

This article was downloaded by:

On: 24 January 2011

Access details: Access Details: Free Access

Publisher Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

Triacylglycerol Analysis by High Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry: *Crepis Alpina* and *Vernonia Galamensis* Seed Oils

W. E. Neff^a; W. C. Byrdwell^a

^a U.S. Department of Agriculture, Food Quality and Safety Research, National Center for Agricultural Utilization Research, Agricultural Research Service, Peoria, Illinois

To cite this Article Neff, W. E. and Byrdwell, W. C. (1995) 'Triacylglycerol Analysis by High Performance Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry: *Crepis Alpina* and *Vernonia Galamensis* Seed Oils', Journal of Liquid Chromatography & Related Technologies, 18: 20, 4165 – 4181

To link to this Article: DOI: 10.1080/10826079508013753

URL: <http://dx.doi.org/10.1080/10826079508013753>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

**TRIACYLGLYCEROL ANALYSIS BY HIGH
PERFORMANCE LIQUID CHROMATOGRAPHY-
ATMOSPHERIC PRESSURE CHEMICAL
IONIZATION MASS SPECTROMETRY:
CREPIS ALPINA AND *VERNONIA
GALAMENSIS* SEED OILS**

W. E. NEFF* AND W. C. BYRDWELL

*Food Quality and Safety Research
National Center for Agricultural Utilization Research
Agricultural Research Service
U.S. Department of Agriculture
1815 N. University Street
Peoria, Illinois 61604*

ABSTRACT

Unusual seed oils having significance for chemical synthesis, *Crepis alpina*, or with fatty acids which contain functional groups important in the preparation of plastics, *Vernonia galamensis*, were analyzed by a new reversed-phase high performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry technique. Using this method, we have identified 16 triacylglycerols in the *Crepis alpina* oil and 18 triacylglycerols in *Vernonia galamensis* oil and showed greater sensitivity for detection of and improved identification of triacylglycerols compared to previous analyses using the techniques of reversed-phase and silver ion high performance liquid chromatography with a flame ionization detector. The most abundant *Crepis alpina* triacylglycerols were: linoleoyldicrepenynoylglycerol (33.0%), tricrepenynoyl (32.3%), palmitoyldicrepenynoyl

(11.5%), dilinoleoylcrepenynoyl (6.7%) glycerols. The remaining triacylglycerols occurred at five or less mole percent abundance. The most abundant *Vernonia galamensis* triacylglycerols were: trivernoloyl (43.3%), linoleoyldivernoloyl (21.3%), oleoyldivernoloyl (7.9%), palmitoyldivernoloyl (8.2%) and stearoyldivernoloyl (6.4%) glycerols. The remaining triacylglycerols occurred at four or less mole percent abundance. These studies provided new knowledge concerning the triacylglycerol composition of these oils and show that the atmospheric pressure chemical ionization technique is suitable for mass spectral identification of neutral molecules which do not contain a chargeable functional group.

INTRODUCTION

In order to fully evaluate the utility of unusual seed oils having significance for chemical synthesis, such as *Crepis alpina* and *Vernonia galamensis*, knowledge of their triacylglycerol composition is important.

Crepis alpina seed oil (CAO) is a source (70-80%) of crepenynic acid [*cis*-9-octadecen-12-ynoic acid; (C) (1)]. This acid is a useful intermediate in the chemical synthesis of deuterium-labeled compounds for human metabolism studies (2,3). Likewise, *Vernonia galamensis* (VGO), a potential source of epoxy fatty acids, is also useful for preparation of deuterium-labeled fats for human metabolic studies (2).

In addition, because VGO contains 70-80% of an unsaturated epoxy fatty acid, vernolic (*cis*-12,13-epoxy-*cis*-9-octadecenoic) (V), there has been much interest in its applications for the manufacture of commercial products (4-10). VGO has potential industrial uses for coating formulations and production of epoxy resins (11,12). Also, VGO is a potential source of raw material for elastomers (13) and chemicals for plastics manufacturing (14,15).

TAG composition data for these oils has been obtained by reversed phase-high performance liquid chromatography (RP-

HPLC) (16) and silver ion-high performance liquid chromatography (Ag-HPLC) (17) with flame ionization detection (FID). While the FID proved satisfactory for quantitation of the eluted TAG, identification of the individual TAG species required the collection of fractions for characterization by proton and carbon nuclear magnetic resonance spectroscopy and conversion to methyl esters for GC analysis to identify the TAG constituent FA (16,17).

We recently reported the development of a RP-HPLC technique, coupled with a quadrupole mass spectrometry (MS) equipped with an atmospheric pressure chemical ionization (APCI) interface for qualitative analysis of standard TAG species eluted from an HPLC column (18). The resultant simple spectra contained only the protonated TAG molecular ion (M+1) and diacylglycerol fragments to conclusively identify TAG (18,19).

We report here the use of the coupled RP-HPLC/APCI-MS technique for qualitative and semi-quantitative analysis of TAG with unusual FA.

EXPERIMENTAL

Material

CAO and VGO were obtained from K. Carlson and R. Kleiman (USDA, ARS, NCAUR, Peoria, IL). Solid-phase extractive purification of TAG to avoid interference by non-TAG during RP-HPLC/APCI-MS was performed by a previously reported procedure (16).

Methods

RP-HPLC equipment consisted of a quaternary pump system with membrane degasser (LDC 4100 MS, Thermo Separation Products, Schaumburg, IL), and two columns in series: An Adsorbosphere C18 25 cm x 4.6 mm, 5 μ m (12% carbon load)

(Alltech Assoc., Deerfield, IL) and an Adsorbosphere UHS C18 25 cm x 4.6 mm, 10 μ m (30% carbon load).

A quadrupole mass spectrometer system (Finnigan MAT SSQ 710C, San Jose, CA) was used which was fitted with an atmospheric pressure chemical ionization source (vaporizer temperature at 400°C, capillary heater temperature of 265°C, corona current of 6 μ A, high purity nitrogen as sheath gas at 60 psi and auxiliary gases at 25 mL/min).

CAO and VGO TAG were separated using a gradient solvent program with propionitrile (PrCN), dichloromethane (DCM), and acetonitrile (ACN) as follows: 45% PrCN throughout, initially 20% DCM and 35% ACN, held for 15 minutes; DCM was increased to 25% and ACN decreased to 30% over 5 minutes, and held for 15 minutes; DCM was further increased to 30% and ACN decreased to 25% over 5 minutes, and held for 35 minutes; the composition was returned to the initial conditions over 5 minutes. The flow rate was 1 mL/min. The effluent was split so that ~600 μ L/min went to an evaporative light scattering detector (ELSD) and ~400 μ L/min went to the APCI interface. In both analyses the sample size injected was 5 μ l of TAG mixture (50 mg solute per 2 mL hexane).

GC Analysis

The purified TAG were transmethylated and the methyl esters analyzed by GC by a previously reported procedure for SBO (20). GC reference standards for crepenynic acid and vernolic acid were obtained by transmethylation of tricrepenynoylglycerol and trivernoloylglycerol, respectively, previously collected by RP-HPLC (16).

RESULTS AND DISCUSSION

RP-HPLC/APCI-MS identification data (mass spectra results) for the TAG are presented in Table 1 (CAO) and

TABLE 1
Crepis alpina Seed Oil Triacylglycerols Determined by Reversed-Phase HPLC Coupled with Quadrupole Mass Spectrometer Via Atmospheric Pressure Chemical Ionization*

TG Name ^{b,c}	Mol. Wt.	DG1 Mass ^d	DG1 Int. %	DG2 Mass	DG2 Int. %	DG3 Mass	DG3 Int. %	TG+1 Mass ^e	TG Int. %	Ret. Time ^f	Mole %
CCCX	870	593 (C,Cx)	4.2	595 (C,C)	0.7			871	100	11:44	0.5
CCC	872	595 (C,C)	15.2					873	100	13:01	32.3
CCL	874	595 (C,C)	7.0	597 (C,L)	13.2			875	100	16:29	33.0
CCM	822	595 (C,C)	N.U.	545 (C,M)	21.3			823	100	18:00	0.2
CCO	876	595 (C,C)	8.4	599 (S,O)	10.6			877	100	20:23	4.1
CLL	876	597 (C,L)	12.8	599 (L,L)	11.0			877	100	21:20	6.7
CCP	850	595 (C,C)	13.3	573 (C,P)	14.2			851	100	21:31	11.5
CC 20:1	904	595 (C,C)	9.1	627 (C,20:1)	3.7			905	100	24:41	0.5
CCS	878	595 (C,C)	9.0	601 (C,S)	10.2			879	100	26:37	4.0
LLL	878	599 (L,L)	9.0					879	100	27:52	1.7
PLC	852	597 (C,L)	12.1	573 (C,P)	4.9	575 (P,L)	11.0	853	100	28:23	2.4
CCA	906	629 (C,A)	11.6	595 (C,C)	9.3			907	100	31:03	0.6
SLC	880	597 (C,L)	6.3	601 (C,S)	12.3	603 (S,L)	9.6	881	100	32:33	1.4
LLP	854	575 (P,L)	21.3	599 (L,L)	31.3			855	100	34:25	0.5
CLA	908	597 (C,L)	5.4	629 (C,A)	12.3	631 (L,A)	14.0	909	100	37:36	0.2
LLS	882	599 (L,L)	44.9	603 (L,S)	50.0			883	100	40:36	0.4

*See Experimental Section for HPLC-Mass Spectrometry Conditions.
^b See Fig. 1 for Reversed-Phase-HPLC Coupled with Quadrupole Mass Spectrometer Via Atmospheric Pressure Chemical Ionization Chromatogram.
^c triacylglycerol. Triacylglycerol fatty acids: C, crepenynic; L, linoleic; O, oleic; S, stearic; M, myristic; A, arachidic; 20:1, twenty carbon fatty acid with one double bond unspecified location; and Cx, crepenynic with one additional double bond unspecified location.
^d specified diacylglycerol fragments remaining after loss of one fatty acid residue from the triacylglycerol during mass spectrometry.
^e Int. % is the abundance of a particular ion with respect to the most abundant ion.

Table 2 (VGO). The RP-HPLC/APCI-MS total ionization curves for the mass spectral identified TAG, with respect to RP-HPLC retention time, are presented in Fig. 1 (CAO) and Fig. 2 (VGO).

Example mass spectra obtained for tricrepenynoyl (same FA), linoleoyldicrepenynoyl (2 same FA, 1 different FA) and palmitoyllinoleoylcrcpenynoyl (all different FA) glycerols are presented in Fig. 3a, 3b, 3c, respectively, and for trivernoloyl (same FA), linoleoyldivernoloyl (2 same FA, 1 different FA) and oleoyllinoleoylvernoloyl (all different FA) glycerols are presented in Figs. 4a, 4b, 4c, respectively.

For each of the CAO TAG, the base peak is the protonated TAG molecular ion. For vernolic acid-containing VGO TAG, except trivernoloyl and linoloyl divernoloyl glycerol, the base peak is one of the diacylglycerol fragments (DG). Other mass spectral peaks are the distinctive diacylglycerol fragment masses which are presented in Tables 1 and 2. For TAG containing only one FA, one DG fragment is required for identification; for TAG with one different and two same FA, two DG are required; and for TAG with three different FA, three DG are required for identification. The diacylglycerol fragments conclusively identify TAG with the same molecular weight (19). Also, protonated molecular ions plus propionitrile (TG+1+55(Pr)) are observed in the CAO mass spectra (Fig. 3a,b,c). The origin of the [M+38]⁺ ions (Fig. 3a,b,c) is not known. Also, through selective ion monitoring, TAG like PLC and LLL, which eluted in the same RP-HPLC peak (Fig. 1), could be identified by the appropriate masses (Table 1, Fig. 3c).

It is important to note that trilinolenoylglycerol produced a mass spectrum in previous RP-HPLC/APCI-MS work (18) similar to the mass spectrum obtained here for

TABLE 2
 Vernonia Galamensis Seed Oil Triacylglycerols Determined by Reversed-Phase HPLC Coupled with Quadrupole Mass Spectrometer Via Atmospheric Pressure Chemical Ionization^a

TG Name ^{a,b}	Mol. Wt.	DG1 Mass ^c	DG1 Int. % ^d	DG2 Mass	DG2 Int. %	DG3 Mass	DG3 Int. %	(TG+1) Mass* Int. %	(TG+1)-18 ^f Mass Int. %	(TG+1)-36 ^f Mass Int. %	(TG+1)-100 ^f Mass Int. %	(TG+1)+102 ^f Mass Int. %	HPLC Ret. Time ^g	Mole %					
VVV ^e	926	631 (V,V)	87.7					927	100	909	62.4	891	22.8	827	11.9	1029	35.8	7:55	43.3
VVL	910	631 (V,V)	56.6	615 (V,L)	95.3			911	100	893	54.7	875	12.3	811	10.1	1013	40.3	10:07	21.3
VVO	912	631 (V,V)	76.0	617 (V,O)	100			913	96.3	895	46.2	877	14.8	813	6.3	1015	46.7	11:47	7.9
VVP	886	631 (V,V)	100	591 (V,P)	84.1			887	91.1	869	36.0	851	14.4	787	5.9	990	42.0	12:21	8.2
LLV	894	615 (L,V)	100	599 (L,L)	69.0			895	79.9	877	53.6			795	5.9	998	33.5	13:28	3.6
VVS	914	631 (V,V)	74.3	619 (V,S)	100			915	97.4	897	44.9	879	20.5	815	8.3	1017	45.6	14:25	6.4
OLV	896	601 (O,L)	97.3	615 (V,L)	100	617 (V,O)	85.0	897	80.8	879	70.3			797	6.2	1000	59.1	16:05	1.5
PLV	870	575 (P,L)	71.9	615 (V,L)	100	591 (V,P)	24.3	871	51.2	853	42.7			771	4.7	974	26.8	17:12	1.9
LLL	878	599 (L,L)	41.3					879	100									18:48	0.4
S1V	898	603 (S,L)	75.8	615 (V,L)	100	619 (V,S)	26.7	899	60.0	881	45.0			799	5.1	1001	34.8	20:14	1.4
POV	872	577 (P,O)	58.4	617 (V,O)	100	591 (V,P)	25.3	873	35.9	855	22.5			773	2.6	976	25.4	20:36	0.8
LLO	880	599 (L,L)	48.0	601 (L,O)	77.9			881	100									23:16	0.3
LAV	926	631 (L,A)	100	647 (A,V)	18.6	615 (V,L)	96.9	927	44.0	909	44.6			827	4.0	1029	27.8	24:02	0.5
LLP	854	599 (L,L)	69.9	575 (L,P)	60.0			855	100									24:43	0.4
SOV	900	605 (S,O)	87.8	617 (V,O)	100	619 (V,S)	22.5	901	51.4	883	35.7			801	2.1	1003	29.8	24:50	1.0
OOA ^h LLS	882	601 (L,O)	53.7	603 (O,O)	57.2	599 (L,S)	67.1	883	100									28:53	0.5
ORV	928	633 (O,A)	69.9	647 (A,V)	15.9	617 (V,O)	100	929	29.9	911	28.6			829	4.3	1031	18.6	29:10	0.3
POL	856	575 (P,L)	100	577 (P,O)	21.7	601 (L,O)	33.7	857	5.8									30:53	0.2

^aMass spectrometer total ionization chromatogram peaks, see Fig. 2. For analysis conditions, see Experimental Section.

^bTG=triacylglycerol. Triacylglycerol fatty acids: S, stearic; P, palmitic; O, oleic; L, linoleic; V, vernolic; and A, arachidic

^cDG 1,2,3 are diacylglycerol fragments remaining after loss of one fatty acid residue from the triacylglycerol during mass spectrometry.

^dInt. % is the abundance of a particular ion with respect to the most abundant ion formed during mass spectrometry.

^eTG+1 is the protonated triacylglycerol molecular ion.

^f(TG+1)-18, (TG+1)-36, (TG+1)-100 and (TG+1)+102 is the loss of one water, two water molecules, hexanal or addition of protonated hexanal to the vernolic acid of the TAG during mass spectrometry.

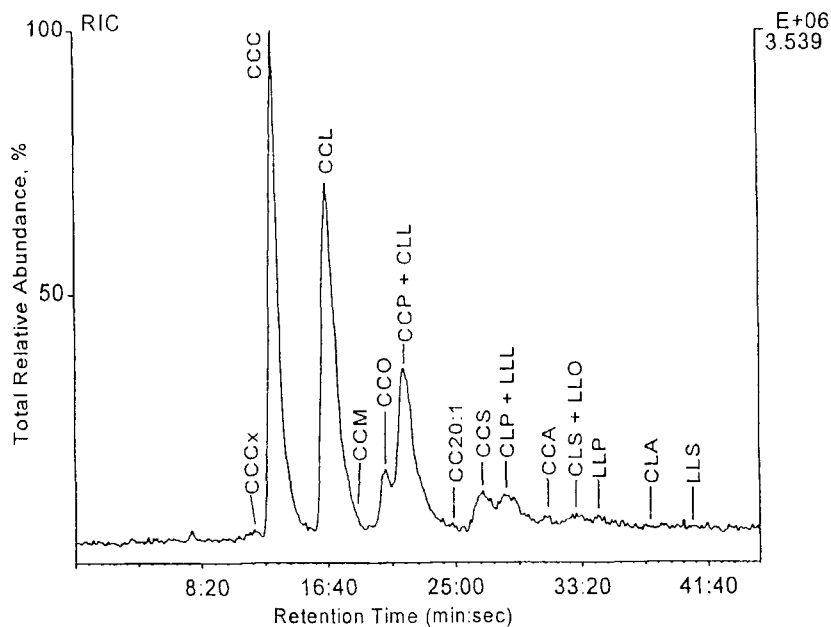


FIGURE 1.

Reconstructed ion chromatogram of *Crepis alpina* seed oil.

Triacylglycerol fatty acids: C, crepenynic; Cx, crepenynic with one additional π bond; L, linoleic; O, oleic; M, myristic; P, palmitic; S, stearic; A, arachidic; 20:1, twenty carbon fatty acid with a double bond at an unspecified location.

triclepenynoylglycerol. However, the CAO contained only 0.3% linolenic acid. Therefore, this potential problem is not of concern for RP-HPLC/APCI-MS analysis of CAO.

In addition to the diacylglycerol and parent ions observed in the APCI spectra for vernolic acid-containing TAG, protonated molecular ions and diglyceride fragments exhibiting loss of water are observed. The number of fragments exhibiting loss of water is dependent on the number of vernoloyl chains present. Also, there are protonated molecular ion fragments which have reduced mass due to loss

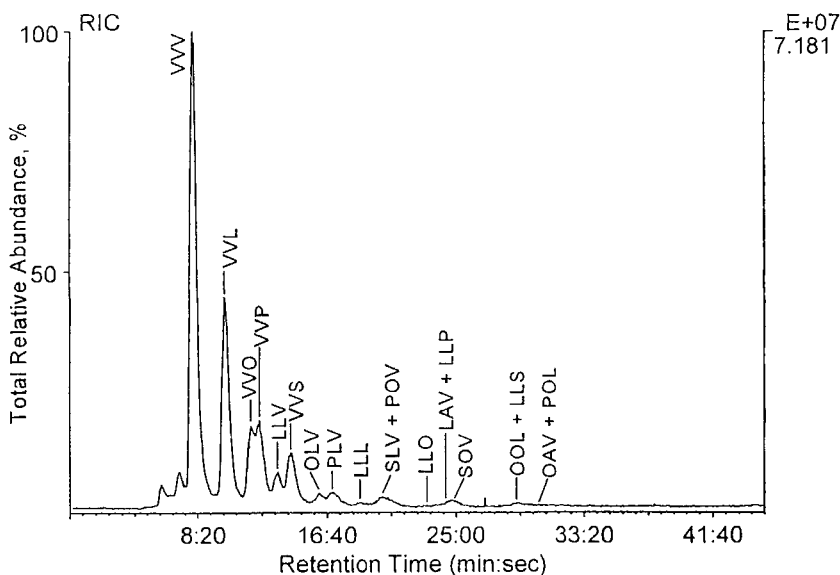


FIGURE 2.

Reconstructed ion chromatogram of *Vernonia galamensis* seed oil. Triacylglycerol fatty acids: V, vernolic; L, linoleic; O, oleic; P, palmitic; S, stearic; A, arachidic.

of hexanal from cleavage possibly due to the proposed fragmentation pattern depicted in Fig. 5 during APCI-MS of the TAG. The corresponding diacylglycerol fragment [V, 12:2], which would result from hexanal loss via the depicted pattern in Fig. 5 is observed in spectra for VVV (Fig. 4a) and VWL (Fig. 4b). In addition, the hexanal adduct of the protonated molecular ion may be observed in the spectra for the three vernolic acid TAG given in Fig. 4. The origin of masses 966 to 1015 for VVV (Fig. 4a), 950 to 1000 for VWL (Fig. 4b) and 927 to 986 for OLV (Fig. 4c) is not known. Also, through selective ion monitoring, TAG like SLV and POV, LLP and LAV, and OAV and POL, which eluted in the same RP-HPLC peaks (Fig. 2), could be identified by the appropriate

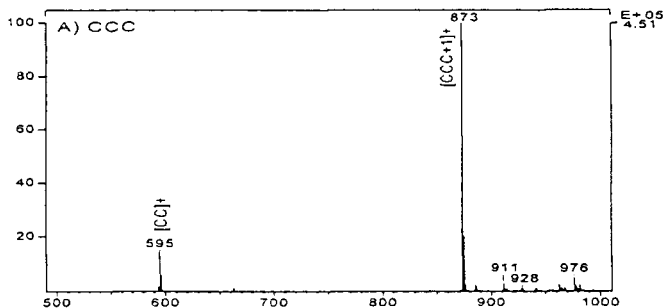


FIGURE 3a.

Mass spectrum of tricrepenynoylglycerol. Identities of fragment ions are shown in brackets. Triacylglycerol fatty acids: C, crepenynic.

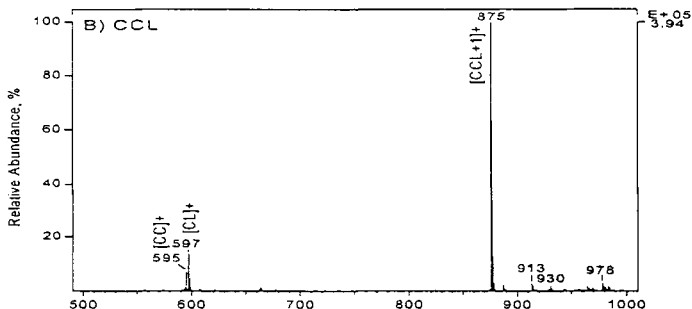


FIGURE 3b.

Mass spectrum of linoleoyldicrepenynoylglycerol. Identities of fragment ions are shown in brackets. Triacylglycerol fatty acids: C, crepenynic; L, linoleic.

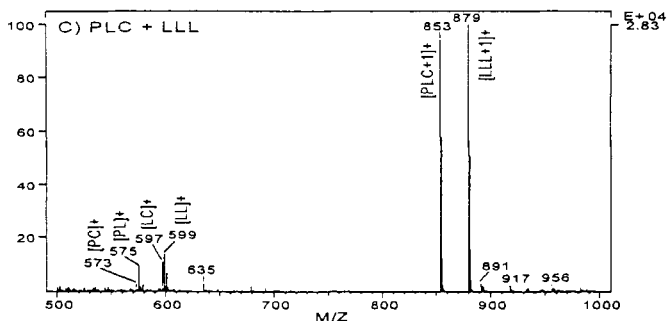


FIGURE 3c.

Mass spectrum of palmitoyllinoleoylcrepenynoylglycerol with minor trilinoleoylglycerol, which coelute during reversed phase-HPLC (Fig. 1). Triacylglycerol fatty acids: C, crepenynic; L, linoleic; P, palmitic.

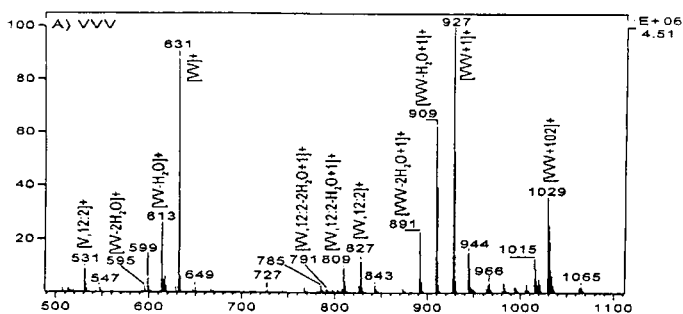


FIGURE 4a.
 Mass spectrum of trivernoloylglycerol. Identities of fragment ions is shown in brackets. Triacylglycerol fatty acids: V, vernolic; 12:2, vernolic minus hexanal fragment (mass=100).

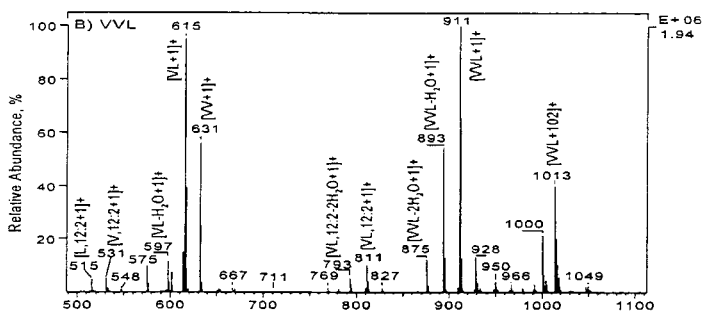


FIGURE 4b.
 Mass Spectrum of linoleoyldivernoloylglycerol. Identities of fragment ions is shown in brackets. Triacylglycerol fatty acids: V, vernolic; L, linoleic; 12:2, vernolic minus hexanal fragment (mass=100).

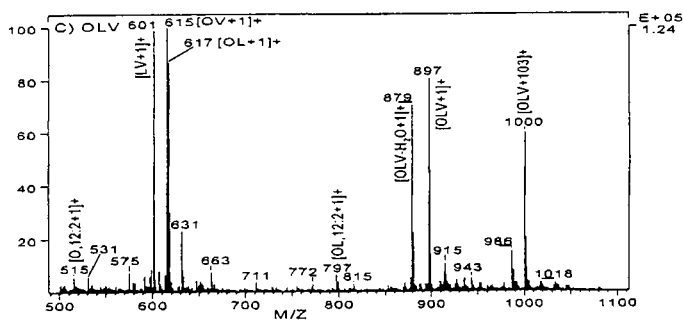


FIGURE 4c.
 Mass spectrum of oleoyllinoleoylvernoloylglycerol. Identities of fragment ions is shown in brackets. Triacylglycerol fatty acids: V, vernolic; L, linoleic; O, oleic; 12:2, vernolic minus hexanal fragment (mass=100).

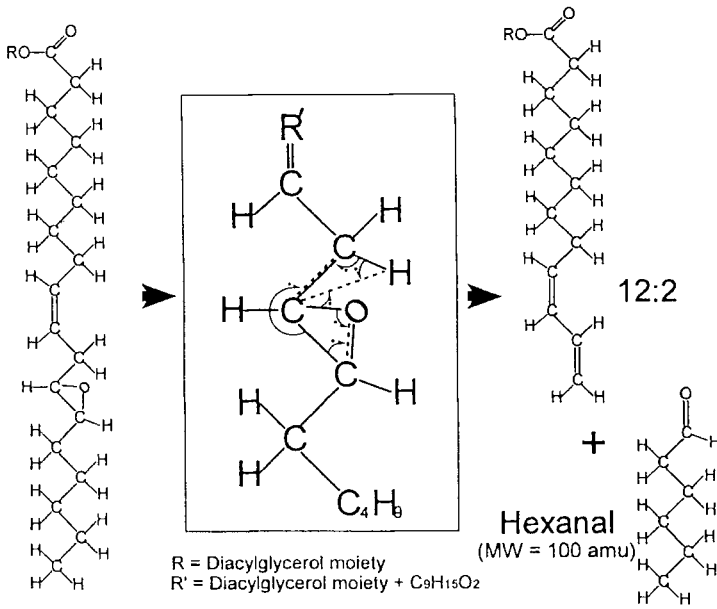


FIGURE 5.

Proposed mechanism for mass spectral cleavage of vernolic acid to yield hexanal during reversed phase-HPLC coupled with mass spectrometer with atmospheric pressure chemical ionization of vernolic acid containing triacylglycerols.

masses (Table 2). Thus, the spectra for VGO TAG (Fig. 4) are more complex and show loss of water and hexanal fragments from V compared to the spectra for *Crepis alpina* oil (Fig 3) and soybean oil (20). RP-HPLC/APCI-MS produced simple spectra for the TAG of CAO, including those TAG with the alkene-alkyne containing FA of crepenynic acid.

This RP-HPLC/APCI-MS technique identified more CAO TAG than the RP-HPLC-FID (16) and Ag-HPLC-FID techniques (17). Even coeluting TAG like CCP and CLL, CLP and LLL, or CLS and LLO (Fig. 1) were differentiated by producing extracted ion chromatograms of individual masses. One TAG identified,

CCCx, contained a crepenynic acid with two other π bonds (probably forming a second acetylene bond) and was not previously identified. In addition, our RP-HPLC/APCI-MS procedure conclusively identified 19 TAG in VGO which were not previously conclusively identified, including OLV, PLV, LLL, SLV, POV, LLO, LLP, LAV, SOV, LOO, OAV and POL (Fig. 2).

Mole percent of the TAG components was determined by summation of selected ion masses (protonated molecular ion and DG fragments) obtained by APCI-MS and listed in Table 1 (CAO) and Table 2 (VGO). By this MS method, the most abundant CAO TAG were: linoleoyldicrepenynoylglycerol (33.0%), tricrepenynoyl (32.3%), palmitoyldicrepenynoyl (11.5%), and dilinoleoylcrcpenynoyl (6.7%). The remaining twelve CAO TAG were found at four or less mole percent abundance.

The most abundant VGO TAG were: trivernoloylglycerol (43.3%), linoleoyldivernoloyl (21.3%), oleoyldivernoloyl (7.9%), palmitoyldivernoloyl (8.2%) and stearoyldivernoloyl (6.4%) glycerols. The remaining 13 VGO TAG occurred at four or less mole percent. Trivernoloyl and linoleoyldivernoloylglycerols were also determined previously to be the most and second most abundant TAG in VGO by RP-HPLC-FID (16). The RP-HPLC/APCI-MS technique for qualitative and quantitative analysis of TAG showed greater sensitivity for detection of and improved identification of TAG compared to the previously reported techniques for the TAG analysis (16,17,22-26). Some TAG could be identified, but not quantitated. In CAO, three TAG were not quantitated: crepenynoyllinoleoylloleoyl glycerol (CLO), crepenynoyllinoleoyl-20:1 (CL,20:1), and dilinoleoylloleoyl glycerol (LLO). These TAG have the same equivalent carbon number as, coeluted chromatographically with, and share common masses with dicrepenynoylstearyl

TABLE 3
Comparison of Fatty Acid Composition of Seed Oils as Calculated from Triacylglycerol Composition^a and as Determined After Transmethylation of the Seed Oil^b

Method	Fatty Acid Percent												UID
	C ^c or V ^d	14:0	16:0	18:0	18:1	18:2	20:0	18:3	20:1	20:2	22:0	24:0	
	<i>Crepis Alpina</i> Seed Oil												
LC/APCI-MS ^e	72.2	0.1	4.4	2.0	1.4	19.3	0.3	0.0	0.2	0.0	0.0	0.0	0.2
GC-FID ^b	75.0	0.5	3.9	1.3	2.5	15.9	0.3	0.3	0.3	0.1	0.1	0.0	0.0
	<i>Vernonia galamensis</i> Seed Oil												
LC/APCI-MS ^e	77.3	0.0	3.4	3.0	4.0	12.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0
GC-FID	73.7	0.0	3.7	3.1	4.7	14.0	0.4	0.1	0.3	0.0	0.1	0.1	0.0

^aTriacylglycerol Composition Determined by Reversed Phase-High Performance Liquid Chromatography Coupled with Atmospheric Pressure Chemical Ionization Mass Spectrometry (RP-HPLC-APCI-MS).

^bGas Chromatography with Flame Ionization Detection (GC-FID).

^cC, crepenynic acid

^dV, vernolic acid

^eThe RP-HPLC/APCI-MS TAG mole percent composition, Tables 1 & 2, was converted to TAG weight percent composition for valid comparison of calculated fatty acid composition with experimental fatty acid which is related to weight percent obtained by GC-FID.

glycerol (CCS), dicrepenynoylarachidoyl glycerol (CCA), and crepenynoyllinoleoylstearoyl (CLS) glycerol, respectively.

Application of the APCI-MS technique for accurate quantitation of individual TAG species may require the use of response factors based on analysis of TAG standard mixtures of known weight. We evaluated the APCI-MS quantitation for CAO TAG (Table 1) and VGO TAG (Table 2) by comparing the FA composition, calculated from the TAG composition, with the experimental FA composition, obtained by GC-FID analysis of the transmethyated TAG mixtures, as presented in Table 3. The mole percent data was converted to weight percent data to make a more valid comparison with GC-FID data, which is related to number of TAG carbons or TAG weight. While the APCI-MS technique is much improved over other MS quantitative methods, there is some variation from the fatty acid composition determined by the GC-FID methods, as reported

previously (18). The disparity of response between TAG is largely due to differences in fragmentation patterns between TG and DG fragments, which depends on different levels of unsaturation within the TAG. The more similar the series of analytes, the less will be the disparity between responses of the TAG. Nevertheless, there is good agreement between the calculated FA composition from RP-HPLC/APCI-MS and the FA composition determined by GC-FID.

The RP-HPLC/APCI-MS technique has conclusively identified 16 TAG in the CAO and 18 TAG in VGO and shows greater sensitivity for detection of and improved qualitative analysis of TAG compared to the previously used RP-HPLC-FID and Ag-HPLC-FID (17,18).

ACKNOWLEDGEMENTS

We are grateful to Ray K. Holloway for gas chromatography of methyl esters of the transmethylated TAG. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

REFERENCES

1. R. Kleiman, in Proceedings of the World Conference on Biotechnology for the Fats and Oils Industry, T.H. Applewhite, ed., American Oil Chemists' Society, Champaign, 1988, p. 73-77.
2. R.O. Adlof, H. Rakoff, E.A. Emken, J. Am. Oil Chem. Soc. 68: 303-306 (1991).
3. R.O. Adlof, E.A. Emken, Ibid. 70: 817-819 (1993).
4. C. Singh, N. Kapur, B.L. Kaul, Res. Ind. 38: 152-153 (1993).
5. C. Singh, B.L. Kaul, Ibid. 38: 1-2 (1993).

6. F.O. Ayorinde, B.D. Butler, M.T. Clayton, J. Am. Oil Chem. Soc. 67: 844-845 (1990).
7. Anon., Agric. Eng. 70: 11-13 (1989).
8. K. Kaplan, Agric. Res. 37: 10-11 (1989).
9. R.E. Perdue Jr., K.D. Carlson, M.G. Gilbert, Econ. Bot. 40: 54-68 (1986).
10. R.E. Perdue Jr., J. Am. Oil Chem. Soc. 63: 405 (1986).
11. S. Latta, INFORM 1: 434-443 (1990).
12. Anon., Ibid 2: 685-690 (1991).
13. O.A. Afolabi, M.E. Aluko, G.C. Wang, W.A. Anderson, F.O. Ayorinde, J. Am. Oil Chem. Soc. 66: 983-985 (1989).
14. F.O. Ayorinde, F.T. Powers, L.D. Streete, R.L. Shepard, D.N. Tabi, Ibid. 66: 690-692 (1989).
15. F.O. Ayorinde, G. Osman, R.L. Shepard, F.T. Powers, Ibid. 65: 1774-1777 (1988).
16. W.E. Neff, R.O. Adlof, H. Konishi, D. Weisleder, Ibid. 70: 449-455 (1993).
17. W.E. Neff, R.O. Adlof, M. El-Agaimy, Ibid. 71: 853-855 (1994).
18. W.C. Byrdwell, E.A. Emken, Lipids 30: 173-175 (1995).
19. W.W. Christie, High Performance Liquid Chromatography and Lipids, Pergamon Press, New York, 1987, p. 188.
20. W.E. Neff, W.C. Byrdwell, In press J. Am. Oil Chem. Soc. (1995).
21. A. Kuksis, J.J. Myher, L. Marai, J. Am. Oil Chem. Soc. 61: 1582-1589 (1984).
22. A. Kuksis, L. Marai, J.J. Myher, J. Chromatogr. 273: 43-66 (1983).
23. J.J. Myher, A. Kuksis, L. Marai, F. Mangano, J. Chromatogr. 283: 289-301 (1984).

24. R.G.J. VanLeuken, G.T.C. Kwakkenbos, *Ibid.* 626: 81-86 (1992).
25. K.D. Carlson, S.P. Chang, *J. Am. Oil Chem. Soc.* 62:934 (1985).
26. M.A. Anderson, L. Collier, R. Dilliplane, F.O.Ayorinde, *Ibid.* 70: 905-908 (1993).

Received: April 20, 1995

Accepted: May 10, 1995