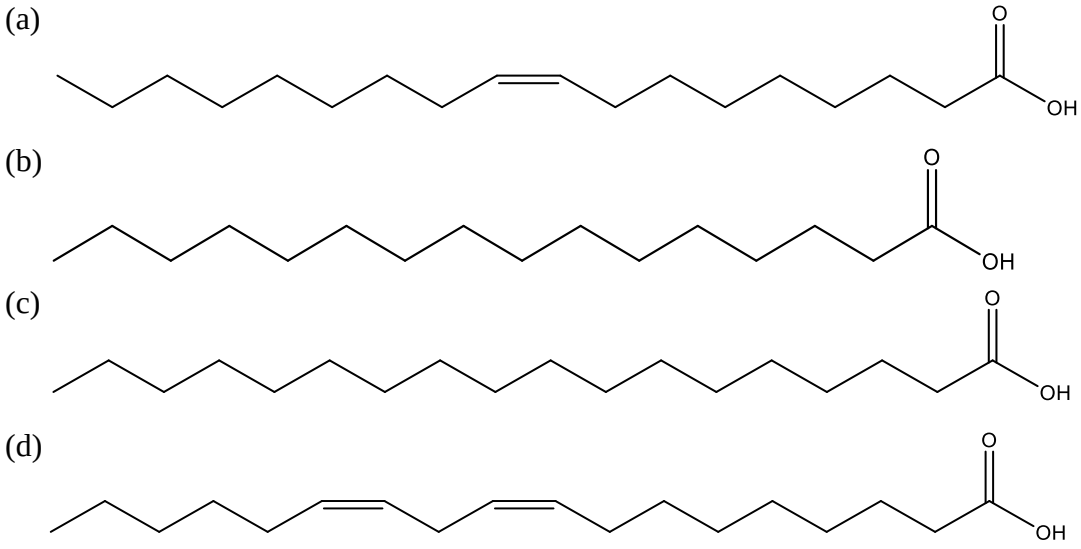
MAGs can be formed from TAGs lipolysis and hydrolysis, so their presence like DAGs is expected to reflect the types of TAGs the oil contains. The structure of monoolein, the MAG made up of oleic acid and glycerol, is shown in **Scheme 1-3**.



Scheme 1-3: Structural representation of monoolein.

1.4.1.4 Fatty Acids

The most abundant fatty acids reported are oleic and palmitic acids, accounting for approximately 75% of the total. Relatively high amounts of stearic (~9% w/w) and linoleic acids (~10% w/w) have also been reported [101, 108]. Small quantities of lauric acid (0.11% w/w) and myristic acid (0.18% w/w) have been reported in some studies [101], but several other studies did not report these fatty acids [80, 104]. The structures of oleic, palmitic, stearic, and linoleic acids are shown in **Scheme 1-4**.



Scheme 1-4: Structural representation of (a) oleic, (b) palmitic, (c) stearic and (d) linoleic acids.

1.4.2 Unsaponifiable Compounds of Crabwood Oil

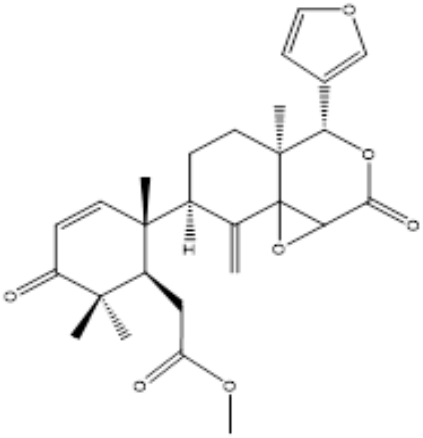
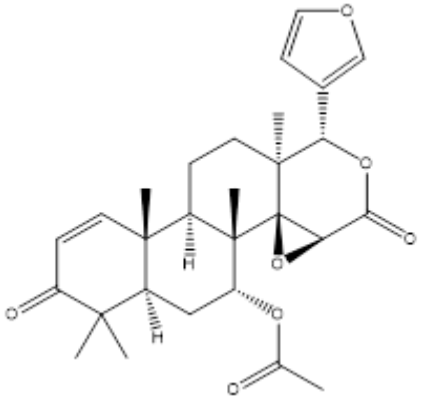
The components of CWO which are not saponified with caustic alkali (unsaponifiable component) include potent bioactive components, mostly highly oxygenated limonoids but also flavonoids, sterols and phenolic compounds, to which are attributed the biological activities of the oil and its bitter taste [39, 76, 109, 110].

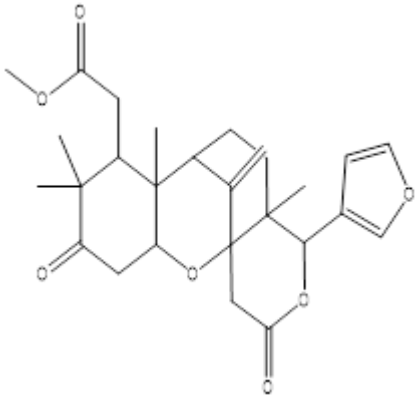
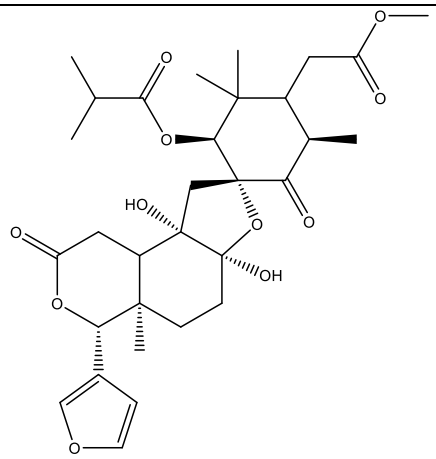
1.4.2.1 Limonoids

Limonoids are a class of tetranortriterpenes that are the chemotaxonomic markers of the *Meliaceae* family [77]. These highly oxygenated compounds common in the Rutaceae family (citrus group) are present in different plant species of the *Meliaceae* family, like Neem (*Azadirachta indica*), and are responsible for the therapeutic properties of these plants and their oils [59, 60, 111]. The limonoids of CWO vary structurally and have diverse activities [77, 112-115]. They are reported to have anti-plasmodial [116], anti-inflammatory [117], antifeedant/antiparasitic [118-121], antioxidant [76, 122], antifungal [33], hepatoprotective [123], and anti-allergic activities [124]. The traditional uses of the "medicine" recognize that the value comes from the entirety of plant/oil and do not attribute the beneficial activity to single or group of chemicals. The value is attributed to the synergies which may come from the interaction of the thousands of chemicals present in the plant/ oil – not just from the most abundant ones. The modern approach relies on identifying specific compounds in a plant/oil and relate them to a given activity or medicinal value. This approach goes as far as *In silico* investigations to establish the potential activity of specific compounds such the study conducted on limonoids against therapeutic target proteins of the

SARS-CoV-2 virus [125, 126]. Of the limonoids identified in CWO, the *Gedunin*-type limonoids are for example extensively individually investigated for diverse biological activities [127-130]. Other limonoids which have been of research interest are *Andirobin* [110, 131], *Methyl Angolensate* [56, 132], and *Carapanolide* limonoids [133, 134]. The IUPAC name, structure, and activity of these limonoids are shown in **Table 1-2**.

Table 1-2: Examples of Limonoids found in Crabwood Oil and their activities.

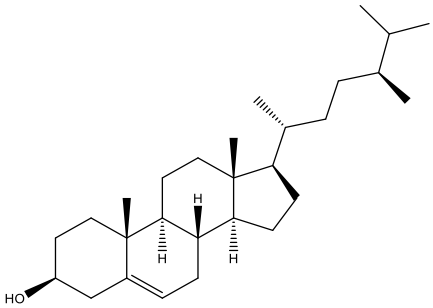
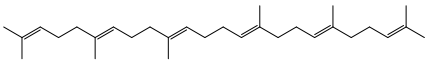
Limonoid	**IUPAC Name**	**Structure**	**Activity**	**Ref.**
Andirobin ($C_{27}H_{32}O_7$)	Methyl 2-[(1R,2R)-2-[(1aS,4S,4aS,7R,8aS)-4-(furan-3-yl)-4a-methyl-8-methylidene-2-oxo-4,5,6,7-tetrahydro-1aH-oxireno[2,3-d] isochromen-7-yl]-2,6,6-trimethyl-5-oxocyclohex-3-en-1-yl] acetate		Antiallergic Antimalarial Antileishmanial Analgesic	[130, 135]
Gedunin ($C_{28}H_{34}O_7$)	[(1S,2R,4S,7S,8S,11R,12R,17R,19R)-7-(furan-3-yl)-1,8,12,16,16-pentamethyl-5,15-dioxo-3,6-dioxapentacyclo[9.8.0.02,4.02,8.012,17]nonadec-13-en-19-yl] acetate		Antiallergic Antimalarial Antileishmanial Collagen synthesis Antifungal Antibacterial Neuroprotective Analgesic	[130, 135, 136]

Limonoid	IUPAC Name	Structure	Activity	Ref.
Methyl angolensate ($C_{27}H_{34}O_7$)	methyl 2-[(1R,3R,7S,8S,9S,12R,13R)-13-(furan-3-yl)-6,6,8,12-tetramethyl-17-methylidene-5,15-dioxo-2,14-dioxatetracyclo[7.7.1.01,12.03,8]heptadecan-7-yl]acetate		Antiallergic Anticancer Anticonvulsant Insecticidal Antidiabetic Antiulcer Antifungal Analgesic	[130, 135, 137]
Carapanolide A ($C_{31}H_{42}O_{11}$)	[(1'S,2R,3aS,4'R,5aR,6R,9bS)-6-(furan-3-yl)-3a,9b-dihydroxy-3'-(2-methoxy-2-oxoethyl)-2',2',4',5a-tetramethyl-5',8-dioxospiro[1,4,5,6,9,9a-hexahydrofuro[3,2-f]isochromene-2,6'-cyclohexane]-1'-yl] 2-methylpropanoate		Anticancer	[130, 134, 135]

1.4.2.2 Sterols

The combined use of chromatography and spectrophotometric methods allows for the successful separation and detection of oil components [101, 108, 138]. LC-APCI-MS was utilized to evaluate the presence of phytosterols in *Carapa procera* oil [139]. Sterol compounds identified in CWO include, Campesterol, β-Sitosterol, Stigmasterol [65], Cholesterol and Squalene [140]. Squalene is a precursor in the biosynthesis of steroidal compounds [141] and Limonoids [142]. **Table 1-3** show examples of Sterols found in Crabwood Oil and their activities.

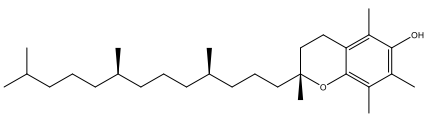
Table 1-3. Examples of Sterols found in Crabwood Oil and their activities.

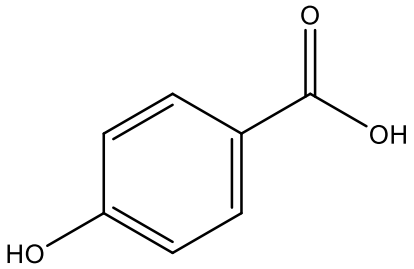
Sterol	IUPAC Name	Structure	Activity	Ref.
Campesterol	(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol		Anti-inflammatory Anticancer Cholesterol lowering	[143]
Squalene	(6*E*,10*E*,14*E*,18*E*)-2,6,10,15,19,23-hexamethyltetracosa-2,6,10,14,18,22-hexaene		Antioxidant Emollient Cholesterol lowering	[144]

1.4.2.3 Other Bioactive Compounds

Phenolic compounds and Tocopherols [92] are detected in small quantities in CWO [115]. **Table 1-4** present the structure and activities of a Phenolic compound and a Tocopherol found in Crabwood Oil.

Table 1-4.Structure, IUPAC name and activities p-hydroxy benzoic acid (PHBA), a Phenolic compound, and ∝-Tocopherols found in Crabwood Oil.

Component	IUPAC Name	Structure	Activity	Ref.
∝-Tocopherol	(2*R*)-2,5,7,8-tetramethyl-2-[(4*R*,8*R*)-4,8,12-trimethyltridecyl]-3,4-dihydrochromen-6-ol		Antioxidant	[92]

Component	IUPAC Name	Structure	Activity	Ref.
p-hydroxy benzoic acid (PHBA)	4-hydroxybenzoic acid		Antimicrobial Antiestrogenic Hypoglycemic Anti-Inflammatory	[145]

1.5 Physical Characteristics of CWO

Key physical properties of CWO have been well characterized, including quality and identity parameters such as acid index, peroxide value, iodine value, free fatty acids, ester Index and viscosity, all critical to its suitability as a raw material for the pharmaceutical and cosmetic products as well as industrial products such as biodiesel [79, 80, 101].

Differential scanning calorimetry (DSC) has been used to determine the thermal transition behavior of CWO. The DSC cooling thermogram of CWO shows three main exotherms corresponding to the crystallization of the saturated fraction (~12 °C) and unsaturated fractions (~-10 and -45 °C) of the oil [101]. The endothermic peak with an offset temperature of 23 °C observed in the DSC heating thermogram of CWO reveals the presence of fat at room temperature. The separation of the high temperature exotherm from the low temperature exotherm occur at ~4 °C, indicating the potential for fractionation of CWO into a “stearin” and “olein” fractions.

Thermogravimetric analysis (TGA) has been used to determine the thermal stability of CWO and it effect on products containing CWO. It has been reported that CWO enhances the thermal stability of microspheres [146] and films of polycaprolactone (PCL) [99]. It has also been reported that CWO positively affects the formation and behavior of polymers and biodiesel [79, 147].

The impact of TAGs and minor components on the physicochemical properties of CWO have been studied but not in detail [43, 103, 148, 149]. The specific contributions of these structures to the function and activity of the oil are still to be established. Such knowledge would assist the development of novel products such as custom designed natural polymeric materials and membranes, emulsions and gels for various cosmetic, medical and pharmaceutical applications spanning from wound healing / tissue regeneration to antiaging formulations.

1.6 Uses of Crabwood Oil

The versatility of Crabwood Oil's physical properties and the presence of lipids and unsaponifiable compounds makes it an excellent candidate for creating materials with biomedical applications and potentially as an alternative energy source [79, 99, 150].

1.6.1 Traditional Uses

CWO has been used to alleviate arthritis, urticaria, acne, ulcerations, insect bites and other common dermatological issues. The oil is taken internally to help with diarrhea, pneumonia and persistent coughs [11]. The oil is mixed with breast milk and warm water to treat ear infections [151]. The CWO is also used to make insect repellents, candles and burnt in lamps for the same purpose.

1.6.2 Modern Uses of Crabwood Oil

CWO is used as an additive in less traditional products such as shampoos and lotions [49, 50, 152]. There are patents that mention Crabwood Oil as an ingredient in formulations to treat dermatological conditions like psoriasis and cellulite and in antiallergic medicine [52-54]. While most research has been on its pharmaceutical and cosmetic applications, there is a patent in which

the CWO is used in an antifungal paint formulation, indicating that the oil can have wider industrial applications such in the construction industry [55].

1.6.3 Potential Uses of CWO

Novel specialty oils and fats products can be developed from Crabwood Oil to meet specific nutritional or functional requirements in foods, pharmaceuticals, and cosmetics. Also, tropical diseases caused by bacterial and parasitic infections, primarily in low-income communities, which do not receive the same attention and funding like other major infectious diseases like HIV/AIDS, malaria and tuberculosis, [153] may benefit from the activity of the Crabwood Oil.

1.6.3.1 Specialty Oils and Fats

Specialty oils and fats are products that are created to meet specific nutritional or functional requirements and are used in the food, pharmaceutical, and cosmetic industries. They are oils and fats modified to enhance their physical-chemical properties, such as melting point, texture and stability [154, 155]. Some common examples of specialty fat use include substitutes for Cocoa Butter, shortening, margarine, butter, and cooking oils [156].

Cocoa Butter (CB) is a major fat predominantly composed of di-saturated TAGs used to produce chocolate. The substitution of CB by specialty fats such as Shea Butter (SB) addresses the fluctuations which often occur in the supply and demand of CB and reduce cost [157]. Chocolate is manufactured to be brittle at room temperature and to melt on contact [158]. This behavior is due to crystallization and melt profiles of CB and its polymorphism [159, 160]. The fat is thermally processed to achieve a crystal-fat network with a structure and mechanical strength which promotes the snap, texture and feel in the mouth sought important to the chocolate experience [161]. Substitution fats are transformed by physical or chemical means such as fractionation or

transesterification to obtain fats having the required physical and chemical properties for making chocolate which they do not naturally have [158, 162].

The molecular profile of Crabwood Oil may allow the production of fractions that can be alternatives to common specialty fats and oil. The presence of bioactive compounds adds a medicinal and nutritional component that gives value to its use and an advantage over other plant oils.

1.7 Crabwood Oil Bioactivity

1.7.1 Crabwood Oil Activity Against Phytopathogenic Fungi

Crabwood Oil was shown to be active against phytopathogenic fungi [29, 33, 163]. The inhibitory effect of *Carapa guianensis* and *Carapa vasquezii*, another *Carapa* species found in Brazil, on the mycelial growth of three phytopathogenic species (*Aspergillus flavus, Fusarium. oxysporium* and *Aspergillus niger*) is reported for different emulsion concentrations. The dose-response curves indicated sigmoidal trends with asymptotes reaching 100% of inhibited mycelial growth. Overall, all three fungi were found to be more sensitive to *Carapa vasquezii* oil than to the *Carapa guianensis* oil, with half minimum inhibitory concentration and asymptotes occurring at ~45% for *Carapa vasquezii* and ~75% for *Carapa guianensis* [29]. This shows that inhibitory effect depends on the oil concentration and the fungus type, and that interspecies variations may play a role in the therapeutic efficacy of the oils. This has been corroborated by a study of phytopathogenic fungi affecting corn [33]. The data collected in that study for *Fusarium, Aspergillus* and *Penicillium* over a 7-day period for concentrations of 10, 20, 30%, and 40% and shown in **Figure 1-2** indicate the importance of the oil concentration and the great dependence of its efficacy on the organism's susceptibility.

Figure 1-2 show that in the 10 to 40% concentration range, the oil was most effective for *Fusarium* (85% *at* 40% and *Penicillium* (90% at 20%) and the least for *Aspergillus* (10% at 30 and 40%). The growth inhibition for *Fusarium* is interesting as it decreased with increasing concentrations until the 40% dose where it reached the highest value.

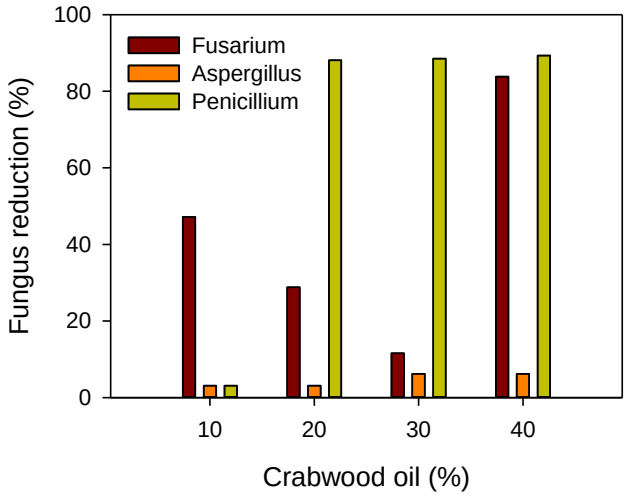


Figure 1-2. The inhibitory effect of Crabwood Oil on three phytopathogenic fungi. Graphed using the data of reference [33].

1.7.2 Crabwood Oil Activity Against Microbes of Public Health Importance

Crabwood Oil has been proven to be active against microbes of public health importance [164, 165]. It has been shown to be effective against tropical parasites such as the malarial protozoa, *Plasmodium falciparum* [166], and the leishmania parasites which cause the neglected tropical disease leishmaniasis [167, 168]. The presence of Gedunin, a compound with antimalarial activity comparable to quinine, justifies the use of the oil against malaria [166] while its derivatives 11β-hydroxygedunin and 6α,11β-diacetoxygedunin are attributed to the antileishmanial activities [167]. Additionally, Crabwood Oil has demonstrated antifeedant activities against, A*edes aegypti*

mosquitos that are vectors for malaria and other infections [169, 170] and other insects [171], suggesting its utility for both prevention and treatment of vector borne infections.

A study evaluating the effects of emulsions formulated with Crabwood Oil on human pathogens *Escherichia coli* (ATCC 22319), *Staphylococcus aureus* (ATCC 21013), *Candida albicans* (ATCC 22019), *Trichophyton rubrum* (ATCC 16322) *and Trichophyton mentagrophytes* (ATCC 13566) showed that the oil is effective in inhibiting the growth of these microorganisms. The positive controls were antifungal substances Ketoconazole (Pfizer), Miconazole (Novartis) and a broad-spectrum antibacterial, Ceftriaxone (Pfizer). The emulsions were formulated using the phase inversion temperature method, using 20% Crabwood Oil and a 5%, mix of non-ionic surfactants. For the antimicrobial studies, two emulsions were evaluated that differed in the surfactant blend, Tween 20 (90%) and Span 20 (10%), or Tween 80 (60%) and Span 20 (40%). Each ingredient was tested separately as a method of control. The findings of the research suggest that the emulsion system improved the antimicrobial properties of the oil. Additionally, it was more effective against the fungal species versus the bacteria [172]. A limitation of this study is that it only looked at one concentration of Crabwood Oil emulsion, as such the dose-dependent inhibition of this emulsion is unknown, but it provides insight to the potential antimicrobial uses of Crabwood Oil formulations.

CHAPTER 2

2 Chapter 2: Materials and Methods

2.1 Materials

The Crabwood Oil (CWO) was purchased on July 2023 in Georgetown, Guyana from A. Leitch for GY$2,000 or ~ US$13 a litre. The CWO produced at Ebini Upper Demerara Berbice, Guyana was extracted using an artisanal method like what is described in **Section** Error! Reference source n ot found., paragraph 2. A liquid and solid sample of Crabwood Oil (CWO) naturally separated and purchased on August 2021 from the same supplier were also studied.

Ethanol, acetone, ethyl acetate (purity> 90%) (Sigma-Aldrich, Oakville, Ontario) and HPLC grade chloroform and methanol were purchased at VWR, Mississauga ON, Canada. Pierce ™ LTQ ESI positive ion calibration solution (ThermoScientific) was obtained from the Trent Water Quality Centre. Cocoa Butter (CB) and Shea Butter (SB) were characterized in the same way as CWO and its fractions to allow for comparison and were purchased on January 2023 from New Directions Aromatics (Mississauga ON, Canada).

2.2 Crabwood Oil Fractionation

The CWO was fractionated using dry fractionation and solvent fractionation with three solvents with varying polarities, namely ethanol acetone and ethyl acetate. Before fractionation, the oil was melted in a water bath at 40-45 ℃.

2.2.1 Dry Fractionation

The Dry fractionation of CWO was performed following a modified method from the literature [173-175]. It was carried out under stirring using a 2L jacketed reactor, fitted with a Isotemp™ temperature regulating system (FisherScientific). The temperature which was first set at 40 °C under stirring at 50 rpm for 10 min, was reduced by 1 °C/h then left at room temperature (18 °C) for 15 hours under stirring. The slurry was then cooled at 1 °C/h to 15 °C and left at this temperature for 17 hours, under 250 rpm stirring. The liquid fraction was filtered using Fisherbrand P8 filter paper under moderate vacuum.

2.2.2 Solvent Fractionation

The fractionation with solvents was performed according to an established method [84-86, 176]. In each solvent fractionation, 4 parts solvent were added to 1 part of the fully melted CWO: 200g CWO were used for fractionations with ethanol and acetone and 50 g CWO for the fractionation with ethyl acetate. The mixture was stored at 4 °C for 72 hours, to allow the stearin to solidify while the olein remained liquid. The relative polarity of the solvents, mass of CWO used, storage time and agitation speed and time used in the fractionation are listed in **Table 2-1**

Table 2-1: Solvent polarities, mass of CWO used, storage time and agitation speed and time.

Solvent	**Relative Polarity**	**Mass of CWO (g)**	**Storage time (h)**	**Agitation**	
				Speed (rpm)	**time** (hours)
Ethyl acetate	0.228	50.1	72	50 rpm	24
Acetone	0.355	200	72	50 rpm	24
Ethanol	0.654	200	72	50 rpm	24

The soluble and the insoluble parts were separated using Fisherbrand P8 filter paper under moderate vacuum. The solvent was removed by rotary evaporation (vacuum= 15 psi, bath temperature (30-40 ℃) and slow rotation (30 rpm). The soluble and insoluble fractions were weighed, stored in closed bottles and kept refrigerated until further analysis.

2.2.3 Nomenclature

The nomenclature shown in **Table 2-2** is used in this work to distinguish between the different fractions. The "soluble fractions" refers to the fractions which dissolved in the solvent and were collected by rotary evaporation. The "Insoluble fractions" refers to the fractions that precipitated and collected by vacuum filtration. The fractions obtained with the dry fractionation are simply labelled "Dry solid" and "Dry liquid" for the solid and liquid fractions, respectively.

Table 2-2. Nomenclature of Crabwood Oil samples and fractions.

	Name	**Abbreviation**
As purchased	Native solid	N Sol
	Native liquid	N Liq
As purchased	Native Crabwood Oil	CWO
Fractions		
Dry	Dry solid	D Sol
	Dry liquid	D Liq
Ethanol (Et)	Soluble in Et	Et Sol
	Insoluble in Et	Et Ins
Acetone (Ac)	Soluble in Ac	Ac Sol
	Insoluble in Ac	Ac Ins
Ethyl Acetate EA	Soluble in EA	Ac Sol
	Insoluble in EA	Ac Ins

2.3 Analytical Methods

2.3.1 Electrospray Ionization Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) was used to identify the lipids and bioactive compounds of CWO. ESI-MS is a method commonly used to characterize forest seed oils like *Carapa guianensis* [2, 102]. The measurements were performed on a QExactive Orbitrap mass spectrometer (ThermoScientific, San Jose, CA, resolution of 140,000 full width at half maximum (FHWM); at 200 *m/z*) located at Trent Water Quality Centre (Trent University, Peterborough, ON, Canada). The Orbitrap was calibrated daily using a Pierce™ LTQ ESI positive ion calibration solution (ThermoScientific). The samples (1ppm w/v) were prepared using chloroform: methanol 70:30 (v/v) [177]. The QExactive Orbitrap generated datafiles that were analyzed using the Qual Browser tool in the Thermo Xcalibur application package (ThermoScientific).

2.4 Physical Methods

The crystal structures of CWO and its fractions were determined by X-Ray Diffraction (XRD) and their microstructures by Polarized Light Microscopy (PLM). Differential Scanning Calorimetry (DSC) was used to determine the crystallization and meting behaviors. The Solid Fat Content (SFC) was measured by pulsed NMR. The thermal stability of the materials was evaluated by Thermal Gravimetric Analysis (TGA).

2.4.1 X-Ray Diffraction

The X-Ray Diffraction (XRD) measurements were performed on an Empyrean X-ray diffractometer equipped with Cu-Kα radiation (λ= 0.1542nm, 45kV and 40mA) and a PIXcel^{3D} detector (PANalytical B.V., Lelyweg, The Netherlands). The melted and homogenized material was loaded into glass capillary tubes (Hampton Research, California USA), left to crystallize at

room temperature (RT= 18 °C) for 8-10 days and measured at RT. The XRD patterns were recorded between 0.5° and 36° (2θ) in 0.013° steps, with 250 s intervals. The procedure was automated and controlled by the PANalytical's Data Collector (V 3.0a) software.

2.4.2 Polarized Light Microscopy

A Leica DM2500P (Leica Microsystems, Wetzlar, Germany) fitted with a Linkam LS 350 temperature-controlled stage (Linkam Scientific Instruments, Tadworth, Surrey, UK) was used to determine the microstructure and crystal habit of the Crabwood Oil and its fractions. The sample was heated to 40 °C to obtain a homogenous melt then a small drop was carefully placed between a preheated glass slide and coverslip to obtain a thin and uniform layer. The samples were then left at room temperature (RT= 18 °C) for 7-10 days to allow the fat to crystallize. The samples were imaged at different magnifications (50×, 100× and 500×) under cross polarizers and captured using a digital camera (DFC 420C, Leica Microsystems, Wetzlar, Germany) mounted to the PLM.

2.4.3 Differential Scanning Calorimetry

The thermal transition behavior was determined using a Q200 DSC (T.A. Instruments, Newcastle, DE, U.S.A.) equipped with a refrigerated cooling system. The sample (4-6 mg) in hermetic aluminum pans was equilibrated at 60 °C for 5 min to erase the thermal history, then cooled at 5 °C/min down to -60 °C where it was held isothermally for 5 min to evaluate the crystallization path, and then heated back to 60 °C at 10°C/min to measure the melting behavior.

2.4.4 Thermogravimetric Analysis

The thermal stability of the CWO and its fractions was analyzed using thermogravimetric analysis (TGA) on a Q500 (TA Instruments, Newcastle, DE, USA). The sample (10-15mg) was heated from 25 °C to 600 °C at 10 °C/min under a nitrogen flow of 60.0 mL/min. The onset temperature

of mass loss was determined at 5 % w/w ($T_{5\%}^{on}$) as is typical in the field. The derivative of the TGA (DTG) was used to assess the mechanisms of mass loss and related parameters such as maximum rates of mass loss (RML).

2.4.5 Solid Fat Content Measurements

The solid fat content (SFC) was measured on a Bruker Minispec mq 20 (Milton, Ontario, Canada) pulsed NMR (pNMR) spectrometer equipped with a combined high and low-temperature probe and a BVT3000 temperature controller (Bruker Ltd, Milton, ON). Liquid nitrogen was used for tempering. Bruker's Minispec V2.58 Rev. 12 and Minispec plus V1.1 Rev. 05 software were used to collect SFC data. The temperature was calibrated with highly unsaturated canola oil, using an external data logger (Oakton, Eutech Instruments, Singapore) and a type K probe (TRP-K, Omega, Stamford, Connecticut) immersed in the oil. The temperature is controlled to better than ± 1 °C.

The fully melted sample was pipetted into the bottom of an NMR tube filling its bottom 1 cm (~0.57 ± 0.05 ml) and measured according to prescribed protocols. In one experiment, the samples were stored at room temperature (18 °C) for 8-10 days, measured at that temperature then melted in situ and progressively cooled down in small steps (2 °C) to allow for the better control of the probe temperature and equilibration of the sample. The SFC is measured at each temperature isothermally until a plateau is reached. In another experiment, the samples were stored at 4 °C for several days and measured at that temperature.

2.5 Statistical Analysis

The statistical analysis of the data which assumptions of normality are met was performed using the Sigmastat module of Sigmaplot 12.5 software. Analysis of variance (ANOVA) followed by Tukey test was carried out to assess the differences in the measured variables between the fractions

and determine the relationship between them. Pearson Product Moment Correlation Analysis which describes the linear relationship between two quantitative variables was carried out to determine the strength and direction of correlation between them.

CHAPTER 3

3 Chapter 3: Results and Discussion

The dry and solvent fractionation of Crabwood Oil (CWO) yielded four solid and four liquid fractions; the solid and the liquid fractions generally referred to as stearin and olein, respectively. The nomenclature provided in **Table 2-2** is used throughout the thesis. The CWO fractionation results including the mass balance and yields are discussed in **Section 3.1**. The MS data, collected according to the method described in **Sections 2.3** are presented and discussed in **Section 3.2**. **Section 3.2.1** presents the lipid and unsaponifiable composition of CWO. **Section 3.2.2** presents the relative abundances of the unsaponifiable compounds found in CWO and its fractions and discusses the partitioning of the unsaponifiable compounds between the solid and liquid fractions as a function of the fractionation methods and solvents. The results of physicochemical functionality analysis, including crystal structure, microstructure, thermal transition behavior, thermal degradation and solid fat content (SFC), determined according to the methods outlined in **Sections 2.4**, are presented and discussed in **Sections 3.3 to 3.7**. The physical functionality of Cocoa Butter (CB) and Shea Butter (SB), commonly used oils in the cosmetic industry, which were similarly studied as reference oils, were compared to CWO and its fractions.

3.1 Fractionation Results

Pictures of the fractions in transparent glass bottles taken after several days at room temperature (RT=18 °C) are shown in **Figure 3-1**. Color, fat/liquid appearance and texture of the fractions are noted below each image.

Stearins	**CB**	**SB**	**N Sol**	**D Sol**	**EA Ins**	**Ac Sol**	**Et Sol**
							
Color	Cream	Light yellow	Light yellow	White	Ivory	Light yellow	Ivory
Appearance	Solid fat	Semi-solid	Semi-solid	Semi solid Fat	Semi solid	Semi-solid	Semi solid Fat
Texture	Melts on skin contact	Melts on skin contact	Melts on skin contact	Melts on skin contact	Melts on skin contact	Melts on skin contact	Melts on skin contact
Oleins	**CWO**		**N Liq**	**D Liq**	**EA Sol**	**Ac Ins**	**Et Ins**
Picture							
Color	Light Amber		Amber	Amber	Ivory	Off white	Light amber
Appearance	Crystals dispersed in the liquid		liquid	Liquid	Semi solid	Semi-solid	Liquid
Texture	Flows freely		Very few sedimented crystals	Very few sedimented crystals	Melts on skin contact	Melts on skin contact	Dispersed and sedimented crystals

Figure 3-1. Physical appearance of the fractions at room temperature (RT=18 ℃)

3.1.1 Mass Balance and Yields

The mass balance and yields from the different fractionations of CWO are listed in **Table 3-1**.

Table 3-1. Mass balance and yield percentages for fractionation of crabwood oil with solvents of varying polarity.

Method	Solvent	CWO mass (g)	Mass loss (g)	Mass loss (%)	Mass (g)			Yield (%)	
					Liquid	Solid	Total	Olein	Stearin
Dry Fractionation	**Relative Polarity**	255.7	3.4	1.3	177.5	74.8	252.3	69.4	29.3
Ethyl Acetate	0.228	50.1	3.5	7.0	43.5	3.1	46.6	86.8	6.2
Acetone	0.355	200.1	5.5	2.7	46.3	148.3	194.6	23.1	74.1
Ethanol	0.654	200.0	4.7	2.4	114.7	80.6	195.3	57.4	40.3

The losses are mainly due to the oil /fat remaining in the filter paper and in the containing glass vessels and in the reactor used for the dry fractionation (405 and 60% respectively). **Table 3-1** demonstrate that the yield of the "soluble" fractions decreased from 86.8% to 40.3% as the polarity of the solvents increased, emphasizing the immense influence of polarity on degree of fractionation. This large drop of the soluble yield with increasing polarity is consistent with the decrease in solubility, in polar solvents, of TAGs which are the main components of CWO [178]. Pearson Product Moment Correlation Analysis indicated a very strong negative correlation between the soluble fraction yields and relative polarities ($r = -0.9994$ and $p = 0.02$).

The yield of the stearin from acetone fractionation is closest to that of the dry fractionation (23.8% vs. 29.6% respectively). Minzangi et al., [15] reported a relatively larger yield (53% vs. 41% for acetone fractionation vs. dry fractionation) for the soluble fraction of *Carapa grandiflora*, a species of Crabwood found in Western Africa [92, 179], obtained by similar methods. This may be

partially explained by the difference in lipid composition between *Carapa guianensis* and *Carapa grandiflora* with approximately 30% and 39% of saturated fatty acids, respectively [101, 180].

The soluble component of CWO in ethanol and acetone presented the characteristics of stearin fractions and their insoluble component the characteristics of olein fractions whereas, the soluble component in ethyl acetate was an olein and the insoluble component a stearin. Given that the conditions of the fractionation were similar, this result is primarily attributed to the decrease in affinity for the oil's components as the polarity decreases.

3.2 Chemical Analysis

The ESI-MS spectra, in positive ion mode, of CWO and its fractions are provided in Appendix A. An example (the ESI-MS spectrum of CWO) is shown in **Figure 3-2**.

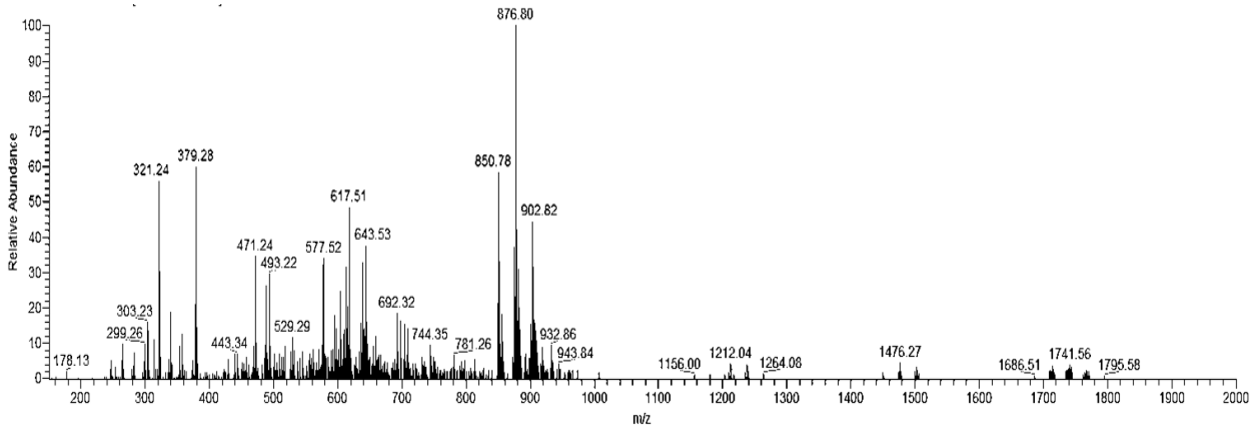


Figure 3-2. ESI-MS spectrum of CWO.

The Thermo XCalibur [181] software was used to process the spectrum data and obtain lists of detected masses for each compound. The putative compounds were identified using online metabolomic and lipidomic tools, namely, MetaboQuest [182] and LIPID MAPS [183]. A putative compound, at a given *m/z* $[M + X= Na, NH_4 \text{ or } H]^+$ value is considered if the difference between

the theoretical and observed values is ±10 ppm or lower as is the normal approach in mass spec analysis [184]. Five (05) classes of lipids were detected: TAG, DAG, MAG, Free Fatty Acids (FFA) and phospholipids. Four (04) classes of unsaponifiable compounds were detected: Sterols, Limonoids, one Flavonoid and one Tocopherol. The relative abundance of a compound is typically represented by the most dominant adduct which is ordinarily (*m/z* $[M + Na]^+$) [106].

The results from MS for CWO are tabulated in **Table 3-2** for (a) TAGs, (b) DAGs and MAGs and (c) free fatty acids. The putative unsaponifiable compounds found in the Crabwood Oil and its fractions are tabulated in **Table 3-3**.

Table 3-2. Relative abundance percentages of assigned lipids in the Crabwood Oil sample. The most predominant compound in each group is highlighted.

(a) Triacylglycerols (TAGs)

TAG#	**Elementary Composition**	**Abbreviated Name**	***m/z* $[M+Na]^+$**	**Relative Abundance (%)**
TAG1	C53H96O6	PPLn	851.7169	4.3
TAG2	C53H98O6	PPL	853.7239	4.4
TAG3	C53H100O6	PPO	855.7383	13.8
TAG4	C53H102O6	PPS	857.7597	3.0
TAG5	C55H98O6	PLL	877.7338	9.1
TAG6	C55H100O6	PLO	879.7347	5.1
TAG7	C55H102O6	POO	881.7672	21.5
TAG8	C55H104O6	POS	883.782	9.3
TAG9	C55H106O6	PSS	885.7788	1.9
TAG10	C57H100O6	LLO, OOLn	903.749	4.4
TAG11	C57H102O6	OOL, LLS	905.7879	6.0
TAG12	C57H104O6	OOO	907.7817	9.0
TAG13	C57H106O6	OOS, SSL	909.7823	7.1
TAG14	C58H108O6	TAG (18:0/18:2/19:0)*	923.8136	0.8
TAG15	C57H110O6	SSS	913.8292	0.2
Total				100

Fatty acid abbreviations: P, Palmitic acid; Po, palmitoleic acid; O, oleic acid; S, stearic acid; L, linoleic acid; Ln, linolenic acid.
* 1-octadecanoyl-2-(9Z,12Z-octadecadienoyl)-3-nonadecanoyl-sn-glycerol.

(b) Diacylglycerols (DAGs) and Monoacylglycerols MAGs. The most predominant compound in each group is highlighted.

DAG/MAG#	Elementary Composition	Putative Compounds	*m/z* $[M+Na]^+$	Relative Abundance %
DAG 1	C36H66O5	1-hexadecanoyl-2-(9Z,12Z-heptadecadienoyl)-sn-glycerol (16:0-17:2)	601.4746	0.3
DAG 2	C36H68O5	1-(9Z-hexadecenoyl)-2-heptadecanoyl-sn-glycerol DG (16:1/17:0/0:0) [iso2]	603.4942	1.7
DAG 3	C37H68O5	1-hexadecanoyl-2-(9Z,12Z-octadecadienoyl)-sn-glycerol (DG (18:2/16:0)	615.4947	6.9
DAG 4	C37H70O5	1-Oleoyl-2-palmitoylglycerol	617.5125	15.9
DAG 5	C37H72O5	1-stearoyl-2-palmitoyl-sn-glycerol (DG (16:0_18:0)	619.5258	2.2
DAG 6	C37H72O4	1-O-hexadecyl-2-(9Z-octadecenoyl)-sn-glycerol DG(O-16:0/18:1)	603.5363	8.5
DAG 7	C37H70O6	1,2-Dipalmitoyl 3-acetyl glycerol	633.5044	0.8
DAG 8	C39H72O5	1,3-Diolein	643.5267	13.1
DAG 9	C39H70O5	1-(9Z-octadecenoyl)-2-(9Z,12Z-octadecadienoyl)-sn-glycerol (DG (18:1/18:2/0:0))	641.5187	4.4
DAG 10	C39H74O5	1-octadecanoyl-2-(9Z-octadecenoyl)-sn-glycerol DG (18:0_18:1)	645.5441	4.4
DAG 11	C41H64O5	1-(6Z,9Z,12Z,15Z-octadecatetraenoyl)-2-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycerol DG (18:4_20:4)	659.4695	0.4
DAG 12	C39H68O5	1,3-dilinoleoyl-sn-glycerol-d5	644.5342	5.5
DAG 13	C34H64O5	1-pentadecanoyl-2-(9Z-hexadecenoyl)-sn-glycerol (DG 15:0-16:1)	575.462	0.5
DAG 14	C34H66O5	1-pentadecanoyl-2-hexadecanoyl-sn-glycerol DG (15:0/16:0/0:0)	577.4835	3.3
MAG 1	C19H38O4	2-Monopalmitin	353.2661	3.6
MAG 2	C21H38O4	rac-1-monolinoleoylglycerol	377.2693	7.4
MAG 3	C21H40O4	2-monoolein	379.2857	19.3
MAG 4	C21H42O4	1-Monostearin	381.2972	1.7
Total				100

(c) Free Fatty Acids (FFA)

FFA #	**Elementary Composition**	**Compound Name**	***m/z* [M+Na]$^{+}$**	**Relative abundance %**
FFA 1	C18H32O2	Linoleic acid (C18:2)	303.2298	11.2
FFA 2	C18H34O2	Oleic acid (C18:1)	305.2479	9.2
FFA 3	C18H34O3	10-keto stearic acid / 2-Hydroxyoleic acid	321.2408	43.7
FFA 4	C18H30O2	Linolenic acid (C18:3)	301.2166	0.1
FFA 5	C18H36O3	15-hydroxy stearic acid	323.2542	24.0
FFA 6	C19H38O3	3-hydroxy-11-methyl-stearic acid	337.2697	3.9
FFA 7	C22H44O2	Behenic acid (C22:0)	363.3261	5.1
FFA 8	C16H30O2	Palmitoleic acid (C16:1)	277.2154	0.2
FFA 9	C16H32O2	Palmitic acid (C16:0)	279.2311	2.6
FFA 10	C20H38O2	Gadoleic acid (C20:1)	333.2758	0.1
FFA 11	C24H48O2	Lignoceric acid (C24:0)	391.3561	0.1
Total				100

Table 3-3. Relative abundance percentages of assigned unsaponifiable components in the Crabwood Oil (a) limonoids, (b) sterols, (c) other unsaponifiable components. The most predominant compound in each group is highlighted.

(a) Relative abundance percentages of assigned limonoids in the Crabwood Oil.

***m/z*[M+Na]$^+$**	**Elemental Composition**	**#**	**Putative compound**	**CWO**	**D Sol**	**D Liq**	**Et Sol**	**Et Ins**	**Ac Sol**	**Ac Ins**	**EA Sol**	**EA Ins**	**N Liq**	**N Sol**
461.1940	C26H30O6	L1	7-Deacetoxy-7-Oxogedunin	3.9	5.3	5.4	3.0	4.9	3.3	5.5	4.8	3.9	4.6	1.4
463.2097	C26H32O6	L2	7-deactylgedunin	0.5	0.7	0.5	0.3	0.3	0.3	0.4	0.6	0.5	0.5	0.2
489.2253	C28H34O6	L3	Deoxygedunin	0.1	3.5	3.5	0.6	2.6	0.9	3.9	0.5	1.1	0.2	0.9
505.2202	C28H34O7	L4	Gedunin	4.2	6.1	5.7	2.7	4.9	3.1	5.7	4.9	4.2	5.2	2.5
563.2257	C30H36O9	L5	6α-acetoxygedunin	4.1	6.1	5.7	3.1	4.7	3.5	5.5	4.5	4.3	8.5	2.9
491.2046	C27H32O7	L6	Andirobin	5.9	6.4	6.9	5.1	6.7	5.4	6.7	5.5	4.8	4.3	5.3
493.2202	C27H34O7	L7	Methyl angolensate	27.9	34.3	35.2	33.1	34.2	33.3	34.8	29.6	34.8	38.7	32.3
509.2151	C27H34O8	L8	Methyl 6- hydroxyangolensate	6.2	4.9	6.4	5.2	5.4	4.8	6.9	6.1	5.6	5.2	4.5
507.1995	C27H32O8	L9	8beta-Hydroxycarapin, 3,8-Hemiacetal	0.04	0.1	0.1	0.04	0.02	0.02	0.03	0.04	0	0	0.1
613.2625	C31H42O11	L10	Carapanolide A	0.9	0.01	0.02	1	0.22	0.6	0.1	0.4	0.5	0.4	1.1
625.2625	C32H42O11	L11	Carapanolide B	0.7	0.01	0.01	0.9	0.1	0.9	0.03	0.5	0.7	0.1	2
747.2993	C39H48O13	L12	Carapanolide K	0.6	0	0	0.02	0	0.01	0	0.1	0	0.02	0
665.2210	C33H38O13	L13	Carapanolide L	1.3	0.9	0.6	1.4	0.8	1.2	0.7	1	1.1	0.4	1.5
735.2265	C36H40O15	L14	Guianolide A	0.4	0.03	0.04	0.5	0.08	0.3	0.04	0.1	0.2	0.1	0.3
693.2159	C34H38O14	L15	Guianolide B	0.01	0	0	0.03	0.01	0.01	0	0	0	0	0
667.2730	C34H44O12	L16	11-Acetoxykhivorin	6.8	5.1	4.8	7.3	6	6.9	5.3	6.9	6.5	4.1	0
697.2836	C35H46O13	L17	Trichilin A	17.9	14.6	14.4	21.3	16.2	19.9	12.9	18.3	16.9	16.5	22.4
473.2304	C28H34O5	L18	Azadiradione	2.8	2.4	2.9	1.3	1.4	1.6	2.4	2	1.5	3.9	0.2
597.2676	C31H42O10	L19	Asclepin	14	8.4	6.6	12.3	9.5	11.7	7.9	12.1	11	6.8	19.6
771.2522	C29H48O22	L20	NCGC00381438-01	1.8	1.2	1.4	0.9	1.7	2.5	1.3	2.1	2.2	0.6	2.8
Total				100	100	100	100	100	100	100	100	100	100	100

(b) Relative abundance percentages of assigned sterols and phosphoglycerols (PG) in the Crabwood Oil.

***m/z*[M+Na]$^+$**	**Elemental Composition**	**#**	**Compound**	**CWO**	**D Sol**	**D Liq**	**Et Sol**	**Et Ins**	**Ac Sol**	**Ac Ins**	**EA Sol**	**EA Ins**	**N Liq**	**N Sol**
709.2965	C33H51O13P	PG 1	PI 24:6 (1-stearoyl-2-arachidonoyl-sn-glycero-3-phospho-(1'-myo-inositol)	43.3	68	66	82.9	68.7	76.9	60.6	60.3	63.6	37.9	64.2
473.3243	C27H46O5	Sterol 1	Certonardosterol L	12.4	0.1	0.4	0	0	0	0	0	0	0	0
421.3082	C27H42O2	Sterol 2	24-keto-25dehydrocholesterol	1	0.9	0.4	2.2	1.1	1.9	0.7	1.8	1.6	0	29.4
527.2621	C28H40O8	Sterol 3	Ixocarpalactone A	2.6	0.1	1.2	0.7	0.6	0.7	0.2	0.9	1.1	0.3	3.1
529.3141	C29H46O7	Sterol 4	2-deoxy-20-hydroxy-5alpha-ecdysone 3-acetate	21.5	26	25.3	13.5	24	19.5	30.3	35.8	32.4	58.2	3
531.3662	C30H52O6	Sterol 5	6beta-acetoxy-24-methylcholestan-3beta,5alpha,22R,24-tetrol	18.2	0.8	0.5	0	0.04	0.03	0.2	0	0	0	0.1
551.3196	C28H48O9	Sterol 6	Campesterol	0.4	3.1	1.4	0.7	0.7	0.6	1.1	1.2	1.3	3.5	0
435.3603	C29H48O	Sterol 7	Stigmasterol	0.6	0.9	4.7	0	4.9	0.4	6.9	0	0	0.1	0.3
Total				100	100	100	100	100	100	100	100	100	100	100

(c) Relative abundance percentages of other unsaponifiable compounds in the Crabwood Oil.

***m/z*[M+Na]$^+$**	**Elemental Composition**	**#**	**Compound**	**CWO**	**D Sol**	**D Liq**	**Et Sol**	**Et Ins**	**Ac Sol**	**Ac Ins**	**EA Sol**	**EA Ins**	**N Liq**	**N Sol**
797.2785	C42H46O14	Flavonoid 1	(2R,3S)-2,3-trans-3-Acetoxy-5-[(2R,3S)-2,3-trans-3-acetoxy-3',4',7,8-tetramethoxyflavan-5-yl]-3',4',7,8-tetramethoxyflavan	77.5	74.6	69.8	75.5	72.1	73.6	76.7	72.9	75.9	0	55
615.4237	C35H60O7	Tocopherol 1	alpha-Tocopherol-beta-D-mannoside	1.5	9.3	2.1	2.3	3.7	2.4	1.4	0.7	0	0	10.9
771.2894	C43H44N2O10	Diterpenoid 1	Milliamine C; 5-Anthraniloyl type moiety, 20-Ac-Ingenol diterpenoid	21.0	16.1	28.1	22.2	24.2	24.0	21.9	26.4	24.1	100	34.2
Total				100	100	100	100	100	100	100	100	100	100	100

3.2.1 Molecular composition of the Crabwood Oil

3.2.1.1 Assessment of the Lipids in the Crabwood Oil

3.2.1.1.1 Triacylglycerols

The relative abundance percentages of TAGs present in CWO are tabulated in **Table 3-2** and presented in **Figure 3-3**, and indicate that the main TAG is 1,2-dioleoyl-3-palmitoyl glycerol (POO: 21.5%) followed by 1,2-dipalmitoyl-3-oleoyl glycerol (PPO: 13.8%) (**Scheme 1-1**). The TAG composition of the Crabwood Oil and their relative distribution are similar to the ranges already reported in the literature [105, 106].

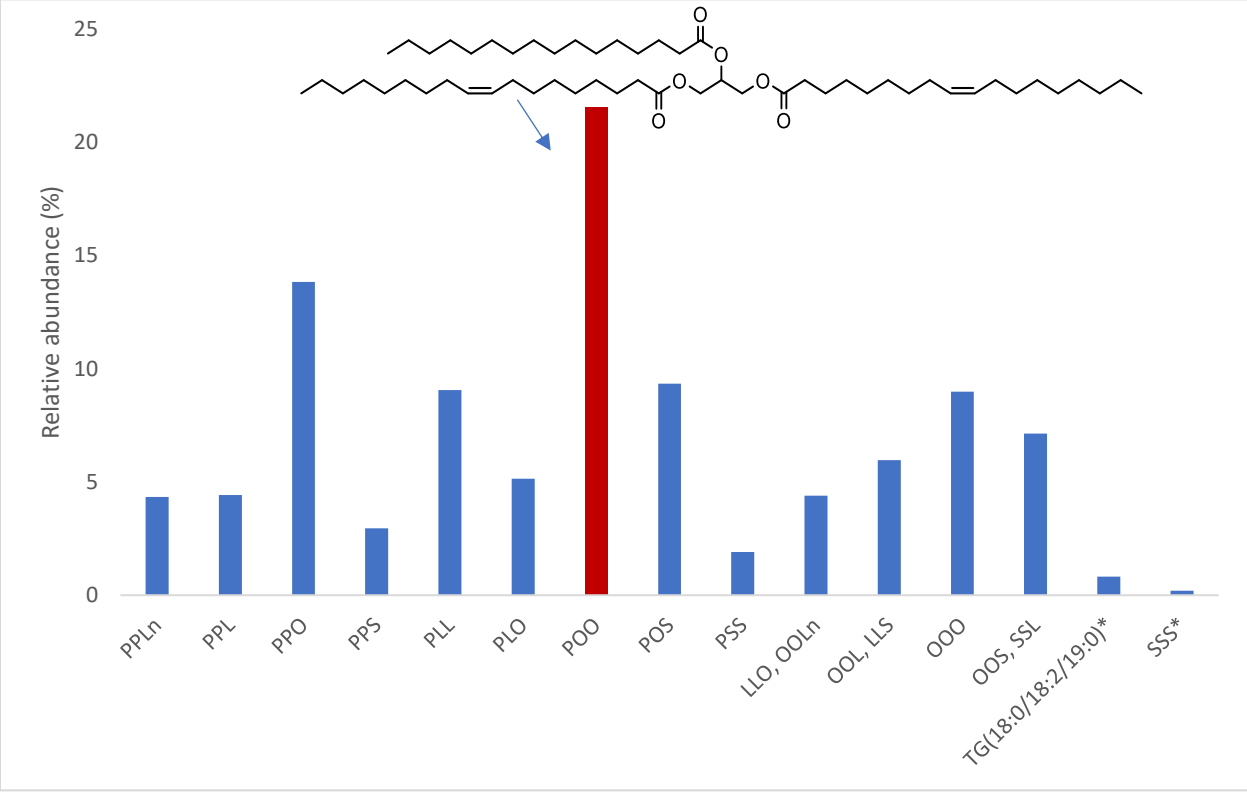


Figure 3-3: Relative abundance percentages of TAGs, found in the Crabwood Oil, the bar in red represents 1,2-dioleoyl-3-palmitoylglycerol (POO) the most predominant TAG.

3.2.1.1.2 Diacylglycerols, Monoacylglycerols and Free Fatty acids

The abundances of the DAGs and MAGs detected in CWO (**Table 3-2**b) are represented in **Figure 3-4**. The most predominant DAG and MAG are 1-oleoyl-2-palmitoylglycerol (PO) and monoolein, respectively. The dominance of PO correlates with POO, the most dominant TAG in CWO. This is understandable because DAGs can be formed from TAG hydrolysis and are also immediate precursors to TAG synthesis [107].

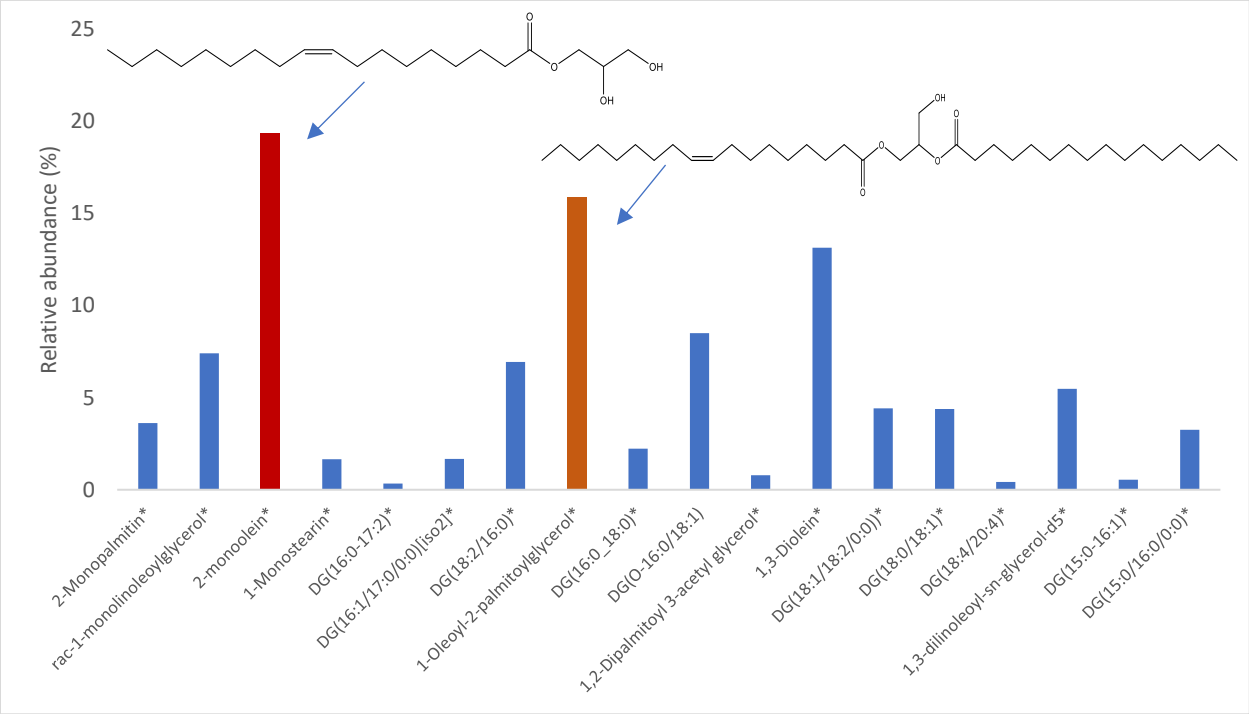
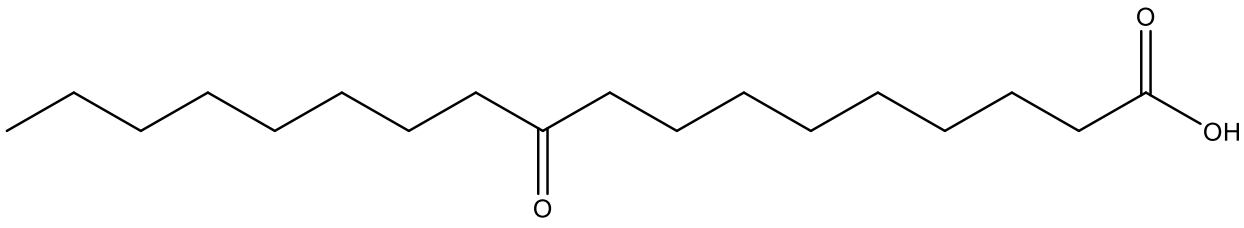


Figure 3-4. Relative abundances of MAGs and DAGs found in CWO.

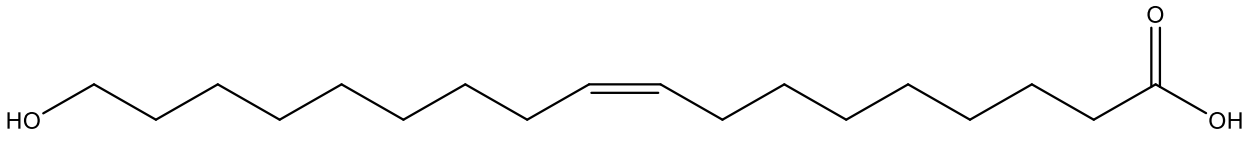
The natural free fatty acid composition of CWO given in **Table 3-2**c is a function of the storage conditions, level of moisture and age of the sample, as well as the means by which the oil was extracted. Saturated FFA account for 35% of the total FFA. **Table 3-2**c shows the combined 10-oxooctadecanoic acid (10-Ketostearic acid)/ ((Z)-18-hydroxyoctadec-9-enoic acid (18-hydroxyoleic acid)) as the most abundant compounds of this group (43.7% of the total FFA). These

could not be discriminated because they have the same molecular mass (***m/z*[M+Na]$^+$** = 321.2408). Their chemical structures are shown in **Scheme 5**. To the best of our knowledge, these very interesting, particularly from an industrial perspective, long fatty acids have not been reported in CWO before. They are also essential nutrients and have been shown to play an important role in the energy metabolism regulation [185]. Ketostearic acid has been identified in *Aloe ferox*, *Gracilariopsis longissima*, and *Galeopsis bifida* [186] and 18-hydroxyoleic acid was found in *Arabidopsis thaliana* [187]. Their presence in CWO may be specific to the Guyanese species of *Carapa guianensis* or due to bacterial biotransformation. Bacteria which could be responsible may be present in the Guyanese *Carapa guianensis* and activated during the fermentation of the seeds. Biotransformation by gut lactic acid bacteria of polyunsaturated fatty acids such as linoleic acid to hydroxy fatty acids and other long chain fatty compounds is well documented [188]. The origin and possible enhancement and separation of these fatty acids in CWO should form the basis of additional research.

The second most abundant FFA (24%) is 15-hydroxyoctadecanoic acid (15-hydroxy stearic acid). Of notable mention is the high relative abundance of linoleic acid (11.2% of the total FFA) which is an Omega 6 fatty acid [189]. Linoleic acid is the parent compound of omega 6 fatty acids such as γ-linolenic acids (GLA) and arachidonic acid [190], and α-linolenic acid (ALA), an omega 3 fatty acid [191].



(a)

(b) 

Scheme 5: (a) 10-oxooctadecanoic acid (10-Ketostearic acid), (b) (Z)-18-hydroxyoctadec-9-enoic acid (18- hydroxyoleic acid).

3.2.1.1.3 Fatty Acid distribution in TAGs, DAGs and MAGs

The relative abundance of the fatty acids in the TAGs, and in the DAGs/ MAGs group is shown in **Table 3-4**. The corresponding percent saturation represented in **Figure 3-5**a-b shows that both groups are largely unsaturated. These results are similar to what has been reported by Lüdtke et al. [101].

Table 3-4. Fatty acid composition of CWO. The most predominant compound is highlighted.

Elementary Composition	**Compound Name**	***m/z* [M+Na]$^+$**	**Relative abundance %**	
			TAGs	**DAGs and MAGs**
C16H30O2	Palmitoleic acid (C16:1)	277.2154	--	1.1
C16H32O2	Palmitic acid (C16:0)	279.2311	32.7	23.0
C18H30O2	Linolenic acid (18:3)	301.2166	1.7	5.7
C18H32O2	Linoleic acid (C18:2)	303.2298	14.1	12.9
C18H34O2	Oleic acid (C18:1)	305.2479	43.0	49.1
C18H36O2	Stearic acid (C18:0)	307.2612	8.2	5.0
C15H30O2	Pentadecyclic acid (C 15:0)	265.2138	--	1.9
C17H34O2	Margaric acid (C 17:0)	293.2451	--	0.8
C17H30O2	Heptadecadienoic acid (C 17:2)	289.2138	--	0.2
C18H28O2	Stearidonic acid (C 18:4)	299.1981	--	0.2
C19H38O2	nonadecanoic acid (C 19:0)	321.2764	0.3	--
C20H32O2	Arachidonic acid (C 20:4)	327.2294	--	0.2
Total			100	100

(a) (b)

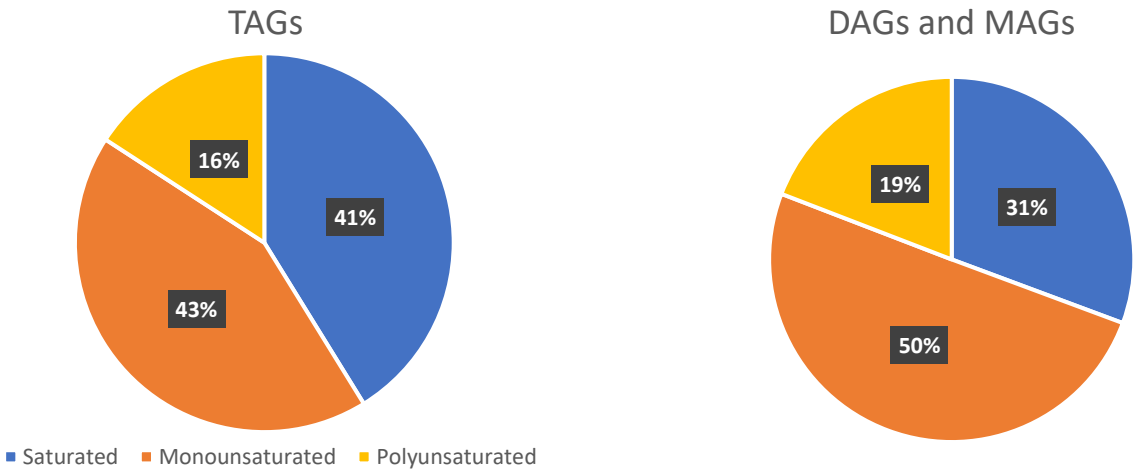


Figure 3-5. Percent saturation in Crabwood Oil detected in (a) TAGs, (b) DAGs/ MAGs.

3.2.2 Assessment of the Unsaponifiable Compounds in the Crabwood Oil Sample and its Fractions

3.2.2.1 Assessment of the Unsaponifiable Compounds in the Crabwood Oil Sample

The limonoids identified in CWO are represented in **Figure 3-6**. As highlighted in **Table 3-3**, the most abundant limonoid in the samples is Methyl angolensate (L7, ~33%), followed by Trichilin A (L17, 17%). Methyl angolensate has cytotoxic [132, 192] and antifungal [137, 193] activities and Trichilin A is a limonoid reported to have major bioactivity including cytotoxic and antifeedant properties. Methyl angolensate has been reported in CWO [110] but Trichilins which have been identified in other Meliaceae species such as Natal mahogany (*Trichilia roka*) [194], have not been reported in CWO. The relative abundance of Methyl angolensate and Trichilin A is large enough to warrant further investigation because of their potential applications related to their bioactivity.

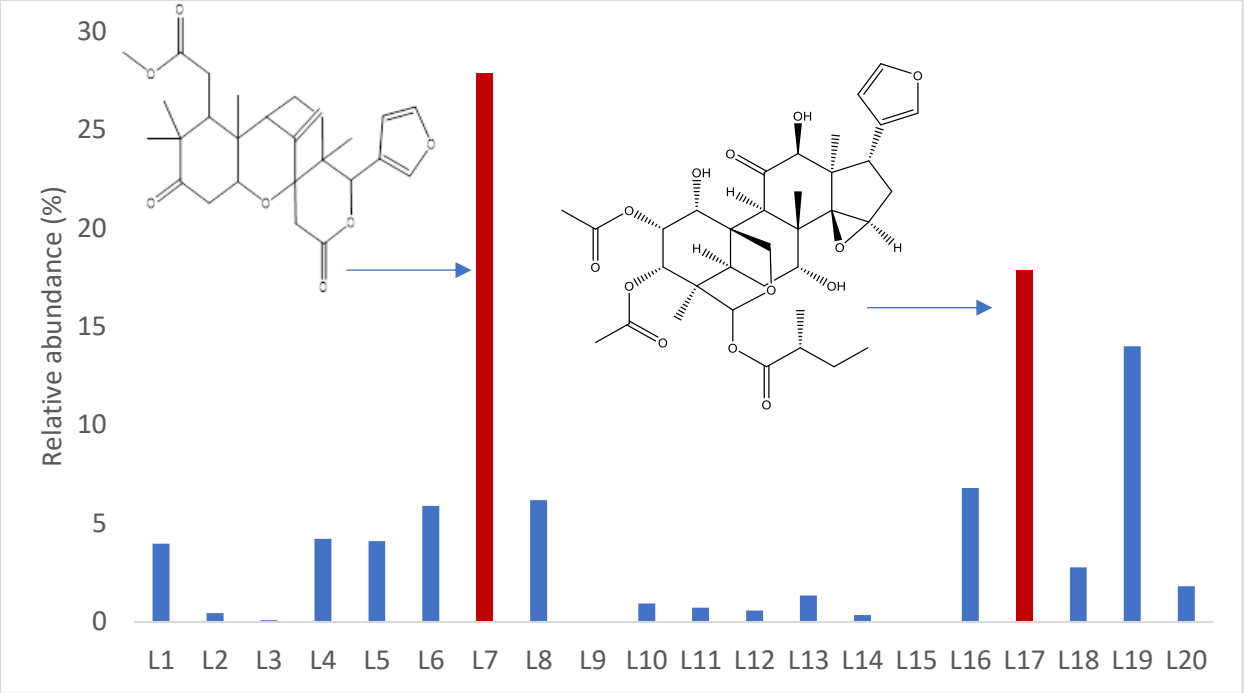


Figure 3-6. Limonoids Identified in the Crabwood oil CWO sample.

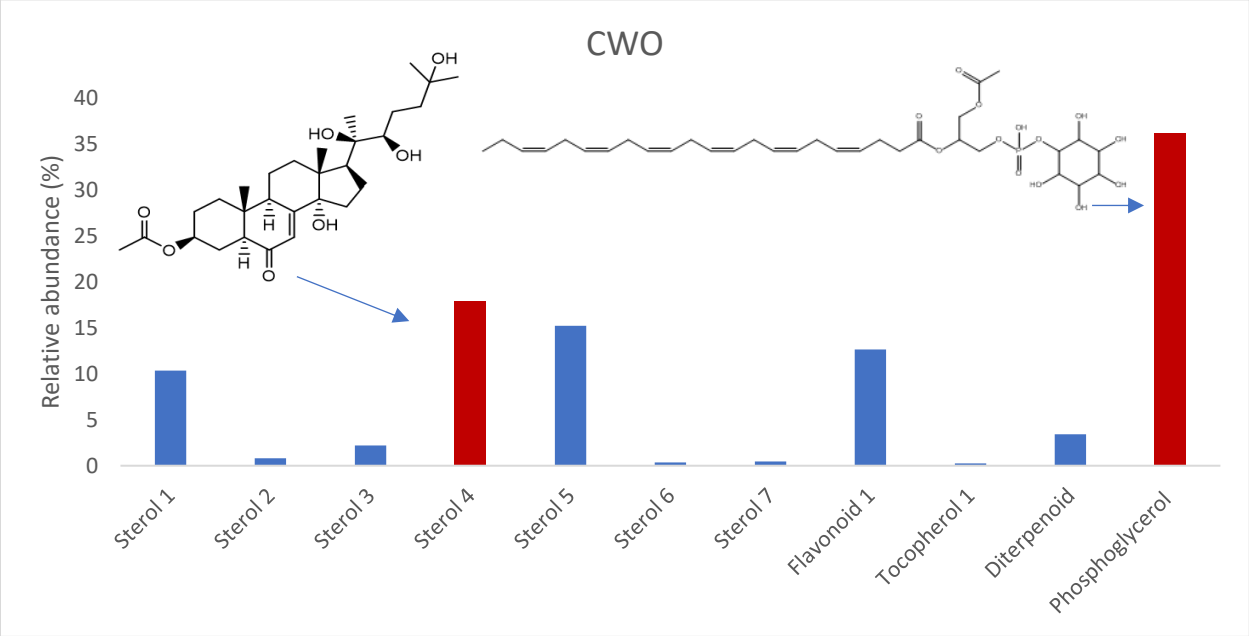


Figure 3-7. Relative abundance of sterols and other unsaponifiable compounds found in the Crabwood oil sample.

The unsaponifiable compounds detected in CWO, listed in **Table 3-3**b-c, include sterols (S1 to S7), a flavonoid, a tocopherol, a diterpenoid and a phosphoglycerol. The relative abundances of these compounds are represented in **Figure 3-7**. The most dominant compound of this group is the phosphoglycerol [1-acetyloxy-3-[hydroxy-(2,3,4,5,6-pentahydroxycyclohexyl) oxyphosphoryl] oxypropan-2-yl] (4*Z*,7*Z*,10*Z*,13*Z*,16*Z*,19*Z*)-docosa-4,7,10,13,16,19-hexaenoate accounting for 36% of the total. This phosphoglycerol has six cis-double bonds [195]. 2-Deoxy-20-hydroxy-5alpha-ecdysone 3-acetate (Sterol 4) is the most predominant in CWO (18%). It belongs to a class called ecdysteroid esters found effective for anticancer activity [196].

3.2.2.2 Assessment of the Unsaponifiable Compounds in CWO Fractions

The unsaponifiable compounds were found in the fractions with differing distribution between the stearin and olein fractions and between the different fractionation methods, particularly those with solvents. The analysis of the relative abundances unambiguously indicate that one can partition important unsaponifiable compounds as a function of fractionation method; in particular there is a direct dependence on solvent polarity.

3.2.2.2.1 Methyl angolensate and Trichilin A

Methyl angolensate (L7) and Trichilin A (L17) are the most predominant limonoids in all the fractions. The partitioning of Methyl angolensate was larger in the fractions from ethyl acetate with 15% more in the stearin compared to the other fractionation method where it was 4 to 3% more abundant in the olein fractions. Asclepin (L19), reported in PubChem [197, 198] with the code NCGC00385378-01) is the third in abundance. It is a compound known as a bioactive including against cancer [199].

3.2.2.2.2 Gedunin class Compounds and Andirobin (L1-L6)

Andirobin and Gedunin class compounds (L1-L6) are listed in **Table 3-3**a and their distribution across the samples shown in **Figure 3-8**. These limonoids are known for their antimicrobial activities including antifungal, antibacterial, antifeedant, insecticidal, antimalarial, anti-allergic, anti-inflammatory, anti-cancer, anti-diabetic, and neuroprotective activities [125, 136].

7-deacetoxy-7-oxogedunin (L1) and Andirobin (L4) are the most predominant in all the fractions. L1 is more prevalent in CWO and L4 is more abundant in the fractions. There were much more Gedunin and Andirobin limonoids in N Liq than in N Sol, in contrast to D Sol and D Liq, which did not display significant difference in total limonoids. Gedunin showed ~7% more in D Sol and Andirobin was ~3% more abundant in D Liq.

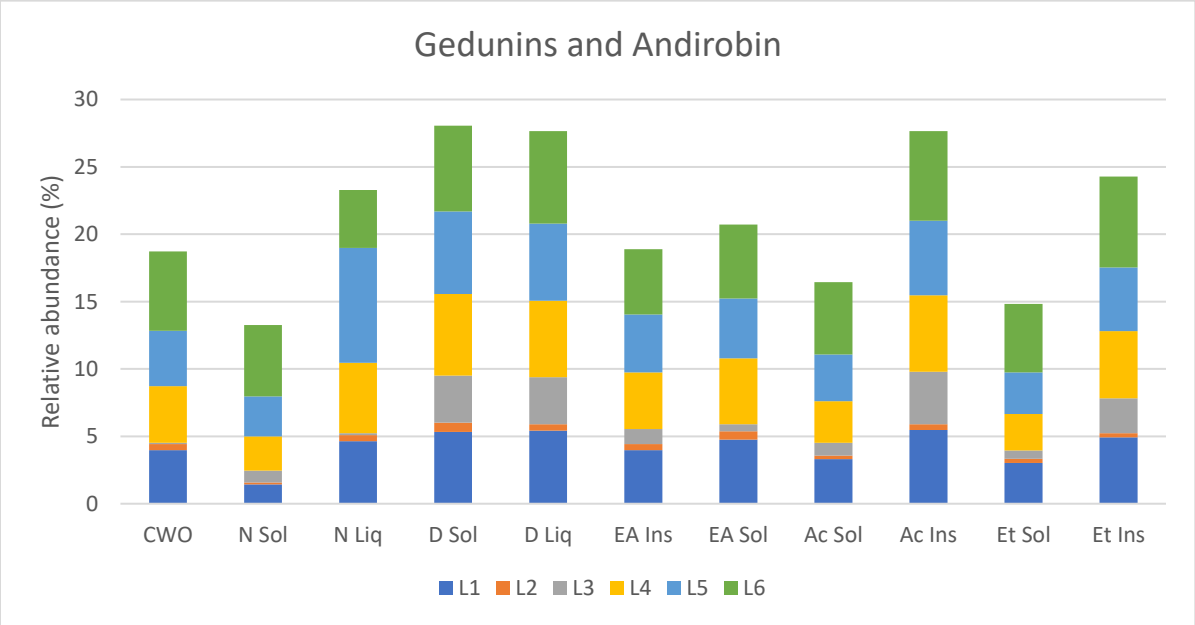


Figure 3-8. Relative percentages of assigned Gedunin and Andirobin limonoids.

In the case of fractionation by solvents, Gedunin and Andirobin were more abundant in the liquid fractions with strong linear correlations with polarity. The difference in their relative abundance

between the olein and stearin fractions significantly increase with increasing polarity. There are 46%, 46% and 13% more Gedunin in the olein fraction from ethanol, acetone and ethyl acetate respectively and 24%, 19% and 13% more Andirobin in the respective olein fractions. The Pearson correlation analysis (**Figure 3-9**) indicated a very strong correlation but weak p-values between the polarity and distribution (r= 0.9912, p= 0.08 for Andirobin and r= 0.9569, p= 0.18 for Gedunin). This data indicate the solubility of these limonoids, while depending on the polarity of the solvent, is influenced by other parameters such as the details of the structures and fractionation conditions such as the temperature [200].

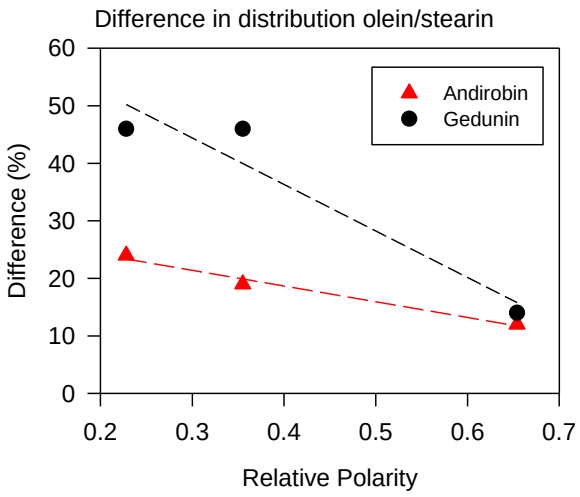


Figure 3-9. Correlation between the difference between the olein and stearin fractions and relative polarity.

3.2.2.2.3 Angolensates and Carapanolides(L7-L15)

Figure 3-10 shows the distribution of Angolensates (L7-L8) and Carapanolides (L9-L15, **Table 3-3**a) in CWO and its fractions. Methyl angolensate (L7: 28 to 39%) and Methyl 6-hydroxyangolensate (L8: 5% to 7%) are the most prominent limonoids of this group. These compounds are interesting because of demonstrated antifungal activity [137]. Methyl angolensate

is distributed with slightly higher amounts (5% or less) in the olein fractions obtained from each fractionation method. The Carapanolides which were present in much smaller amounts (0 to 1.3%) present large partitioning with a preference for the olein fractions. Their relative amounts in the stearin fractions decrease with decreasing solvent polarity. Given the small amounts detected a Pearson correlation analysis was not attempted.

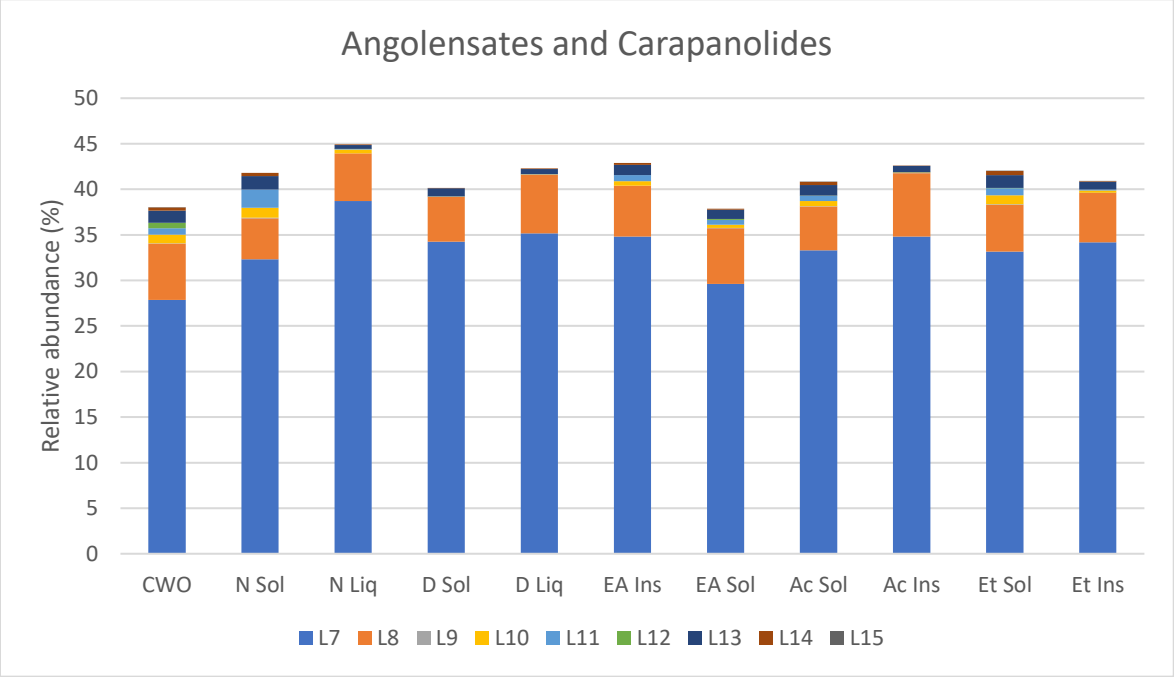


Figure 3-10. Distribution of Angolensates and Carapanolides in CWO and its fractions.

3.2.2.2.4 Other Putative Limonoids (L16-L20)

The other putative limonoids (L16-L20, **Table 3-3**c) are represented in **Figure 3-11**. The L16 - L18 limonoids have been reported in other plants of the Meliaceae family [128, 137].

Figure 3-11 shows that there is significant difference in the partitioning of these compounds between the stearin and olein fractions obtained from solvent but not the dry fractionation. The distribution depended on the type of solvent used but no correlation was found. For example, the

difference in Trichilin A (L17) abundance between the stearin and olein fractions from ethyl acetate, acetone and ethanol was 7%, 35% and 24%, respectively.

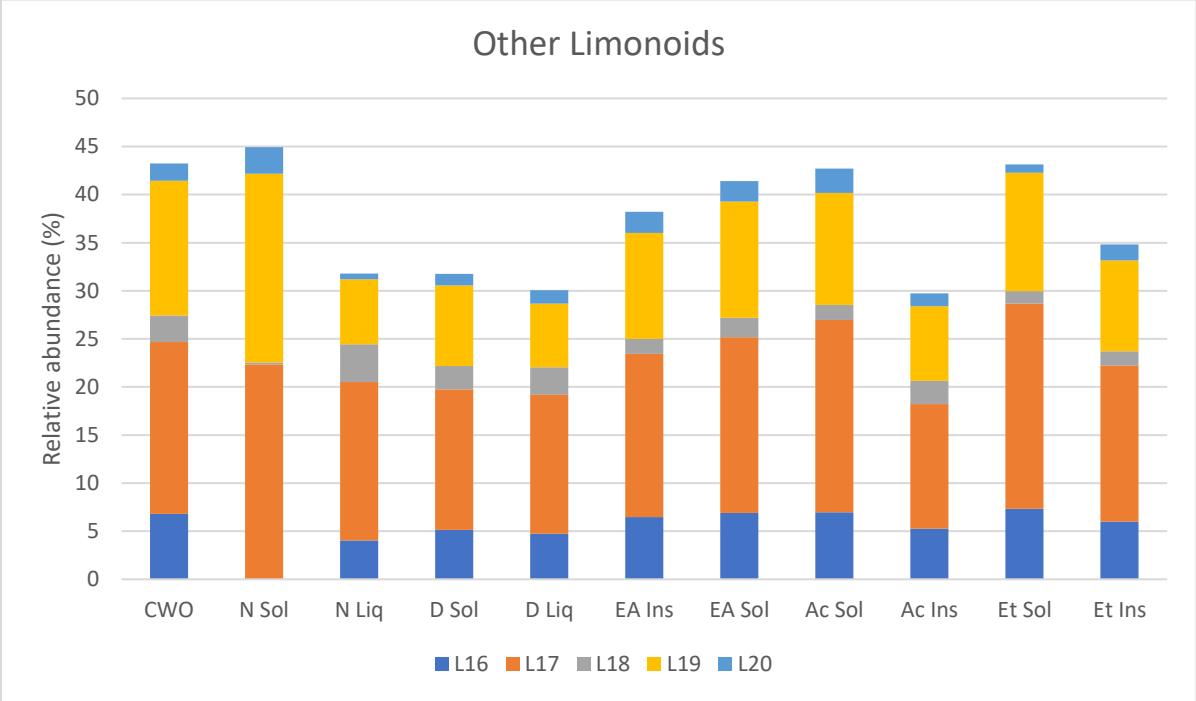


Figure 3-11. Relative percentages of other limonoid types.

3.2.2.2.5 Sterols

Seven sterols were identified in CWO, the dry stearin and all olein fractions; however, Sterol 1 was not detected in all fractions obtained from solvent, probably because it remained in the solvent. Sterol 4 which was the most prevalent in all the fractions (**Figure 3-12**) is similarly abundant in the olein fractions and the dry stearin (± 0.6%).

In the case of the solvent fractions the variation for solvent fractionation between stearin and olein, Sterol 4 is 47%, 38% and 12% more predominant in the olein than in the stearin fractions obtained from ethanol, acetone and ethyl acetate, respectively. The correlation between the polarity and

difference in distribution of Sterol 4 as determined by Pearson correlation analysis (**Figure 3-13**) was strong (r= 0.8615, p= 0.34).

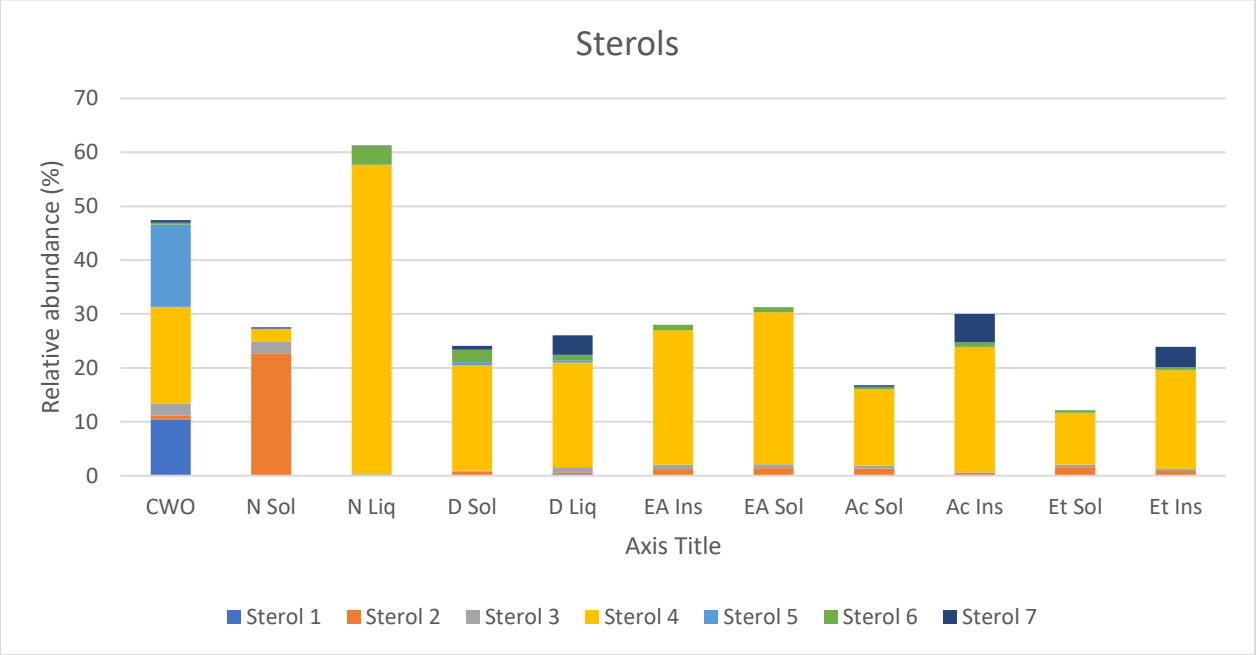


Figure 3-12. Relative percentages of assigned Sterols in CWO and its fractions.

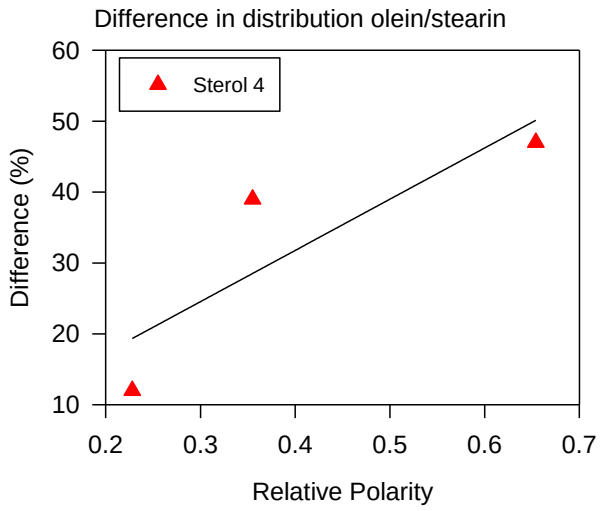


Figure 3-13. Correlation between the olein and stearin difference in abundance and relative polarity.

3.2.2.2.6 Other Unsaponifiable Compounds in CWO and its Fractions

The distribution of the other unsaponifiable compounds detected in CWO and its fractions is represented in **Figure 3-14**.

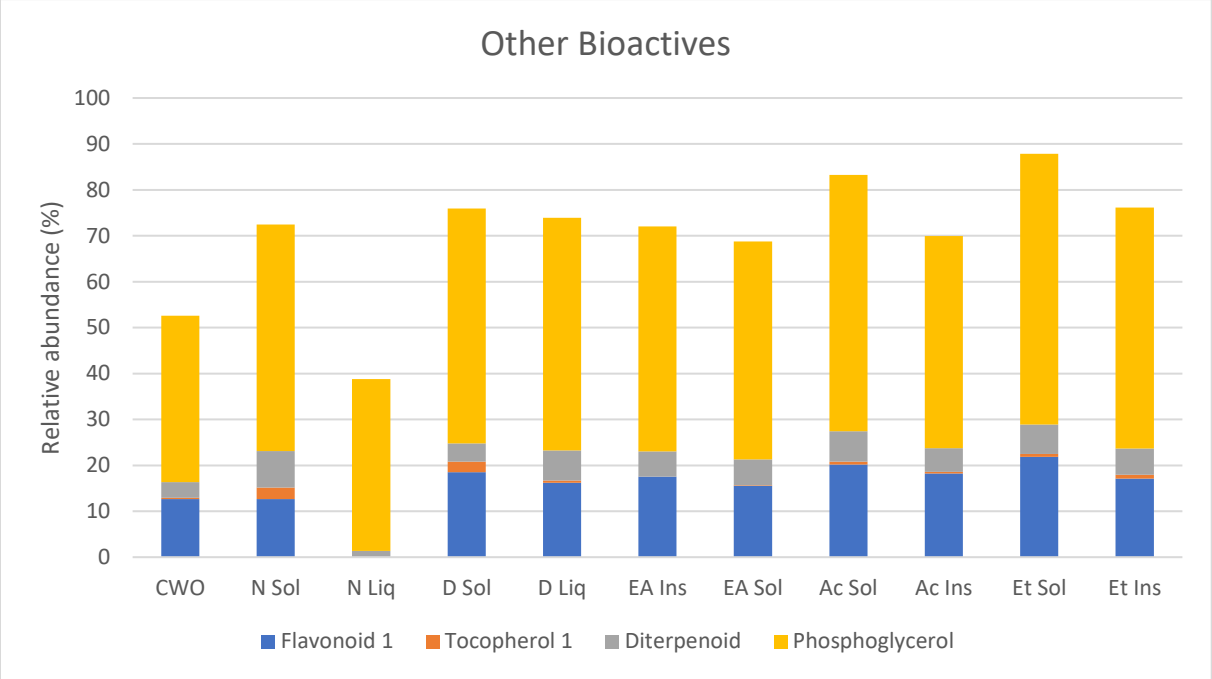


Figure 3-14. Relative percentages of assigned Other unsaponifiable compounds in CWO and its fractions.

The phosphoglycerol (PI 24:6) was the most abundant in all samples. Its distribution did not vary significantly between the stearin and olein fractions obtained by dry and ethyl acetate fractionation methods (±3%). The stearin fractions obtained by ethyl acetate, acetone and ethanol expressed higher abundance levels of PI 24:6, with differences of 3%, 17% and 11% respectively. There was no significant correlation between these differences and polarity ($r= 0.368$, $p= 0.760$). The same trend was observed for the diterpene with coefficient of correlation = 0.290 and p-value= 0.812. The differences observed for the flavonoid and tocopherol were not statistically significant.

3.3 Crystal Structure of CWO, CWO fractions, Cocoa, and Shea Butters

The XRD data are presented in two 2θ-regions as typically done for fatty materials and hydrocarbons: (a) the small-angle scattering (SAXD) region (long Bragg's d-spacing region), where generally 2θ <15° and (b) the wide-angle scattering (WAXD) region (short Bragg's d-spacing region) where generally 2θ >15°. These regions fingerprint the subcell structure and the molecules layer order along the normal direction [201, 202]. The XRD data was analyzed similar to fatty materials including triacylglycerols (TAG) and fatty acids, waxes and related aliphatic compounds [202-204].

The olein fractions from the dry fractionation as well as solvent did not show any crystal peaks indicating liquid or amorphous phases. The normalized SAXD and WAXD spectra obtained for CWO, CB and SB are shown in **Figure 3-15** and those of CWO stearin fractions in **Figure 3-16**. The d-spacings and the related Miller indices are provided in **Appendix B**: XRD Data. The wide background halo observed in the XRD spectra is characteristic of the presence of a liquid phase.

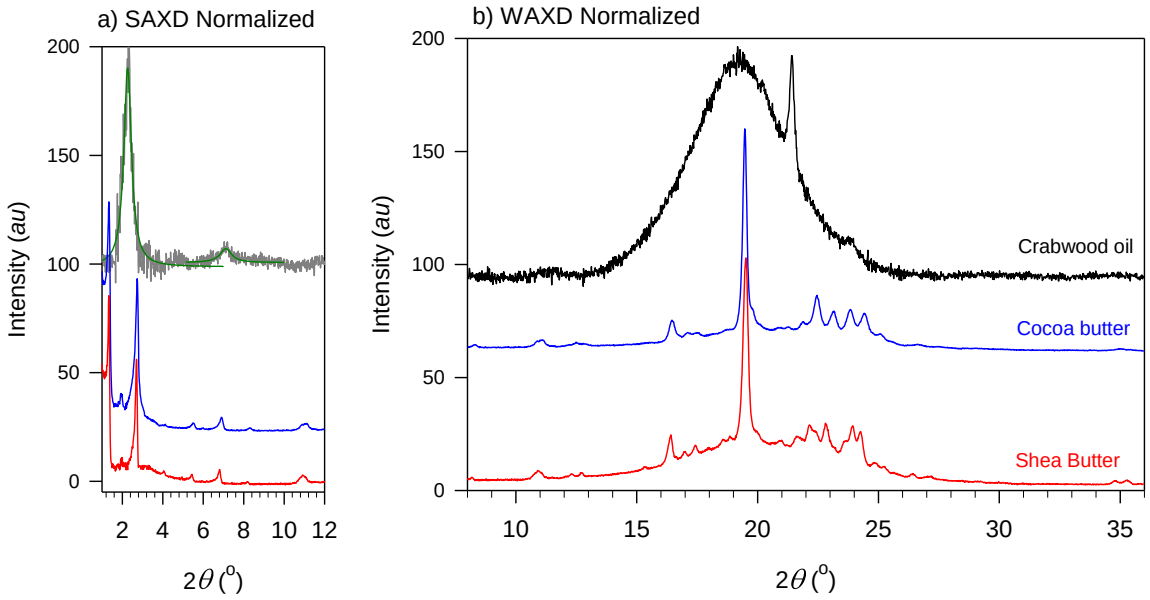


Figure 3-15. XRD of CWO, CB and SB

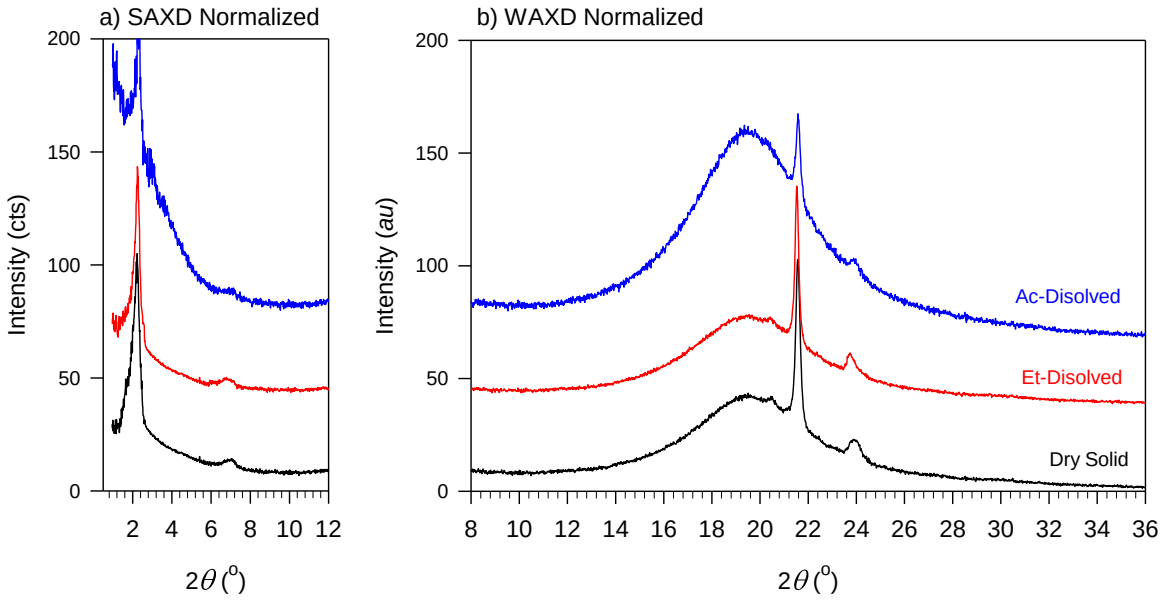


Figure 3-16. XRD of CWO solid fractions

3.3.1 Subcell Structure

The XRD analysis indicate a large difference between the subcell patterns of CWO and its fractions and those of CB and SB. The solid phase in the CB and SB samples consists of a mixture of two distinguishable phases, the triclinic β- phase with characteristic diffraction peaks at 4.6 Å (010) and 3.74 Å, and the orthorhombic β′- phase with characteristic diffraction peaks at 4.16 Å (110) and 3.74 Å (200). CWO and its fractions presented only an orthorhombic subcell structures in the β'-form within a substantial amorphous/liquid phase.

3.3.2 Chain Length Packing

The SAXD data for the β' polymorphs of CWO and its fractions is consistent with a C16 - DCL packing (fork configuration). The SAXD data for the β′ polymorphs of CB and SB is consistent with a C18 triple chain length (TCL) packing (chair configuration) and the SAXD data for the β′ polymorph for CB with a C18 double chain length (DCL) packing (fork configuration). The

packing of CWO and its fractions is consistent with the MS results evidencing TAGs containing predominantly 16-carbon fatty acids stacked. The packing of CB and SB is consistent with homogeneous TAGs containing predominantly 18-carbon fatty acids stacked in a bilayer and trilayer configurations [205].

3.3.3 Crystallinity

The XRD analysis of all the liquid fractions did not show any crystal peaks. The crystallinity [χ(%)], as estimated by the relative intensity of the crystalline peaks to the total intensity in the wide-angle region of CB, SB, CWO and its solid fractions obtained by dry, ethanol and acetone fractionation is shown in **Figure 3-17**. The ratio of β' -form to the total crystal phase is estimated at 13% in CB and 8% ±2% in CS.

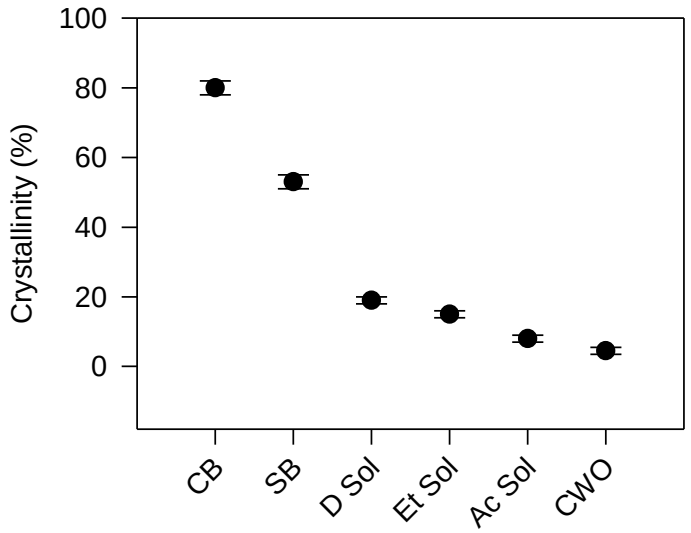


Figure 3-17. Crystallinity of CB, SB, CWO and its solid fractions.

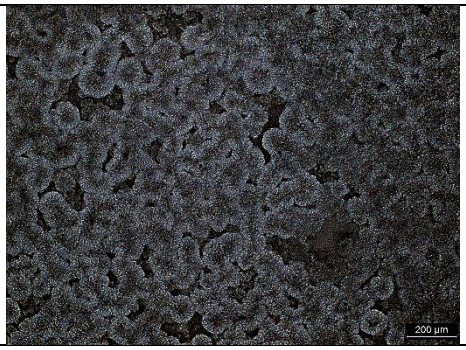
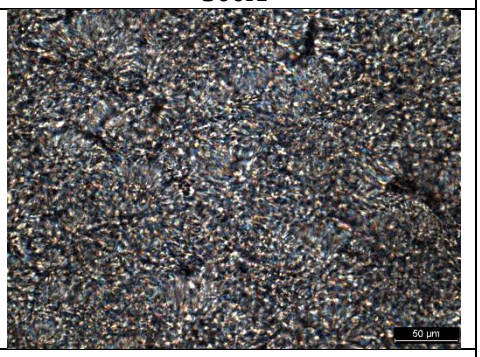
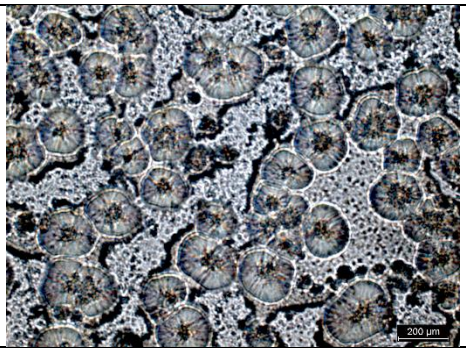
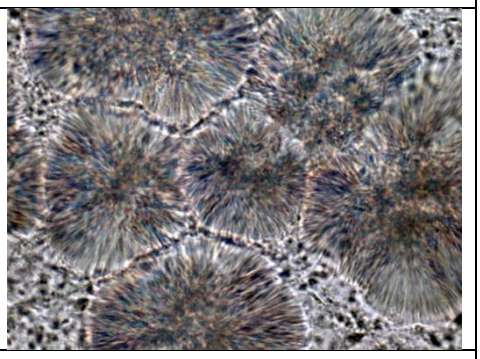
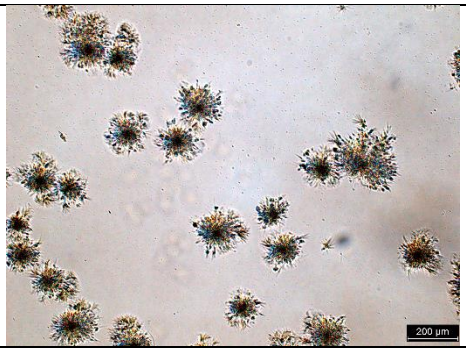
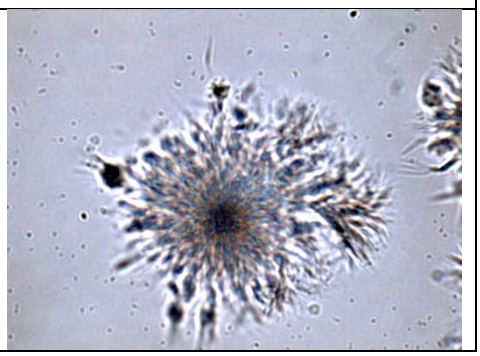
The difference in crystallinity between CB and CWO is significant and attributed to the unsaturated composition of CWO vs. the saturated composition of CB. The crystallinity of D Sol

which is the highest determined for CWO and its fractions (χ(D Sol) = 19%) is a quarter that of CB and approximately one-third that of SB.

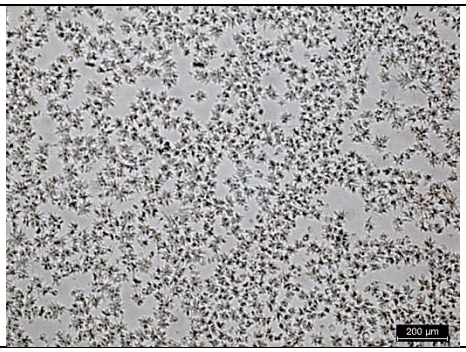
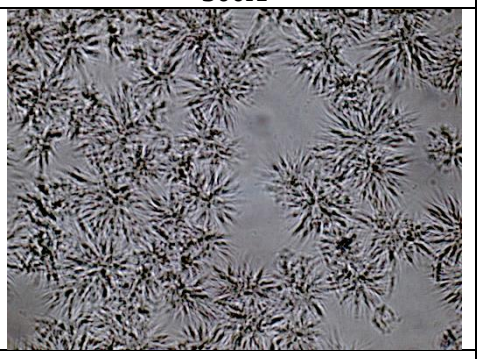
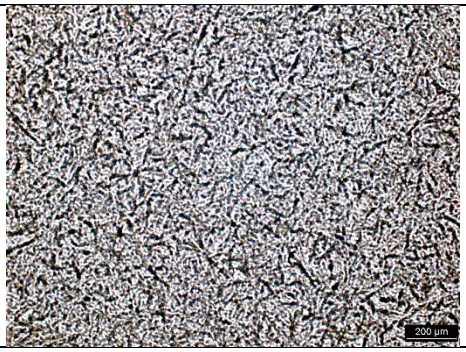
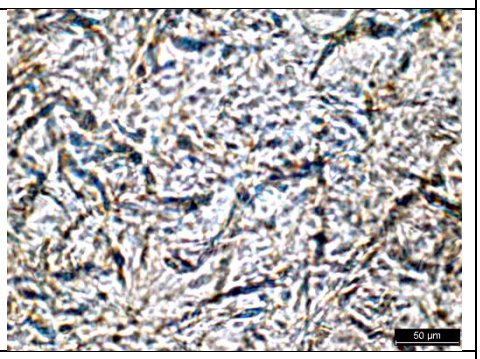
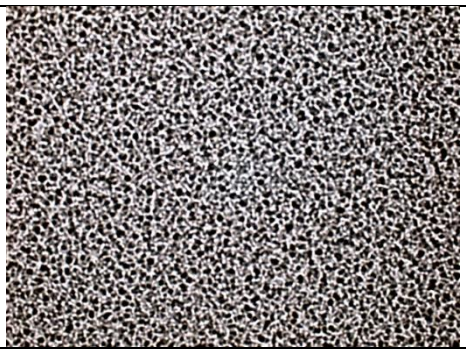
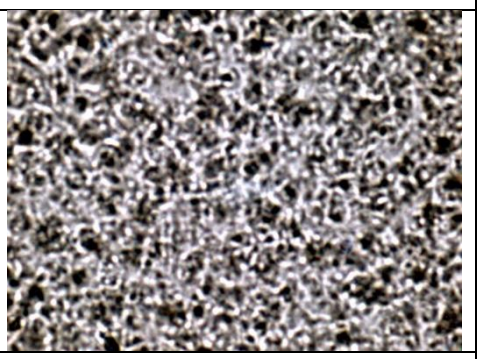
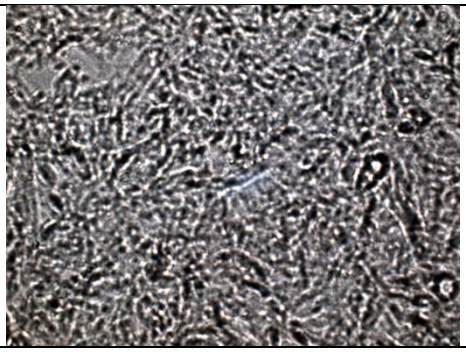
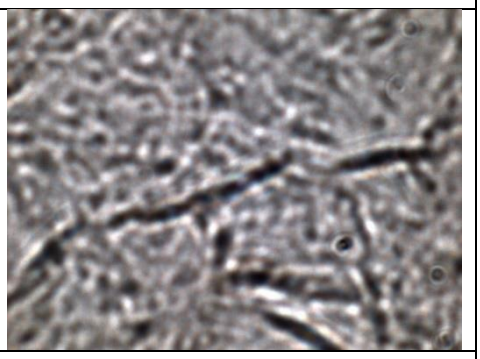
3.4 Microstructure

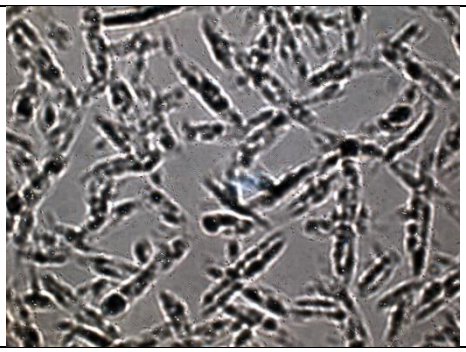
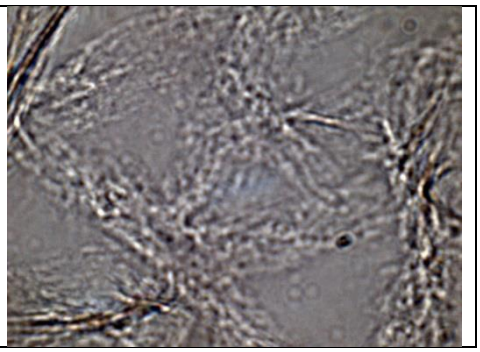
Figure 3-18 shows the PLM images of CWO and its fractions. The solid fractions, liquid fraction, soluble fractions, and insoluble fractions are compared. Cocoa Butter and Shea Butter are presented for comparison.

(a) CB, SB and CWO Compared

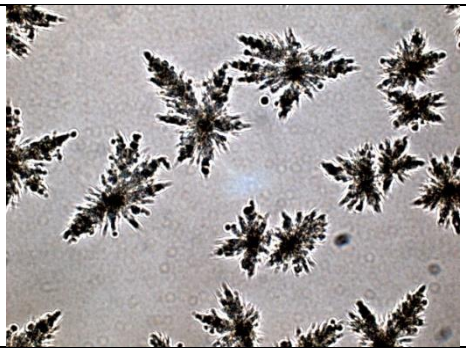
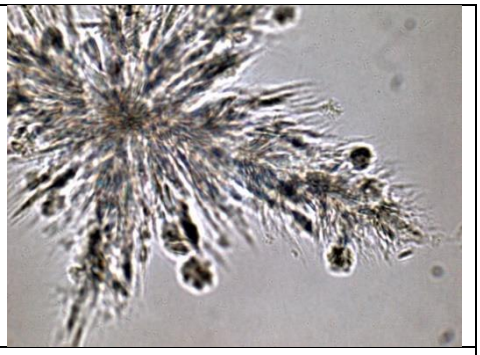
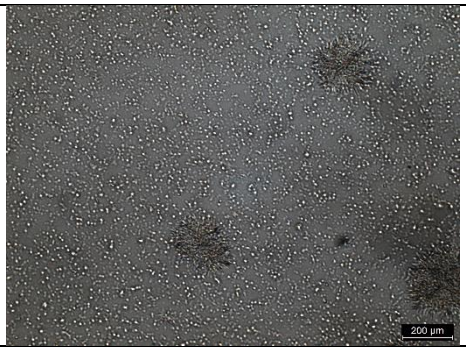
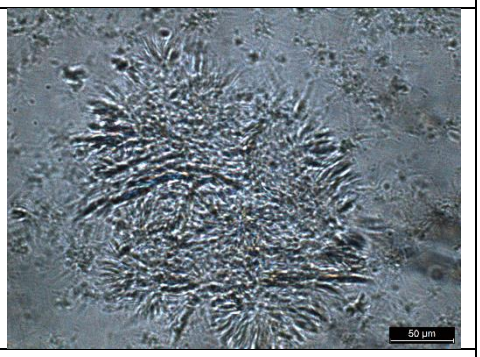
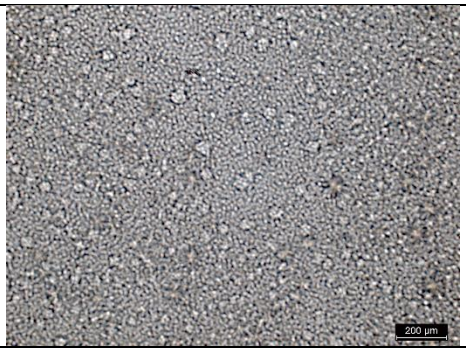
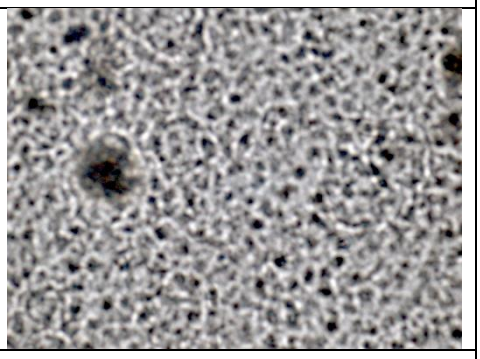
	100X	**500X**
Coca B		
Shea B		
CWO		

(b) Stearin Fractions Compared

	100X	**500X**
N Sol		
D Sol		
EA-Ins		
Et-Sol		

Ac-Sol		

(c) Olein Fractions Compared

EA-Sol		
Et-Ins		
Ac-Ins		

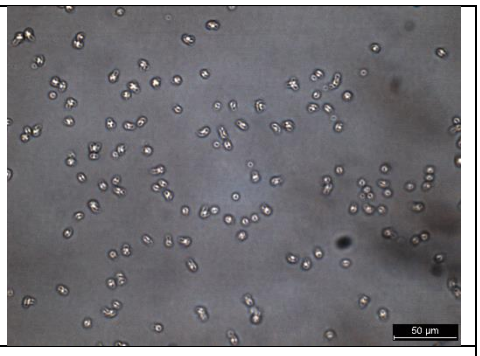
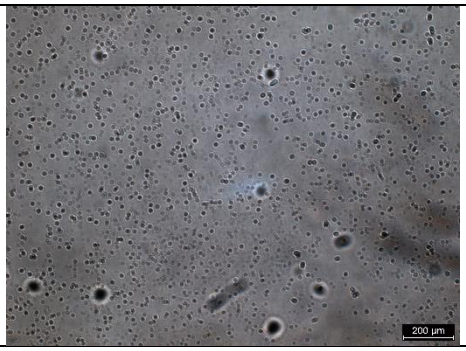
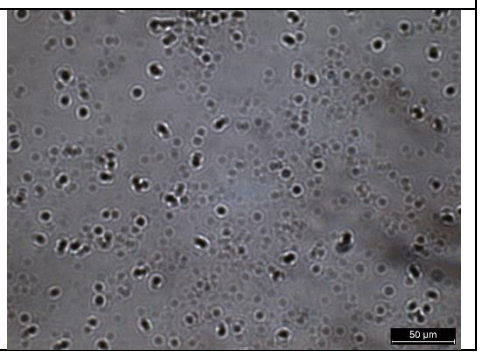
N Liq		
D Liq		

Figure 3-18: PLM images of Crabwood Oil and its fractions taken at room temperature (18°C).

Except for the EA Sol fraction, all the fractions displayed different microstructures compared to CWO. The microstructure of CWO is characterized by relatively large spherical crystals (~175 nm) sparsely distributed in a large liquid phase. EA Sol shows the same crystal distribution, but its crystals grow radial dendrites which can reach ~100 nm in length. The PLM of the stearin fractions consisted of generally very small crystals organized in relatively compact, dense networks. The liquid phase is trapped within the solid network structure. The PLM of CB and SB is closer to the stearin fractions in terms of density but differ in shape and size. The olein fractions also show very small crystals (less than 20 µm) homogeneously distributed in the liquid. The PLM images indicate a high rate of crystallization for all fractions. The fractions are, however, distinguishable by the different shape which vary from well-organized spherulites with radial needle-like to rod-like crystals, size varying from 5 to 200 µm and distribution of their crystals.

The diversity of the microstructures suggests that the fractions are likely to have different flow characteristics and different feel to the skin and mouth. This also suggests that they will confer different texture, consistency and mouthfeel to fat products. This is often determined by both the shape and size of the crystals and aggregates with the large microstructures promoting grainy texture [206]. The smaller crystals would lead to firmer products and larger size crystals to sandy mouth feel [207]. Their mechanical properties would be different because they are mostly controlled by the material's networks of polycrystalline microstructures and the SFC [205].

The microstructure exhibited by the fractions are dependent on the lipid chemical features as well as unsaponifiable compounds in a complex manner [208]. The respective roles of the actual TAG, DAG, MAG and other minor component's structure, nature of fatty acid composition, degree of unsaturation, position of double bonds, and cis/trans contents are still to be determined.

3.5 Thermal Transition Behavior

The crystallization and melting DSC data collected for this work and discussed below is presented in **Appendix C**: Differential Scanning Calorimetry.

3.5.1 Thermal Transition Behavior of Crabwood Oil and Reference CB and SB Butters

The cooling thermogram of the CWO is characterized by a well-defined separation at ~4 °C between a high (12 °C) and three sub-zero exotherms (-3.4, -24 and -47.4°C, **Figure 3-19**a) associated with a stearin and olein fractions, respectively. The crystallization path is mirrored on heating by two groups of endotherms (**Figure 3-19**b) indicating the melting of the two fractions.

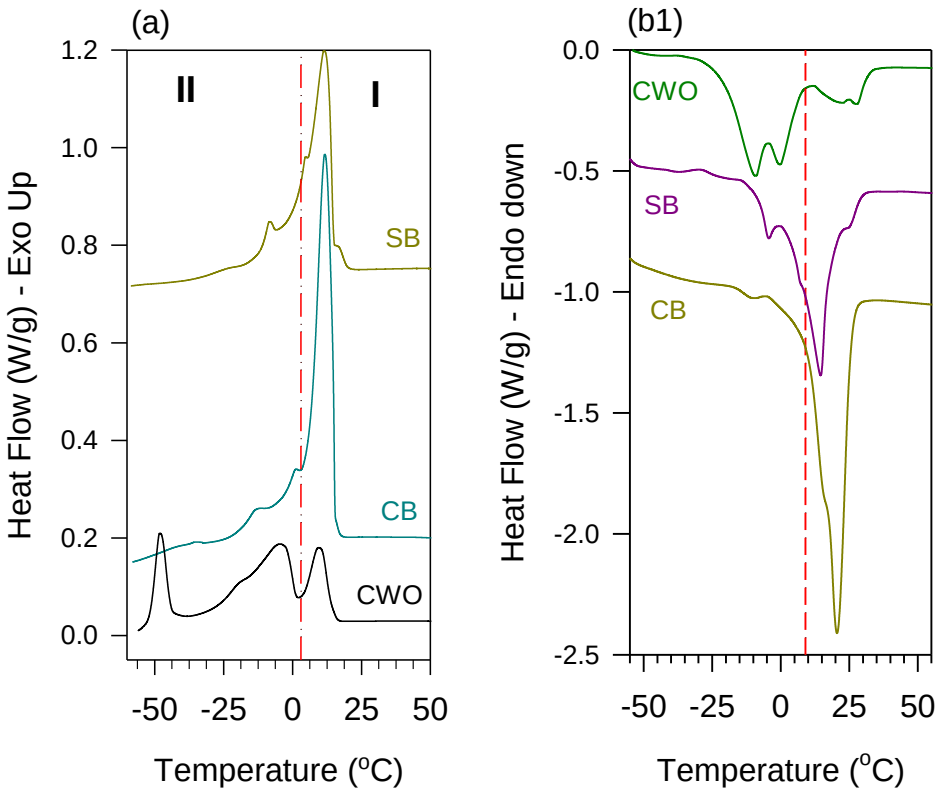


Figure 3-19. (a) Cooling (5° C/min) and (b) heating (10° C/min) thermograms of crabwood oil, Cocoa Butter and Shea Butter.

The enthalpy of crystallization of the stearin phase of CWO (ΔH_{cs} = 13 J/g) is 25% of its total enthalpy of crystallization (ΔH_c). Like CWO, the crystallization thermograms of CB and SB (ΔH_c= 84 and 68 J/g, respectively) started with a very large leading peak at the same temperature as CWO (12 °C) associated with a stearin component followed by very small low temperature peaks (-13 °C for CB, and -8 °C and -22 °C for SB, enthalpies ~15 ± 3 J/g each) associated with an olein component. The enthalpy of crystallization of the stearin phase of CWO which is ~33% and 20% of that of SB and CB, respectively, indicates a much more disorganized crystal phase and a lower solid fat content above freezing, as discussed in **Section 3.7**.

3.5.2 Thermal Transition of the Crabwood Oil Fractions

The cooling (5°C/min) and heating (10°C/min) thermograms of the CWO fractions are shown in **Figure 3-20**a and b, respectively.

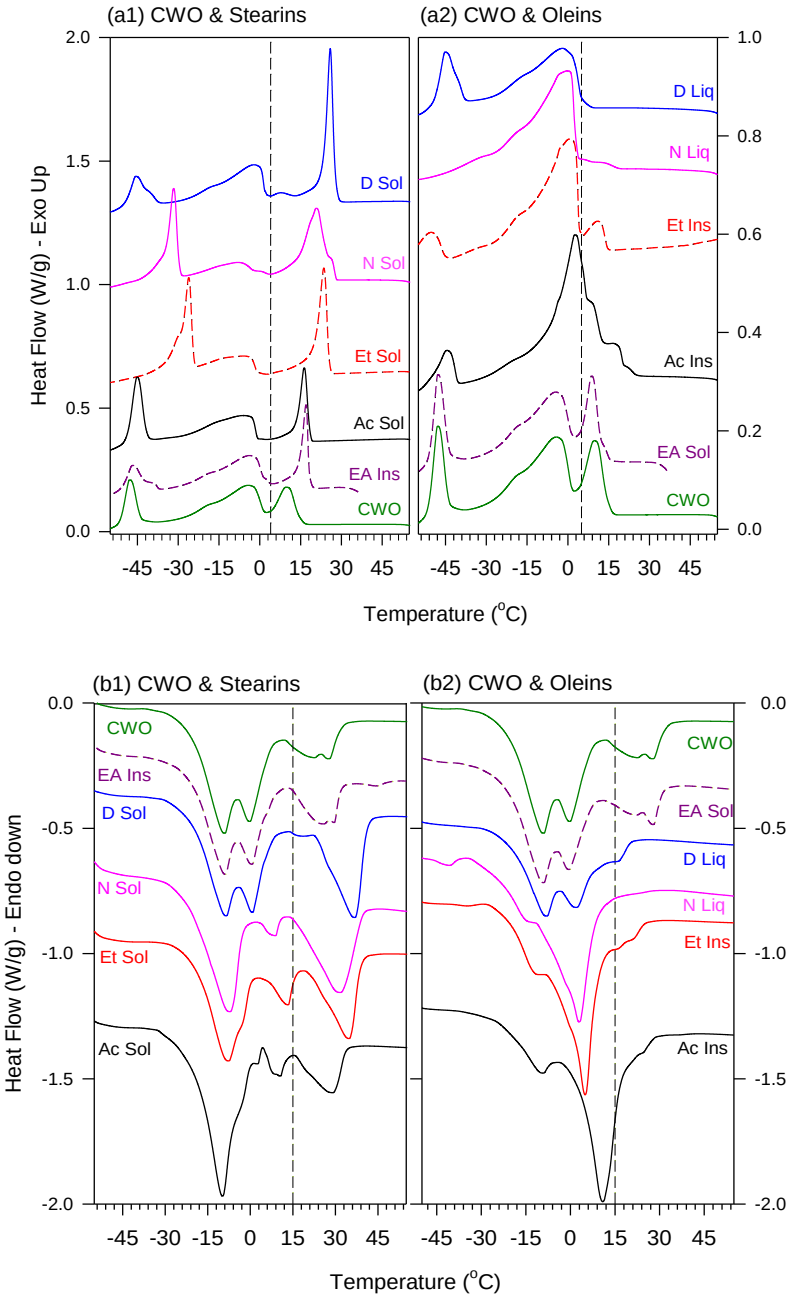


Figure 3-20. (a) Cooling (5° C/min) and (b) heating (10° C/min) thermograms of stearin and olein fractions of CWO.

All samples exhibit broad and overlapping melting and crystallization peaks because of the complex lipid profile (TAG and other minor components). Although the minor components affect greatly the crystallization, the TAGs are the predominant compounds that form the structure of the fat crystal network [209] The resolved peaks can be directly associated with the low, middle, and high melting groups of TAGs and the shoulder peaks may be due to the formation of unstable crystal structures that transform to form more stable crystal structures as the temperature is changed.

Three main exothermic events appear for the CWO fractions during cooling (5° C/min) (**Figure 3-20**a). There are also small shouldering events which indicate a relatively complex thermal transition behavior. The fractions characterized by a large leading crystallization event having an onset as high as 28 °C and offset just above sub-zero temperatures are called stearin fractions for convenience (T ~ 4°C; **Figure 3-20**a1). The crystallization thermograms of the fractions where this peak is absent or dramatically decreased indicate that the high melting components such as the saturated (PSS, PPS and SSS) and desaturated TAGs (PPO, POS, SSL, PPL) were partially removed. These are called olein fractions. The intensity and enthalpy associated with the leading exotherm in the olein fractions vary depending on the fractionation method (**Figure 3-20**a2) indicating the relative depletion in the molecules responsible for the solid fat, mainly the most saturated lipids.

The heating (10° C/min) thermograms of the stearin fractions is characterized with four main endotherms (**Figure 3-20**b1). Except the olein fraction obtained with ethyl acetate, the heating thermograms of the olein fractions were missing the high temperature melting peak (T_{m1}, **Figure 3-20**b2). T_{m1} of the stearin fractions decreases from 37 °C (*D Sol.*) to 29 °C (CWO) indicating

decreasing stabilities of the solid phases. This is consistent with the trend observed for the leading crystallization event, which decreased from 26 to 12 °C. The prominent peaks at T_{m3} and T_{m4} ccur at the same temperatures (0±2°C and -10±2°C, respectively) for all the fractions indicating low temperature crystal phases of similar stabilities. The relatively small peak which appears at 14±2°C (T_{m2}) for fractions obtained with the solvents is probably the melting of a small recrystallized olein phase.

3.5.3 Correlation between the Type of Fractionation and Thermal Transition Behavior

The differences in thermal transition behavior between the CWO fractions is related to the way the fractions were obtained. The first observation is that the fractions obtained using ethanol and acetone (Et Ins and Ac Ins) present distinctive thermograms indicative of a stearin (soluble component) and olein (insoluble component) fractions whereas the fractions obtained from ethyl acetate are almost similar with a slightly higher olein character for the soluble component (EA Sol) than the insoluble (**Figure 3-20**a).

The olein fractions show quite different crystallization behaviors in the high temperature side of the cooling thermograms (T> 0 °C) depending on the fractionation method. The leading exothermic event in these fractions can be used to qualitatively assess the relative separation efficiency of the saturated elements. The olein fraction obtained by dry fractionation did not present the high temperature peak. The crystallization data can be explained by the complex dependence of the solubility of the CWO constituents on the type and the selectivity associated with polarity. As the polarity decreases from ethanol to ethyl acetate, the affinity for the saturated components such PSS, PPS and SSS, which constitute ~5% of the TAGs in CWO, and the di-saturated components such as PPO, POS, SSL, PPL, which constitute ~35% of the TAGs,

decreases to the point where it reverses for ethyl acetate, as discussed in **Section 3.1.1**. The main peak temperatures (T_c) of the stearin and olein fractions are presented in **Figure 3-21**a and b, respectively.

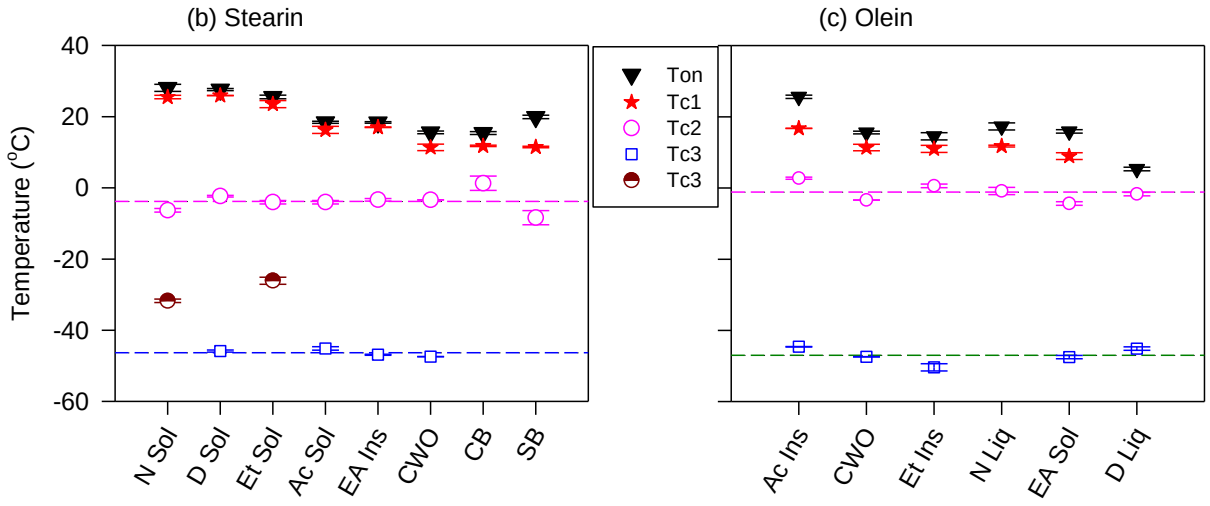


Figure 3-21. Crystallization peak temperatures (T_c) of (a) stearin and (b) olein fractions.

The fat crystals of stearin which were obtained from dry fractionation crystallized at the highest temperature (26 °C) followed by those from ethanol, acetone and ethyl acetate following the decrease in polarity. Et Ins fraction presented one small exotherm at ~11 °C reminiscent of CWO followed by a large cascading exotherm peaking at ~1°C whereas the Ac Ins fraction presented four (04) convoluted exotherms (23, 17, 9 and 3 °C). The leading peaks of both the soluble and insoluble parts obtained from ethyl acetate present similarities with CWO.

Generally, the leading exothermic peaks of the olein fractions are much smaller and their onset and peak temperatures shifted to lower temperatures compared with those of CWO because of much larger unsaturated moieties.

The enthalpy of crystallization of the leading peak of the stearin (ΔH_{1s}) and olein (ΔH_{1o}) fractions are shown in **Figure 3-22**a and b, respectively. As shown in **Figure 3-22**c, ΔH_{1s} decrease with increasing solvent polarity. In fact, Pearson correlation analysis shows that ΔH_{1s} is strongly correlated with polarity (r= 0.9979; p= 0.04).

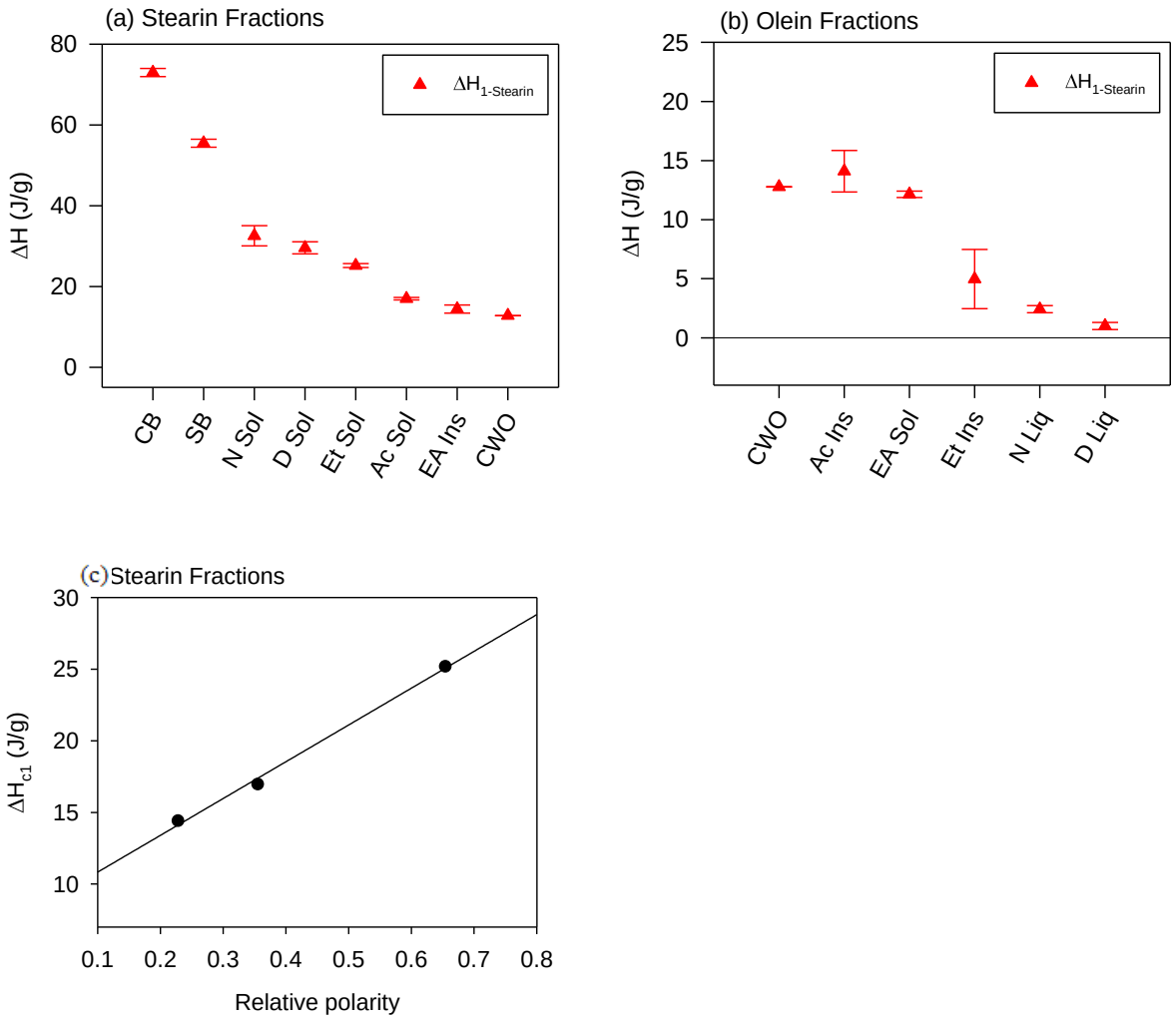


Figure 3-22. Enthalpy of crystallization of the leading peak of the (a) stearin and (b) olein fractions. (c) Enthalpy of the leading peak of the stearin fractions versus relative polarity.

Overall ΔH_{1o} is much lower than ΔH_{1s} with those of Et Ins, N Liq and D Liq being very small (<4 ± 3 J/g). ΔH_{1s} of CB and SB (73 and 55 J/g, respectively) is much higher than that of the fraction

obtained from dry fractionation (N Sol, D Sol) (**Figure 3-22**a). The similarity of the DSC cooling thermograms of EA Ins and EA Sol with those of CWO indicates that the fractionation with ethyl acetate separate the molecules more along the lines of polarity rather than saturation. This is also what is suggested when comparing the position and enthalpy of crystallization leading peaks. The leading exotherm associated with EA Sol (9 °C, ΔH_{c1}= 12 J/g) is only slightly lower than that of CWO (~12 °C, ΔH_{c1}= 13 J/g) indicating close saturation levels. Both are significantly lower than that associated with EA Ins (17 °C, ΔH_{c1}= 27 J/g), a shift that can be attributed to differences in high melting components and the influence of the minor components such as DAGs and MAGs which may have acted like seeds for crystallization.

The resolved melting peak temperatures (T_m) of the stearin and olein fractions are presented in **Figure 3-23**a-b. There are several shoulder peaks in the heating thermograms not represented in the figure to keep a clear representation. The transformation path during heating is more complex than the crystallization behavior because of the several recrystallizations mediated by melting that occurred.

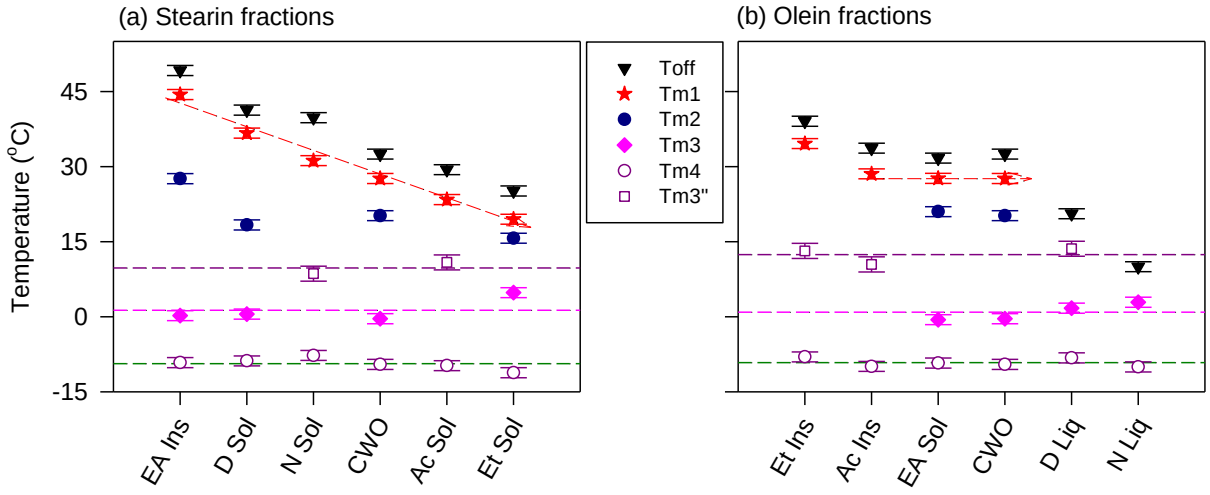


Figure 3-23. Melting peak temperatures (T_c) of (a) stearin and (b) olein fractions.

The steady decrease of T_{m1}, the representative of the most stable phase in each stearin fraction, from 45 °C (EA Ins) to 19 °C (Et Sol) indicates a decrease in stability of the highest temperature phases achieved by the fractions. The prominent peaks at T_{m3} (0±2°C) and T_{m4} (-10±2°C) are associated with the most saturated elements, predominantly the saturated and di-saturated TAGs. The melting behavior of the fractions from ethyl acetate is special. EA Ins displayed 6 melting events spanning from -22 °C to 50 °C suggesting the possibility of achieving highly stable phases by manipulating the processing of the material. EA Sol displayed 4 melting events with T_{m1} (32 °C) matching those of CWO, CB and SB.

3.6 Thermal Gravimetric Analysis

The TGA and DTG curves and the corresponding data of all the samples are provided in **Appendix D**: Thermal Degradation Data. TGA and DTG curves representative of the solid, liquid, soluble and insoluble fractions are shown in **Figure 3-24**a and b, respectively. The onset temperature of mass loss as determined at 5% ($T_{5\%}^{on}$) of the stearin fractions (**Figure 3-24**c) are not significantly different (195 ± 2 °C). Also, $T_{5\%}^{on}$ of Ac Sol and N Liq. are not significantly different from CWO (205 ± 2 °C). $T_{5\%}^{on}$ of the insoluble fractions increase with increasing solvent polarity. $T_{5\%}^{on}$ which is highest for Et Ins (225 °C) is significantly lower than that of SB (299 °C) and CB (336 °C).

The DTG curves of **Figure 3-24**b indicating the CWO fraction experience the same two main mass loss mechanisms (T_{D1}= 233 ± 6 °C and T_{D2} = 393 ±8 °C). A very small event centered at T_{D3} = 435 8 °C showed for some samples but involved less than 2% of mass loss. The degradation of CWO can be understood in light of the mechanisms by which TAGs degrade. TAGs are thermally degraded at elevated temperatures via β-hydrogen elimination starting at ~250-380 ℃ and by γ-

hydrogen transfer starting at ~450°C [210-212]. Therefore, the DTG of **Figure 3-24**b indicates that CWO degradation is dominated by the β-hydrogen elimination mechanism.

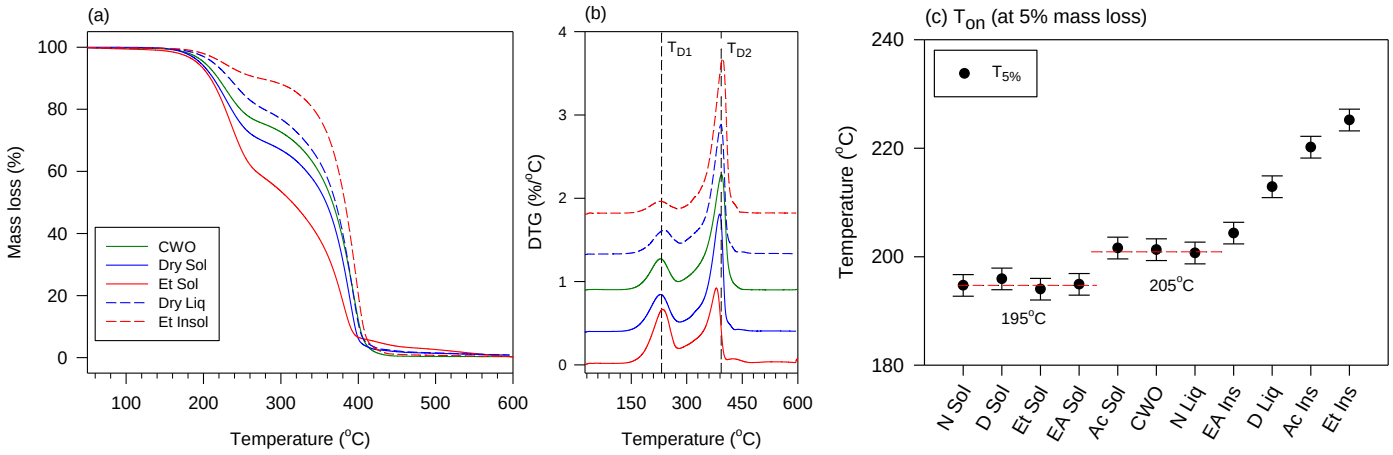


Figure 3-24. (a) TGA and (b) DTG stacks of the crabwood oil and its fractions; (c) Onset temperature of degradation as determined at 5% mass loss ($T_{5\%}^{on}$). Horizontal dashed lines in panel c indicate crossed points are not significantly different (ANOVA).

The ~140 °C and 300 °C range of the first DTG peak was identified as the range where the volatilization of free fatty acids occur. Niu et al., [213] reported that gaseous products from thermal degradation of oleic acid involve alkanes in large proportion, but also alkenes, aldehydes, ethers, and CO_2. It is safe to assume that all the free fatty acids and other minor light components were lost at the offset temperatures of the first DTG peak (~300 °C). However, because the *β*-hydrogen elimination starts at ~240 °C, this peak may also involve the degradation of some MAGs, DAGs, and TAGs.

The 300 °C - 450 °C temperature range is where the degradation of the TAGs involving the saturated as well unsaturated fatty acids is reported to occurs such as in rapeseed oil [214] and virgin olive oil [215]. In a study of the thermal degradation of pure simple TAGs with C18:0,

C18:1, C18:2 and C18:3 fatty acids, one DTG step was observed for C18:0, C18:2 and C18:3 and two partly separated steps were observed for C18:1 suggesting that it start to degrade before all the other fatty acids [215].

The distinct two-step degradation of CWO and its fractions and their association with two distinct groups of molecules can be used to estimate the major lipid compounds of CWO and its fractions. The heating rate and gas employed can be varied to allow the TGA to discriminate further between groups of components based on kinetics of degradation. The refined technique can potentially be used as a first simple tool of composition analysis.

3.7 Solid Fat Content

The SFC of CWO and its fractions measured at different temperatures in situ while the sample is cooled from the melt by 5°C steps followed exponential rise to a maximum function ($SFC(t) = SFC_0 + a\left(1 - e^{-\frac{t}{t_0}}\right)$; R^2= 0.8625 - 0.9976). The evolution of the SFC of the different samples at 0 °C is represented in **Figure 3-25**a. The maximum SFC values at 25, 10 and 0 °C of CWO and its fractions are presented in **Figure 3-25**b. The final SFC values of all the fractions are well below 40% at 0 °C, indicating that these compounds form semi-solid badly organized plastic fats. The measured SFC of the fractions is also well below that of CB at all the measurement temperatures. Ac Sol is the only fraction that matches SB at low temperature (below 10 °C).

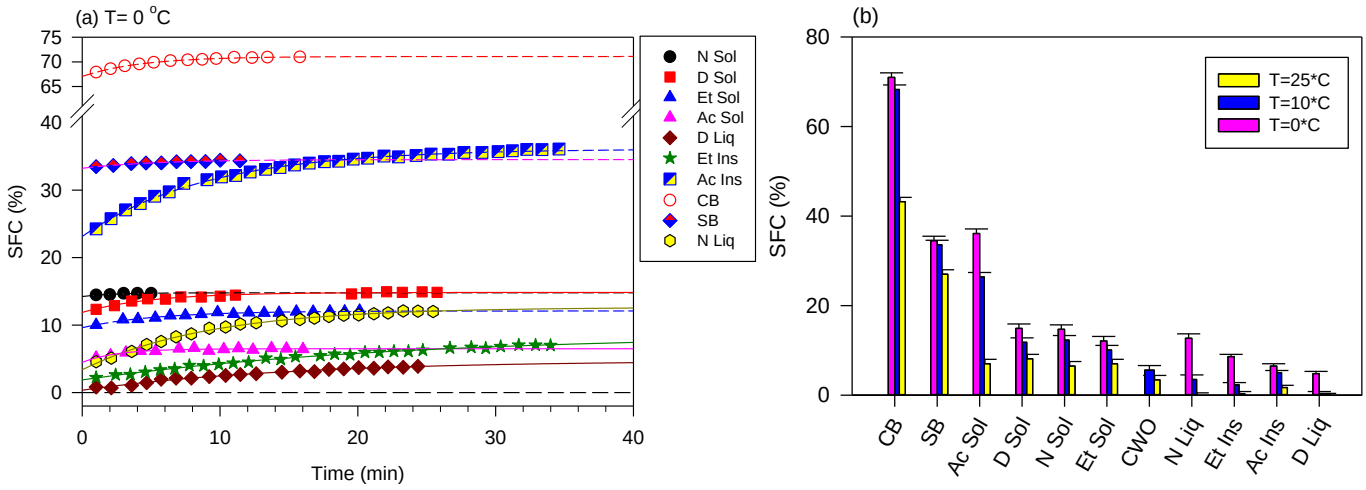


Figure 3-25. (a) Evolution of the SFC of CWO and its fractions measured at 0 °C. (b) SFC of CWO and its fractions measured at 25, 10 and 0 °C.

The SFC values of the solid fractions at room temperature are not significantly different ($p < 0.05$) and are much higher than those of the liquid fractions because of significant differences in FA and TAG compositions. The group of molecules which are susceptible to crystallize at this temperature are those comprising most saturated moieties such the saturated PSS, PPS and SSS and the di-saturated TAGs such as PPO, POS, SSL, PPL which form which form ~40% of the TAGs in CWO. The SFC of the olein fractions which is very low at room temperature increases to match that of the stearin fractions at 0°C, indicating the crystallization in these fractions of a second group of molecules, most probably mainly the di-unsaturated TAGs such as SOO, POO, PLO, PLL which form approximately 43% of the TAGs of CWO.

The SFC-temperature data show that the solidification behavior is strongly affected by kinetics. The SFC versus temperature curves of the dry and native fractions which were measured in detail are presented in **Figure 3-26**.

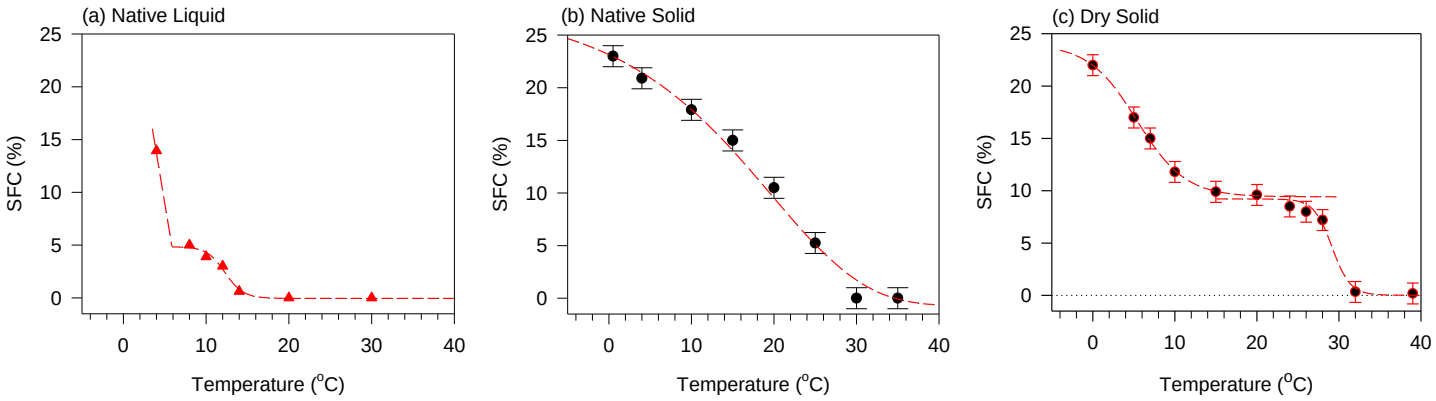


Figure 3-26. SFC versus temperature of the Dry solid (D Sol) and Native solid fractions.

The SFC versus temperature of D Sol (**Figure 3-26**c) shows two distinct segments each of which can be described by a sigmoidal function indicating a two-step crystallization process. The SFC of N Liq also appear to follow the same pattern (**Figure 3-26**a). Each segment starts initially with a slow crystallization followed by a rapid increase in the solid development. The first sigmoid is the expression of the crystallization of the stearin part, which is revealed by the leading event observed in the DSC crystallization thermogram (T> 4 °C), and the second segment is related to one group of molecules of a remaining olein part which crystallization occurs below 4 °C and which is revealed in the DSC by a smaller exotherm at T_{c2}~-2 °C. Measurements at temperatures below freezing which would evidence the contribution of the group of molecules melting at (T_c ~-10 and -50 °C) were not performed.

Despite a crystallization path like D Sol, the SFC of N Sol displayed one sigmoid indicating different competition between growth and activated secondary nucleation. The relative contributions of the different processes to the overall growth rate can result in different apparent activation energies and hence explain the measured SFC trends [216-218]. The differences in microstructures between D Sol and N Sol (**Figure 3-18**) may be correlated to their SFC behavior.

One can note that N Sol consisted of spherulitic crystals distributed in the liquid phase whereas D Sol microstructure consisted of a rod-like crystal distributed in an undelaying network of small crystals which would explain its two-step crystallization. The longer storage time of N Sol (purchased two years ago) may have altered its lipid structure and hence its crystallization behavior.

The role of kinetics in the crystallization behavior of CWO and its fractions is further evident from the SFC result obtained at 4 °C under two different thermal protocols (**Figure 3-27**a): (a) cooling from the melt in small steps (2 °C) and isothermal measurement, and (b) long storage time at 4 °C then direct measurements. The first protocol resulted in a much higher SFC with gains varying from ~65% (Et Ins) to 14% (Ac Ins) (**Figure 3-27**b) indicating the possibility to achieve by processing levels of SFC like SB.

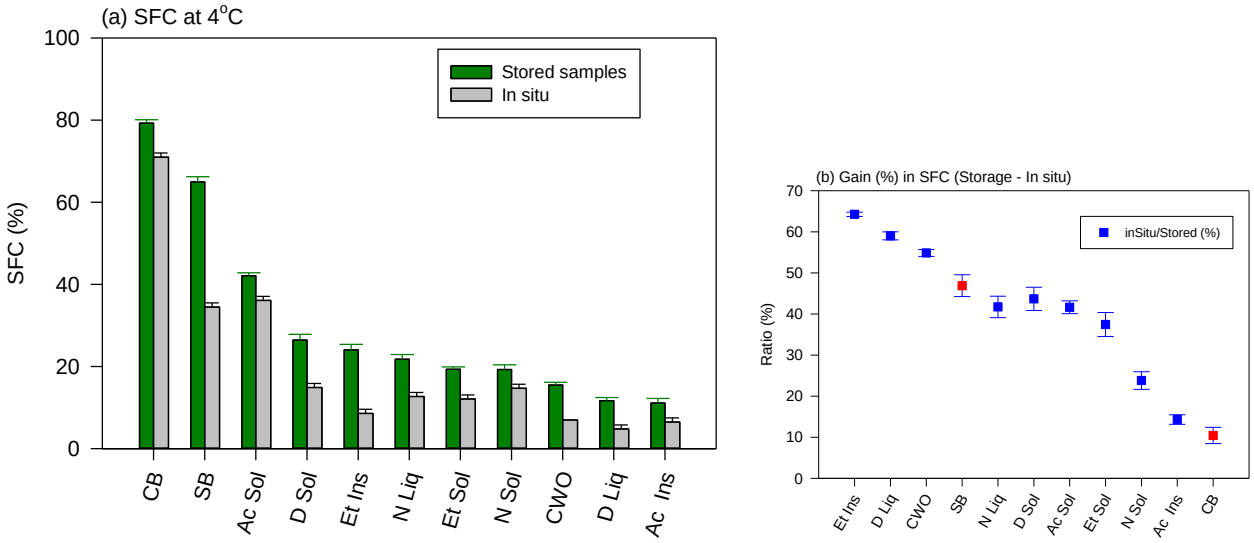


Figure 3-27. (a) SFC of CWO and its fraction for samples cooled in situ from the melt and measured isothermally in small steps (2 °C), and directly after a long storage time at 4 °C. (b) Gain (%) in the SFC obtained by storage over in situ successive isothermal measurements.

The gains in SFC obtained for CB with the storage protocol is more than 6 times smaller than that of Et Ins and 1.4 times that of Ac Ins, indicating the relative differences with the fast CB kinetics. The gains measured for Et Sol, Ac Sol, D Sol and N Liq are similar (4 times) and not significantly different from that of SB (4.5 times) suggesting similarly slow crystallization kinetics relative to CB. The differences observed in their DSC thermograms would be therefore the expression of similar crystallization kinetics with measurable subtle differences driven by different molecular compositions.

CHAPTER 4

4 Conclusion Chapter

This thesis involves the study of the oil from the seeds of *Carapa guianensis,* known as crabwood oil (CWO) and its fractionation. The oil is mainly composed of lipids (95-98%) and unsaponifiable compounds (2-5%) comprising limonoids, sterols, and phenols. The goal of the fractionation was to obtain narrower composition ranges having altered physical properties and meeting specific nutritional or functional requirements for use in the food, pharmaceutical, and cosmetic industries. A diversity in the starting CWO material would result in fractions with slightly diverse compositions. Dry and solvent fractionation with ethanol, acetone and ethyl acetate were used to recover stearin (solid) and olein (liquid) fractions. Established methods of phytochemical and physicochemical functionality analysis were used to compare the composition and functionality of the fractions. In addition, Cocoa Butter (CB) and Shea Butter (SB), commonly used oils in the cosmetic industry, were similarly studied as reference oils and their physical functionality compared to CWO and its fractions. Statistical analysis including Analysis of variance (One way ANOVA) and Pearson Correlation Analysis were carried out to determine the relationship between the measured variables and the strength and direction of correlation between them, respectively.

The thesis has the following important findings:

a. The different methods of fractionation yielded significantly different stearin and olein fractions depending on the fractionation method.

The yield of the stearin fraction decreased with increasing solvent polarity in a predictive manner. Pearson Correlation analysis indicated very strong (negative) correlation of the yield with the

relative polarity with a Correlation coefficient r= -0.9994 and p-value p= 0.02. A conclusion was supported that fractionation of CWO using solvents can be further directed by using other solvents, mixtures of solvents and by varying the process parameters such solvent to oil ratio, crystallization temperature, agitation, cooling rate and crystallization time.

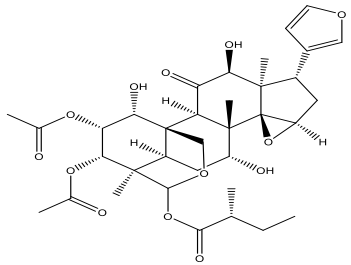
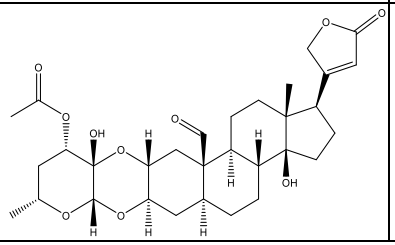
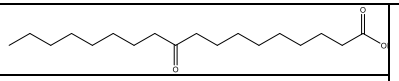
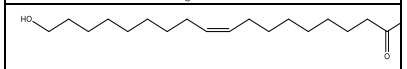
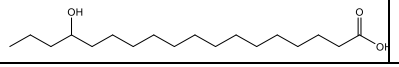
b. Detection of large concentration of major bioactives including bioactive compounds not reported for CWO before

Methyl angolensate, a major limonoid credited with cytotoxic [132, 192] and antifungal [137, 193] activities and previously reported in CWO [110], was the most abundant with ~33% of the total limonoids. Trichilin A, a limonoid with important bioactivity including cytotoxic and antifeedant properties was present with 17% of the total. Trichilins which has been identified in other Meliaceae species such as Natal mahogany (*Trichilia roka*) [194], has not been reported in CWO before.

Ketostearic acid/ hydroxyoleic acid which have the same molecular mass (***m/z*[M+Na]$^+$ =** 321.2408) were the most abundant of the total naturally occurring FFA at 43.7%. These are essential nutrients which play important roles in the energy metabolism regulation [185]. Very industrially interesting long fatty acids have not been reported in CWO before. Ketostearic acid has been identified in some plants of the Asphodelaceae family [186] and hydroxyoleic acid was found in *Arabidopsis thaliana* [187] but not in CWO. Their presence in CWO may be related to transformation related to the fractionation process or specific to the Guyanese species of *Carapa guianensis* or due to biotransformation by bacteria present in the Guyanese *Carapa guianensis*. The origin and possible enhancement and separation of these fatty acids in CWO should form the basis of additional research.

The list of the major compounds identified in this thesis and which to the best of our knowledge have not been reported before in CWO are listed in **Table 4-1**.

Table 4-1. Bioactive compounds identified in this thesis which have not been reported before in CWO.

Elementary composition	IUPAC name	Synonym	*m/z* $[M+Na]^+$	Structure	Relative abundance (%) in CWO	Ref
Unsaponifiable compounds						
C35H46O13	[(1S,2R,4S,5R,6S,8R,10S,11S,12R,14R,15R,19R,20R,21S)-20,21-diacetyloxy-6-(furan-3-yl)-4,12,19-trihydroxy-5,11,15-trimethyl-3-oxo-9,17-dioxahexacyclo[13.3.3.01,14.02,11.05,10.08,10]henicosan-16-yl] (2R)-2-methylbutanoate	Trichilin A	697.2836		17.9	[219, 220]
C31H42O10	[(1S,3R,5S,7R,9S,10S,12R,14R,15S,18R,19R,22S,23R)-14-formyl-10,22-dihydroxy-7,18-dimethyl-19-(5-oxo-2H-furan-3-yl)-4,6,11-trioxahexacyclo[12.11.0.03,12.05,10.015,23.018,22]pentacosan-9-yl] acetate	Asclepin	597.2676		14	[198, 221]
Fatty acids						
C18H34O3	10-oxooctadecanoic acid	10-ketostearic acid	321.2408		43.7	[186, 222]
C18H34O3	(Z)-18-hydroxyoctadec-9-enoic acid	18-hydroxyoleic acid				[187]
C18h3603	15-hydroxyoctadecanoic acid	15-hydroxy stearic acid	323.2542		24	[223]

c. One can partition important bioactive compounds as a function of fractionation method, in particular there is a direct dependence on solvent polarity.

The partition of the bioactive components is particularly evident for the Andirobin and Gedunin-type limonoids. Gedunin showed a preference for the liquid fractions of all solvents and the solid fraction of the dry fractionation. In the case of Methyl angolensate, it was more prevalent in all the liquid fractions except for ethyl Acetate, where it is found predominantly in the solid fraction. The difference between the dry liquid and solid fractions was much less than in the solvent fractions, indicating the success of the solvents in increasing the partitioning of these compounds to enrich one fraction.

The partition of the bioactive components depended on the type of solvents. The high polarity ethanol and the mid-polar acetone enriched the liquid fraction with Methyl angolensate, Andirobin, and Gedunin-type limonoids. The least polar solvent (ethyl acetate) enriched the solid fraction with methyl angolensate and the liquid fractions with Andirobin and Gedunin. Additionally, as the polarity increases, the partition of Andirobin and Gedunin improves, indicating the affinity of these limonoids for high polarity solvents. The partition of Methyl angolensate was moderate, with an enrichment of approximately 3 to 5% when ethanol and acetone were used and was 15% when ethyl acetate was used, indicating much higher affinity for the low polarity solvents.

d. Can change the crystallization temperature and melting temperature of CWO fractions as a function of solvent polarity.

The composition of the CWO fractions was determined by the fractionation method which dictates the crystallization behavior. is characterized by exothermic events indicating their solid and liquid parts. The intensity and position of the exotherm associated with the solid fractions can be

unambiguously related to the fractionation method. The thermal transition behavior including enthalpy, and onset temperature of crystallization and melting of the fractions obtained by solvents is complex but is correlated to the polarity of the solvents. For example, enthalpy of crystallization of the exotherm associated with the solid part of the fractions is very strongly correlated with polarity ($r = 0.9979$ and $p = 0.04$).

e. Can change the solid fat content (SFC) profile of CWO fractions as a function of solvent polarity.

The final SFC values are well below 40% at 0 °C, indicating that all the fractions form semi-solid fats. The SFC values of the solid fractions at room temperature (RT= 18 °C) are not significantly different (p-value < 0.05) and are below that of CB and SB.

Furthermore, the SFC result obtained at 4 °C under isothermal cooling from the melt in small steps and after long storage resulted in gains in SFC varying from ~65% (Et Ins) to 14% (Ac Ins).

The role of kinetics in the SFC of CWO fractions is significant and plays differently depending on the solvent polarity as evident from the SFC obtained at 4 °C under: (a) cooling from the melt in small steps (2 °C) and isothermal measurement, and (b) long storage time at 4 °C then direct measurements. The first protocol resulted in a much higher SFC with gains varying from ~65% for Et Ins to 14% for Ac. The gains obtained for CB is more than 6 times smaller than that of Et Ins and 1.4 times that of Ac Ins. The gains measured for Et Sol, Ac Sol, D Sol and N Liq are similar (4 times) and not significantly different from that of SB (4.5 times). The SFC data suggests significant opportunity for improving the SFC of the fraction to at least SB like levels by processing.

f. Can change the microstructure of CWO fractions as a function of solvent polarity.

The PLM of samples left 7-10 days at room temperature indicates that the microstructure of the fractions can be changed as a function of solvent polarity. The fractions displayed much smaller crystals than CWO. The crystals of the fractions are all distinguishable by shape, size and density of the crystal networks. The crystals of the stearin fractions are generally larger and organized in relatively compact, dense networks compared to the crystals of the olein fractions which are homogeneously distributed in a large liquid phase. The microstructure of the stearin fractions is close to that of CB and SB in terms of density but significantly differ in shape and size.

g. As a result of 1 – 6: one can tailor physical properties such as crystallization, melt and solid fat content and possibly texture (through drastic modification of microstructure) through fractionation, using polarity as a predictive tool.

Furthermore, can concentrate important bioactive components in fractions by using solvent polarity as a tool for selective partitioning. The bioactive rich olein fractions can be used to make improved emulsions creams and salves with specific physical properties, with enhanced antimicrobial and antifungal efficacy due to the enhancement of the concentration of the bioactive components through fractionation. The stearin fractions which have palmitic acid as the major fatty acid which would be effective in topical applications, alleviating skin dehydration and facilitating the absorption of bioactive compounds can be used for specialized products desirable in topical cosmetic and pharmaceutical applications.

4.1 Challenges and Future Perspectives

The development and manufacture of specialty products based on CWO which would take advantage of the lipids and bioactive components is a very promising area. The opportunities and challenges to the sustainability of CWO from land management to seed collection, oil extraction and processing and efficient use are considerable. However, from the Crabwood tree to the seed oil and the useable material, the research that addresses the physicochemical properties from a structure perspective is largely insufficient and dispersed. The present work shows the potential to make unique compositions with significantly different properties through fractionation of CWO, using polarity as a predictive tool. Its results invite for further investigations and optimization which would establish robust predictable relationships between processing conditions, chemical structure and function. The study of other solvents / solvent mixtures and fractionation conditions (solvent to oil ratio, crystallization temperature, agitation, cooling rate and crystallization time) that affect the yield and quality can result in controlled narrower fractions and partition of the of both the lipids and unsaponifiable components of CWO.

4.1.1 Challenges

Crabwood oil is a relatively abundant feedstock facing many problems because the tree is unsustainably logged for its mahogany-like timber quality [5, 6]. Given the tree is being logged already, bark and foliar extractions of valuable phenolics may be the most urgent to explore to add the very valuable non-timber uses of the tree. Currently CWO is mostly used traditionally for medicinal purposes and increasingly in cosmetics and personal care product. Understanding the variability of the oil at the seed level although challenging given the many factors at its origin is a critical step for a reliable and stable supply. The novel technologies that may be needed for an economical exploitation would add to cost and stave off the traditional market which is critical to

the indigenous and small local dealers of the oil. The ideas that are floated for its use in even larger industrial applications may cause severe restrictions on the access of the indigenous people to the commodity and therefore frustrate them of a significant source of income.

Traceability and adulteration of CWO are major concerns for CWO-based products. CWO-based pharmaceuticals and cosmetics are more expensive than synthetics and their environmental benefits, particularly those related to renewability, side effects and toxicity compared need to be popularized.

4.1.2 Perspectives for CWO and CWO fractionation

There are several research directions from a structure-property perspective that can be pursued to further improve the thermophysical and bioactivity properties of CWO-based products. These are related to (*i*) development of more effective extraction and processing techniques, (*ii*) production of novel separation techniques to obtain functional materials with narrower controlled molecular compositions and (*iii*) use of modification agents to direct and control phase behavior. Properties of importance to consider include the viscosity and flow properties, and hydrolytic and oxidative stability which influence the shelf and use life. The major challenges involved in these directions and potential solutions to overcome them are:

4.1.2.1 Making new molecular compositions

Unlike most vegetable oils, CWO is a TAG oil that contains essential fatty acids and potent bioactive components in sizeable amounts. There exists the possibility of achieving quite different CWO-based products through benign and environmentally friendly separation techniques. The lipid structure and unsaponifiable components can be controlled to direct the phase behavior, solid content and bioactivity in a significant and predictable manner. For example, CWO fractions with

thermal properties susceptible of demonstrating some properties of popular fats and oils can be achieved. Carefully designed processes which would allow for the controlled distribution of the unsaponifiable components in these products could provide a powerful platform to explore specific factors that contribute to varied functionality based on phase and bioactivity behaviors. The use of seeding agents such as diacylglycerol, monoacylglycerol, hard fat, sugar, etc. common in the fat and oils industries to influence fractionation, is an opportunity that can greatly benefit the crabwood oil industry.

4.1.2.2 Using the Current Oil and Fat Industry Techniques and Devising Specific Ones

The work on novel derivatization, transformation and purification processes to improve efficiency in the production of novel CWO-based materials is inexistant. There are efforts to improve on the extraction and purification processes to produce CWO materials. Several studies report on the use of mechano-chemical treatments, microwaves, or ultrasound to improve process yields under mild conditions. The physical and chemical refining to remove undesirable molecules to obtain target useful structures, is an area yet to be explored.

All the performance enhancement techniques that are routinely used on fats and oils can benefit CWO also. CWO and its fractions obtained in this thesis have a low solid fat content due to very large amounts of unsaturated molecules that remained in the stearin fractions. Beside more targeted fractionation methods, a solution to improves SFC so it can be used in specialty fats is possibly to use blending of known property enhancers, or to use the fractions as additives. Encapsulation techniques which have yielded some good results [51, 146, 224] but are still not sufficiently tested in many ways can lead to innovative solutions and tangible improvements in the delivery of the bioactive as well as essential lipids.

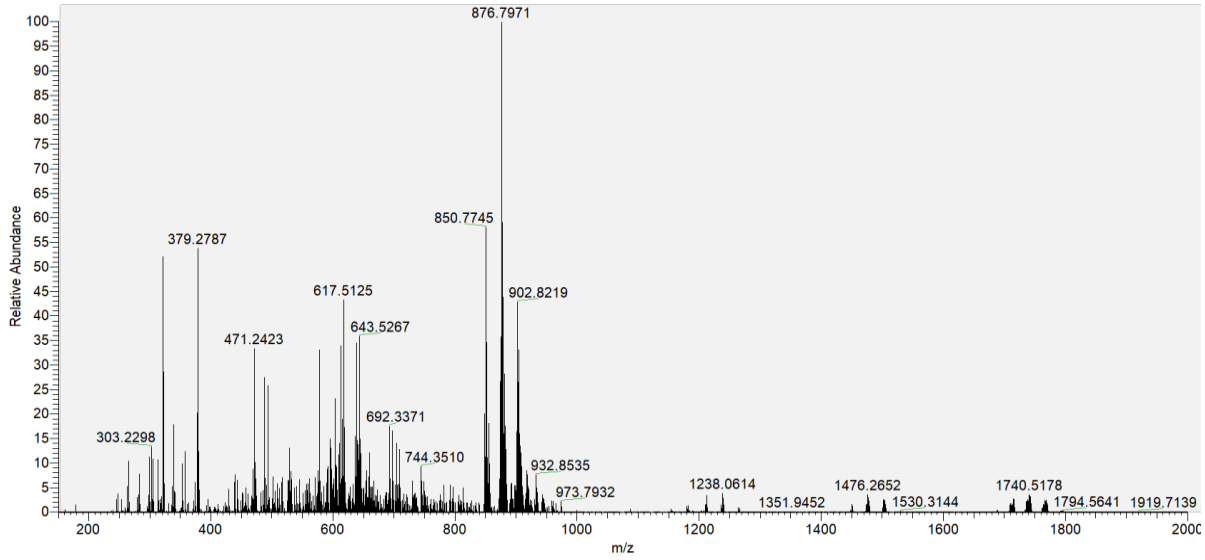
The preparation of these materials would be multifaceted and the ensuing phase behavior very complex. All aspects related to these materials as well as the techniques used to make them need careful investigation to determine their phase behavior and establish predictable structure-function relationships. Understanding the fundamental mechanisms driving the behavior of these materials and interactions at play would be instrumental in finding means to adjust the processes to make molecular systems with optimal performance.

APPENDICES

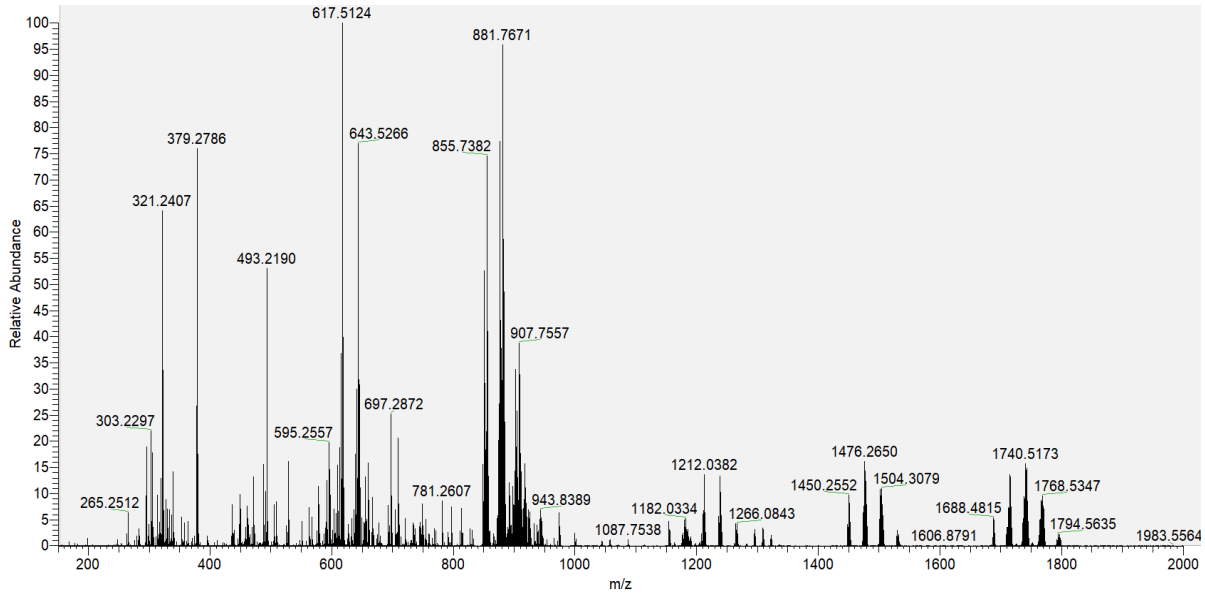
Appendix A: ESI-MS

Appendix A1. ESI-MS spectrums of Crabwood Oil and its fractions.

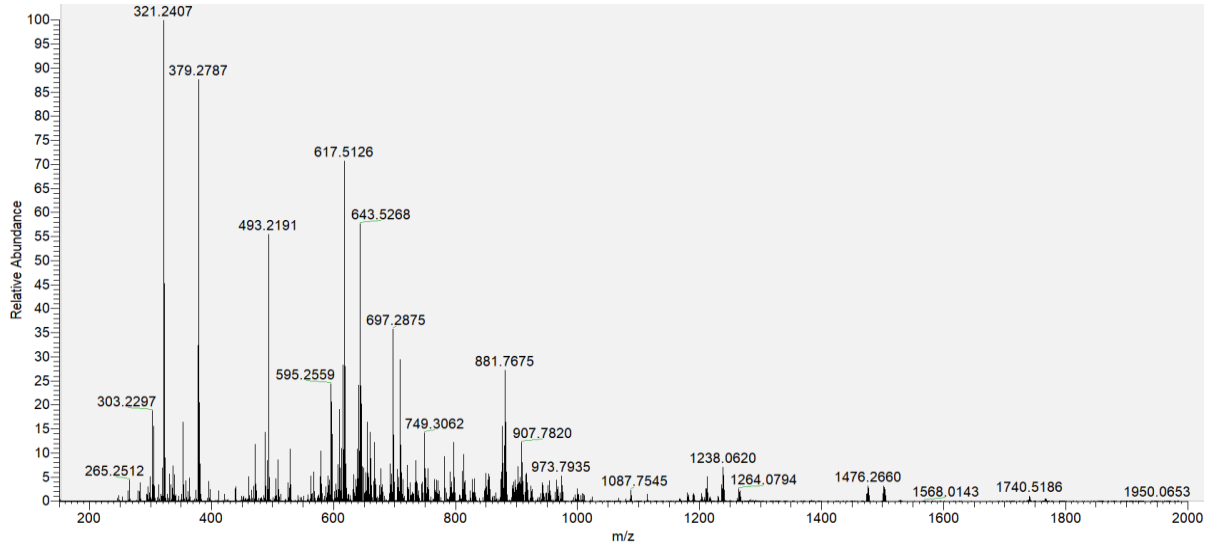
CWO



Et Ins



Et Sol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379.2787
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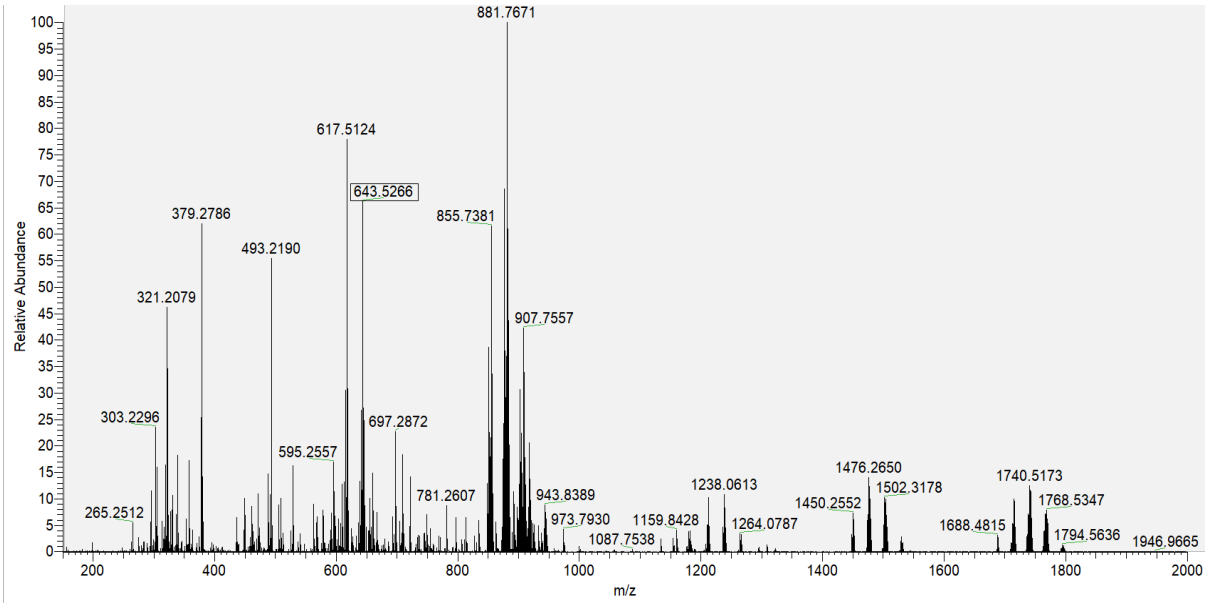
643.5268
493.2191
697.2875
881.7675
595.2559
303.2297
749.3062
907.7820
973.7935
265.2512
1087.7545
1238.0620
1264.0794
1476.2660
1568.0143
1740.5186
1950.0653
Relative Abundance
m/z

D Liq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595.2557
781.2607
943.8389
265.2512
973.7930
1159.8428
1087.7538
1238.0613
1264.0787
1476.2650
1502.3178
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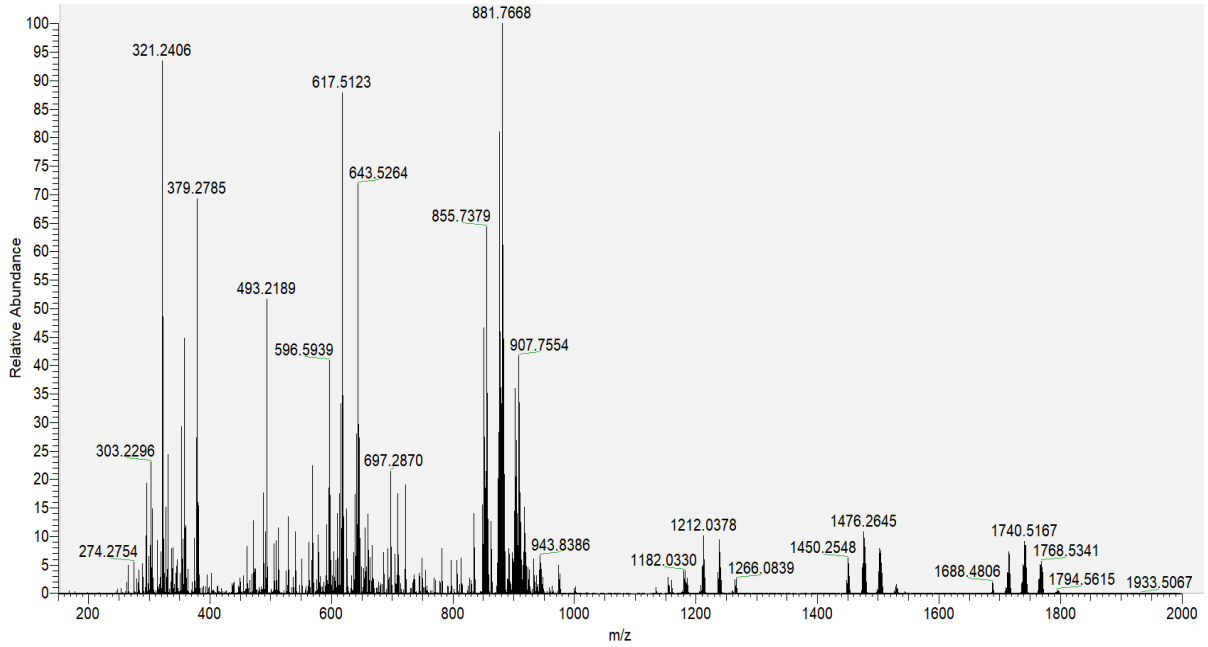
1768.5347
1688.4815
1794.5636
1946.9665
Relative Abundance
m/z

D Sol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907.7554
303.2296
697.2870
274.2754
943.8386
1212.0378
1476.2645
1740.5167
1450.2548
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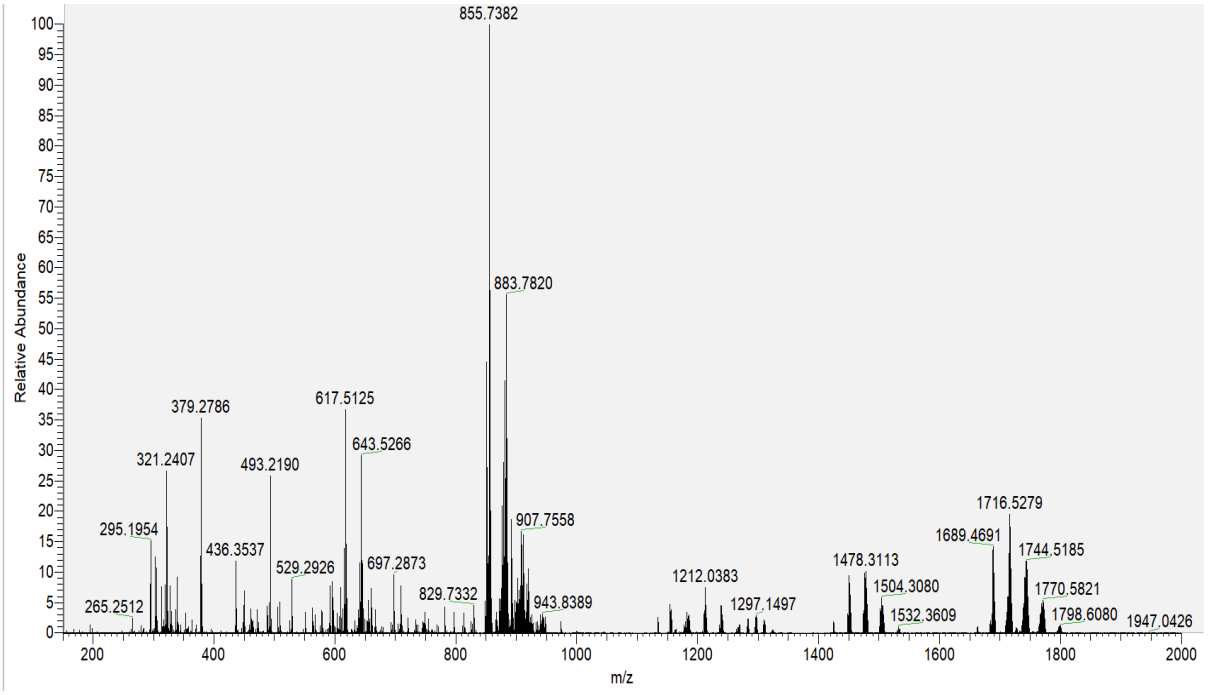
1266.0839
1688.4806
1794.5615
1933.5067
Relative Abundance
m/z

Ac Sol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379.2786
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643.5266
321.2407
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436.3537
1744.5185
1478.3113
529.2926
697.2873
1212.0383
1504.3080
829.7332
943.8389
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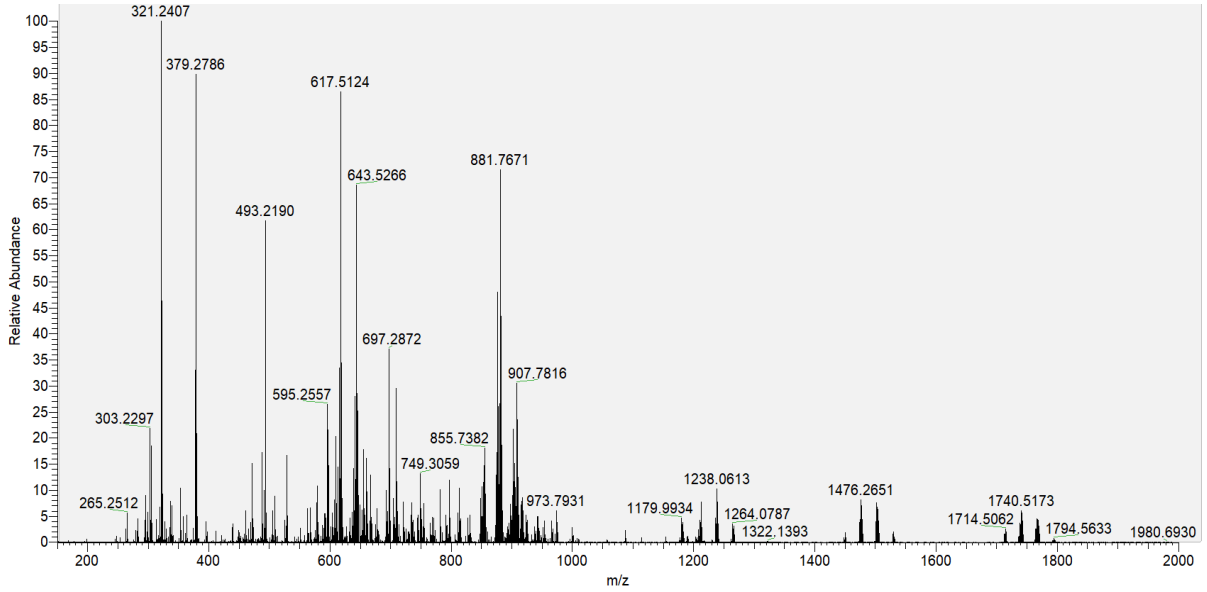
1947.0426
Relative Abundance
m/z

Ac Sol


Relative Abundance
m/z
321.2407
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617.5124
643.5266
881.7671
493.2190
697.2872
907.7816
595.2557
303.2297
855.7382
749.3059
265.2512
973.7931
1238.0613
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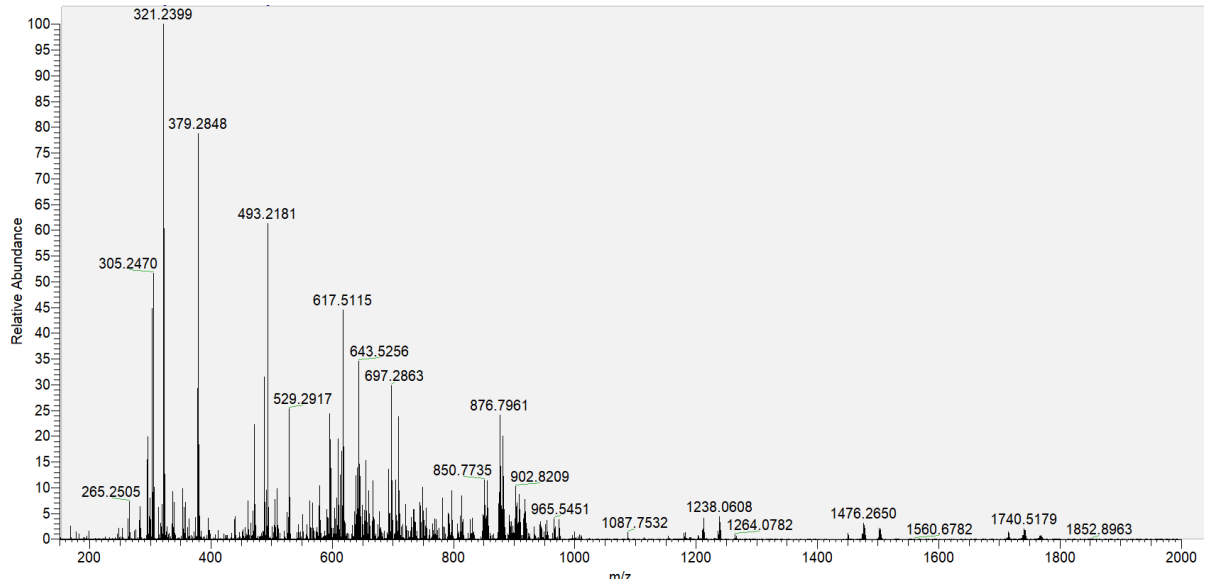
1794.5633
1980.6930

EA Ins


Relative Abundance
m/z
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617.5115
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697.2863
529.2917
876.7961
850.7735
902.8209
265.2505
965.5451
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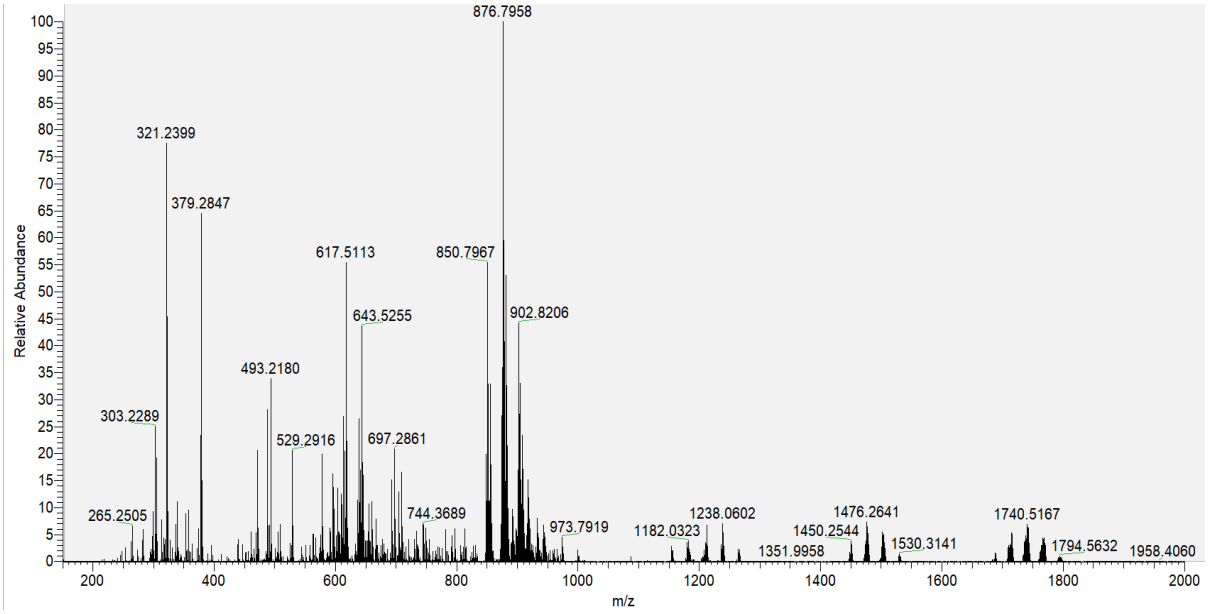
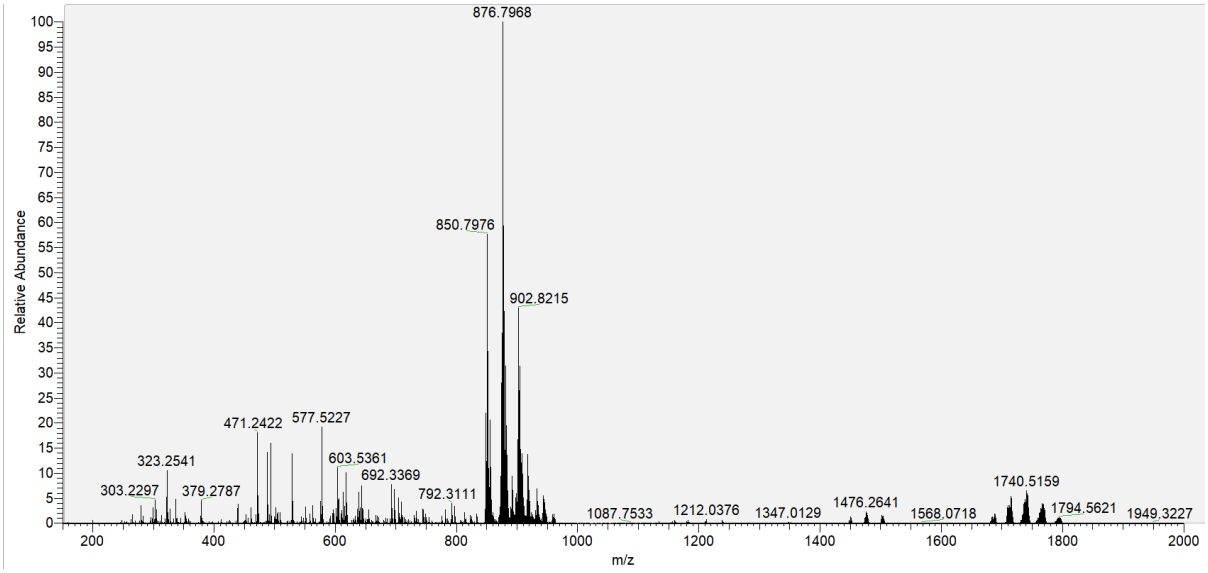
1476.2650
1560.6782
1740.5179
1852.8963

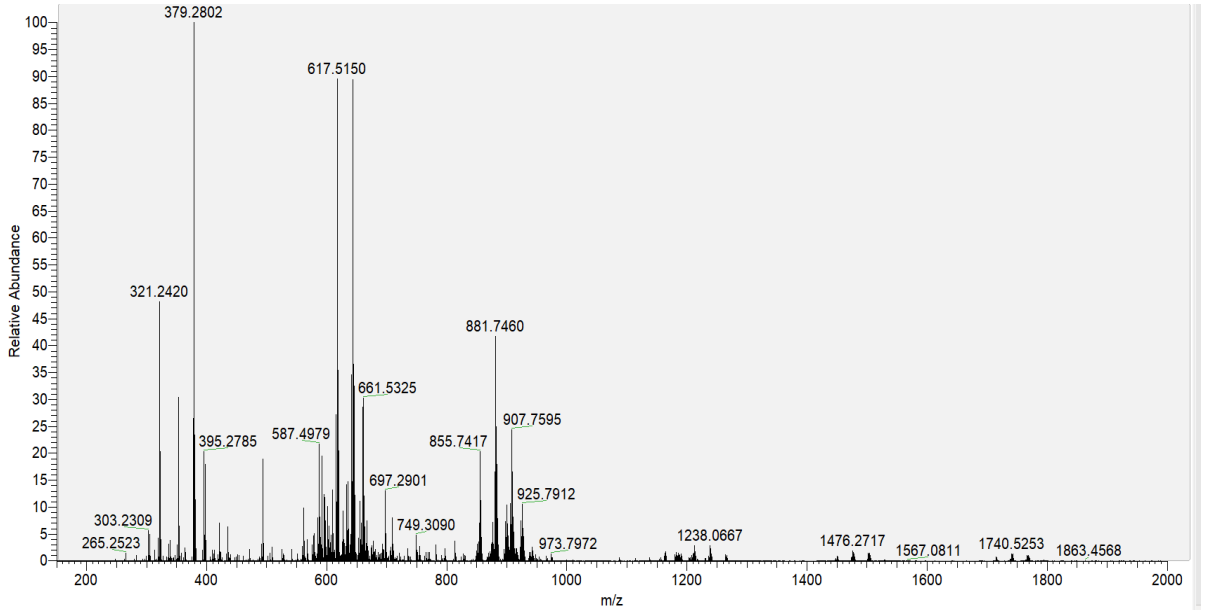
EA Sol



Native Liquid



N Sol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303.2309
749.3090
265.2523
973.7972
1238.0667
1476.2717
1567.0811
1740.5253
1863.4568
Relative Abundance
m/z

Appendix A2. Bioactive profile of Crabwood oil and its fractions.

***m/z*[M+Na]$^+$**	**Elemental Composition**	**#**	**Putative compound**	**CWO**	**D Sol**	**D Liq**	**Et Sol**	**Et Ins**	**Ac Sol**	**Ac Ins**	**EA Sol**	**EA Ins**	**N Liq**	**N Sol**
461.1940	C26H30O6	L1	7-deacetoxy-7-oxogedunin	3.0	4.5	4.5	2.4	4.0	2.6	4.5	3.7	3.2	3.7	1.2
463.2097	C26H32O6	L2	7-deactylgedunin	0.3	0.6	0.4	0.2	0.3	0.2	0.4	0.5	0.4	0.4	0.1
489.2253	C28H34O6	L3	Deoxygedunin	0.1	3.0	2.9	0.5	2.1	0.8	3.2	0.4	0.9	0.1	0.7
505.2202	C28H34O7	L4	Gedunin	3.1	5.1	4.7	2.1	4.1	2.5	4.7	3.8	3.4	4.2	2.0
563.2257	C30H36O9	L5	6α-acetoxygedunin	3.0	5.2	4.7	2.4	3.9	2.8	4.6	3.5	3.4	6.8	2.4
491.2046	C27H32O7	L6	Andirobin	4.4	5.4	5.7	4.0	5.5	4.3	5.5	4.3	3.9	3.4	4.3
493.2202	C27H34O7	L7	Methyl angolensate	20.7	28.9	29.1	26.2	27.8	26.5	28.9	23.1	27.8	30.9	26.1
509.2151	C27H34O8	L8	Methyl 6-hydroxyangolensate	4.6	4.2	5.3	4.1	4.4	3.8	5.8	4.8	4.4	4.2	3.6
507.1995	C27H32O8	L9	8beta-hydroxycarapin, 3,8-hemiacetal	0.03	0.1	0.0	0.03	0.02	0.02	0.02	0.03	0.0	0.0	0.04
613.2625	C31H42O11	L10	Carapanolide A	0.7	0.01	0.02	0.8	0.2	0.5	0.1	0.3	0.4	0.3	0.9
625.2625	C32H42O11	L11	Carapanolide B	0.5	0.01	0.01	0.6	0.1	0.5	0.02	0.4	0.5	0.1	1.6
747.2993	C39H48O13	L12	Carapanolide K	0.4	0	0	0.02	0	0.01	0	0.1	0.0	0.0	0.0
665.2210	C33H38O13	L13	Carapanolide L	1.0	0.7	0.5	1.1	0.7	0.9	0.6	0.8	0.9	0.3	1.2
735.2265	C36H40O15	L14	Guianolide A	0.3	0.03	0.04	0.4	0.1	0.3	0.03	0.1	0.2	0.0	0.3
693.2159	C34H38O14	L15	Guianolide B	0.01	0	0	0.02	0.01	0.01	0.0	0	0	0	0
667.2730	C34H44O12	L16	11-Acetoxykhivorin	5.1	4.3	3.9	5.8	4.9	5.5	4.4	5.4	5.1	3.2	0.0
697.2836	C35H46O13	L17	Trichilin A	13.3	12.3	11.9	16.8	13.2	15.9	10.8	14.2	13.6	13.1	18.1
473.2304	C28H34O5	L18	Azadiradione	2.1	2.0	2.4	1.0	1.2	1.2	2.0	1.6	1.2	3.1	0.2
597.2676	C31H42O10	L19	Asclepin	10.4	7.1	5.5	9.7	7.7	9.3	6.5	9.4	8.8	5.4	15.9
771.2522	C29H48O22	L20	NCGC00381438-01	1.3	1.0	1.2	0.7	1.4	2.01	1.1	1.7	1.8	0.5	2.2
709.2965	C33H51O13P	PG 1	PI 24:6	9.3	7.9	8.8	12.4	9.7	11.4	7.8	10.4	9.9	7.6	9.5
473.3243	C27H46O5	Sterol 1	Certonardosterol L	2.7	0.02	0.1	0	0	0	0	0	0.0	0.0	0.0
421.3082	C27H42O2	Sterol 2	24-keto-25dehydrocholesterol	0.2	0.1	0.1	0.3	0.2	0.28	0.1	0.3	0.2	0.0	4.3

527.2621	C28H40O8	Sterol 3	Ixocarpalactone A	0.6	0.02	0.2	0.1	0.1	0.1	0.02	0.2	0.2	0.1	0.5
529.3141	C29H46O7	Sterol 4	2-deoxy-20-hydroxy-5alpha-ecdysone 3-acetate	4.6	3.0	3.4	2.0	3.4	2.9	3.9	6.2	5.0	11.6	0.4
531.3662	C30H52O6	Sterol 5	6beta-acetoxy-24-methylcholestan-3beta,5alpha,22R,24-tetrol	3.9	0.1	0.1	0	0.01	0	0.02	0	0	0	0.01
551.3196	C28H48O9	Sterol 6	Campesterol	0.1	0.4	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.7	0
435.3603	C29H48O	Sterol 7	Stigmasterol	0.1	0.1	0.6	0	0.7	0.1	0.9	0	0	0.02	0.04
797.2785	C42H46O14	Flavonoid 1	(2R,3S)-2,3-trans-3-Acetoxy-5-[(2R,3S)-2,3-trans-3-acetoxy-3',4',7,8-tetramethoxyflavan-5-yl]-3',4',7,8-tetramethoxyflavan	3.3	2.9	2.8	4.6	3.2	4.1	3.1	3.4	3.5	0	2.4
615.4237	C35H60O7	Tocopherol 1	alpha-Tocopherol-beta-D-mannoside	0.1	0.4	0.1	0.1	0.2	0.1	0.1	0.03	0	0	0.5
771.2894	C43H44N2O10	Diterpenoid 1	Miliamine C; 5-Anthraniloyl type moiety, 20-Ac-Ingenol diterpenoid	0.9	0.6	1.1	1.3	1.1	1.4	0.9	1.2	1.1	0.3	1.5

Appendix B: XRD Data

Appendix B1: (a) WAXD and (b) SAXD data.

(a) WAXD		**χ (%)**	**Typical Peaks for phase identification (Å)**					**Secondary peaks (Å)**						
			β			**β′**								
			010	011	-100	(-110) (110)	200	020	100					
CB	β	80±2	4.56	4.26	3.84			4.06	3.64	5.73	5.37	5.19	5.05	4.74

	β′					4.18	3.73			3.96	3.55	3.35	3.24	3.07
SB	β +	53±2	4.55	4.23	3.90			4.02		5.78	5.40	5.22	5.10	4.78
	β′					4.11	3.71			3.97				
CWO	β′	5±1				4.14	3.73							
D Sol	β′	19±1				4.12	3.72			4.33				
Et Sol	β′	15±1				4.13	3.75			4.35				
Ac Sol	β′	5±1				4.12	3.72							

(b) SAXD (Å)	**Form**	**001**	**002**	**003**	**004**	**005**	**006**	**008**	**009**	**Configuration**
CB	β	65.5	32.2	21.2	16.06	12.76	10.63	8.12	7.28	TCL - C18 chair configuration
	β′	45.21		14.80						DCL - C18 fork configuration
SB	β	66.10	32.51	21.77	16.25	12.97	10.79	8.10	7.19	TCL - C18 chair configuration
Crabwood oil	β′	38.1		12.4						DCL - C16 fork configuration
CWO	β′	38.8		12.8						DCL - C16 fork configuration
D Sol	β′	38.1		12.7						DCL - C16 fork configuration
Et Sol	β′	38.2		12.6						DCL - C16 fork configuration

Appendix C: Differential Scanning Calorimetry

Appendix C1. DSC Crystallization data. (Temperatures in ℃, Enthalpy ΔH in J/g)

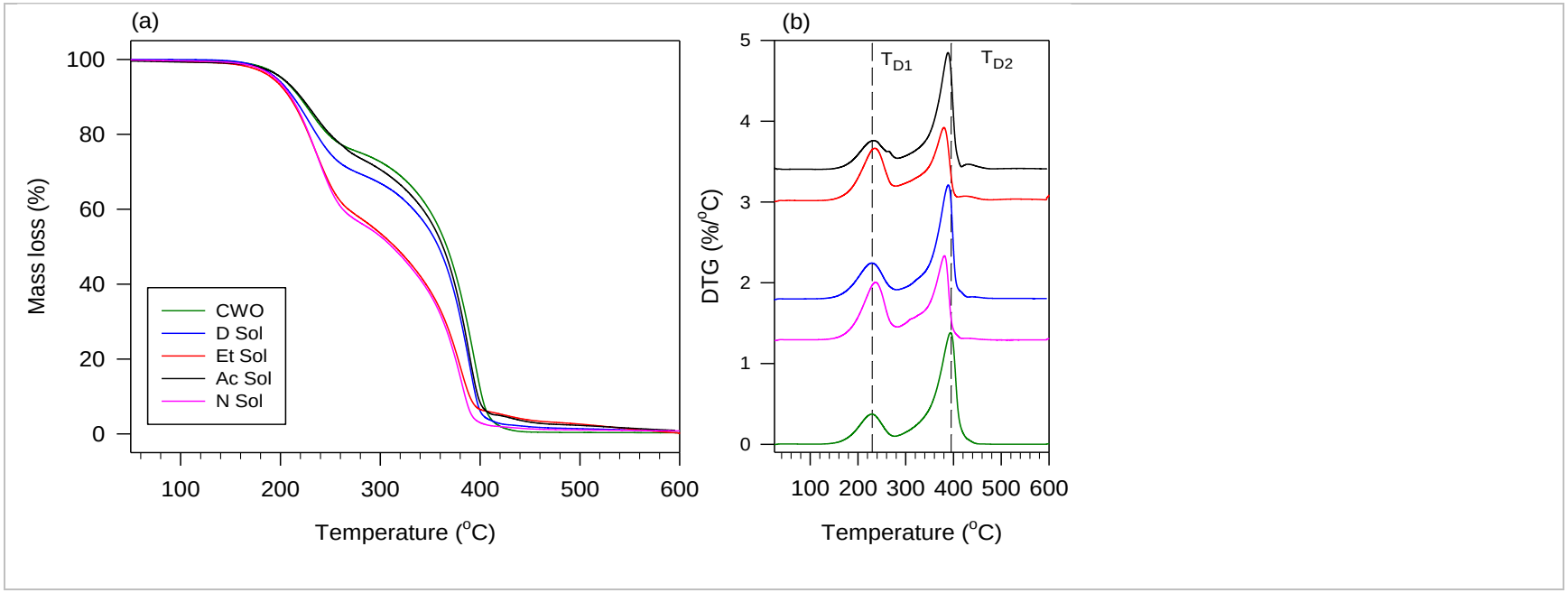
Sample	T_{ON}	T_{c1}	ΔH_1	T_{c2}	ΔH_2	T_{c3}	ΔH_3	T_{OFF}	ΔH_{tot}
		Zone I ~ (up to 3 ℃)		**Zone II ~ (up to -35 ℃)**		**Zone II ~ (up to -60 ℃)**			
CWO	15.6	11.4	12.8	-3.4	27.2	-47.4	12.3	-51.5	52.3
N Sol	28.1	25.5	32.6	-6.3	24.7	-31.7	29.9	-51.7	87.2
N Liq	17.3	11.8	2.4	-0.8	43.4	-32.2	4.9	-49.9	50.7
D Sol	27.6	26.0	29.6	-2.3	29.2	-45.8	12.6	-51.3	71.4
D Liq	5.3	--	0	-1.7	30.3	-45.1	11.7	-50.1	42
Et Sol	25.5	23.5	25.2	-4.0	18.9	-26.1	32.3	-37.4	76.4
Et Ins	14.5	11.0	4.9	0.6	52.8	-50.4	7.3	-58.8	65
Ac Sol	18.4	16.3	16.9	-4.0	37	-45.1	19.6	-49.4	73.5
Ac Ins	25.6	16.8	14.1	2.8	22.2	-44.6	6.7	-54.6	43
EA Sol	15.9	9.0	12.2	-4.4	23.9	-47.5	11.7	-51.5	47.8
EA Ins	18.4	17.0	14.4	-3.3	26.9	-46.9	10.9	-52.2	52.2
SB	19.9	11.5	55	-8.4	12.5	0	0	-34.6	67.5
CB	15.4	11.8	73	1.3	9	0	0	-21.	82

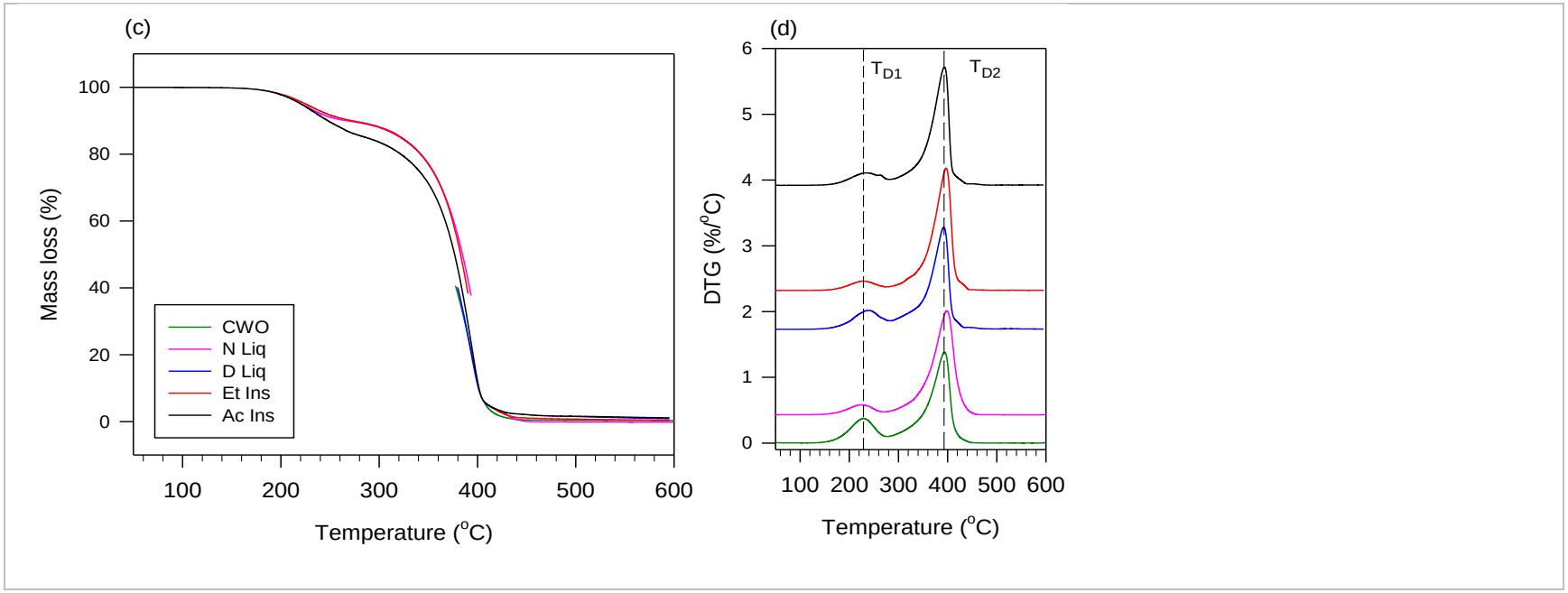
Appendix C2. DSC Melting data (Temperature in ℃, Enthalpy ΔH in J/g).

Sample	T_{ON}	Temperature range										T_{OFF}	ΔH_{tot}
		High Temperature					Low temperature						
		60℃ to 25℃		25℃ to 10℃		ΔH_I	10℃ to -5℃		-5℃- to 60℃		ΔH_{II}		
		T_{m1}	ΔH_1	T_{m2}	ΔH_2		T_{m3}	ΔH_3	T_{m4}	ΔH_4			
CWO	-21.4	27.1	5.5	20.2	9.8	15.3	-0.4	24.7	-9.5	33.5	58.1	32.5	73.2
N Sol	-22.5	31.2	33.3	0	0	33.3	8.6	7.9	-7.7	40.8	48.7	39.8	82
N Liq	-23.4	0	0	0	0	0	2.9	40.5	-10	8.6	49.1	10.0	49.6
D Sol	-21.4	36.7	20.8	18.4	4.7	25.5	0.6	24.3	-8.8	32.9	57.2	41.3	82.70
D Liq	-20.4	0	0	0	3.6	3.6	1.7	20.1	-8.2	23.6	43.7	20.6	47.3
Et Sol	-21.4	19.5	24.8	15.7	13.5	38.3	4.8	0	-11.2	40.0	40	25.1	78.2
Et Ins	-19.8	34.6	4.9	0	0	4.9	13.2	49.9	-11.2	15.2	65.1	39	70
Ac Sol	-23.2	23.4	14.7	0	6.3	21	10.8	1.5	-9.8	50.8	52.3	29.4	73.3
Ac Ins	-19.8	28.6	2.9	0	54	56.9	10.5	0	-9.9	18.1	18.1	33.7	75
EA Sol	-21.8	27.7	15.1	21	-	15.1	-0.6	54.4	-9.24	-	54.4	31.7	69.5
EA Ins	-21.5	44.4	0.9	27.6	14.6	15.5	0.2	20.5	-9.2	30.9	51.4	49.2	67.1
SB	-8.7	0	0	0	0	0	14.5	58.4	-4.4	9.5	67.9	29.7	67.9
CB	-16.7	0	0	0	0	0	20.5	85.8	-10.8	1.9	87.7	25.8	87.7

Appendix D: Thermal Degradation Data.

Appendix D1: TGA and DTG stacks of Crabwood Oil and its stearin and olein fractions.




(c)
Mass loss (%)
100
80
60
40
20
0
CWO
N Liq
D Liq
Et Ins
Ac Ins
100
200
300
400
500
600
Temperature (°C)
(d)
DTG (%/°C)
6
5
4
3
2
1
0
T_D1
T_D2
100 200 300 400 500 600
Temperature (°C)

Appendix D2. DTG and TGA data for Crabwood Oil and its fractions with Cocoa Butter and Shea Butter for comparison.

	DTG							TGA			
Sample	**TD1 (°C)**	**Rm1 (%/°C)**	**TD2 (°C)**	**Rm2 (%/°C)**	**TD3 (°C)**	**Rm3 (%/°C)**	**Ton (°C)**	**1%**	**5%**	**10%**	**Ash %**
CWO	229.2	0.37	393.9	1.38	0	0	173.8	165.9	201.3	218.9	0.3
N Sol	237.1	0.7	381.1	1.0	430.7	0.02	181.8	157.0	194.7	210.5	0.8
N Liq	224.9	0.15	398.3	1.58	0	0	169.7	180.5	200.7	217.2	0
D Sol	229.0	0.4	389.1	1.41	438.6	0.02	169.8	165.3	195.9	212.7	0.8
D Liq	239.8	0.28	397.3	1.5	441.9	0.03	181.5	173.4	212.9	233.4	0.9
Et Sol	234.5	0.65	380.0	0.91	431.3	0.1	177.1	142.1	192.0	208.9	0.3
Et Ins	226.6	0.1	396.9	1.8	431.34	0.06	172.7	182.9	225.2	272.3	0.4
Ac Sol	232.4 [265.9]	0.36 [0.23]	388.6	1.45	453.9	0.02	175.5	148.6	201.6	220.4	0.9
Ac Ins	234.7 [262.8]	0.19 [0.16]	393.6	1.79	431.3	0.07	171.1	183.6	220.2	247.7	1.1
EA Sol	224.8	0.3	386.5	1.3	432.9	0.05	164.1	156.2	194.9	214.3	0
EA Ins	232.4	0.3	382.5	1.6	425	0.1	170.5	171.3	204.4	223.4	1.2
CB	220.9	0.02	403.8	2.23	0	0	150.3	236.4	335.7	355.0	0
SB	240.7	0.06	403.2	1.89	0	0	179.4	210.1	298.6	340.8	0.1

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