Matrix-Assisted Laser Desorption/ Ionization Time-of-Flight Mass Spectrometry of *Vernonia galamensis* Oil

Folahan O. Ayorinde*, Eisa Elhilo, Caswell Hlongwane, and Kamal A. Saeed

Department of Chemistry, Howard University, Washington, DC 20059

ABSTRACT: Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) has been used to identify eight triacylglycerols (TAG) in *Vernonia galamensis* oil (VO). The TAG include trivernolin, divernoloylstearoylglycerol, divernoloyloleoylglycerol, divernoloyllinoleoylgylcerol, divernoloylpalmitoylglycerol, vernoloyllinoleoylstearoylglycerol, vernoloyllinoleoyloleoylglycerol, and vernoloyldilinoleoylglycerol. Samples for the MALDI/TOF/MS analysis were prepared with alpha-cyano-4-hydroxycinnamic acid (matrix) in an acetonitrile/tetrahydrofuran solvent system. A mol ratio of matrix/VO (44:1) gave reproducible composite spectra, resulting in resolutions greater than 8,000 and signal-to-noise ratios of above 1000 for the most abundant molecular species. 1,3-Dioleoyl-2-stearoylglycerol and 1,2-dioleoyl-3-palmitoylglycerol were used as calibration standards.

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KEY WORDS: α-Cyano-4-hydroxycinnamic acid, MALDI/TOF/MS, mass spectrometry, triacylglycerols, *Vernonia galamensis*, vernonia oil, vernolic acid.

Seed oils often consist of complex mixtures of closely related triacylglycerols (TAG), thus requiring time-consuming and demanding processes for their analysis. Determination of the fatty acid composition of these oils generally involves transmethylation, followed by chromatographic and mass spectrometric analysis (1,2). TAG are generally analyzed by thin-layer chromatography (TLC) and/or high-pressure liquid chromatography (HPLC) (3,4).

Matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF)/mass spectrometry (MS) is an emerging and powerful tool that enables the analysis of complex mixtures of biomolecules such as proteins, peptides, and carbohydrates (5,6). Even though there are increasing reports of the use of MALDI TOF MS in the analysis of a variety of molecules, including synthetic polymers and oligomers (7,8), there is a major drawback in that it requires the use of an ultraviolet-absorbing molecule as a crystalline matrix for the sample preparation. Furthermore, the choice of matrix and the mode of sample preparation could be analyte-dependent, thus necessitating the development of specific sample preparation protocols for different types of molecules. However, in spite of such impediments, MALDI TOF MS could provide a simple and efficient characterization of the molecular species that are found in vegetable oils, once the sample preparation methodologies are developed.

Vernonia galamensis oil (VO) is a new industrial oil which has recently been investigated for its potential use in coatings, polymer formulations, cosmetic formulation, and oleochemicals (9,10). VO is unique in that it consists mostly of naturally epoxidized triacylglycerols in which the major acid is *cis*-12,13-epoxy-*cis*-9-octadecenoic (vernolic) acid (11). We recently published the desorption chemical ionization/mass spectrometry (MS)/MS investigation of VO in which seven TAG were identified (12).

Consequently, the present study is aimed at the development of a simple and time-saving sample preparation method for the characterization of vernonia oils using MALDI TOF MS.

EXPERIMENTAL PROCEDURES

The matrix, α-cyano-4-hydroxycinnamic acid (CHCA), acetonitrile, and tetrahydrofuran (THF) were purchased from Aldrich Chemical Company (Milwaukee, WI). Crude VO was purchased from IXTT Corporation (Culver, IN). 1,3-Dioleoyl-2-stearoylglycerol (SOO) and 1,2-dioleoyl-3-palmitoylglycerol (POO), obtained from Sigma Chemical Co. (St. Louis, MO), were used as calibration standards. MALDI TOF MS experiments were performed on a Perkin Elmer Biosystems Voyager-DE STR workstation (Norwalk, CT). Positive-ion MALDI spectra (100 acquisitions) were acquired in the delayed-extraction (150 ns) and reflector modes, with accelerating voltage at 20,000 V, grid voltage at 75%, nitrogen laser (337 nm) with the attenuator setting at 2,300, and the low-mass gate set at 500 Da. Postsource decay (PSD) experiments were performed under similar conditions except for a 50-ns delay time and laser attenuator setting at 2,500. All solutions were prepared in 2-mL Eppendorf microcentrifuge tubes, and vortexed for about 15 s prior to use.

Preparation of matrix solution. CHCA (1.0 mg, 5.29 μmol) was dissolved in 1 mL CH_3CN/THF (7:1, vol/vol), stored in a 4°C refrigerator, and used as necessary over a

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*To whom correspondence should be addressed at Department of Chemistry, Howard University, 525 College Street, NW, Washington, DC 20059.
E-mail: fayorinde@howard.edu

TABLE 1
Eight Triacylglycerols Identified in *Vernonia galamensis* Oil Using MALDI/TOF/MS[a]

Triacylglycerols	Mass of sodiated molecular ion (*m/z*)
Trivernolin (VVV)	949.7
Divernoloylstearoylglycerol (VVS)	937.7
Divernoloyloleoylglycerol (VVO)	935.7
Divernoloyllinoleoylgylcerol (VVL)	933.7
Divernoloylpalmitoylglycerol (VVP)	909.7
Vernoloyllinoleoylstearoylglycerol (VLS)	921.7
Vernoloyllinoleoyloleoylglycerol (VLO)	919.7
Vernoloyldilinoleoylglycerol (VLL)	917.7

[a] MALDI TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

2-wk period. It was necessary to vortex for about 4 min in order to completely dissolve the matrix.

Preparation of analyte solution. Crude VO (1.0 mg, 1.08 μmol) was dissolved in 1 mL CH$_3$CN/THF (3:1, vol/vol), stored in a 4°C refrigerator, and used as needed.

Preparation of calibration solution. Both 1,3-dioleoyl-2-stearoylglycerol (SOO) and 1,2-dioleoyl-3-palmitoylglycerol (POO) were separately dissolved (1.0 mg/mL) in CH$_3$CN/THF (3:1 vol/vol). The solutions were stored in a 4°C refrigerator and used as needed.

Preparation of sample solution for MALDI analysis. The matrix solution (90 μL) was added to the analyte solution (10 μL). The calibration sample was similarly prepared, except that 20 μL of each calibrant solution was added to a 90-μL matrix solution. About 1 μL of each sample solution was placed on the 100-well sample plate. The deposited samples crystallized within 30 s upon evaporation of the solvent. The sample plate was then loaded into the MALDI ion source.

RESULTS AND DISCUSSION

Given in Table 1 are the eight triacylglycerols that were identified in *V. galamensis* oil. All the TAG, trivernolin (VVV), divernoloylstearoylglycerol (VVS), divernoloyloleoylglycerol (VVO), divernoloyllinoleoylglycerol (VVL), divernoloylpalmitoylglycerol (VVP), vernoloyllinoleoylstearoylglycerol (VLS), vernoloyllinoleoyloleoylglycerol (VLO),

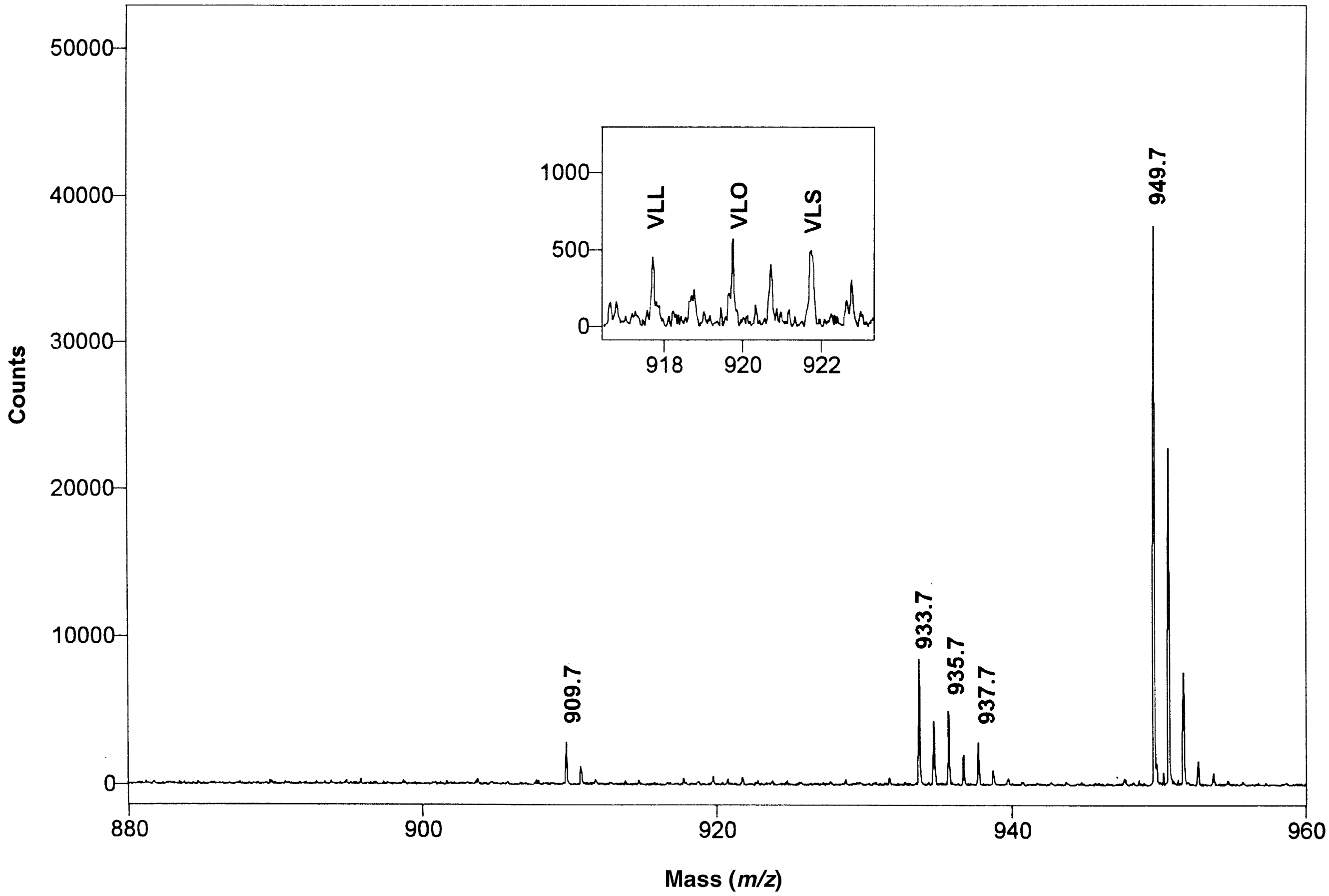

FIG. 1. Matrix-assisted laser desorption/ionization time-of-flight (MALDI TOF) mass spectrum of vernonia oil showing sodiated molecules at *m/z*: 949.7 = trivernolin (VVV); 937.7 = divernoloylstearoylglycerol (VVS); 935.7 = divernoloyloleoylglycerol (VVO); 933.7 = divernoloyllinoleoylglycerol (VVL); 909.7 = divernoloylpalmitoylglycerol (VVP). Insert showing minor components: vernoloyllinoleoylstearoylglycerol (VLS) = 921.7; vernoloyllinoleoyloleoylglycerol (VLO) = 919.7; vernoloyldilinoleoylglycerol (VLL) = 917.7.

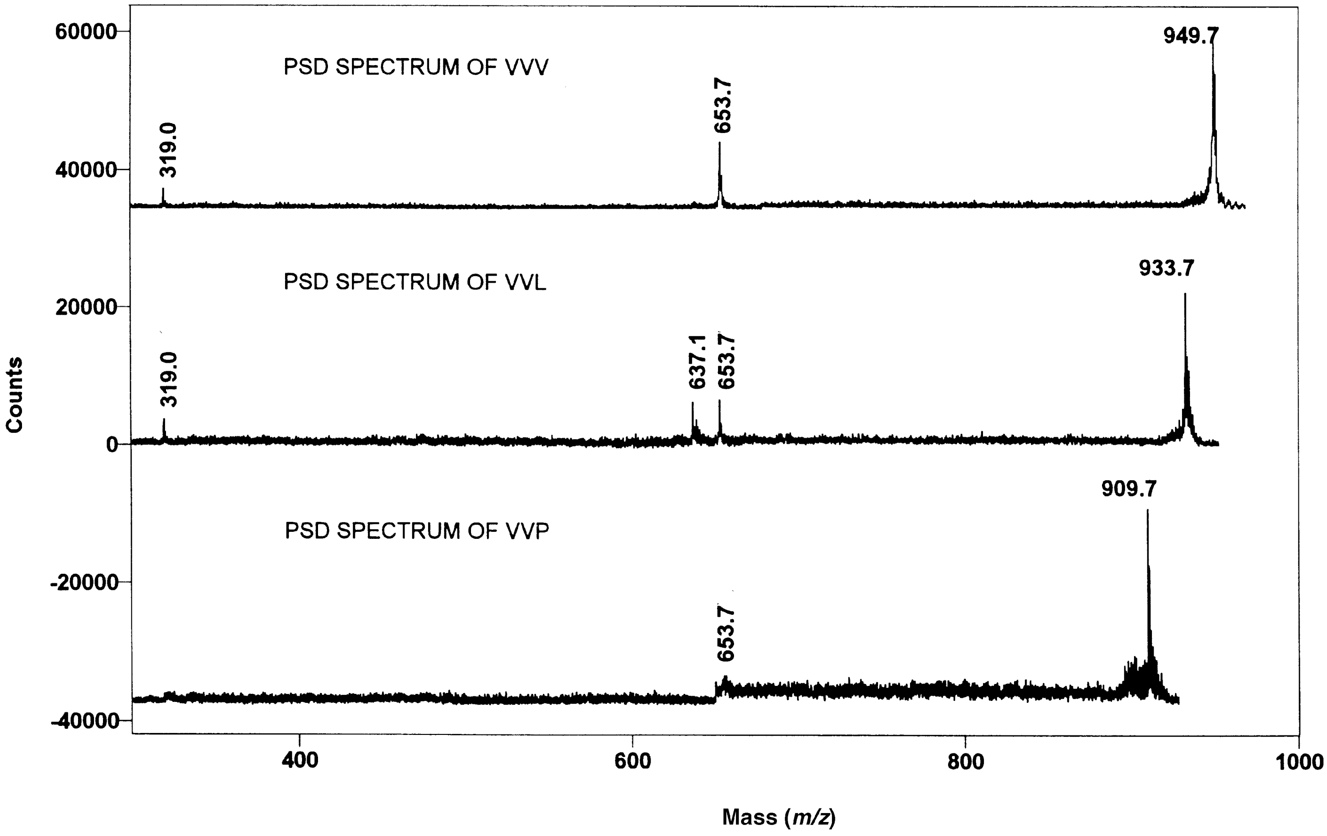

FIG. 2. Post-source decay (PSD) spectra of sodiated VVV, VVL, and VVP fragment ion at *m/z* 653 due to loss of vernolic, linoleic, or palmitic acids from the corresponding sodiated molecules. Fragment ion at *m/z* 637 due to loss of vernolic acid from the sodiated molecules at *m/z* 933.7. Fragment ion at *m/z* 319 = sodium vernolate. For abbreviations see Figure 1.

and vernoloyldilinoleoylglycerol (VLL), contain the vernoloyl moiety. This is consistent with previous studies on the composition of VO (12). In order to obtain reproducible MALDI TOF composite spectra with acceptable signal/noise ratio (>1,000) for the most abundant molecular species (*m/z* 949.7), we found that a matrix/VO mole ratio of 44:1 was optimum. With this mole ratio, more than 20 composite spectra were obtained (100 shots per composite spectrum), and each composite spectrum gave a resolution greater than 8000. A matrix/VO ratio less than 40:1 resulted in high noise level, making it difficult to identify the minor TAG component (VLS, VLO, VLL). On the other hand, a matrix/VO ratio of greater than 50:1 produced poor sample crystallization, resulting in low and unreproducible ion count. The identified TAG gave sodiated molecular ions (Fig. 1) rather than protonated, thus providing very uncomplicated MALDI TOF spectra. To further confirm that cationization was the norm under our experimental conditions, we tried two other matrices, sinapinic acid and dithranol, both of which gave spectra similar to that with CHCA.

Additional confirmation of our TAG assignments was obtained by performing PSD experiments on the sodiated VVV (*m/z* 949.7), VVL (*m/z* 933.7), and VVP (*m/z* 909.7), the results of which are shown in Figure 2. Sodiated VVV gave only one fragment at *m/z* 653 indicating the loss of vernolic acid from the sodiated molecular ion. On the other hand, sodiated VVL gave two fragment ions at *m/z* 653 and 637, due to the loss of vernolic acid and linoleic acid from the sodiated molecular ion, respectively. The PSD spectrum from sodiated VVP gave a very weak fragment ion at *m/z* 653 attributed to the loss of vernolic acid, and a weaker ion intensity at *m/z* 613 due to the loss of palmitic acid from the sodiated molecular ion. All the PSD experiments gave a fragment ion at *m/z* 319 (sodium vernolate).

In preparing the sample for MALDI TOF analysis, the choice of solvent mixture was dictated by the solubility and crystallization efficiency of the matrix. CHCA and VO are both soluble in THF, but insoluble in acetonitrile. However, using THF as the sole solvent resulted in rapid dispersion of the sample on the sample plate, giving rise to poor crystallization. Thus the ratio (7:1, vol/vol) CH_3CN/THF for the matrix solution and (3:1, vol/vol) for the VO solution was the best combination of CH_3CN/THF in that it gave fast solvent evaporation and reproducible crystallization.

In the case of the calibration sample, the optimal mole ratio of matrix to each of the calibrants (SOO, POO) was found to be 20:1 (Fig. 3), for the same reasons as indicated above. We also attempted to use distearoylpalmitoylglycerol

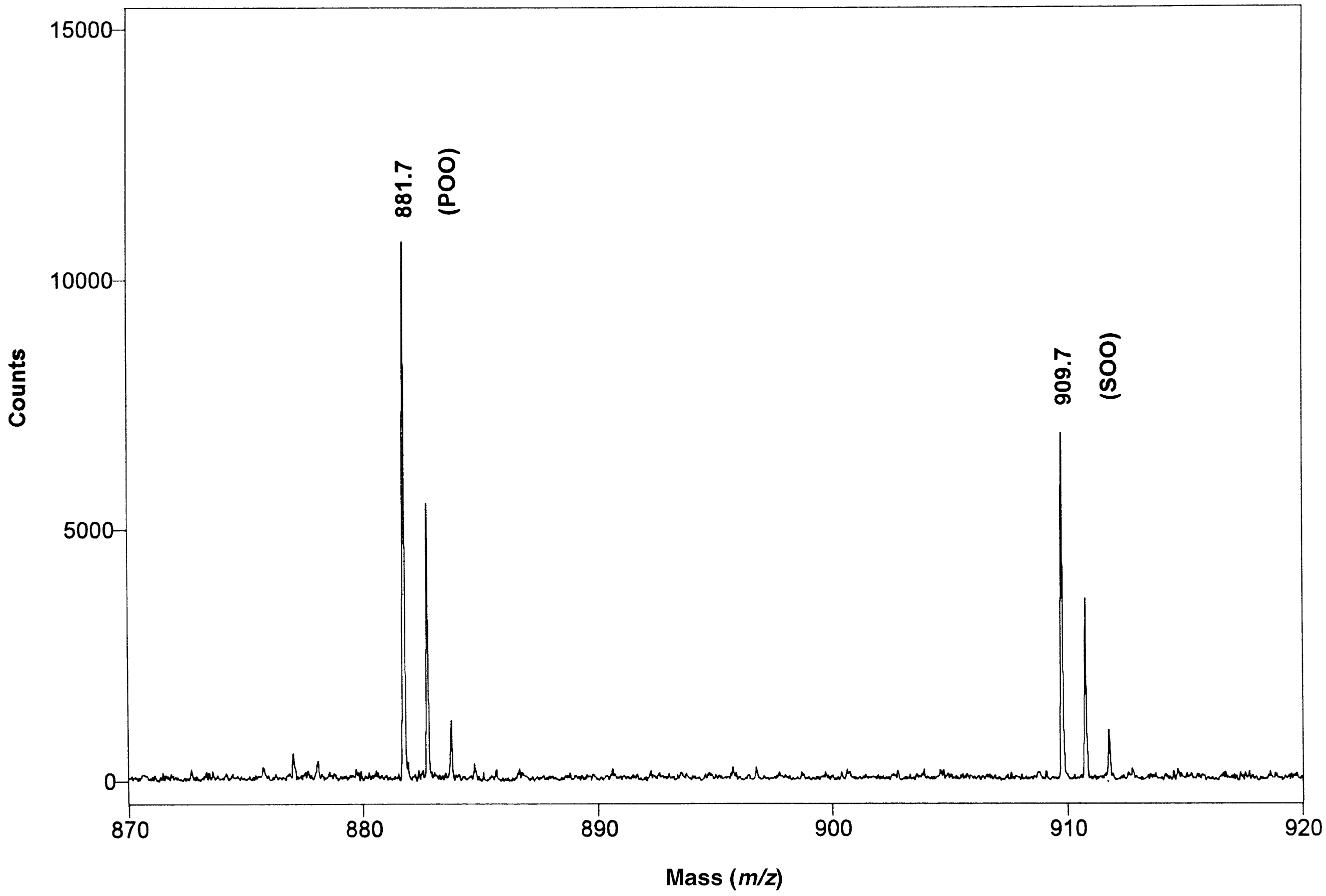

FIG. 3. MALDI TOF mass spectrum of calibration standand showing sodiated molecules at *m/z* 909.7 for stearoyldioleoylgylcerol (SOO) and *m/z* 881.7 for palmitoyldioleoylglycerol (POO). See Figure 1 for other abbreviation.

(PSS) as one of the calibrants, and found PSS was insoluble in the CH_3CN/THF solvent system. This suggests that the sample preparation method that is ideal for VO will not be applicable to those vegetable and/or animal fats that contain highly saturated fatty acids.

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