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Rapid Analysis of Microalgal Triacylglycerols with Direct-Infusion Mass Spectrometry

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ABSTRACT: Cultivation of microalgae has the potential to provide lipid-derived feedstocks for conversion to liquid transportation fuels. Lipid extracts from microalgae are significantly more complex than those of traditional seed oils, and their composition changes significantly throughout the microalgal growth period. With three acyl side chains per molecule, triglycerides (TAGs) are an important fuel precursor, and the distribution of acyl chain composition for TAGs has a significant impact on fuel properties and processing. Therefore, determination of the distribution of microalgal TAG production is needed to assess the value of algal extracts designed for fuel production and to optimize strain, cultivation, and harvesting practices. Methods utilized for TAG speciation commonly involve complicated and time-consuming chromatographic techniques. We present a method for TAG speciation and quantification based on direct-infusion mass spectrometry, which provides rapid characterization of TAG profiles without chromatographic separation. Specifically, we utilize Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to provide a reference library of TAGs for the microalgae *Nannochloropsis* sp. that provides the basis for high-throughput TAG quantitation by time-of-flight mass spectrometry (TOF MS). We demonstrate the application of this novel approach for lipid characterization with respect to TAG compound distribution, which informs both immediate and future strain and process optimization strategies.

INTRODUCTION

Triacylglycerols (triglycerides or TAGs) are an important food source and are also used extensively in the production of oleochemicals and renewable fuels. Large-scale renewable fuel production is sought as a means of improving U.S. energy independence and security, partially offsetting environmental impacts of fossil carbon combustion, and addressing government mandates for renewable fuel use. Currently, the two most widely used renewable fuel products are ethyl alcohol produced via fermentation and renewable distillate fuels produced from lipids found in oils and fat feedstocks. TAGs can be converted into fuel products via transesterification with an alcohol to produce fatty acid monoalkyl esters (i.e., biodiesel),[1] or they can be converted to hydrocarbons through deoxygenation followed by isomerization producing renewable diesel or jet fuel.[2]

Utilization of biodiesel has increased significantly in the United States with 1.3 billion gallons produced in 2013 according to the U.S. Department of Energy's Energy Information Administration.[3] This production volume is just more than 2% of the total diesel fuel used in the United States in 2012.[4] Increased production of fuel from fats and oils to offset a larger portion of fuel use will require increased production of oil feedstocks. One source for the production of renewable oils containing TAGs for renewable fuels is microalgae, which have the potential to produce oils at a capacity vastly higher than that of terrestrial feedstocks.[5,6] Harvesting algae for oil has potential advantages over terrestrial oil production, including less land area needed for cultivation and lower levels of water usage.[7−9] However, often the oils consist of more than just TAGs, and significant levels of polar lipids such as phospholipids and glycolipids can be present, which have implications for the downstream processing efficiency as well as catalyst longevity. A rapid and direct characterization of the feedstock prior to catalytic upgrading is imperative in the research, development, and deployment stages of algal biofuels. The full lipid profile characterization aids with the choice of catalyst or even cleanup operations, whereas the TAG quantitative content and profile can provide insight into downstream fuel options or even guide the prior separation of high-value TAGs (e.g., those containing highly unsaturated side chains) from the crude oils for coproduct development.[10] For example, during biodiesel production by transesterification, the presence of polar lipids can cause the formation of an emulsion during the removal of the alkali catalyst and glycerol, which slows production and reduces yields.[11] In the case of renewable diesel production, basic nitrogen groups present in polar lipid classes can lead to isomerization catalyst deactivation.[12] To enhance the value of algal oils as fuel feedstocks, polar lipids may need to be removed prior to fuel production. There is a major incentive to maximize the TAG content of oils while minimizing the production of other lipid classes, because of their high energy content and relatively high respective fuel yield. The fatty acid composition of algal oils can be rapidly assessed by esterification followed by gas chromatography;[13] however, if these fatty acids are present as part of other lipid classes, this analysis will not accurately determine oil value. High-

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throughput measurement of the TAG content and profile can support a rapid screening approach to accelerate the development and selection of ideal oil feedstocks for future biofuel production.[7,14]

The technical and economic considerations for the growth, harvesting, and processing of microalgae to produce oils for fuel and chemical production involve a range of research questions and challenges.[15,16] Strategies for increasing microalgal TAG content and utilization require the capability to rapidly monitor TAG composition and quantity. The fatty acid carbon chain length and degree of unsaturation dictate the properties of biodiesel such as cold weather operability and oxidation stability,[17−22] and knowledge of the fatty acid composition of TAGs as well as overall TAG content is needed to assess the value of microalgal lipids as biodiesel feedstocks. The composition of the TAGs with respect to the degree of unsaturation can have a dramatic influence on the hydrotreating conditions for renewable diesel production. During hydrotreatment, double bonds are saturated prior to oxygen removal; therefore, a greater degree of unsaturation in a feedstock requires more hydrogen for the production of hydrocarbons.[23] Hydrogen is the third largest variable cost in renewable diesel production identified in the National Renewable Energy Laboratory's recent techno-economic analysis[24] (after the cost of the biomass and the hexane solvent for extraction), and the presence of elevated levels of polyunsaturated TAGs, such as those found in *Nannochloropsis* strains, can have a significant negative economic impact. Microalgal lipids can have concentrations of polyunsaturated TAGs considerably higher than those found in traditionally utilized feedstocks, and the TAG concentration and composition can vary greatly between species and growth conditions and can provide novel avenues to high-value coproducts to be generated alongside fuels in a biorefinery.[25−28] Therefore, knowledge of the TAG concentration and profile is required for a number of steps in the production of microalga-derived biofuel and bioproducts.

Identification and quantification of TAGs can be accomplished with several techniques, the most common being liquid chromatography/mass spectrometry (LC/MS)[25,29,30] with retention time and fragment ion mass spectra being used to provide identification. However, these traditional methods for collecting detailed information about the TAG content and profile over physiological changing growth conditions take considerable time and effort. Alternative methods include matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS).[31] Gas chromatography-based quantitation of lipid acyl side chains involves transesterification of the lipids to more volatile fatty acid methyl esters (FAMEs)[13] and does not allow for speciation of intact TAGs or other lipid molecules. An alternative gas chromatography (GC) method is described in ASTM International (ASTM) method ASTM D6584, which utilizes a high-temperature column for separation of TAGs from other lipids.[32] That method does not allow for detailed speciation of TAG isomers but can be used to quantify total TAG content. Liquid chromatography is preferred for analysis of TAGs without conversion to FAMEs and, when coupled with high-resolution mass spectrometry, can provide detailed TAG profiles.[25]

Alternatively, detailed lipid compositional analysis can be accomplished without chromatography using direct-infusion mass spectrometry. This technique has been demonstrated by Han et al. using electrospray ionization with two-dimensional (2D) MS to deconvolute spectra using both positive and negative ionization to provide lipidomic information about biological samples.[33] A similar approach was demonstrated by Gomez-Ariza et al. using both electrospray and photoionization with 2D MS to authenticate olive oils.[34] However, most of the 2D MS approaches rely on the use of chromatography to reduce the level of co-elution and confusion of fragmentation patterns, with the exception of a recent report on direct-infusion polar lipid identification and quantification based on an internal standard [e.g., dinitrophenyl-phosphatidylethanolamine (DNP-PE)].[35] Direct-infusion mass spectral data can also be collected by ultra-high-resolution FT-ICR MS. This approach hinges on ultrahigh resolution to avoid inaccuracies associated with overlapping mass spectral peaks observed with complex, unfractionated lipid extracts. Additionally, the technique provides sub-part-per-million mass measurement accuracy, which in conjunction with Kendrick mass sorting and isotopic fine structure analysis allows for the determination of elemental composition directly from the measured mass to charge ratio. This technique has been utilized with highly complex hydrocarbon mixtures such as petroleum crude and fuel products.[36−40] Via observation of Kendrick mass defects in broadband mass spectra, series of compounds that differ by CH_2 units can be readily identified.[41,42] For example, one TAG molecule may have three fatty acids of the form C18:1, while a second may have two C18:1 fatty acids and one C20:1 fatty acid. The first TAG molecular weight of 907.7731 Da is converted to a Kendrick mass of 906.7594 by multiplying by 14/14.01565. The second mass of 935.80436 Da would have a Kendrick mass of 934.7594. The Kendrick mass defect (nominal Kendrick mass minus exact) of both molecules is equal to 0.2406. Other compounds detected having this Kendrick mass defect can be identified on the basis of knowledge of the elemental composition of a smaller compound in the homologous series. Complex lipid profiles of algae have been elucidated using this technique.[43−46]

Currently, FT-ICR MS instruments are somewhat uncommon. Here we utilize a detailed FT-ICR MS-based lipidomic profile of microalgal lipid extracts as a reference to inform rapid TAG characterization for similar extracts (i.e., from the same species) by lower-resolution mass spectrometers. Specifically, we explore the use of FT-ICR MS to provide a lipid component library for *Nannochloropsis* sp. to develop a high-throughput methodology for generating microalgal TAG profiles and content measurement with direct-infusion electrospray ionization (ESI) time-of-flight (TOF) MS. With this approach, the more common, lower-resolution mass spectrometer may be utilized to rapidly monitor changes in TAG content and composition during research and development of strains of algae and harvest technologies with quantitative performance that exceeds that provided by FT-ICR MS. The lipid component library developed with FT-ICR MS for this study can be easily expanded and disseminated to advance algal lipid research efforts. Ultimately, access to a larger alga specific database will allow for use of high-throughput mass spectrometry as a tool to assist in the development of algal biofuels.

■ EXPERIMENTAL SECTION

Growth and Harvesting of Algae. Biomass from one microalgal strain, *Nannochloropsis* sp. (LRB-MP-0209), was provided by Arizona State University (courtesy of J. McGowen and T. Dempster) and represents harvests taken in early, mid, and late cultivation stages or ranging from high-protein [>30% dry weight (DW) of protein] to

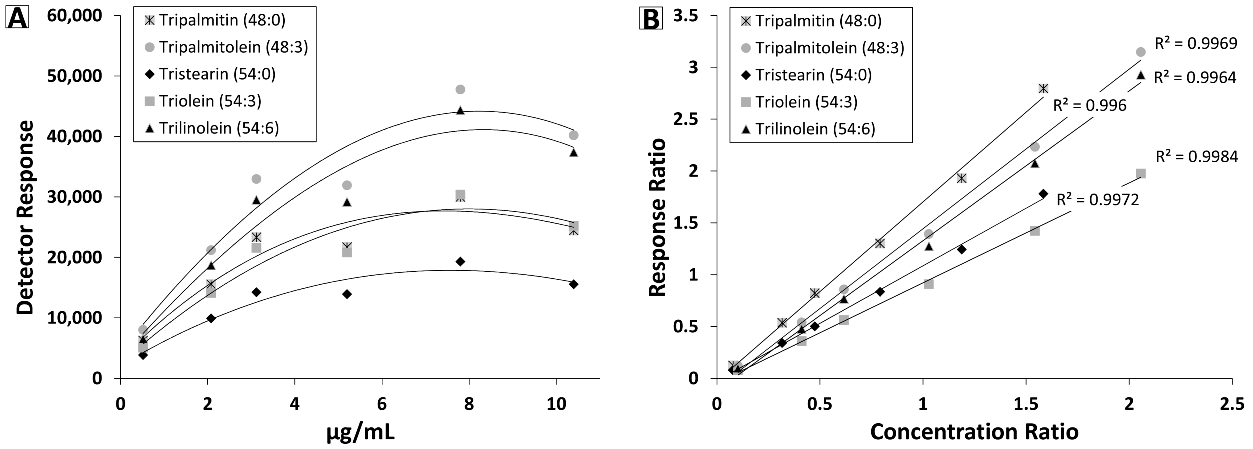

Figure 1. Direct-infusion TOF MS response for TAG standards with increasing concentrations (A) and linear calibration with internal standards (B).

high-lipid (>30% DW of total lipid) content biomass. Details of the growth characteristics and overall biomass composition have been reported previously.[47] By timing the harvest, we obtained biomass of different compositions in a controlled fashion in outdoor flat panel (650 L) photobioreactors in nitrate-depleted cultivation media. The cultivation time after reaching nutrient deplete conditions depended on the final target biomass composition desired, which, depending on the growing season, was 3−5 days for the midpoint harvest biomass and 6−9 days for the high-lipid (late harvest) biomass. The high-protein (early harvest) biomass was obtained by harvesting prior to nutrient depletion.

Lipid Extraction. Lyophilized algal biomass samples were extracted using a pressurized fluid system, ASE 200 Accelerated Solvent Extractor (Thermo-Fisher, Waltham, MA). Biomass (200 mg) was added to a stainless steel cell with a capacity of 11 mL, covered with glass fiber filters on both sides and 3 mm glass beads to fill the extra space in the cell, and extracted with a chloroform/methanol solvent [2:1 (v/v)]. The extraction system was set to reach 50 °C and 1500 psi with a 5 min solvent residence time and five cycles for each extraction. Three consecutive extractions were conducted on each biomass sample and combined to ensure complete extraction. The recovered extract was evaporated under a stream of nitrogen at 40 °C using a TurboVap solvent evaporator (Biotage, Uppsala, Sweden), and the extract weight was recorded after further drying overnight in a vacuum oven at 40 °C.

ESI FT-ICR MS. Direct-infusion ESI FT-ICR MS was employed to provide detailed characterization of algal lipid extracts. Stock lipid extracts were prepared for analysis by dissolution in a chloroform/methanol solvent [2:1 (v/v)] to a concentration of 5 mg/mL. Odd chain fatty acid-containing phosphatidylethanolamine (C17:0/C17:0) (PE) (Avanti Polar Lipids, Alabaster, AL) was added as an internal standard at a concentration of 1 mg/mL. The samples were further diluted to 0.05 mg/mL in a chloroform/methanol solvent [1:2 (v/v)] containing 0.5 mM aqueous sodium acetate. All solvents were high-performance liquid chromatography (HPLC) grade and purchased from Sigma (St. Louis, MO).

Analyses were performed with a hybrid linear ion trap FT-ICR MS system (LTQ FT, Thermo, San Jose, CA) equipped with a Triversa NanoMate chip-based electrospray ionization (ESI) source (Advion, Ithaca, NY). The mass resolving power ($m/\Delta m_{50\%}$) was 400000 at m/z 400. Mass spectra were collected in positive ion mode, and 200 time domain transients were co-added for each sample prior to fast Fourier transformation and frequency to m/z conversion.

Mass spectra were internally calibrated to achieve sub-part-per-million mass measurement accuracy, and elemental compositions were assigned to the observed ions based on the exact mass. The assigned elemental compositions were searched against the Lipid Maps database (Nature, Lipidomics Gateway)[48] using software developed in house. TAGs were isolated from the list of assigned compounds, and Kendrick mass defect analysis was then used to identify TAGs in the mass spectra that were not included in the database. This compiled list of TAGs was then used as a search list for analysis of high-resolution TOF MS spectra.

ESI TOF MS. Direct-infusion ESI TOF MS analyses were conducted using an AccuTOF JMS-T100LC instrument (JEOL, Peabody, MA) equipped with an ESI source. Samples were diluted to a concentration of 0.01 mg/mL in a chloroform/methanol solvent [2:1 (v/v)] and directly infused into the ESI TOF MS instrument using an Agilent Technologies (Santa Clara, CA) 1200 Series automatic liquid sampler to deliver liquid at a constant flow rate of 100 μL/min. Analysis times for each injection were 3 min, including time for the autosampler to make an injection. The direct-infusion solvent was the chloroform/methanol solvent [2:1 (v/v)] containing 50 μM sodium acetate. The ESI desolvating chamber and orifice temperatures were set at 350 and 200 °C, respectively. The capillary voltage was set to 2 kV; the orifice potentials were 65 V for orifice 1 and 5 V for orifice 2, and the ring lens was 10 V. Ultra-high-purity nitrogen was used for both nebulizing and drying gases at flow rates of 1 and 2 L/min, respectively. The scan range for the mass spectrometer was m/z 250−1000. The TOF MS instrument was calibrated using a standard solution of reserpine in acetonitrile to achieve a mass resolving power ($m/\Delta m_{50\%}$) of 7000 at m/z 609 (protonated reserpine). Prior to injection of each sample, a solution of sodium iodide (Waters, Milford, MA) was injected for mass calibration. An internal standard solution containing trinonadecanoin and trinonadecenoin (NuCheck Prep, Elysian, MN) was added to each sample and used as a lock mass to adjust for instrument drift prior to ion assignments. TOF MS spectra were searched for ions identified as TAGs by FT-ICR MS using Mass Mountaineer MS interpretation software (RBC Software, Peabody, MA).

A mixture of TAGs (NuCheck Prep) containing tripalmitin [48:0; acyl carbon:double bond equivalent (DBE)], tripalmitolein (48:3), tristearin (54:0), triolein (54:3), and trilinolein (54:6) was infused in a concentration range of 0.5−10 μg/mL to determine the TOF MS linearity. The instrument response was found to be nonlinear with an increasing concentration; additionally, the response of saturated TAGs was lower than that of unsaturated TAGs at the same concentration (Figure 1A). Two internal standards were added to the TAG mixture, trinonadecanoin (57:0, C19:0/C19:0/C19:0) and trinonadecenoin (57:3, C19:1/C19:1/C19:1), to provide response ratios. The saturated TAGs in the mixture were related to the response of TAG 57:0 and the unsaturated TAGs to 57:3. Linearity was significantly improved with use of internal standards (Figure 1B). This calibration was used to quantify TAGs detected in lipid samples. Calibration curves of standards were applied as follows: acyl carbon range of 42−52 quantified with 48:*X* standards, DBE 0−1 quantified with 48:0, DBE >1 quantified with 48:3, acyl carbon >52 quantified with 54:*X*, DBE 0−1 quantified with 54:0, DBE 2 and 3 quantified with 54:3, and DBE >3 quantified with 54:6.

The resolving power of the TOF MS instrument utilized is insufficient to isolate ion signals of monoisotopic TAGs from $^{13}C_2$ nuclide ions of TAGs with one fewer molecular DBE. For example, the two TAGs 54:0 and 54:1 produce $[M + Na]^+$ ions of m/z 913.820 and 911.804, respectively. The $^{13}C_2$ heavy nuclide ion signal of sodiated 54:1 would be observed at m/z 913.811. Speciation of these two ions requires a resolving power $(m/\Delta m_{50\%})$ of >100000, well beyond the capability of the TOF instrument. Responses of TAGs detected within series separated by a single DBE were corrected by subtracting the parent ion response multiplied by the relative abundance of its $^{13}C_2$ heavy nuclide ion from the next TAG response. In the example given above, the response of 54:0 would be corrected by subtracting the response of 54:1 multiplied by the relative abundance of $^{13}C_2$ heavy nuclide ion (0.2082).

Gas Chromatography. Total TAGs in lipid samples were quantified by GC following ASTM Method D6584 for residual glycerin and glycerides in biodiesel. This method utilizes high-temperature GC to separate TAGs from other lipids for quantification.[32] The concentration of residual TAGs in biodiesel is typically well below 1 wt %;[49] therefore, the method was modified to measure higher concentrations of TAGs. The sample weight was reduced from 100 to <10 mg to keep the TAG concentration within the calibrated range. Other than this modification, the method was followed as written. Analyses were conducted using an Agilent 7890A gas chromatograph equipped with a flame ionization detector, a cool on-column injection port, and a Restek (Bellefonte, PA) MXT-Biodiesel TG capillary column (5% phenyl polydimethyl-siloxane column, 14 m × 0.53 mm, 0.16 μm d_f).

RESULTS AND DISCUSSION

Triglycerides detected with these two mass spectrometric methods were compared by analysis of a sample of commercial soybean oil. Results of these analyses are provided in Figure 2 along with comparison to published results for soybean oil analyzed by HPLC.[50] These HPLC data are provided as a reference for the TAGs known to be present in soy oil; concentrations of individual TAGs are not expected to match the MS data given that these are not identical samples of oil. FT-ICR MS results are presented as percentages of the total observed signal observed for all TAG ions, while TOF and HPLC results are presented as the weight percent of oil. Analysis of the oil by FT-ICR MS detected 28 ions that matched TAGs ranging from 50 to 58 acyl carbons present at a ≥0.1% total signal magnitude of TAGs identified.

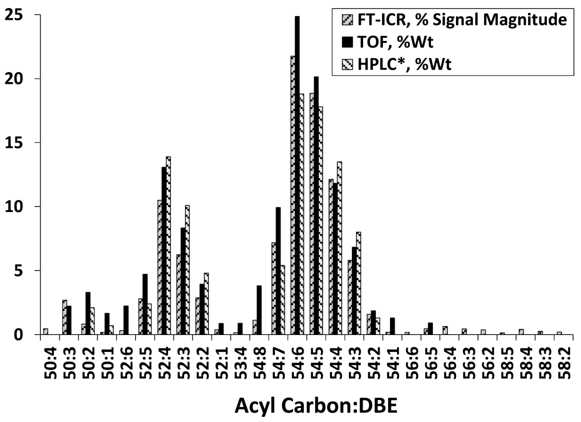

Figure 2. Comparison of TAG profiles of soybean oil determined by direct-infusion FT-ICR MS, TOF MS, and HPLC.

The TOF MS data shown in Figure 2 were generated by searching the soybean oil spectrum for ions corresponding to TAGs detected with FT-ICR MS using the Mass Mountaineer MS interpretation software. For TOF data, m/z-based searches were conducted using ranges of ±5, ±10, and ±15 mDa from the monoisotopic mass of the individual TAGs. The three searches were conducted to determine the mass accuracy range that would allow for assignment of ions to the majority of TAGs detected by FT-ICR MS while minimizing false positives. Searching within 5 mDa accounted for only 60% of the total signal magnitude of the FT-ICR MS results. Increasing the search range up to 10 mDa accounted for 87% of the total signal magnitude. A search range of 15 mDa resulted in assignment of 19 ions that encompassed 97% of the total signal magnitude of the FT-ICR MS data. Many of the TAGs detected by FT-ICR MS are present at low concentrations (14 of the 28 TAGs included in Figure 2 have total abundances of <0.5%) and were not detected by TOF MS. The results of these searches highlight the utility of the FT-ICR MS data as a reference list for the identification of the TAGs found in the TOF MS data. Even though the mass accuracy of the TOF instrument is not as high as that of FT-ICR, a reference list of potential TAGs identified via FT-ICR MS helped to identify molecular TAG ions that would not otherwise be directly identified because of the greater mass measurement error of the TOF instrumentation, which illustrates the high value of using FT-ICR MS data to function as a reference database.

TOF MS quantification of soybean oil TAGs using spectra from triplicate sample preparations resulted in a total TAG measurement of 108 ± 3 wt %. This overestimation is primarily due to the lack of standards for many of the TAGs detected. As shown in Figure 1, the acyl carbon number (fatty acid chain lengths) and DBE influence the response factors of individual TAGs. The presence of many TAGs containing DBE greater than the calibration standard used for quantification as well as mixed fatty acid chain lengths results in application of response factors that do not accurately quantify the TAGs detected. This inaccuracy could be corrected with use of more representative TAG standards; however, many of these compounds are not currently available for purchase. Despite overestimation by 8 wt % total TAGs, direct-infusion TOF MS shows promise for the rapid determination of TAG profiles, especially for monitoring TAG compositional change for a given species across a multisample set, such as in a cultivation or growth time course sample set, for example.

The methodology used for analysis of soybean oil was applied to lipid extracts of *Nannochloropsis* sp. harvested at three stages of growth: early, mid, and late. These extracts were also analyzed by GC to measure the total TAG content to provide a comparison to TOF MS quantification. GC and TOF MS results for total TAG content are provided in Figure 3. There was reasonable agreement between the two methods for the early and midharvest samples; however, the late stage sample was found to have considerably higher TAG content as determined by TOF MS. As noted with soybean oil, the TOF method may overestimate TAGs due to a lack of standards for calibration response factors; alternatively, the GC methodology for TAG quantification may underestimate the total TAG content because of co-elution of major contributing components.

To compare data generated by TOF MS expressed as the weight percent of the lipid extract to the percent TAG signal magnitude data from FT-ICR MS, the TOF MS results were normalized to total TAG weight percent (Figure 4). Most TAGs identified by FT-ICR MS in the algal extracts were also detected with TOF MS, similar to soybean oil. More than 95%

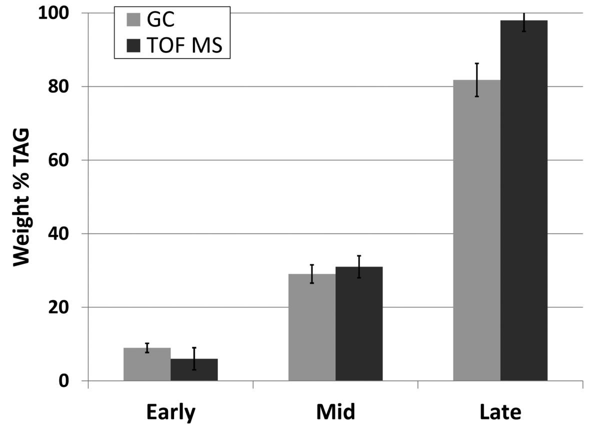

Figure 3. Comparison of total TAGs determined by GC and TOF MS. Error bars for GC results are calculated from the published repeatability of ASTM D6584 (95% confidence interval). Error bars for TOF MS are estimated from triplicate analyses of soybean oil.

of the total FT-ICR MS mass spectral signal of the mid and late harvest samples can be ascribed to TAG ions that are also observed by TOF MS. The early harvest extract had the largest number of TAGs that were not detected by TOF MS where TOF-observed TAGs correspond to 81% of the FT-ICR MS signal derived from TAG. That difference arises primarily from low-abundance compounds that were not observed by TOF MS because of the much higher limit of detection relative to that of the FT-ICR.

The FT-ICR MS analyses detected a total of 61 monoisotopic TAG ions in the three algal lipid extracts. The number of TAG molecular species present in these extracts is undoubtedly higher given that stereoisomers cannot be differentiated by mass. These algal extracts are considerably more complex than soybean oil, which contained only half as many unique TAG ions.

Comparing TAG profiles across the growth stages shows not only that the total TAG concentration increased with time but also that the TAG composition changed dramatically (Figure 4). During early growth, the lipid extract contains numerous TAGs composed of long chain, highly unsaturated fatty acids. For example, TAG 60:15 is prominent in the early harvest extract, which likely contains three C20:5 fatty acids, eicosapentaenoic acid. It has been noted that the composition of total fatty acids found in *Nannochloropsis* biomass harvested from late or high-lipid growth conditions favors a reduced level of unsaturation.[27,51] It was thought that highly unsaturated fatty acids are associated with cell membranes as phospho- and galactolipids, and as the cells become more stressed, lipid composition changes to favor the accumulation of TAGs with concomitant degradation of the photosynthetic apparatus and associated glycolipids, which supported the overall lower degree of unsaturation.[26] The data we present here are the first demonstration that the relative contribution of short chain fatty acids to TAG molecules increases with nutrient deprivation. There have been reports that indicate minor reductions in the contribution of C20:5 and C20:4 fatty acids to TAGs in favor of the short chain C16:1 and C16:0 fatty acids upon nutrient starvation.[27,28,52,53] This can be attributed to a lower energetic cost associated with the biosynthesis of C16-saturated fatty acids, when the overall energy equation of the cells is reduced due to high-metabolic stress conditions. The dramatic shifts observed in our data, e.g., the complete absence of the 60:15, 60:14, and 60:13 TAGs in the last harvest point, indicate considerable architectural modification of the TAG biosynthetic machinery during nitrogen starvation. With downstream processing of TAGs to renewable diesel in mind, accumulation of TAGs with C16 chain fatty acids with a lower degree of unsaturation is preferable. The shorter chain hydrocarbons produced from hydrotreating C16 fatty acids will have improved cold weather operability compared to that with longer chain fatty acids.[54] In addition, lower degrees of

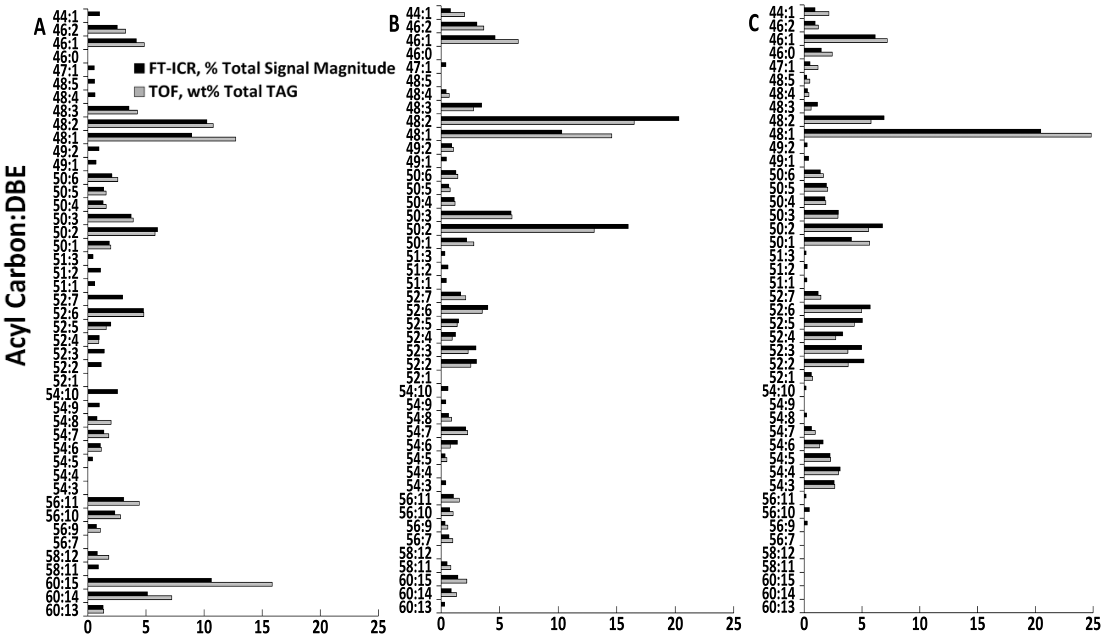

Figure 4. TAG profiles of *Nannochloropsis* sp. lipid extracts determined by FT-ICR and TOF MS for early, mid, and late harvest (A−C, respectively). FT-ICR MS data expressed as a percent of the total TAG ion signal magnitude and TOF MS data expressed as the normalized weight percent of total TAGs. TAGs are identified by their total acyl carbon and DBE values.

unsaturation will require less hydrogen to saturate fatty acids, thus improving the economics of hydrotreating.

■ CONCLUSIONS

Rapid determination of TAG concentration and composition is desirable for comparing strains of algae as well as changes in TAG profiles during growth and in biofuel-bound feedstocks. Direct-infusion TOF MS serves as a high-throughput methodology that takes <3 min to generate detailed TAG profiles. With the assistance of FT-ICR MS, a database of microalgal TAGs can be used to deconvolute and assign TOF mass spectral data for lipid extracts of these samples. Although this lower-resolution mass spectrometry is unable to provide the level of detail that can be gathered with ultra-high-resolution FT-ICR MS, the compounds making the greatest contributions can be rapidly speciated and compared between samples with this more common technology in a rapid, high-throughput manner. Currently existing lipid databases such as the Lipid Maps database are available to provide a TAG library for searching mass spectra; however, these are mainly human- and medical lipid-focused and may be inadequate for algal lipid research. An alga specific library of TAGs identified by FT-ICR MS using Kendrick mass sorting reduces the likelihood of assigning false positives or failing to identify alga specific TAGs when utilizing lower-resolution mass spectrometers. Development and publication of an alga TAG database, including other strains and growth conditions, will allow for the use of high-throughput mass spectrometric methodology for algae-related fuel development research without the need for access to FT-ICR technology.

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Notes

The authors declare no competing financial interest.

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