UNIVERSITY OF WATERLOO
FACULTY OF APPLIED HEALTH SCIENCES
Department of Kinesiology

Untargeted Macrolipidomic Profiling of Plant-Based Oils

Juan J. Aristizabal Henao[1], Ningombam Sanjib Meitei[2], Ken D. Stark[1]

[1]Laboratory of Nutritional Lipidomics, Department of Kinesiology, University of Waterloo, Waterloo, ON, Canada

[2]PREMIER Biosoft, Palo Alto, CA, USA

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Overview

- **Purpose**: Plant based oils are a major source of dietary fat and they are largely triacylglycerols (TAG). TAG can be challenging to measure due to fatty acyl diversity, therefore a dual column serial-coupling setup for chromatographic separation was explored.
- **Methods**: Macrolipidomic profiling of 10 plant oils completed by UHPLC-MS/MS on a Waters Synapt G2S*i* QTOF and data processed using SimLipid software. Fatty acid composition was also completed.
- **Results**: The oils were mainly TAG molecules although diacylglycerols were also abundant. Coconut oil, flaxseed oil and DHASCO had the greatest diversity of types of lipid molecules.

Introduction

The analysis of TAG in plant-based oils can be particularly challenging to measure due to their high structural diversity encompassing various fatty acyl chain lengths and isobaric/isomeric configurations. While TAG constitute most of the weight of vegetable oils, the measurement of other lipid species, such as diacylglycerols, phospholipids and sterols is relatively limited in the literature. Dual column chromatography was used for untargeted macrolipidomics of 10 commercially-available plant-based oils. Lipids were identified using automated identification software and confirmed by manual inspection and by fatty acid analysis using gas chromatography.

Results

Figure 6. Principal Component Analysis of the Plant Oils

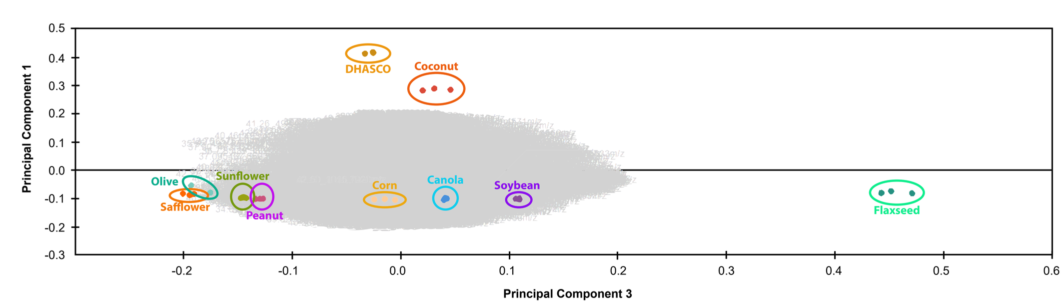
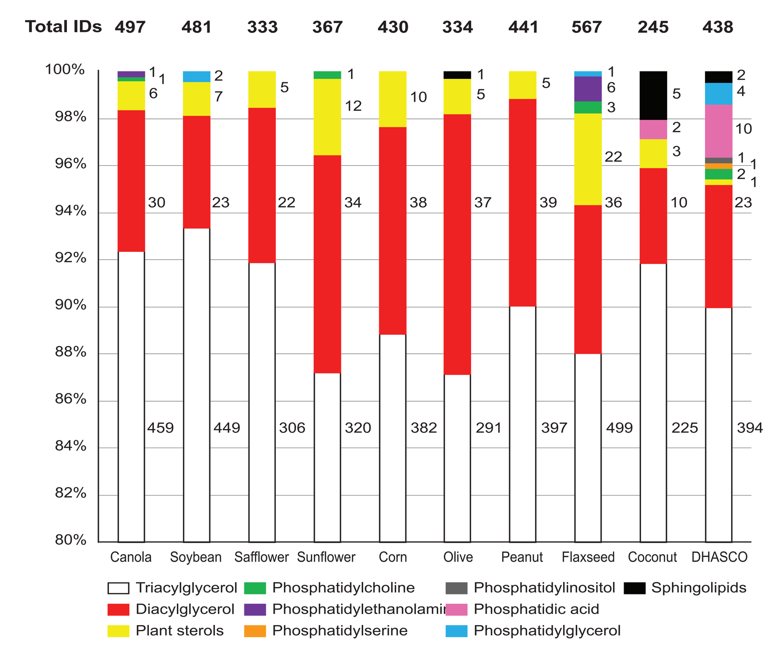


Table 1. Number of Brutto Species in the Oils

Lipid Class	Canola	Soybean	Safflower	Sunflower	Corn	Olive	Peanut	Flaxseed	Coconut	DHASCO
TAG	79	76	61	63	70	51	68	85	56	114
DAG	13	10	11	13	16	15	15	16	10	19
Plant sterols	5	4	4	3	4	4	4	7	1	1
PC	1	0	0	1	0	0	0	3	0	2
PE	1	0	0	0	0	0	0	5	0	0
PS	0	0	0	0	0	0	0	0	0	1
PI	0	0	0	0	0	0	0	0	0	1
PA	0	0	0	0	0	0	0	0	2	9
PG	0	2	0	0	0	0	0	1	0	4
Sphingolipids	0	0	0	0	0	1	0	0	4	2
Total	99	92	76	80	90	71	87	117	73	153

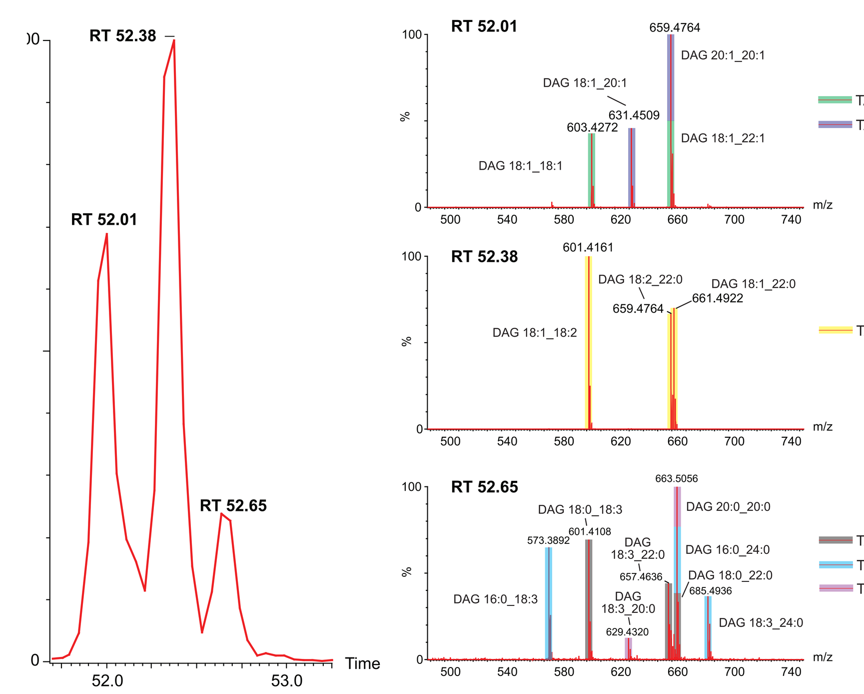
PG, phosphatidylglycerol; PA, phosphatidic acid; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DAG, diacylglycerol; TAG, triacylglycerol.

Figure 7. Possible Number of Medio Species in the Oil



Number of possible medio level species is listed beside each class bar with total species in bold at the top. MS/MS data was used and identifications were filtered for a base peak for sterols, a head group for phospholipids, and each acyl fragment of glycerolipids.

Figure 8. Manual Confirmation of Medio Species in Canola Oil



The extracted ion chromatogram for m/z 958.8797 ± 0.05 Da and the MS/MS Spectra of each peak. Six TAG species out of the 15 possible medio species were confirmed for the brutto species of TAG 58:3

Conclusions

- Each oil formed distinct clusters by principal component analysis with DHASCO, coconut and flaxseed oils being particularly isolated. These latter oils had unique TAG species containing 22:6, 12:0 and 18:3, respectively. While still unique, olive, safflower, sunflower and peanut oils had the most overlap.
- TAG molecules were the most abundant lipids at the brutto and medio level. Olive oil and coconut oil had the lowest number of TAG species. Flaxseed, canola, and soybean had high brutto and medio level TAG. DHASCO had the highest number of brutto TAG species, but the fifth highest number of possible medio species.
- Diacylglycerols were also common in the oils followed by plant sterols. Oils with the most diverse lipid class profile were DHASCO, followed by coconut and flaxseed oil. Interestingly, flaxseed oil had the most individual lipids while coconut had the least.
- Oleic acid (18:1n-9) was the most abundant fatty acid across the ten oils and then followed by linoleic acid (18:2n-6). Palmitic acid (16:0) and stearic acid (18:0) were also common in most oils but at lower levels. Distinct fatty acid profiles were determined for coconut oil (10:0, 12:0 and 14:0) and DHASCO (22:6n-3).

Table 2. Ten Most Abundant Lipids (TAG Species) in Each Oil

Canola TAG	wt%	Soybean TAG	wt%	Safflower TAG	wt%	Sunflower TAG	wt%	Corn TAG	wt%
18:1_18:2_18:3	7.4	*18:1_18:2_18:3*	6.6	*18:1_18:2_18:2*	8.9	*18:2_18:2_18:2*	10.5	*18:2_18:2_18:2*	8.6
18:1_18:1_18:1	7.3	*18:2_18:2_18:3*	6.3	*18:1_18:2_18:3*	8.6	*18:1_18:1_18:2*	9.4	*18:2_18:2_18:3*	6.8
18:1_18:2_18:2	7.1	*18:2_18:3_18:3*	5.6	*18:1_18:1_18:2*	8.4	*18:1_18:2_18:2*	7.7	*18:1_18:2_18:2*	6.3
18:1_18:3_18:3	6.6	*18:1_18:1_18:1*	4.8	*18:0_18:1_18:1*	5.8	*18:0_18:1_18:1*	6.0	*18:1_18:1_18:1*	5.2
18:1_18:1_20:1	3.8	*18:0_18:2_18:2*	3.6	*18:1_18:2_20:0*	4.1	*18:1_18:1_22:0*	4.5	*16:1_18:1_18:2*	4.4
18:0_18:1_18:3	3.6	*18:1_18:2_18:2*	3.6	*18:1_20:1_22:1*	3.9	*18:1_18:1_24:0*	3.7	*18:0_18:1_18:1*	3.3
18:2_18:3_18:3	3.2	*18:0_18:1_18:1*	3.5	*18:1_20:1_22:0*	3.7	*16:1_18:1_18:2*	3.5	*18:0_18:2_18:2*	3.3
18:1_18:1_18:2	3.1	*16:0_18:2_18:2*	2.9	*18:1_18:1_22:0*	3.6	*18:2_18:2_18:3*	3.2	*16:0_18:1_18:1*	2.6
18:0_18:1_18:1	2.6	*16:0_18:0_18:2*	2.4	*18:1_18:1_20:0*	3.5	*18:1_18:1_20:0*	3.1	*16:0_18:1_18:2*	2.6
18:1_18:1_20:0	2.4	*18:1_18:2_18:2*	2.4	*16:0_18:2_18:2*	3.5	*18:1_18:1_20:1*	3.0	*18:1_18:1_18:2*	2.5
Total wt%	47.0		41.7		53.9		54.7		45.7

Olive TAG	wt%	Peanut TAG	wt%	Flaxseed TAG	wt%	Coconut TAG	wt%	DHASCO TAG	wt%
18:1_18:1_18:1	9.7	*18:2_18:2_18:2*	6.7	*18:3_18:3_18:3*	5.1	*12:0_12:0_16:0*	10.0	*14:0_22:6_22:6*	6.3
18:1_18:2_18:2	6.3	*18:1_18:1_18:1*	6.1	*18:2_18:3_18:3*	5.0	*12:0_12:0_18:1*	6.8	*22:6_22:6_22:6*	5.9
18:0_18:1_18:1	6.3	*18:1_18:2_18:2*	6.0	*18:0_18:1_18:2*	4.8	*12:0_14:0_18:1*	6.5	*16:0_22:6_22:6*	4.9
18:1_18:1_18:2	6.2	*18:1_18:1_18:2*	5.5	*18:1_18:1_18:2*	4.7	*12:0_12:0_18:0*	6.3	*18:1_18:1_18:1*	3.4
16:0_18:1_18:2	5.3	*18:1_18:1_20:1*	3.7	*18:2_18:2_18:3*	4.6	*12:0_16:0_18:1*	5.1	*16:0_16:0_22:6*	3.2
16:0_18:1_18:1	4.1	*18:1_18:1_22:0*	3.7	*18:0_18:3_18:3*	3.7	*12:0_18:1_18:1*	5.0	*16:0_16:1_22:6*	3.0
18:1_18:1_20:0	3.5	*18:1_20:1_22:0*	3.6	*18:0_18:1_18:1*	2.8	*14:0_18:1_18:1*	4.7	*16:0_18:1_22:6*	2.7
16:0_18:1_18:3	3.2	*18:2_18:2_18:3*	3.5	*18:1_18:2_18:3*	2.7	*14:0_16:0_18:1*	4.3	*14:0_16:0_22:6*	2.7
18:1_18:1_20:1	2.9	*18:1_18:2_22:0*	3.5	*18:1_18:2_18:2*	2.7	*16:0_16:0_18:1*	3.6	*18:0_18:1_18:1*	2.7
18:2_18:2_18:2	2.9	*18:1_18:1_20:0*	3.1	*18:1_18:3_18:3*	2.5	*16:0_18:1_18:1*	3.6	*16:1_22:6_22:6*	2.3
	50.4		45.3		38.7		55.8		37.1

The top ten most abundant lipids of each oil were determined and identified. For each oil, the top ten were TAG species. TAG species appearing in the top ten for four or more oils are highlighted with the same colour while those identified in three or less are not highlighted.

Table 3. Fatty Acid Composition of Plant Oils

Fatty acid	Canola	Soybean	Safflower	Sunflower	Corn	Olive	Peanut	Flaxseed	Coconut	DHASCO
10:0	0.005 ± 0.005	0.002 ± 0.003	0.005 ± 0.005	0.005 ± 0.005	0.005 ± 0.005	0.006 ± 0.004	0.003 ± 0.003	0.003 ± 0.003	5.86 ± 0.14	1.17 ± 0.32
12:0	0.01 ± 0.01	0.007 ± 0.007	0.01 ± 0.01	0.004 ± 0.005	0.01 ± 0.01	0.006 ± 0.004	0.01 ± 0.01	0.007 ± 0.002	51.43 ± 1.36	5.66 ± 0.33
14:0	0.045± 0.045	0.053 ± 0.053	0.063 ± 0.062	0.047± 0.047	0.023 ± 0.022	0.010 ± 0.005	0.03 ± 0.01	0.034 ± 0.004	19.69 ± 0.54	14.08 ± 0.48
16:0	3.88 ± 0.02	5.19 ± 0.07	4.59 ± 0.03	5.34 ± 0.02	11.25 ± 0.02	9.91 ± 0.03	8.98 ± 0.05	5.19 ± 0.07	8.49 ± 0.23	11.98 ± 0.08
18:0	1.66 ± 0.08	3.96 ± 0.16	1.78 ± 0.01	3.39 ± 0.01	1.38 ± 0.01	3.18 ± 0.01	2.19 ± 0.06	3.96 ± 0.16	2.98 ± 0.09	0.53 ± 0.04
20:0	0.49 ± 0.05	0.10 ± 0.01	0.307 ± 0.004	0.193 ± 0.005	1.375 ± 1.378	0.30 ± 0.01	0.93 ± 0.05	0.10 ± 0.01	0.05 ± 0.01	0.06 ± 0.01
22:0	0.21 ± 0.02	0.08 ± 0.01	0.18 ± 0.01	0.51 ± 0.01	0.287± 0.287	0.07 ± 0.01	2.15 ± 0.19	0.08 ± 0.01	0.01 ± 0.01	0.11 ± 0.01
24:0	0.07 ± 0.01	0.04 ± 0.01	0.09 ± 0.01	0.12 ± 0.01	0.06 ± 0.01	0.020 ± 0.004	0.90 ± 0.12	0.04 ± 0.01	0.01 ± 0.01	0.03 ± 0.03
Total SFA	***6.37 ± 0.14***	***9.42 ± 0.23***	***7.02 ± 0.03***	***9.61 ± 0.04***	***13.07 ± 0.02***	***13.50 ± 0.04***	***15.19 ± 0.36***	***9.42 ± 0.23***	***88.52 ± 2.32***	***33.62 ± 0.38***
14:1	0.005 ± 0.003	0.002 ± 0.002	0.004 ± 0.003	0.004 ± 0.004	0.004 ± 0.005	0.003 ± 0.003	0.002 ± 0.003	0.005 ± 0.005	0.01 ± 0.01	0.30 ± 0.02
16:1	0.19 ± 0.02	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.60 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.01 ± 0.01	2.66 ± 0.06
18:1n-7	3.45 ± 0.05	1.54 ± 0.02	0.89 ± 0.04	0.80 ± 0.03	0.63 ± 0.01	2.02 ± 0.04	0.87 ± 0.04	0.79 ± 0.02	0.07 ± 0.01	0.22 ± 0.01
18:1n-9	62.16 ± 0.86	22.83 ± 0.55	79.26 ± 0.30	39.13 ± 0.09	27.54 ± 0.03	76.93 ± 0.24	56.18 ± 0.43	18.05 ± 0.50	4.48 ± 0.13	19.16 ± 0.80
20:1n-9	0.95 ± 0.05	0.13 ± 0.02	0.22 ± 0.01	0.11 ± 0.01	0.185 ± 0.004	0.20 ± 0.01	1.24 ± 0.04	0.09 ± 0.01	0.016 ± 0.005	0.04 ± 0.01
22:1n-9	0.03 ± 0.01	0.014 ± 0.005	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.006 ± 0.004	0.09 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01
24:1n-9	0.04 ± 0.03	0.01 ± 0.01	0.04 ± 0.04	0.006 ± 0.005	0.006 ± 0.003	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.006 ± 0.003	0.09 ± 0.09
Total MUFA	***66.82 ± 0.85***	***24.62 ± 0.55***	***80.49 ± 0.23***	***40.13 ± 0.10***	***28.44 ± 0.03***	***79.79 ± 0.22***	***58.46 ± 0.43***	***19.01 ± 0.51***	***4.60 ± 0.13***	***22.51 ± 0.77***
18:2n-6	18.57 ± 0.45	54.19 ± 0.77	12.04 ± 0.10	49.53 ± 0.13	57.38 ± 0.04	5.91 ± 0.03	25.48 ± 0.69	14.82 ± 0.08	0.65 ± 0.03	0.62 ± 0.02
18:3n-6	0.008 ± 0.003	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.004	0.09 ± 0.15	0.009 ± 0.010	0.006 ± 0.001	0.01 ± 0.01	0.01 ± 0.01	0.007 ± 0.008
20:2n-6	0.036 ± 0.005	0.02 ± 0.01	0.007 ± 0.003	0.01 ± 0.01	0.014 ± 0.003	0.005 ± 0.004	0.03 ± 0.05	0.035 ± 0.004	0.005 ± 0.004	0.002 ± 0.001
20:3n-6	0.01 ± 0.01	0.010 ± 0.004	0.005 ± 0.005	0.005 ± 0.003	0.004 ± 0.003	0.006 ± 0.007	0.01 ± 0.01	0.02 ± 0.02	0.006 ± 0.003	0.005 ± 0.005
20:4n-6	0.01 ± 0.01	0.008 ± 0.003	0.005 ± 0.004	0.004 ± 0.003	0.004 ± 0.004	0.006 ± 0.005	0.006 ± 0.004	0.03 ± 0.01	0.005 ± 0.004	0.007 ± 0.007
22:4n-6	0.01 ± 0.01	0.01 ± 0.01	0.010 ± 0.003	0.008 ± 0.003	0.011 ± 0.004	0.01 ± 0.01	0.014 ± 0.005	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01
22:5n-6	0.01 ± 0.01	0.01 ± 0.01	0.009 ± 0.005	0.007 ± 0.005	0.008 ± 0.004	0.01 ± 0.01	0.01 ± 0.01	0.009 ± 0.003	0.01 ± 0.01	0.01 ± 0.01
Total n-6 PUFA	18.67 ± 0.46	54.28 ± 0.77	12.09 ± 0.10	49.57 ± 0.12	57.52 ± 0.16	5.96 ± 0.03	25.56 ± 0.68	14.94 ± 0.06	0.70 ± 0.02	0.68 ± 0.02
18:3n-3	7.52 ± 0.39	6.00 ± 0.17	0.12 ± 0.01	0.22 ± 0.01	0.66 ± 0.01	0.45 ± 0.01	0.46 ± 0.03	56.20 ± 0.77	0.01 ± 0.01	0.04 ± 0.01
20:5n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.004 ± 0.003	0.006 ± 0.003	0.005 ± 0.004	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.004	0.01 ± 0.01
22:5n-3	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.005 ± 0.002	0.004 ± 0.005	0.006 ± 0.005	0.005 ± 0.005	0.008 ± 0.003	0.01 ± 0.01	0.34 ± 0.01
22:6n-3	0.03 ± 0.03	0.01 ± 0.01	0.05 ± 0.04	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.006 ± 0.004	42.36 ± 0.43
Total n-3 PUFA	***7.57 ± 0.41***	***6.03 ± 0.18***	***0.19 ± 0.03***	***0.24 ± 0.01***	***0.68 ± 0.01***	***0.48 ± 0.01***	***0.49 ± 0.04***	***56.25 ± 0.77***	***0.03 ± 0.02***	***42.75 ± 0.43***
Total FA (mg/mL)	1011 ± 72	1050 ± 96	1025 ± 68	1030 ± 27	1028 ± 46	946 ± 54	952 ± 63	1064 ± 104	910 ± 18	939 ± 98

Pink highlights indicate fatty acids of high abundance in a few oils. Blue highlights indicate fatty acids that are of high abundance and relatively common across the oils. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids, PUFA, polyunsaturated fatty acids.

Methods

Study Design

Nine plant-based oils (flaxseed oil, soybean oil, coconut oil, corn oil, sunflower oil, canola oil, peanut oil, safflower oil, and olive oil) were purchased locally and DHASCO was donated from DSM Company (**Figure 1**).

Figure 1. Ten Plant Oils Analyzed

Sample Preparation

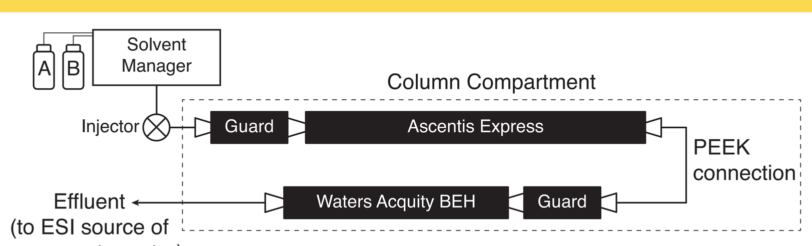
For macrolipidomic profiling, lipids were extracted from the oils using 2:1 chloroform/methanol (v/v) with TAG 17:0/17:0/17:0 as an internal standard (**Figure 2**). The lipid extracts were dried under N_2 gas and reconstituted in 65:35:5 acetonitrile/isopropanol/water (v/v/v) +0.1% formic acid and stored in vials at 4°C until analysis by UHPLC-MS/MS (n = 3).

For fatty acid compostion analysis, fatty acid methyl esters were prepared from the oils by direct transesterification using 14% boron trifluoride in methanol with hexane and heating for one hour at 100°C. TAG 17:0/17:0/17:0 was again included as an internal standard (n = 8).

Macrolipidomic Profiling

Ultra-high-performance liquid chromatography (UHPLC) was completed using a Ascentis Express C18 column, 2.0µm x 2.1mm I.D. x 15cm and a Waters Acquity BEH C18 column, 1.7µm x 2.1mm I.D. x 10cm dual column set-up (**Figure 3**). The mobile phase used a gradient of 60:40 acetonitrile/water (v/v) +10mM ammonium formate +0.1% formic acid, with 90:10 isopropanol/acetonitrile (v/v) +10mM ammonium formate +0.1% formic acid. Spectra were acquired in positive electrospray ionization mode under top-5 data-dependent acquisition conditions (Waters Synapt G2Si Quadrupole-Time-of-Flight mass spectrometer). Compound identifications were completed with SimLipid (v6.02 PREMIER Biosoft, Palo Alto, CA, USA). Quantitations were completed with Progenesis QI (v2.3, Newcastle upon Tyne, UK).

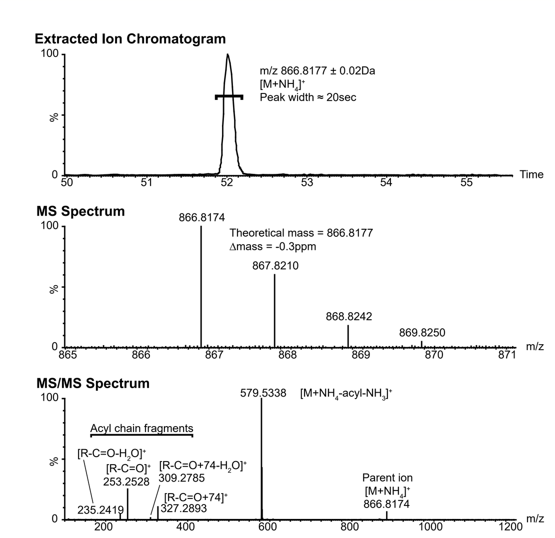
Figure 3. Dual Column Set-Up



Fatty Acid Composition Analysis

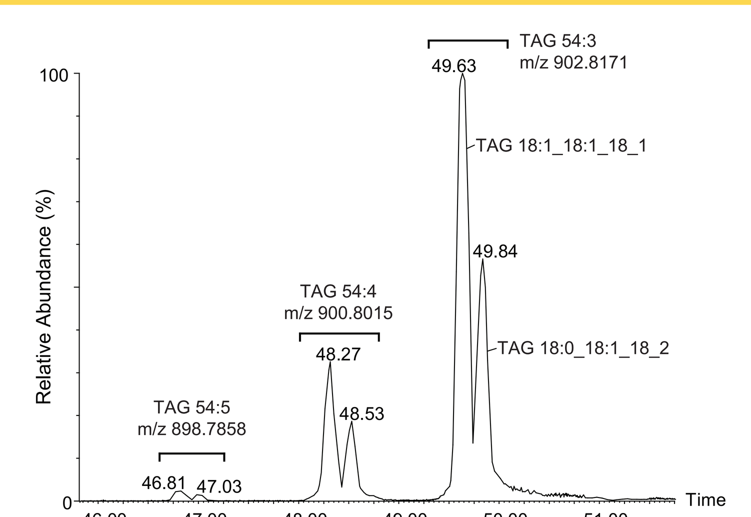
The prepared fatty acid methyl esters in hexane were analyzed by gas chromatography (Varian 3900) equipped with a DB-FFAP 15m × 0.1mm internal diameter × 0.1µm film thickness capillary column (Agilent Technologies, Mississauga, ON) with hydrogen as the carrier gas and flame ionization detection.

Figure 2. Internal Standard



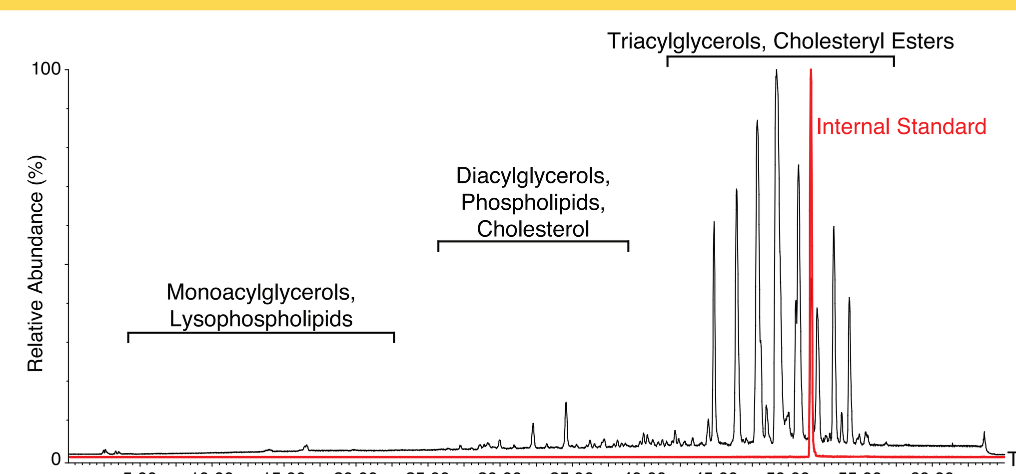
Extracted ion chromatogram, MS spectrum, and MS/MS spectrum of TAG 17:0/17:0/17:0

Figure 5. Representative Extracted Chromatogram



Three sets of two peaks each were detected with different isotopic contributions for the m/z 902.9871±0.02 Da extraction. Inspection of tandem mass spectra of parent molecular ions of TAG 54:3 revealed TAG 18:1_18:1_18:1 and TAG 18:0_18:1_18:2 acyl species.

Figure 4. Representative Chromatogram (Sunflower Oil)



Total ion chromatogram of sunflower oil.

The approximate retention times of some of the major lipid classes are indicated.

The internal standard, TAG 17:0/17:0/17:0, is overlaid (shown in red).