Lipid Fractionation and Physicochemical Characterization of *Carapa guianensis* Seed Oil from Guyana

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Abstract: The seed oil of *Carapa guianensis*, known as crabwood oil (CWO), is distinguished for its medicinal and cosmetics applications, attributed to its bioactive components and lipid profile. CWO and its dry and solvent fractionation were studied, with a focus on physicochemical functionality and the partitioning of known bioactive compounds, such as limonoids and sterols. Important bioactive components, including limonoids and sterols, were partitioned depending on the fractionation method; in particular, there is a direct dependence on solvent polarity. There was a very strong solid fraction yield–solvent polarity with a high linear slope of −121.3%. The partitioning of the lipids is significant enough to drive measurable and predictable changes in the physical properties. Palmitic (P: C16:0) and oleic (O: C18:1) fatty acids account for about 60% of the total fatty acid composition of the TAGs of CWO and its fractions. The most abundant limonoid is methyl angolensate (from 28 to 39%), followed by Trichilin A (from 13% to 22%). Gedunin and Andirobin were more abundant in the liquid fractions, whereas Carapanolides (less than 1.3%) were more present in the olein fractions. The crystallization and melting temperatures of the solid fractions were up to 26 °C, compared to 11 °C for CWO, and were particularly strongly correlated to the polarity of the solvents. The SFC profile indicated semi-solid fats, with the solid fractions showing up to 19% at 18 °C, twice the SFC in CWO. The fractions demonstrated a wide range of distinguishable microstructures. The shapes include well-organized spherulites and needle-like and rod-like crystals with sizes varying from 5 to 250 µ, suggesting that they are likely to have different flow characteristics and feel to the skin and mouth. There is a potential to make unique compositions with significantly different properties, with antimicrobial and antifungal efficacy due to the bioactive components of CWO through fractionation, using polarity as a predictive tool.

Keywords: *Carapa guianensis*; crabwood oil (CWO); dry fractionation; solvent fractionation; chemical profile; lipids; bioactive compounds; physical properties

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 8000 species, of which 6500 are identified [1]. Amid this biodiversity, the *Carapa* Aublet species, commonly referred to as Crabwood and Andiroba, from the *Meliaceae* family of flowering plants, receives special attention because of its potentially high economic value [2,3]. Crabwood trees are native to tropical and subtropical regions and thrive in the rich soils found in swamps, alluvial flats, marshes and uplands of South and Central America, the Caribbean and tropical Africa [4–6]. The following three species of Crabwood are found in Guyana: *Carapa guianensis*, the most predominant, *Carapa akuri* and *Carapa surinamensis* [7].

The extracts from the crabwood tree, especially the oil extracted from its seeds, known as crabwood oil (CWO), have been recognized in traditional medicine for several centuries [4]. The extract from the leaves, bark and seeds is traditionally used to improve
wound healing and treat various conditions, such as fungal infections [8], malaria [9] and fever [10]. CWO has been used as an itch-relieving, insect-repelling [11], anti-inflammatory [12], antimalarial [13] and skin-protecting medicine [14]. CWO is actively investigated in the scientific community, reaching beyond the ethno-medicinal uses to less traditional applications. The pharmaceutical and homeopathic industries sell the oil in capsules advertised to treat various conditions, including diabetes and rheumatism [13]. Some pharmacies in Georgetown, Guyana, process the oil into soap, candles and insecticidal washes [3]. CWO is also used in modern-day formulations, including in shampoos, salves, creams and other emulsified cosmetics [15,16]. Guyana’s National Institute of Applied Science and Technology has formulated a medicated face wash from CWO, widely used to treat chronic acne [17].

Chemically, CWO is 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 [18]. The remaining 2–5% non-saponifiable component includes structurally varied secondary metabolites, mostly highly oxygenated limonoids, but also flavonoids, sterols and phenolic compounds, which are attributed to the oil’s biological activities and its bitter taste [19]. The presence and yields of the oil’s constituents may be affected by interspecies variations, environmental conditions and extraction methods. However, these variations are not always manifested visibly [20].

The lipid component of CWO is comprised of primarily triacylglycerols (TAGs) and other minor components, including free fatty acids (FFAs), diacylglycerols (DAGs), monoacylglycerols (MAGs) and phospholipids [18,20,21]. The unsaturated fatty acids percentages have been reported to be up to 60% [22]. The reported lipid fraction consists of up to 67% TAG, 10% DAG, 20% MAG and FFA. 1,2-dioleoyl-3-palmitoyl-glycerol (POO) is the most abundant TAG, accounting for approximately 20% of all the TAGs present. Other reported TAGs include 1-palmitoyl-2-palmitoyl-3-oleoyl-glycerol (PPO), 1-palmitoyl-2-stearoyl-3-oleoyl-glycerol (POS) and triolein (OOO). Together, these make up less than half the content of POO in CWO [18].

The medicinal and other technological applications of CWO have been an active area of research [23]. For example, biocatalysis and enzymatic catalysis have been used to transform CWO into materials suitable for biomedical and industrial applications [24,25]. Free fatty acid amides (FFAA) with anticonvulsant properties have been produced using biocatalysts [25]. CWO has been used for the preparation of biodiesel by enzymatic catalysis [24]. Excellent physicochemical properties and activity against Aedes aegypti have been reported for nanoparticles made of silk fibroin with esters obtained from the oil of Carapa guianensis [26]. The waste products of CWO have also been tested for the production of sustainable new materials, such as adsorbents or microporous activated carbon for CO₂ capture [27,28].

The physical properties of CWO have been well characterized, including quality and identity parameters, such as acid index, peroxide value, iodine value, FFAs, ester index and viscosity [18]. Studies that consider the plant ecology, seed harvest and processing conditions are scarce and dispersed, even though they are potentially important to the oil’s chemical composition, bioactivity and physicochemical properties, and hence its efficient and sustainable use [18,29–31].

Like other tropical oils, such as palm oil [32], coconut oil and shea butter [33], which are commonly fractionated to make specialty materials [34], CWO can be fractionated into solid (stearin) and liquid (olein) fractions. The fractionation of CWO is a potential avenue to amplify the uses and bioactivity of the oil, but it has been reported only once for Carapa grandiﬂora, a species of crabwood found in Western Africa [35]. The authors used dry fractionation and with acetone reported yields of 41% for the solid fraction from dry fractionation and 53% for the soluble fraction from acetone. Carapa guianensis, which has approximately 30% total saturated fatty acids compared to 40% for Carapa grandiﬂora [18–36], is expected to provide very different stearin and olein fractions. CWO fractionation, as a
means to extend and amplify the use of the oil, is more challenging than the other tropical
oils because of its high unsaturation levels.

This communication describes the fractionation of C. Guianensis seed oil (CWO)
sourced from Guyana using dry and solvent fractionation with the following three differ-
ent solvents of varying polarity: ethanol, acetone and ethyl acetate. The selection of the
three solvents was based on polarity/ pKa and selectivity criteria. A targeted fractionation
of CWO, which comprises lipids and bioactive compounds, is challenging because of the
delicate balance between crystallization and selective extraction using solvents. Ethyl ac-
etate, acetone and ethanol are included in a range of polarities, which would allow to
measurably adjust fractionation yield and chemical composition. This approach is also
intended to improve our understanding of polarity’s impact on the partitioning of the
different compounds by fractionation and possibility of using polarity or pKa as a pre-
dictability tool.

The hazard risks and toxicity of the solvents are considered. Ethyl acetate, acetone
and ethanol are flammable volatile organic compounds. Ethyl acetate vapor/air mixtures
are explosive, its inhalation can lead to irritations and impaired coordination [37], cause
organ damage, central nervous system depression and can even be lethal in cases of severe
exposure [38]. Acetone and ethanol are both safer when compared to ethyl acetate, with
ethanol being the less harmful solvent unless consumed in high quantities [39,40].

Sustainability, availability and cost are also to be considered in the case of industrial
scale use. In this regard, ethanol would be advantageous for solvent-mediated fractiona-
tion processes. Ethanol is manufactured in Guyana, widely available and relatively afford-
able making it an effective and sustainable solvent choice for solvent-mediated fractiona-
tion for the local cosmetic and pharmaceutical industries.

Established phytochemical and physicochemical functionality analysis methods
were utilized to compare the composition and physical functionality of the solid (stearin)
and liquid (olein) fractions. The chemical compositions of the fractions were determined
by ESI-MS and metabolomic analyses, as typically performed for the characterization of
similar seed oils [41].

2. Materials and Analytical Methods

2.1. Materials

Two samples of naturally separated crabwood oil, so-called Native SOLID (NSOLID)
and Native LIQUID (NILIQUID), were purchased in August 2021 in Georgetown, Guyana,
from A. Leitch (Georgetown, Guyana) for GYD 2000 or ~USD 13/L. They were obtained
by letting CWO sit at ambient temperature and then decanting the liquid from the solid.
Unseparated CWO was purchased from the same supplier in July 2023. The native and
separated CWO samples were produced from seeds collected by hand from the forest
floor at Ebini Upper Demerara Berbice, Guyana and extracted there using an artisanal
method. In this method, the seeds are cleaned of debris with stream water, boiled in fresh
water for about 12 h, then removed from the water and stored in a cool and dry area for
2–3 weeks. The seeds are then shelled and the kernel is directly exposed to sunlight on
galvanized roofing sheets and hand-kneaded to collect the oil in bottles. A semi-porous
bag is eventually used to press the remaining oil [29,42]. Ethanol, acetone and ethyl acetate
(purity >90%), HPLC grade chloroform and methanol (Sigma-Aldrich, Oakville, ON, Can-
da) were purchased from VWR, Mississauga, ON, Canada.

2.2. Crabwood Oil Fractionation

CWO was fractionated using dry fractionation and solvent fractionation with etha-
nol, acetone and ethyl acetate. Before fractionation, the oil was fully melted using a water
bath at 40–45 °C.
2.2.1. Dry Fractionation

Dry fractionation was performed following a method from the literature [43]. It was conducted under stirring using a 2-L jacketed reactor fitted with an Isotemp™ temperature regulating system (Fisher Scientific, Waltham, MA, USA). The temperature, which was first set at 40 °C under stirring at 50 rpm for 10 min, was reduced by 1 °C/h to room temperature (RT= 18 °C) and then left at RT for 15 h under 50 rpm stirring. The resulting slurry was then cooled at 1 °C/h to 15 °C and kept at 15 °C under 250 rpm stirring for 17 h. The liquid fraction was then 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 [44]. In each solvent fractionation, 4 parts solvent were added to 1 part of the fully melted CWO: 200 g CWO was 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 h to allow the stearin to solidify while the olein remained liquid. The mixture was stirred at 50 rpm for 24 h before filtration. The soluble and the insoluble components were separated using Fisherbrand P8 filter paper under moderate vacuum. The solvent was removed by rotary evaporation (vacuum = 15 psi, bath temperature = 30–40 °C) and slow rotation (30 rpm). The fractions were weighed, stored in closed glass bottles and kept refrigerated at 4 °C until further analysis.

2.3. Methods

2.3.1. Electrospray Ionization Mass Spectrometry

Electrospray ionization mass spectrometry (ESI-MS) was performed on a QExactive Orbitrap mass spectrometer (Thermo Scientific, San Jose, CA, USA, resolution of 140,000 full width at half maximum (FHWM); at 200 m/z). The Orbitrap was calibrated daily using Pierce™ LTQ ESI positive ion calibration solution (Thermo Scientific). The samples (1 ppm w/v) were prepared using chloroform: methanol (70:30 v/v). The data were processed using the Qual Browser tool of the Thermo Xcalibur software version 3.1 (Thermo Scientific).

2.3.2. Differential Scanning Calorimetry (DSC)

The thermal transition behavior was determined using a Q200 DSC (TA Instruments, Newcastle, DE, USA) 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, and then heated back to 60 °C at 10 °C/min.

2.3.3. Solid Fat Content Measurements

The solid fat content (SFC) was measured on a Bruker Minispec mq 20 (Bruker, Milton, ON, Canada) pulsed NMR spectrometer equipped with a combined high- and low-temperature probe and a BVT3000 temperature controller (Bruker Ltd.). Liquid nitrogen was used for tempering. The fully melted sample was pipetted into the bottom of an NMR tube filling its bottom 1 cm (~0.57 ± 0.05 mL), stored at RT (18 ± 1 °C) and at 4 °C both for 8–10 days and measured at these temperatures. The samples were also melted in situ and progressively cooled down in 2 °C steps and measured at each temperature isothermally until a plateau is reached. Bruker’s Minispec V2.58 Rev12 and Minispec plus V1.1 Rev05 software were used to collect the SFC data.

2.3.4. 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 image the microstructure 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 RT (18 °C) for 7–10 days to allow the fat to crystallize. The samples were imaged under cross-polarizers and captured using a digital camera (DFC 420C, Leica Microsystems) mounted to the PLM.

2.3.5. X-ray Diffraction

The X-ray diffraction (XRD) measurements were performed on an Empyrean X-ray diffractometer equipped with Cu-Kα radiation operated at 45 kV and 40 mA, and a PIXcel3D detector (PANalytical B.V., Lelyweg, The Netherlands). The sample was melted at 40 °C, then loaded into preheated glass capillary tubes (Hampton Research, Aliso Viejo, CA, USA), left to crystallize at RT= 18 °C for 7–10 days, then 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 PANalytical’s Data Collector (V 3.0a) software. The regions of wide-angle X-ray diffraction scattering (WAXD, 2θ >15°) and small-angle X-ray diffraction scattering (SAXD, 2θ <15°), which fingerprint the subcell structures and the molecules layer order along the normal direction, respectively, were analyzed similar to other fatty compounds [45,46].

2.3.6. Thermogravimetric Analysis

The thermogravimetric analysis (TGA) of CWO and its fractions was conducted on a Q500 (TA Instruments, Newcastle, DE, USA). The sample (10–15 mg) 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 onset) as is typical in the field [47]. The derivative of the TGA (DTG) was used to assess the mechanisms of mass loss.

2.4. Statistical Analysis

One sample of NSOLID and NLIQUID, and CWO were used for the experiments. The fractionations were not performed in replicates. The physical properties were performed, at least in duplicates. Analysis of variance (ANOVA) followed by the Tukey test was conducted to assess group differences and determine the relationship between the variables measured in each experiment. Pearson product-moment correlation analysis, which describes the linear relationship between two quantitative variables, was conducted to determine the strength and positive or negative nature of the correlation. The statistical analysis was performed using the Sigmastat module of Sigmaplot 12.5 software.

3. Results and Discussion

The solid and liquid fractions of CWO obtained by dry and solvent fractionation are conventionally referred to as stearins and oleins, respectively. “Soluble Fraction” refers to the component, which dissolved in the solvent and was collected by rotary evaporation, and “Insoluble Fraction” refers to the component that precipitated and was collected by vacuum filtration.

As verified by visual observation and confirmed by DSC (see Section 3.3 below), the soluble component of CWO in ethanol and acetone presented the characteristics of stearin (SOLID) fractions and their insoluble component, the characteristics of olein (LIQUID) fractions, whereas the soluble component in ethyl acetate was an olein and the insoluble component a stearin. Given that the fractionation conditions were similar, this result is primarily attributed to the decrease in affinity for the oil’s components as the polarity decreases. The preferential distribution of the lipids according to polarity is confirmed by the MS analysis of these fractions (see Section 3.2.1).

The nomenclature used in this work is shown in Table 1.
Table 1. Nomenclature of crabwood oil samples and fractions.

<table>
<thead>
<tr>
<th>Name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>As purchased</td>
<td></td>
</tr>
<tr>
<td>Native Solid CWO</td>
<td>N\textsubscript{SOLID}</td>
</tr>
<tr>
<td>Native Liquid CWO</td>
<td>N\textsubscript{LIQUID}</td>
</tr>
<tr>
<td>Unseparated Crabwood Oil</td>
<td>CWO</td>
</tr>
<tr>
<td>Fractions</td>
<td></td>
</tr>
<tr>
<td>Dry Fractionation</td>
<td></td>
</tr>
<tr>
<td>Dry Solid</td>
<td>D\textsubscript{SOLID}</td>
</tr>
<tr>
<td>Dry Liquid</td>
<td>D\textsubscript{LIQUID}</td>
</tr>
<tr>
<td>With Ethanol (Et)</td>
<td></td>
</tr>
<tr>
<td>Soluble in Et</td>
<td>E\textsubscript{T SOLID}</td>
</tr>
<tr>
<td>Insoluble in Et</td>
<td>E\textsubscript{T LIQUID}</td>
</tr>
<tr>
<td>With Acetone (Ac)</td>
<td></td>
</tr>
<tr>
<td>Soluble in Ac</td>
<td>A\textsubscript{C SOLID}</td>
</tr>
<tr>
<td>Insoluble in Ac</td>
<td>A\textsubscript{C LIQUID}</td>
</tr>
<tr>
<td>With Ethyl Acetate (EA)</td>
<td></td>
</tr>
<tr>
<td>Soluble in EA</td>
<td>E\textsubscript{A LIQUID}</td>
</tr>
<tr>
<td>Insoluble in EA</td>
<td>E\textsubscript{A SOLID}</td>
</tr>
</tbody>
</table>

3.1. Fractionation Results

Pictures of the fractions in transparent glass bottles taken after 8–10 days at RT (18 °C) are shown in Figure 1. The color of the fractions is noted below each image.

<table>
<thead>
<tr>
<th>Picture</th>
<th>CWO</th>
<th>N\textsubscript{SOLID}</th>
<th>D\textsubscript{SOLID}</th>
<th>E\textsubscript{A SOLID}</th>
<th>A\textsubscript{C SOLID}</th>
<th>E\textsubscript{T SOLID}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Light Amber</td>
<td>Light yellow</td>
<td>White</td>
<td>Ivory</td>
<td>Light yellow</td>
<td>Ivory</td>
</tr>
<tr>
<td>Texture</td>
<td>Flows freely</td>
<td>Melts on skin contact</td>
<td>Melts on skin contact</td>
<td>Melts on skin contact</td>
<td>Melts on skin contact</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Picture</th>
<th>N\textsubscript{LIQUID}</th>
<th>D\textsubscript{LIQUID}</th>
<th>E\textsubscript{A LIQUID}</th>
<th>A\textsubscript{C LIQUID}</th>
<th>E\textsubscript{T LIQUID}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Amber</td>
<td>Amber</td>
<td>Ivory</td>
<td>Off white</td>
<td>Light amber</td>
</tr>
<tr>
<td>Texture</td>
<td>sedimented crystals</td>
<td>sedimented crystals</td>
<td>Melts on skin contact</td>
<td>Melts on skin contact</td>
<td>Dispersed and sedimented crystals</td>
</tr>
</tbody>
</table>

Figure 1. Physical appearance of CWO and the fractions at room temperature (RT = 18 °C).
Mass Balance and Yield

The yields obtained from the different fractionations of CWO are presented in Table 2. The oil and fat losses were weighed from the filter paper and the glass vessels. The losses from the filters and vessels represented approximately 40% and 60% of the total loss, respectively.

Table 2. Mass balance and yields for crabwood oil dry and solvent (ethyl acetate, acetone and ethanol) fractionation. Relative polarity and pKa values [48] NA: not applicable.

<table>
<thead>
<tr>
<th>Method</th>
<th>Relative Polarity</th>
<th>pKa</th>
<th>CWO Mass (g)</th>
<th>Mass Loss (g)</th>
<th>Mass Loss (%)</th>
<th>Mass Result (g)</th>
<th>Solid</th>
<th>Liquid</th>
<th>Total</th>
<th>Solid Fraction Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry</td>
<td>NA</td>
<td>NA</td>
<td>258</td>
<td>3.4</td>
<td>1.3</td>
<td>74.8</td>
<td>177.5</td>
<td>252.3</td>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>Ethyl Acetate</td>
<td>0.228</td>
<td>25</td>
<td>50</td>
<td>3.5</td>
<td>7.0</td>
<td>3.1</td>
<td>43.5</td>
<td>46.6</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>0.355</td>
<td>20</td>
<td>200</td>
<td>5.5</td>
<td>2.7</td>
<td>148.3</td>
<td>46.3</td>
<td>194.6</td>
<td>74.1</td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>0.654</td>
<td>15.9</td>
<td>200</td>
<td>4.7</td>
<td>2.4</td>
<td>80.6</td>
<td>114.7</td>
<td>195.3</td>
<td>40.3</td>
<td></td>
</tr>
</tbody>
</table>

The yields of the soluble fraction increased steadily from the fractionation with ethanol, the most polar, to ethyl acetate, the least polar. The uncertainty about the measurements is less than 5%. Given all the other conditions of the fractionation were similar, this result is primarily attributed to a monotonous variation in the affinity of the solvent for the oil’s components as the polarity decreases. The decrease in the yield with increasing polarity is consistent with the decrease in solubility, in polar solvents, of TAGs, which are the main components of CWO [49]. The Pearson analysis indicated a very strong negative linear correlation between the yields of the soluble fractions and relative polarities (r=−0.9994, Figure 2). The slope of the correlation is high (−121.3%/relative unit), and given all other fractionation conditions are the same, it is associated with the rate of decrease in solubility of the TAGs with the polarity increase [49].

![Figure 2](image)

The pKa values (see Table 2) strongly correlate negatively with the polarities of the solvents (r = −0.9954), which provides a similarly strong but positive correlation with the fractionation yields and physical properties.

3.2. Chemical Analysis—Assessment of the Lipids of CWO and its Fractions

The ESI-MS spectra of CWO and its fractions are provided in the Supporting Information (SI) in Figure S1. An example, the ESI-MS spectrum of CWO, is shown in Figure 3.
The lipid and non-lipid (non-saponifiable) compounds of CWO and its fractions were identified using MetaboQuest [50] and LIPID MAPS [51]. The relative abundances of the putative compounds were determined considering m/z [M + Na]+, which is, typically in the field, the most dominant adduct [52]. The abundance and distribution of individual compounds were assessed relative to their class rather than to the total lipids or non-lipids. The differences between the fractions expressed in relative abundances for each class of lipids were measurable and depended on the crystallization behavior and solubility of the lipids in the solvents. The MS results for the lipids and non-lipids are provided in Table S1 and Table S2, respectively.

3.2.1. Assessment of the Lipids

The following four (04) classes of lipids were detected: TAG, DAG, MAG and occurring FFAs. The lipid assessment corresponds to the literature [52,53].

The chemical structures of the most abundant lipids detected in CWO are shown in Scheme 1.
Scheme 1. Representative molecules detected in the lipid group. (a) Palmito- diolein (PO\textsubscript{2}), the predominant TAG detected in CWO via ESI-MS, (b) 1-oleoyl-2-palmitoylglycerol, the predominant DAG detected in CWO via ESI-MS, (c) Predominant FFA detected in CWO via ESI-MS (c) 10-oxooctadecanoic acid (10-Ketostearic acid), (d) (Z)-18-hydroxyoctadec-9-enoic acid (18-hydroxyoleic acid).

Assessment of the Triacylglycerols

Seventeen (17) different TAGs were identified in CWO and in the fractions. Palmitic (P: C16:0) and oleic (O: C18:1) fatty acids account for about 60% of the total fatty acid composition of the TAGs. Because MS does not discriminate between positional isomers, the TAGs were identified without specification of the fatty acids position on the glycerol backbone. TAG symmetry is an important factor when considering crystallization; however, disregarding sn-2 acyl migration, which can occur depending on processing, temperature and time, mono- and di-unsaturated symmetrical or asymmetrical TAGs in CWO may be like those of palm oil, which occur in constant relative proportion [54]. No attempt was made to determine the relative proportion of symmetrical to asymmetrical TAGs in the samples. The relative abundances of the identified TAGs in CWO and its fractions are presented in Figure 4. The calculated molecular weight based on the TAG analysis is provided in Table 3.

Table 3. Calculated molecular weight based on this analysis.

<table>
<thead>
<tr>
<th>Sample</th>
<th>MW (g/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWO</td>
<td>858.8</td>
</tr>
<tr>
<td>DSOLID</td>
<td>862.1</td>
</tr>
<tr>
<td>DLIQUID</td>
<td>859.1</td>
</tr>
<tr>
<td>ETSOLID</td>
<td>863.7</td>
</tr>
<tr>
<td>ETLIQUID</td>
<td>857.1</td>
</tr>
<tr>
<td>ACSOLID</td>
<td>862.5</td>
</tr>
<tr>
<td>ACLIQUID</td>
<td>850.0</td>
</tr>
<tr>
<td>EA LIQUID</td>
<td>858.4</td>
</tr>
<tr>
<td>EA SOLID</td>
<td>858.5</td>
</tr>
<tr>
<td>NSOLID</td>
<td>863.0</td>
</tr>
<tr>
<td>NLIQUID</td>
<td>858.2</td>
</tr>
</tbody>
</table>

The assessed TAG composition of the crabwood oil is within the ranges reported in the literature [52,53]. The main TAG in CWO is palmito-diolein (PO\textsubscript{2}: 21.5%) followed by oleo-dipalmitin (P\textsubscript{2}O: 13.8%). Except for AC\textsubscript{LIQUID}, in which P\textsubscript{2}O was prevalent (33.0% of the total), the main TAG detected in CWO and its fractions was also PO\textsubscript{2}. The relative abundance of PO\textsubscript{2} was maximum in ET\textsubscript{SOLID} with 32.8% of the TAGs and minimum in AC\textsubscript{LIQUID} with 13.7%.

To better clarify the partitioning, the TAGs are classified according to their saturation as follows: tri-saturated (S\textsubscript{3}), mono-unsaturated (S\textsubscript{1}U), di-unsaturated (S\textsubscript{2}U) and tri-unsaturated (U\textsubscript{3}). The main components of each group and their relative distribution may be emphasized to elucidate the differences in behavior between the fractions. Generally, stero-dipalmitin (P\textsubscript{3}S) is the main trisaturated TAG, whereas oleo-dipalmitin (P\textsubscript{2}O), palmito-diolein (PO\textsubscript{2}) and triolein (OOO) are the most abundant TAGs of the S\textsubscript{1}U, S\textsubscript{2}U and U\textsubscript{3} classes, respectively. Figure 5 shows that the fractions are particularly enriched or depleted in S\textsubscript{3}, S\textsubscript{1}U, S\textsubscript{2}U or U\textsubscript{3}. 
Figure 4. Relative abundance sorted in the descending order of TAGs in CWO and its fractions from dry and solvent (ethanol, acetone and ethyl acetate) fractionations. Abundances determined from the relative intensities of the ESI-MS sodium adducts.

The distribution of the different groups between the solid and liquid fractions ranges from equivalent to twice larger depending both on the crystallization behavior of the TAG systems and solubility in the case of solvent fractionation. The dry fractionation enriched the solid fraction in S3 and SU2 and depleted it of S2U. The fractionation with ethyl acetate distributed each TAG group almost evenly in the solid and liquid fractions. ET LIQUID and AC LIQUID were significantly enriched in S3 and S2U and depleted of SU2 and U3, indicating a distribution dominated by solubility effects.
Figure 5. Distribution of tri-saturated (S₃), mono-unsaturated (S₂U), di-unsaturated (SU₂) and tri-unsaturated (U₃) TAGs detected in CWO and its fractions. Relative abundances determined from the relative intensities of the ESI-MS sodium adducts of the TAGs.

Assessment of the Diacylglycerols and Monoacylglycerols

The most abundant MAG detected in CWO and its fractions is monoolein (O), followed by monolinoleate (L). The most abundant DAG is 1-oleoyl-2-palmitoylglycerol (PO) followed by diolein (OO) in all the samples (Table S1b).

Noticeably, except for the ethyl acetate fractionation, which enriched the solid fraction in 8 of the 13 DAGs, the fractionations either distributed evenly or enriched the olein fractions by an average of 1.5 of most DAGs. EASOLID was also enriched with MAGs by an average of 1.5 times compared to EALIQUID. The distribution of TAGs and these data indicates that the fractionation with ethyl acetate separated the molecules more along the lines of polarity, particularly the DAGs and MAGs components, rather than saturation.

Assessment of the Free Fatty Acids

Fourteen FFAs were detected in CWO and its fractions. Saturated FFA in CWO accounts for 36% of the total FFA. The molecular mass sodium adducts (m/z[M+Na]⁺ = 321.2408) corresponding to both 10-oxooctadecanoic acid (ketostearic acid) and (Z)-18-hydroxyoctadec-9-enoic acid (hydroxyoleic acid) were detected as the most abundant (43.7% of the total FFA). The two compounds cannot be discriminated with MS. These long fatty acids occur naturally, but to the best of our knowledge, they have not been reported in CWO before. They are essential nutrients and are known to play an important role in energy metabolism regulation [55]. Ketostearic acid is found in Aloe ferox, Gracilariopsis longissima and Galeopsis bifida [56], and 18-hydroxyoleic acid in Arabidopsis thaliana [57]. The presence of ketostearic and hydroxyoleic acids in CWO may be a result of the transformation that occurs during the fractionation process, or they might be specific to the Guyanese species of Carapa guianensis or even due to biotransformation by bacteria present in the Guyanese Carapa guianensis. The biotransformation of polyunsaturated fatty acids by gut lactic acid bacteria—such as linoleic acid to hydroxy fatty acids and other long-chain fatty compounds—is well-documented [58]. The origins, possible enhancement and separation of these fatty acids should form the basis of additional research in CWO.

The second most abundant FFA (24%) is 15-hydroxyoctadecanoic acid (hydroxy stearic acid). The relative abundance of linoleic acid is notable, with 11.2% of the total FFA. Linoleic acid is an omega-6 fatty acid parent compound of omega-6 fatty acids, such as γ-linolenic acids (GLA) and arachidonic acid [59] and omega-3 fatty acids, such as α-linolenic acid (ALA) [60].

As the pKa increased, and because of increased solubility, the soluble fractions were increasingly enriched in weak acids, such as free fatty acids. The role of the pKa although prominent and the results can further enhance the design of specialized fractions, is more complex and needs further research work, particularly in the case of limonoids, and other bioactive compounds.

3.2.2. Assessment of the Unsaponifiable Compounds

The non-lipids (unsaponifiable) compounds detected in CWO and its fractions consisted of 20 limonoids (Table S2a), seven sterols (Table S2b), one flavonoid, one tocopherol, one diterpenoid and one phosphoglycerol, labelled together “other unsaponifiable compounds” (Table S2c). These compounds have been reported in other plants of the Meliaceae family [61,62]. The chemical structures of the most abundant limonoids detected in CWO are shown in Scheme 2.
Scheme 2. Representative molecules detected in the limonoid group: (a) Methyl Angolensate, (b) Trichilin A, (c) Gedunin and (d) Andirobin.

Assessment of the Limonoids

The portioning of the most abundant limonoids in CWO and its fractions is presented in Figure 6. As can be seen, the most abundant limonoid in the samples is methyl angolensate (from 28 to 39%) followed by Trichilin A (from 13% to 22%). The angolensates are noteworthy because of their demonstrated cytotoxic [63,64] and antifungal [62,65] activities. Trichilin A is a limonoid reported to have important bioactivity, such as cytotoxic and antifeedant properties. Antifeedants are natural compounds produced by the plant to prevent the attack of pests, such as Spodoptera eridania (Southern armyworm), which feeds heavily on the leaves of young plants [66,67]. Although methyl angolensate has been reported in CWO [68], Trichilins have not, even though they have been identified in other Meliaceae species, such as Natal mahogany (Trichilia roka) [69]. The relative abundance of methyl angolensate and Trichilin A is large enough to warrant further investigation because of potential applications related to their bioactivities.

Figure 6. Distribution of the most abundant limonoids in CWO and its fractions. Relative abundances determined from the relative intensities of the ESI-MS sodium adducts of the limonoids.

The Andirobin and Gedunin class compounds are known for their antimicrobial activities, including antifungal, antibacterial, antifeedant, insecticidal, antimalarial, anti-allergic, anti-inflammatory, anti-cancer, anti-diabetic and neuroprotective activities [70,71]. Figure 6 shows that 7-deacetoxy-7-oxogedunin and Andirobin are the most predominant in all the fractions. The analysis of the relative abundances indicates that one can partition
important unsaponifiable compounds as a function of the fractionation method; in particular, there is a direct dependence on solvent polarity. Gedunin and Andirobin, for example, were more abundant in the liquid fractions. The correlation between polarity and Gedunin and Andirobin abundances was negative ($r = 0.957$ and slope $= -81\%$ per unit, and $-0.991$ and slope $= -27\%$ per unit, respectively, Figure 7).

![Figure 7](image)

**Figure 7.** Difference in distribution of Gedunin and Andirobin between the olein and stearin fractions obtained from ethyl acetate, acetone and ethanol fractionations versus relative polarities. Dashed lines are fits to straight lines ($R^2 = 0.982$ and 0.916 for Andirobin and Gedunin, respectively).

The Carapanolides, which were in low amounts (less than 1.3%), were more present in the olein fractions. The other putative limonoids listed in Table S2 have been reported in other plants of the Meliaceae family [61,62]. The partitioning of these compounds between the stearin and olein fractions obtained from the solvent was significant. The distribution of these limonoids depended on the type of solvent used, but no correlation was found with the polarities.

**Assessment of the Sterols**

Seven sterols were identified in CWO. 2-Deoxy-20-hydroxy-5α-ecdysone 3-acetate was the most prevalent in CWO (18%) in all the fractions and is similarly abundant in the olein fractions and the dry stearin (±0.6%). This sterol belongs to a class called ecdysteroid esters found effective for anti-cancer activity [72].

**Assessment of the Other Unsaponifiable Compounds**

The most dominant compound of the group is the phosphoglycerol, which accounts for 36% of the total. Its distribution did not vary significantly between the stearin and olein fractions obtained by the dry fractionation methods (±2%), whereas the stearin fractions obtained by ethyl acetate, acetone and ethanol expressed 3%, 17% and 11% more phosphoglycerol, respectively. The differences observed for the flavonoid and the tocopherol were not statistically significant.

As $pK_a$ increased and because of increased solubility, the soluble fractions were increasingly enriched in weak acids, such as free fatty acids. The role of $pK_a$ although prominent, its role is more complex and needs further research, particularly for the limonoids, and other bioactive compounds.

**3.3. Thermal Transition Behavior**

The cooling (5 °C/min) and heating (10 °C/min) thermograms of CWO and its fractions are shown in Figure 8(a1–2) and (b1–2), respectively. The corresponding crystallization and melting data are provided in the SI in Table S2 and Table S3, respectively.
Figure 8. (a) Cooling (5 °C/min) and (b) heating (10 °C/min) DSC thermograms of CWO and its stearin and olein fractions. Dashed lines separate the high- and low-temperature thermal transition zones.

The cooling thermogram of CWO is characterized by a relatively sharp high temperature leading event $T_{L1}$ at 11.4 °C and two prominent subzero exotherms (~−3.4 and ~−47.4 °C, Figure 8(a1–2)), indicating high, medium and low melting components. The high melting group of molecules distinctly separates from the low melting groups at 4 °C, indicating the possibility of fractionating CWO into a stearin fraction (SOLID comprising mostly high melting TAG components) and an olein fraction (LIQUID mainly comprising the mid and low melting TAG components).

The DSC thermograms of the CWO fractions are altered versions of CWOs. The enrichment of the stearin fractions in the saturated components (S 3) manifested in the high temperature leading exotherms, whereas the corresponding depletion of the olein fractions in these elements manifested as a weaker or even absent high temperature leading peak. The transformation paths recorded during heating included recrystallizations mediated by melting and resolved endotherms, indicating there was a melting of different crystal phases.

3.3.1. Crystallization Behavior

The stearin fractions comprised the two subzero crystallization peaks (Figure 8(a1)), indicating the presence of liquid phases. The stearin fractions presented leading events at temperatures higher than CWO ($T_{C1}= 11.4$ °C), with $D_{SOLID}$ presenting the highest (26 °C) followed, in order, by the following: $ET_{SOLID}$ at 23.5 °C, $EA_{SOLID}$ at 17.0 °C and $AC_{SOLID}$ at 16.3 °C. Conversely, in that range of temperatures, the olein fractions had smaller or even, like $DLIQUID$, absent peaks above 3 °C (Figure 8(a2)). The leading peaks of $EA_{LIQUID}$ (9.0 °C) and $ET_{LIQUID}$ (11.0 °C) are similar to those of CWO, whereas $AC_{LIQUID}$ presented four distinct but non-resolved exotherms (23, 17, 9 and 3 °C).

The soluble components from solvent fractionation gradually lose their stearin character as the polarity decreases from ethanol to acetone ($ET_{LIQUID}$ and $AC_{LIQUID}$) and become an olein for ethyl acetate ($EA_{LIQUID}$) (Figure 8(a1–2)). The cooling thermograms of $EASOLID$ and $EA_{LIQUID}$ are like CWO in shape, but they present differences in peak temperatures and enthalpies. The leading exotherm associated with $EA_{LIQUID}$ (9.0 °C, $\Delta H_{L1} = 12$ J/g) is only slightly lower than that of CWO (~11.4 °C, $\Delta H_{L1} = 13$ J/g), indicating a close saturation distribution. Both are significantly lower than $EASOLID$ (17 °C, $\Delta H_{L1} = 27$ J/g).
enrichment of EA\text{SOLID} with most of the DAGs, known to act as seeds for early crystallization, may explain the high crystallization peaks.

The enthalpies of the stearin fractions can be associated with a solid fat content. Overall, as shown in Figure 9a,b, the enthalpy of crystallization of the leading event for the stearin fractions ($\Delta H_{1s}$) was much higher than that of the olein fractions ($\Delta H_{1o}$).

**Figure 9.** Enthalpy of crystallization from DSC of the leading peak ($\Delta H$) of the (a) stearin and (b) olein fractions of CWO.

Note that $\Delta H_{1s}$ of ET\text{LIQUID}, N\text{LIQUID} and D\text{LIQUID} are the lowest ($<4 \pm 3$ J/g). The differences in the intensity of the leading peaks are caused by the fractionation method, and solvent polarity and this is measurable, hence, the olein fractions, which reflect the depletion of the most saturated components, can be used to assess the relative separation efficiency of the fractionation methods.

The correlation between $\Delta H_{1s}$ and the relative polarity of the solvents is strong and statistically significant ($r = 0.9979$; Figure 10). The slope of the correlation line $25.7 \pm 1.7$ J/g per relative unit is a quantification of the effect of polarity on the partitioning of the lipids, which can be used to control fractionation and select specific solid and liquid fractions of CWO by solvents.

**Figure 10.** Enthalpy of crystallization from DSC of the leading peak of the stearin fractions (●) obtained by ethyl acetate, acetone and ethanol fractionations versus relative polarities. Dashed line is a fit to a straight line ($R^2 = 0.996$).
3.3.2. Melting Behavior

The heating thermograms of the stearin fractions are characterized with four main endotherms (Figure 8(b1)), and, except for EALIQUID, those of the olein fractions were missing the highest temperature melting peak ($T_{m1}$) (Figure 8(b2)). This indicates that the most stable phases generally available for CWO did not form in the olein fractions.

The most stable phases of the stearin fractions, as indicated by their $T_{m1}$, were markedly different. $T_{m1}$ of EASOLID (44.4 °C), D SOLID (36.7 °C) and NSOLID (31.2 °C) were higher than $T_{m1}$ of CWO (27.6 °C), AC SOLID (23.4 °C) and ET SOLID (19.5 °C). This decrease in $T_{m1}$ mirrors the trend observed for the leading crystallization event indicating that it was those crystals formed during cooling that transformed and melted. The significant differences in $T_{m1}$ (up to 23.9 °C) can be associated with the partitioning of the most saturated elements, including the saturated and di-saturated TAGs, as well as the minor lipid components and the non-saponifiable components. The prominent peaks observed at $0 \pm 2$ °C and $-10 \pm 2$ °C indicate that the same low-temperature crystal phases occur in all the fractions. Despite being overwhelmed by the endotherms, recrystallizations mediated by melt are evident in the heating thermograms of all the samples. This is a common occurrence in fats and oils [73].

The heating thermogram of the fractions from ethyl acetate is singular. EA LIQUID displayed four melting events, of which the highest at $T_{m1} = 23.4$ °C indicates a lower stability phase than that of CWO. EASOLID shows six distinct melting events, including a large endotherm at 27.6 °C, which matches the highest of CWO (27.6 °C). A small endotherm shows in EA SOLID at 44.4 °C, probably because of the sizable amounts of saturated FFAs and DAGs.

3.3.3. Thermal Transition Behavior and Lipid Profile

The categorization into different saturation groups (Figure 5) helps explain the behavior of CWO and its fractions. The behavior of these complex palmitic/oleic TAG systems is generally dictated by the behavior of the main components and their interactions. The influence of fatty acid unsaturation is critical, driving the properties in both metastable and stable forms [74,75]. The main components of the different saturation groups, namely, P:S, P:O, PO2 and OOO form binary and higher-order systems with specific interactions through the acyl chains leading to the formation of particular transition behaviors, including monotectic behavior, eutectics and molecular compounds. These systems that contain palmitic and oleic acids are relatively well-studied [76].

Table S1 shows that the solid fractions (stearin) are particularly enriched in S3 compounds at the detriment of the liquid fractions. Although the stearin fractions were depleted in S:U (P:O being the most abundant), a sizable amount remained, which suggests that it played an essential role with the TAGs of S3 (PSS/PPS/PPP) in the physical characteristics of the CWO fractions. A minimal amount of PPS in the olein fractions seems necessary for their crystallization. The tendency of POP, POO, OPO, OPP and POP blends to form molecular compounds, which release significant heat when crystallizing, can explain the typical crystallization behaviors of the olein [76].

3.4. Solid Fat Content

The SFC versus time curves of CWO and its fractions from the in situ step and isothermal measurements followed an exponential rise to a maximum ($SFC(T) = SFC_0 + a (1 - \exp (- t/t_0))$; $R^2 > 0.8625$). The final SFC obtained at 25, 18, 10 and 0 °C is shown in Figure 11.
Figure 11. SFC of CWO and its fractions measured by pulsed NMR at (25, 18, 10 and 0) °C.

The SFC of the liquid fractions was much smaller than those of the solid fractions above 4 °C. The SFC of the fractions obtained at 25 °C was not significantly different ($p < 0.05$), averaging 7 ± 1% for the stearins and 3% for the oleins. Below 25 °C, AC_SOLID achieved the highest SFC (19% at 18 °C and 36% at 0 °C) and ET_SOLID the lowest (10% at 18 °C and 12% at 0 °C). D_SOLID and N_SOLID achieved similar SFCs (12% at 18 °C and 15% at 0 °C). The SFC of the liquid fractions increased substantially at 0 °C, indicating the involvement of molecules of a higher unsaturation.

The understanding gained from the crystallization behavior of palmitic-oleic TAGs is applied here to explain the SFC at the different temperatures. The main groups of molecules, which are susceptible to crystallizing above 4 °C, are those comprising the most saturated components, such as the saturated PS2 and P2S and their interaction with the disaturated TAGs, such as P2O. At the freezing temperature, the crystallization of the diunsaturated TAGs, such as PO2, PLO and PL2 (which together form approximately 43% of the TAGs of CWO), may be involved in a significant manner.

The detailed examination of N_LIQUID, N_SOLID and D_SOLID presented in Figure 12, indicates important differences in the evolution of the SFC due to different crystallization kinetics. The SFC versus temperature curves of N_LIQUID (Figure 12a) and D_SOLID (Figure 12c) show two distinct segments, indicating a two-step crystallization process. This indicates that the crystallizations of the most saturated components and the mid melting components occur separately when processed in the MNR machine, similar to what is observed in the DSC between the leading crystallization exotherm and the exotherm at $T_{x2} = -2$ °C.

Despite a crystallization path like D_SOLID, the SFC of N_SOLID displayed one sigmoidal segment, indicating different relative contributions of the activated secondary nucleation processes to the overall growth rate and apparent activation energies [77–79]. Measurements below freezing, which would show the contribution of the components of CWO, which crystallize at lower temperature (~35 °C), were not performed.
The role of kinetics in the SFC of CWO and its fractions is further evident from the measurements of the samples at 4 °C under two different thermal protocols (Figure 13). This is demonstrated by (a) cooling from the melt in small steps (2 °C) and isothermal measurement, and (b) after 8–10 days storage time. Protocol (a) resulted in a much higher SFC with gains varying from ~65% (ETLIQUID) to 14% (ACLIQUID).

![Figure 12](image-url)  
**Figure 12.** SFC measured by pulsed NMR versus temperature of the solid fraction obtained by dry fractionation of CWO (DSOLID) and the solid and liquid fractions obtained by decanting CWO (NSOLID and NLIQUID, respectively).

![Figure 13](image-url)  
**Figure 13.** SFC measured by pulsed NMR at 4 °C of CWO and its fractions for samples (i) cooled in situ from the melt and measured isothermally in small steps (2 °C) and (ii) after an 8–10-day storage time.

3.5. Microstructure of Crabwood Oil and Its Fractions

The PLM images of CWO (Figure 14a) show large spherulitic crystals of long radial dendrites dispersed in a sizeable liquid phase. The microstructures of both the stearin and olein fractions (Figure 14b,c) were significantly altered by the fractionation method and solvent used. EA LIQUID (Figure 14c) displayed microstructures with crystals resembling those of CWO but with longer dendrites (250 nm vs. 175 nm on average) and much less liquid phase. The size of the crystals of all the other fractions was reduced to less than 20 µm, and their number increased, indicating high nucleation and crystallization rates.
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(b) Stearin Fractions
Figure 14. PLM images taken after 7–10 days at room temperature (18 °C) of (a) CWO, and the (b) stearin fractions and (c) olein fractions obtained from dry, ethyl acetate, acetone and ethanol fractionations.

The crystals of the stearin fractions were either homogeneously distributed in relatively compact, dense networks in which the liquid phase is trapped (D\textsubscript{SOLID}, E\textsubscript{SOLID}, ET\textsubscript{SOLID}; Figure 14b), or they were closely and homogeneously dispersed in a small liquid phase (N\textsubscript{SOLID}, ACSOLID; Figure 14b). The crystals of the olein fractions were all dispersed in a large liquid phase (Figure 14c).

The details of the microstructures, such as shape, size and distribution of the crystals of the different fractions, are measurably distinguishable. The sizes vary from 5 to 250 µm. The shapes include well-organized spherulites, and needle-like and rod-like crystals. The distribution of the crystals varies from highly dispersed to compact networks. Because of the predominance of the liquid phase, the microstructures of the olein fractions differ measurably in all these parameters but in a more subtle manner than in the stearin
fractions. The different microstructures of the stearin fractions depended on the solvent. 

\(EA_{\text{solid}}\) formed a dense network of small spherulitic crystals, \(AC_{\text{solid}}\) formed an open network of long (20 nm on average) rod-like crystals, whereas \(ET_{\text{solid}}\) comprised both types of crystals organized in a dense network. The microstructures of the stearins from solvent also measurably differ from those obtained by dry fractionation (\(DS_{\text{solid}}\) and \(NS_{\text{solid}}\)) in crystal type, size and distribution.

The diversity of the microstructures suggests that the fractions are likely to have different flow characteristics and different feels to the skin and mouth. This also suggests that they will confer different textures, consistencies and mouthfeels 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 [80]. The smaller crystals would lead to firmer products and larger size crystals to sandy mouth feel [81]. Their mechanical properties would be different because they are mostly controlled by the material’s networks of polycrystalline microstructures and the SFC [82].

3.6. Crystal Structure of CWO, CWO Fractions

The XRD of the olein fractions did not present crystal peaks because of the overwhelming liquid phase. The normalized SAXD and WAXD spectra obtained for CWO and its stearin fractions are shown in Figure 15. The d-spacings and the related Miller indices are provided in the SI in Table S1.

CWO and its fractions presented their diffraction peaks at 4.16 Å (110) and 3.74 Å (200), a characteristic of an orthorhombic subcell structure in the \(\beta’\)-form and a substantial wide background halo at approximately 4.8 Å, indicative of the liquid phase in the sample. Their SAXD is consistent with the double chain length (DCL) packing in the fork configuration of TAGs containing predominantly C16 fatty acids. The C16-DCL packing reflects the predominance of CWO and its fractions of high melting temperature TAGs containing palmitic acids, such as PPS, PPO and POS.

![Figure 15. (a) Small-angle X-ray diffraction (SAXD) and (b) Wide-angle X-ray diffraction (WAXD) of crabwood oil (CWO) and its solid fractions. ET_{\text{solid}}, AC_{\text{solid}} and DS_{\text{solid}}: solid fractions obtained from acetone and dry fractionation, respectively.](image)

3.7. Thermal Gravimetric Analysis

The TGA and DTG curves and the corresponding data of all the samples are provided in the (SI) in Figure S2 and Table S1, respectively. TGA and DTG curves representative of the solid, liquid, soluble and insoluble fractions are shown in Figure 16a and b, respectively.

The onset temperature of mass loss as determined at 5% \((T_{\text{on}}^{5\%})\) of the stearin fractions (Figure 16c) are not significantly different \((195 \pm 2 ^\circ \text{C})\). Also, \(T_{\text{on}}^{5\%}\) of \(AC_{\text{solid}}\) and \(N_{\text{liquid}}\).
are not significantly different from CWO (205 ± 2 °C). $T_{5\%}^{on}$ of the insoluble fractions increase with increasing solvent polarity.

![Figure 16](image_url)

**Figure 16.** (a) TGA and (b) DTG stacks of crabwood oil (CWO) and its fractions from dry and ethanol fractionations; (c) onset temperature of degradation as determined at 5% mass loss ($T_{5\%}^{on}$) of CWO and its fractions from dry, ethanol, acetone and ethyl acetate fractionations.

Figure 16b indicates that the CWO fractions experience the same two main mass loss mechanisms ($T_{D1}= 233 \pm 6$ °C and $T_{D2} = 393 \pm 8$ °C). A small event showed centered at $T_{D3} = 435 \pm 8$ °C but involved less than 2% of mass loss. The degradation of CWO and its fractions can be understood considering the mechanisms by which TAGs degrade, i.e., via $\beta$-hydrogen elimination starting at 250–380 °C and $\gamma$-hydrogen transfer starting at 450 °C [83–85].

The ~140 °C–300 °C range of the first DTG peak was identified as the range in which the volatilization of free fatty acids occurs. Niu et al. [86] reported that the gaseous products from the thermal degradation of oleic acid involve alkanes in large proportions, 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 ~300 °C, the offset temperatures of the first DTG peak. However, because the $\beta$-hydrogen elimination starts at ~240 °C, this peak may also involve the degradation of MAGs, DAGs as well as TAGs.

The 300–450 °C temperature range involves the degradation of the saturated as well as the unsaturated fatty acids of the TAGs, as has been reported to occur, such as in rapeseed oil [87] and virgin olive oil [88]. In a study 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 starts to degrade before all the other fatty acids [88].
4. Conclusions and Perspectives

This work involves the study of the oil from the seeds of *Carapa guianensis*, known as crabwood oil (CWO) and its fractionation. The oil is composed of lipids (95–98%) and non-saponifiable compounds (2–5%) comprising limonoids, sterols and phenols. Dry and solvent fractionation with ethanol, acetone and ethyl acetate were used to obtain narrower composition ranges that have altered physical properties and meet specific functional requirements for use in the food, pharmaceutical and cosmetic industries. The established methods of phytochemical and physicochemical functionality analysis were used to determine the functionality of the fractions.

The fractionation work yielded stearin and olein fractions that have measurable and, in some instances, predictably significant differences in the studied chemical and physical characteristics that depend on the fractionation method and solvent polarity. A conclusion was supported that fractionation of CWO using solvents has great potential to be further directed by using other solvents, mixtures of solvents and by varying the process parameters, such as solvent-to-oil ratio, crystallization temperature, agitation, cooling rate and crystallization time.

The work achieved the following important specific findings:

1. Fractionation resulted in a changed microstructure: the fractions displayed smaller crystals than CWO, which were distinguishable in shape, size and density of the crystal networks;
2. Fractionation changed the crystallization and melting temperatures: The partitioning of the lipids is significant enough to drive measurable and predictable crystallization and melting behaviors. The solid fractions transition parameters were particularly very strongly correlated to the fractionation method and polarity of the solvents;
3. Fractionation changed the solid fat content (SFC) profile of CWO fractions: CWO fractions are shown to form semi-solid fats. The role of kinetics is significant and manifests differently depending on the fractionation method and solvent polarity. The data suggest that it is possible to improve the SFC of the fractions by changing the processing methods;
4. 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.

This work shows the potential to make unique compositions with significantly different properties through the fractionation of CWO by using polarity as a predictive tool. The olein fractions can be used to make improved emulsions, creams and salves with specific physical properties, with antimicrobial and antifungal efficacy due to the bioactive components of CWO. The stearin fractions can be used for specialized products desirable in topical cosmetic and pharmaceutical applications.

*Carapa guianensis* seed oil is important for Guyana. It is currently sold in local markets and pharmacies for its medicinal and cosmetic attributes. The reported methods would first provide high-value fractions with targeted enriched compositions suitable for developing materials with customized lipid and bioactive compositions. Bio-potent liquids and solid fractions would be valuable in specific pharmaceutical and cosmetic applications. The antimicrobial and antifungal compounds of CWO can be administered via adequate methods (liquid of fat) to fend off many diseases, such as malaria and vaginosis, endemic to Guyana and tropical countries. With proper safety measures, one can expect the emergence of strong and consequential Guyanese pharmaceutical and cosmetic sectors based on CWO and other similar oils. At a large scale, the less valuable products, if any, because of a potentially large supply, would possibly allow for the development of a more traditional oleochemical industry and a sustainable administration of this very valuable tree, which presently is heavily logged in Guyana.

The results call for further investigations and optimization, which would establish robust, predictable relationships between the processing conditions and the 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 both the lipids and non-saponifiable components of CWO.

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

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11092565/s1, Figure S1: ESI-MS spectra of the Crabwood Oil (CWO) and its fractions; Figure S2: TGA and DTG stacks of CWO and its stearin and olein fractions Table S1: Relative abundance percentages of assigned lipids in CWO and its fractions ; Table S2: Relative abundance percentages of assigned unsaponifiable components in CWO and its fractions; Table S3: (a) Crystallization and (b) Melting data of CWO and its fractions; Table S4: (a) WAXD and (b) SAXD data. \( \chi \% \): crystallinity; and Table S5: DTG and TGA data for CWO and its fractions.

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