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Characterization of Cyclopropenoic Fatty Acid Containing Triacylglycerols of Monguba Oil by MALDI-TOF and High-Energy CID MALDI-TOF/TOF Mass Spectrometry

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Overview

We present the analysis of lipids from Monguba (*Pachira aquatica Aubl.*) seeds by MALDI-(TOF/)TOF MS. To the best of our knowledge, this is the first time MALDI-TOF/TOF has been used for the structural analysis and confirmation of triacylglycerols containing cyclopropenoic fatty acid e.g., sterculic acid and derivatives.

1. Introduction

Monguba (*Pachira aquatica Aubl.*) is a tree native to tropical regions, which is traditionally used in folk medicine. Its seeds are rich in oil containing saturated, monounsaturated and polyunsaturated fatty acids ranging from 46.67–84.87%, 6.62–39.30% and 5.24–11.81%, respectively. Beside these common fatty acids (FAs), the oil is characterized by varying amounts of cyclopropenoic fatty acids (CPFAs), i.e., sterculic, dihydrosterculic, 2-hydroxy-sterculic, malvalic acid (Figure 1), which are of great interest because of their anti-cancer potential. On the other hand, due to the toxicity for humans, their contents are of importance for potential food applications of the oil. Our work is intended to provide a method for the rapid detection and structural analysis of triacylglycerols (TAGs) of Monguba oil using a MALDI-TOF-(MS/)MS approach, based on a previous gas chromatography study.

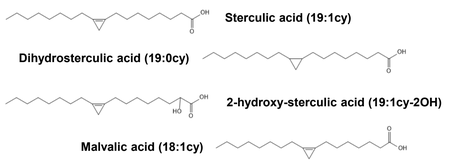

Figure 1. Structures of cyclopropenoic fatty acids associated with this work.

2. Methods

The oil was extracted from Monguba seeds using supercritical fluid extraction (60° C, 350 bar, supercritical CO_2 as solvent). The first sample (MO1) was not heat-treated, and the second (MO2) was heated to 90° C for 10 consecutive times. Gas chromatography (GC) was performed by using a GC-FID following AOCS official method to obtain the fatty acid profiles. For the MALDI analyses, oil samples (5 mg/mL oil in chloroform) were prepared with $Na_4Fe(CN)_6$ (for MS) and 2,5-dihydroxybenzoic acid (DHB) containing 10 mM NaTFA (for MS/MS) matrices. MALDI-MS analyses were conducted in positive ion mode on a benchtop linear MALDI-TOF mass spectrometer (MALDI-8030, Shimadzu) to obtain the TAG profiles. High-energy (HE)-CID MALDI-MS/MS analyses of individual TAGs were conducted in positive ion mode on a MALDI-TOF/TOF mass spectrometer (MALDI-7090, Shimadzu). The sample analysis workflow is illustrated in Figure 2.

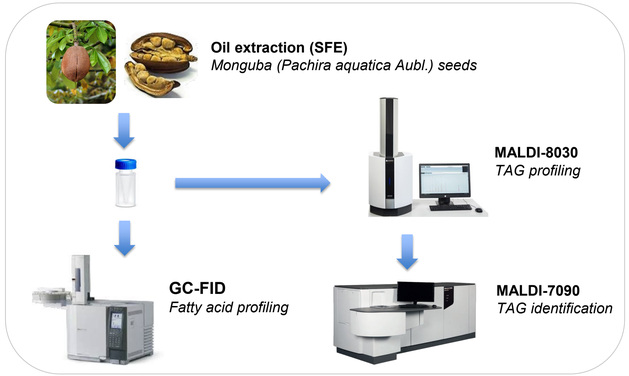

Figure 2. Analysis workflow of native Monguba (*Pachira aquatica Aubl.*) oil.

3. Results

3-1. MALDI-MS analyses

The TAG profiles of Monguba oil were obtained on the MALDI-8030 in positive ion mode. Figure 3 shows the TAG profiles of the un-heated (red trace) and heated (blue trace) oils. TAG identification was carried out by HE-CID MALDI-MS/MS analyses (section 3-2).

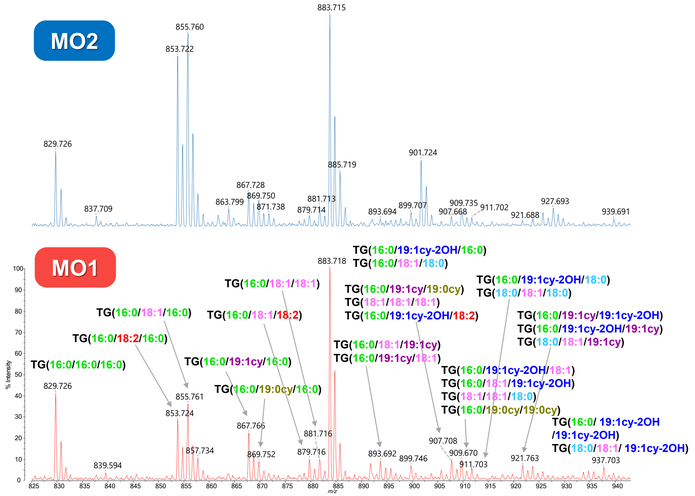

Figure 3. MALDI-MS spectrum of Monguba oil: unheated (MO1; red trace) and heated (MO2; blue trace). TAG identification was carried out by high-energy CID MALDI-MS/MS.
16:0 = palmitic; 18:0 = stearic; 18:1 = oleic; 18:2 = linoleic; 19:1cy = sterculic; 19:0cy = dihydrosterculic; 19:1cy-2OH = 2-hydroxy-sterculic.

3-2. High-energy CID MALDI-MS/MS analyses

High-energy-CID MALDI-MS/MS analyses were conducted on all visible, high- and low-abundant TAG peaks, with particular focus on those species containing CPFAs. MS/MS spectra were carefully interpreted for the presence of fragments indicative of: 1) the *sn*-positions of the fatty acids in the TAGs; 2) monoacylglycerols (MGs) and diacylglycerols (DAGs); 3) charge-remote fragments (CRFs) for the presence and position of the cyclopropene ring and double bond(s). Figure 4 shows the HE-CID MS/MS spectrum of *m/z* 883, which resulted in the combination of TG(16:0/19:1cy-2OH/16:0) and TG(16:0/18:1/18:0). Figure 4A shows the DAG fragment region, where all FAs composing the two TAGs were confirmed. Figure 4B shows the region of the MS/MS spectrum of the free FAs ([M + Na]$^+$), MGs ([M] + H]$^+$), and diagnostic ions for the *sn*-1/3 and *sn*-2 positions. The fragments at *m/z* 359 and 331 indicate that the 19:1cy-2OH and 18:1 FAs are in the *sn*-2 position, respectively. Figure 4C shows the CRF region for a full, detailed structural characterisation of the FA chains. For the 2-hydroxy-sterculic acid (19:1cy-2OH), the diagnostic CRF at *m/z* 717 was successfully detected (blue series) along with the 66 Da unit, indicating the cyclopropene ring is present. For the oleic acid (18:1), the diagnostic CRF at *m/z* 729 was successfully detected (pink series), and the 40 Da unit, characteristic of a double bond, is present. CRFs of palmitic (16:0) and stearic (18:0) acids can also be observed (green and cyan series, respectively). Figure 5 shows the CRFs region of the HE-CID MS/MS spectrum of the TAG at *m/z* 867, which was identified as TG(16:0/19:1cy/16:0). Once again, the CRFs diagnostic of the sterculic acid were found (*m/z* 659, 687, 701; purple series).

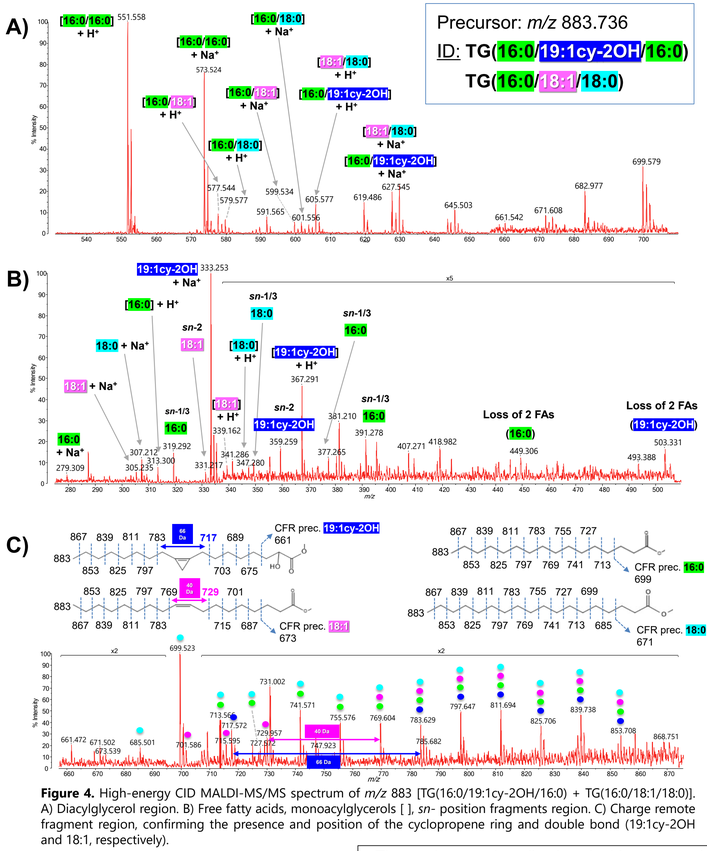

Figure 4. High-energy CID MALDI-MS/MS spectrum of *m/z* 883 [TG(16:0/19:1cy-2OH/16:0) + TG(16:0/18:1/18:0)]. A) Diacylglycerol region. B) Free fatty acids, monoacylglycerols [ ], *sn*- position fragments region. C) Charge remote fragment region, confirming the presence and position of the cyclopropene ring and double bond (19:1cy-2OH and 18:1, respectively).

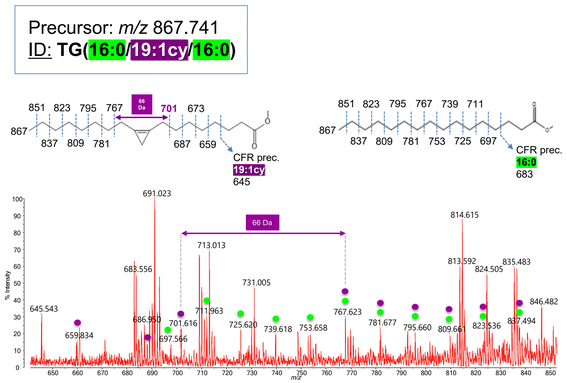

Figure 5. Charge remote fragment region of the high-energy CID MALDI-MS/MS spectrum of *m/z* 867 [TG(16:0/19:1cy/16:0)], confirming the presence and position of the cyclopropene ring in the sterculic acid.

Conclusions

This work demonstrates the powerful combination of a benchtop linear MALDI-TOF instrument and a high-performance MALDI-TOF/TOF mass spectrometer for the rapid profiling and detailed characterisation of the TAGs in native Monguba oil. The high-sensitivity, high-resolution MS/MS capability of the MALDI-7090 allowed: 1) the FA composition of the TAGs to be obtained; 2) the presence of CPFAs, such as sterculic, dihydrosterculic and 2-hydroxy-sterculic acid to be confirmed; 3) positional information of the FAs in the TAG to be obtained, as well as a full characterisation of the FA chains including cyclopropene ring and double bond positions based on the detection of CRFs. The differences in the TAG profiles of the un-heated and heated oils will be the subject of a future investigation.